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[1]Passed away on October 20, 2007
[2]Passed away on June 11, 2007
[3]Passed away on June 14, 2008

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Contents

Contents

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Contents

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EDITORIAL

Hepatitis C virus lymphotropism and peculiar immunological phenotype: Effects on natural history and antiviral therapy

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Abstract

Hepatitis C virus (HCV) has been recognized to be both a hepato- and lymphotropic virus. HCV lymphotropism represents an essential lap in the pathogenesis of virus-related autoimmune and lymphoproliferative disorders, ranging from clonal expansion of B-cells with organ- and non-organ-specific autoantibody production up to overt non-Hodgkin's lymphoma along a continuous step-by-step model of B-cell lymphomagenesis, where the intermediated mixed cryoglobulinemia could be considered as a stage of suppressible antigen-driven lymphoproliferation. HCV infection of lymphoid cells could set up privileged reservoirs able to interfere with the host viral clearance efficiency and may be implicated in viral recurrence after apparently successful antiviral therapy. The HCV long-lasting extrahepatic replicative state generates an abnormal systemic immunological response, easily detectable by searching simple laboratory and clinical parameters, mainly represented by vasculitis-like skin features and hypocomplementemia. The presence or absence of this hypersensitivity pattern seems to correlate with the antiviral response and could be identified as a novel immunological cofactor. Further research is required to fully verify the real impact on therapeutic choice/regimen.

Key words: Hepatitis C virus; Lymphotropism; Natural history; Antiviral therapy; Immunological co-factor

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INTRODUCTION

Hepatitis C virus (HCV) infection has been currently identified as the leading cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma, in Western countries. However, despite its large diffusion (with over 170 million of people infected world-wide), the lack of symptoms during the acute phase, together with the indolent course of the disease over time, hampers the difficulties to assess the natural history of the disease. This complexity can also be argued from the wide heterogeneity of disease complications' rate observed when different methodological approaches were used. Moreover, the progression of the disease could also be dramatically affected by many variables related to the host, the virus and the environment. The global socioeconomic burden of HCV is magnified by hundreds of thousands of infections identified every year. Finally, in the last few years, the long-term outcome of the infected subjects has been deeply modified by the use of efficient antiviral therapy.

HCV has been recognized to be both hepato- and lymphotropic virus; HCV lymphotropism represents an essential lap in the pathogenesis of virus-related immunological disorders[1], being responsible for poly-oligoclonal expansion and consequent wide organ- and non-organ-specific autoantibody production, including rheumatoid factor (RF) and cryo- and non-cryoprecipitable immune complexes.

IMMUNOLOGICAL MECHANISMS

HCV, belonging to the Flaviviridae family, is a positive single-stranded RNA virus, without a DNA intermediate of replication, unable to integrate into the host genome[2].

Otherwise, HCV affects cellular functions modulating the immune response, cell proliferation or apoptosis, so facilitating the clonal B-lymphocyte spread[3-5]. HCV may exert an antigen-driven chronic stimulus on the immune system through several viral proteins[6]. An important pathogenetic step is the interaction between the HCV envelope protein E2 and the CD81 molecule, a fairly ubiquitary tetraspanin present on both hepatocytes and B-cells surface[7], ending up to a strong and sustained polyclonal stimulation of B-cell compartment. CD81 is a part, on the B-cell, of a complex with CD21, CD19 and Leu 13; such complex lowers the threshold for B-cell activation by bridging antigen-specific recognition and CD21-mediated complement recognition[8]. Then, the interaction between HCV-E2 and CD81 could increase the frequency of VDJ rearrangement in antigen reactive B-cells[4,5,9], with possible bcl-2 proto-oncogene activation[4,5]. Again, the latter stage could be secondary to t(14; 18) translocation alone, repeatedly observed in B-cells of HCV-infected individuals, particularly in those with type Ⅱ mixed cryoglobulinemia (MC)[4,6]. Bcl-2 proto-oncogene is able to inhibit apoptosis, leading to abnormal B-cell survival[6]. Besides, the prolonged B-cell survival could prompt, in presence of additional factors (genetic, epigenetic, hormonal and immunological), other genetic aberrations up to overt non-Hodgkin's lymphoma (NHL), as late complication of the MC syndrome[1,9]. The critical question remains whether HCV replication occurs in normal B-cells and is directly lymphomagenic or is lymphomagenesis a stochastic process accompanying HCV-driven proliferation of B-cells[10]. At all, given its biological characteristics, HCV may be involved in several autoimmune and lymphoproliferative disorders, and the multifaceted HCV syndrome can fit in a continuous step-by-step model of B-cell lymphomagenesis, whereby MC could be viewed as marker of antigen-driven lymphoproliferation and frank NHL as loss of antigen-dependence[11].

CLINICAL IMPLICATIONS

At this point, the most scheming and open issue is the definite weight placed on the HCV natural history by its lymphotropism. Firstly, HCV infection of lymphoid cells could condition HCV persistence. In fact, lymphoid cells, and particularly long-living subsets and/or bone-marrow elements, may represent privileged reservoirs able to interfere with host viral clearance efficiency, by impairing the capability of immune response and/or by facilitating selection of distinctive viral variants[12]. Nowadays, it remains indefinite how infection of the immune cells by HCV may alter their functions, although impairment in the allostimulatory capability of HCV-infected dendritic cells derived from patients with chronic hepatitis C has been reported[13]. Interestingly, the entity of this extrahepatic reservoir seems to be correlated with the length of infection[12] and may be implicated in HCV recurrence after apparently successful antiviral therapy[1], the peripheral blood mononuclear cells (PBMC) being a potential viral tank resistant to interferon (IFN). In fact, in several researches PBMC infection appeared as a negative factor of patient on-IFN response[14-18] or predictive for relapse off-IFN monotherapy[15,18-20] or -combined (plus ribavirin) antiviral treatment[21]; on the other hand, such prognostic value was not observed in other studies and is still a controversial question[22-25]. Previous literature data report sustained response (SR) in a near 10% of patients with chronic HCV infection after IFN alone. Classical predictors of response include viral load and genotype, as well as histological (fibrosis score) and metabolic[26] features or alcohol as cofactor. Also the immunological background is associated with antiviral response, the cellular immune functions being essential to the elimination of HCV-infected hepatocytes. A basal low T-helper type 1 and type 2 ratio predicted a higher SR rate in a Japanese cohort[27]. Nevertheless, the immunological pattern remains poorly explored. Conflicting data have been reported on the prevalence of MC in chronic HCV patients, ranging from less than 5% up to 50 %[28,29]. In most cases type M immunoglobulins with RF activity have been found in cryoprecipitates[30], inducing the deposition of immune complexes in small vessels (vasculitis)[31]. The MC-related clinical manifestations, including purpura, arthralgias and weakness, and complications, as glomerulonephritis, neuropathic lesions, B-cell NHL, reflect a systemic involvement that may lessen the chance of viral eradication. In a recent effort[32], it was retrospectively confirmed that this immunological phenotype, also labelled as type Ⅲ or hypersensitivity disorder, is significantly associated with a higher risk of viral persistence after IFN monotherapy, with skin involvement and hypocomplementemia being independent predictors of lack of response; conversely, this study suggested that in the absence of common negative predictors, such as this last immunological cofactor, SR could be reached also by a therapeutic approach based on IFN monotherapy. What practical implications does HCV lymphotropism suggest? No factor is currently available to predict the productive HCV infection of PBMC, but such event is likely to be time-dependent during the natural history of HCV infection. In other words, we observe the serological response and the clinical effects of a long-lasting extrahepatic replicative state, i.e. an abnormal immunological status. This picture can be easily detected by looking for cryoglobulins, RF, antinuclear antibody, complement fractions, circulating immune complexes (C1q protein and C1q binding), mono-oligoclonal gamma-globulin expansion or vasculitis-related clinical manifestations including skin lesions (palpable purpura or hyperpigmented macule of the lower limbs), sensory-motor peripheral neuropathy (gait impairment associated with paresthesia and cramps), arthralgias, as well as urinary changes suggestive of glomerular derangement, i.e. microalbuminuria (in the absence of hypertension and diabetes), all variously combined (at least four out of the above mentioned laboratory and clinical parameters)[32]. This approach is reliable and less expensive or hard than direct detection of HCV in PBMC. The antiviral therapy (IFN plus ribavirin) significantly counteracts the

exaggerated immune response, also independently from the viral outcome[33], through different mechanisms: IFN could affect the intrahepatic T-cell response and inhibit interleukin (IL) 10 production[34], meanwhile ribavirin suppresses IL 10, IL 12 and tumor necrosis factor-alpha[35]; since the hypersensitivity disorders are the expression of a polyclonal activation of B-cells, due to stimulation by T-cells, it could be hypothesized that the changes induced by the combined therapy in the cytokine pattern determine a down-regulation of the mechanism of stimulation T-cells/B-cells. Sometimes, polyclonal B-cell hyperactivity partially escapes from the immune modulation effects of the antiviral treatment, so that the immunological spectrum persists after HCV clearance and suppression of the antigenic stimulus[33]. On the other hand, the undetectability of serum HCV RNA does not mean complete viral clearance, since genomic material has been found in PBMC of SR patients[12], and, therefore, an ongoing immune-stimulation cannot be excluded; interestingly, in those same patients with occult PBMC infection, a persistence of the MC syndrome, even if to a lesser degree with respect to the pre-treatment period, was observed, suggesting a potential advantage of using a prolonged course of antiviral treatment to obtain a sufficient consolidation. The discovery of occult HCV infection has challenged the paradigm that apparent complete resolution of hepatitis C, either spontaneously or therapeutically-induced, would be indicative of eradication of HCV[36]; persistent HCV replication in hepatocytes and PBMC would likely drive the continuous antigenic stimulation of the immune system in immunocompetent patients, which, in turn, allows the host to keep this silent infection under relative control[37]; again, such prolonged HCV replication associated with the chronic presentation of HCV antigens by infected B-cells and monocytes could contribute to the immune tolerance of HCV, thus supporting even further HCV persistence[37]. Finally, negativization of anti-HCV antibody, unrelated to an immunosuppression context, occurs in a percentage of long-term (on average 4 years) SR patients showing a weaker CD4+-specific HCV reactivity; such lessened immunological spur could mirror a full disappearance of the minimal, residual viral amounts, although the potential localization of a further load within PBMC, without release of viral particles into the serum, cannot be ruled out[38]. Only a prolonged follow-up will be able to verify the definitive clearance.

In conclusion, the treatment of hepatitis C is expensive, often demanding, from the patients' perspective, and difficult as far as the decision about whom, when and for how long to treat. Another predictor of response to antivirals is recognized, as immunological cofactor, i.e. the HCV lymphotropism. Further studies are warranted to evaluate alternative antiviral schedules depending on the presence or the absence of this additional cofactor.

REFERENCES

1 **Zignego AL**, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, Maggi E, Romagnani S, Gentilini P, Bréchot C. Infection of peripheral mononuclear blood cells by hepatitis C virus. *J Hepatol* 1992; **15**: 382-386

2 **Gatza ML**, Chandhasin C, Ducu RI, Marriott SJ. Impact of transforming viruses on cellular mutagenesis, genome stability, and cellular transformation. *Environ Mol Mutagen* 2005; **45**: 304-325

3 **Alisi A**, Giannini C, Spaziani A, Anticoli S, Caini P, Zignego AL, Balsano C. Hepatitis C virus core protein enhances B lymphocyte proliferation. *Dig Liver Dis* 2007; **39** Suppl 1: S72-S75

4 **Zignego AL**, Ferri C, Pileri SA, Caini P, Bianchi FB. Extrahepatic manifestations of Hepatitis C Virus infection: a general overview and guidelines for a clinical approach. *Dig Liver Dis* 2007; **39**: 2-17

5 **Rosa D**, Saletti G, De Gregorio E, Zorat F, Comar C, D'Oro U, Nuti S, Houghton M, Barnaba V, Pozzato G, Abrignani S. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102**: 18544-18549

6 **Zignego AL**, Ferri C, Giannelli F, Giannini C, Caini P, Monti M, Marrocchi ME, Di Pietro E, La Villa G, Laffi G, Gentilini P. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas. *Ann Intern Med* 2002; **137**: 571-580

7 **Pileri P**, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998; **282**: 938-941

8 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Pileri SA, Zignego AL. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** Suppl 1: S13-S21

9 **Machida K**, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, Levine AM, Lai MY, Lai MM. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. *Proc Natl Acad Sci USA* 2004; **101**: 4262-4267

10 **Agnello V**, De Rosa FG. Extrahepatic disease manifestations of HCV infection: some current issues. *J Hepatol* 2004; **40**: 341-352

11 **Suarez F**, Lefrere F, Besson C, Hermine O. Splenic lymphoma with villous lymphocytes, mixed cryoglobulinemia and HCV infection: deciphering the role of HCV in B-cell lymphomagenesis. *Dig Liver Dis* 2007; **39** Suppl 1: S32-S37

12 **Zignego AL**, Giannini C, Monti M, Gragnani L. Hepatitis C virus lymphotropism: lessons from a decade of studies. *Dig Liver Dis* 2007; **39** Suppl 1: S38-S45

13 **Bain C**, Fatmi A, Zoulim F, Zarski JP, Trépo C, Inchauspé G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. *Gastroenterology* 2001; **120**: 512-524

14 **Qian C**, Camps J, Maluenda MD, Civeira MP, Prieto J. Replication of hepatitis C virus in peripheral blood mononuclear cells. Effect of alpha-interferon therapy. *J Hepatol* 1992; **16**: 380-383

15 **Saleh MG**, Tibbs CJ, Koskinas J, Pereira LM, Bomford AB, Portmann BC, McFarlane IG, Williams R. Hepatic and extrahepatic hepatitis C virus replication in relation to response to interferon therapy. *Hepatology* 1994; **20**: 1399-1404

16 **Basaras M**, de las Heras B, García Bengoechea M, Gallego L, Arrese E, Cisterna R. Detection of hepatitis C virus RNA in serum and peripheral blood mononuclear cells in patients with chronic hepatitis C treated with interferon alpha. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 887-890

17 **Cribier B**, Uhl G, Schmitt C, Doffoël M, Vetter D, Kirn A, Stoll-Keller F. Follow-up of hepatitis C virus RNA in peripheral blood mononuclear cells during interferon therapy. *Arch Virol* 1999; **144**: 355-364

18 **Gong GZ**, Lai LY, Jiang YF, He Y, Su XS. HCV replication in PBMC and its influence on interferon therapy. *World J Gastroenterol* 2003; **9**: 291-294

19 **Kusaka S**, Okusa T, Araki A, Fujiki K, Takashimizu I, Okayasu I, Yamamoto N, Sato C. Prediction of relapses after interferon-alpha therapy by hepatitis C virus RNA in peripheral blood mononuclear cells. *J Med Virol* 1995; **46**: 265-268

20 **Taliani G**, Badolato C, Lecce R, Poliandri G, Bozza A, Duca F, Pasquazzi C, Clementi C, Furlan C, De Bac C. Hepatitis C virus RNA in peripheral blood mononuclear cells: relation with response to interferon treatment. *J Med Virol* 1995; **47**: 16-22

21 **Kessler HH**, Pierer K, Santner BI, Vellimedu SK, Stelzl E, Marth E, Fickert P, Stauber RE. Evaluation of molecular parameters for routine assessment of viremia in patients with chronic hepatitis C who are undergoing antiviral therapy. *J Hum Virol* 1998; **1**: 314-319

22 **Peerlinck K**, Willems M, Sheng L, Nevens F, Fevery J, Yap SH, Vermylen J. Rapid clearance of hepatitis C virus RNA in peripheral blood mononuclear cells of patients with clotting disorders and chronic hepatitis C treated with alpha-2b interferon is not a predictor for sustained response to treatment. *Br J Haematol* 1994; **86**: 816-819

23 **Moonka DK**, Henzel BS, Gutekunst K, O'Brien CB. Quantitative assessment of hepatitis C virus RNA in peripheral blood mononuclear cells during therapy with interferon-alpha2a. *J Viral Hepat* 1998; **5**: 27-33

24 **Magalini AR**, Facchetti F, Salvi L, Fontana L, Puoti M, Scarpa A. Clonality of B-cells in portal lymphoid infiltrates of HCV-infected livers. *J Pathol* 1998; **185**: 86-90

25 **García-Bengoechea M**, Basaras M, Barrio J, Arrese E, Montalvo II, Arenas JI, Cisterna R. Late disappearance of hepatitis C virus RNA from peripheral blood mononuclear cells in patients with chronic hepatitis C in sustained response after alpha-interferon therapy. *Am J Gastroenterol* 1999; **94**: 1902-1905

26 **Tarantino G**, Conca P, Sorrentino P, Ariello M. Metabolic factors involved in the therapeutic response of patients with hepatitis C virus-related chronic hepatitis. *J Gastroenterol Hepatol* 2006; **21**: 1266-1268

27 **Shirakawa H**, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, Ichijo T, Yoshizawa K, Kiyosawa K, Tanaka E. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; **48**: 1753-1760

28 **Persico M**, De Marino FA, Di Giacomo Russo G, Persico E, Morante A, Palmentieri B, Torella R. Prevalence and incidence of cryoglobulins in hepatitis C virus-related chronic hepatitis patients: a prospective study. *Am J Gastroenterol* 2003; **98**: 884-888

29 **Horcajada JP**, García-Bengoechea M, Cilla G, Etxaniz P, Cuadrado E, Arenas JI. Mixed cryoglobulinaemia in patients with chronic hepatitis C infection: prevalence, significance and relationship with different viral genotypes. *Ann Med* 1999; **31**: 352-358

30 **Sansonno D**, Lauletta G, Nisi L, Gatti P, Pesola F, Pansini N, Dammacco F. Non-enveloped HCV core protein as constitutive antigen of cold-precipitable immune complexes in type II mixed cryoglobulinaemia. *Clin Exp Immunol* 2003; **133**: 275-282

31 **Sansonno D**, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. *Lancet Infect Dis* 2005; **5**: 227-236

32 **Tarantino G**, Conca P, Ariello M, Arena A. Exaggerated immune reactions predict the outcome of interferon therapy in patients with chronic hepatitis C. *Int J Immunopathol Pharmacol* 2007; **20**: 837-840

33 **Riccio A**, Conca P, Marzocchella C, Tarantino G. Rheumatoid factor after antiviral therapy in patients with HCV chronic hepatitis. *Clin Exp Rheumatol* 2008; **26**: 926-928

34 **Rahman F**, Heller T, Sobao Y, Mizukoshi E, Nascimbeni M, Alter H, Herrine S, Hoofnagle J, Liang TJ, Rehermann B. Effects of antiviral therapy on the cellular immune response in acute hepatitis C. *Hepatology* 2004; **40**: 87-97

35 **Barnes E**, Salio M, Cerundolo V, Medlin J, Murphy S, Dusheiko G, Klenerman P. Impact of alpha interferon and ribavirin on the function of maturing dendritic cells. *Antimicrob Agents Chemother* 2004; **48**: 3382-3389

36 **Carreño V**, Bartolomé J, Castillo I, Quiroga JA. Occult hepatitis B virus and hepatitis C virus infections. *Rev Med Virol* 2008; **18**: 139-157

37 **Pham TN**, Michalak TI. Occult persistence and lymphotropism of hepatitis C virus infection. *World J Gastroenterol* 2008; **14**: 2789-2793

38 **Marinho RT**, Pinto RM, Santos ML, de Moura MC. Lymphocyte T helper-specific reactivity in sustained responders to interferon and ribavirin with negativation (seroreversion) of anti-hepatitis C virus. *Liver Int* 2004; **24**: 413-418

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EDITORIAL

Clinicopathological features of early gastric cancer with duodenal invasion

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Abstract

The incidence of early gastric cancer (EGC) with duodenal invasion is extremely low, although advanced gastric cancer that arises in the antrum occasionally invades the duodenum. We investigated the clinicopathological features of EGC with duodenal invasion and provided strategies for clinical management. A Medline search was performed using the keyword "early gastric cancer" and "duodenal invasion". Additional articles were obtained from references within the papers identified by the Medline search. We revealed that EGC with duodenal invasion was of the superficial spreading type of tumor. Tumors > 60 mm in size invaded the duodenum more extensively, and the distance of duodenal invasion from the pyloric ring was further in the elevated type than in the depressed type of tumor. There was no significant difference between the length of duodenal invasion and the histological type of the tumor. Gastric cancer located adjacent to the pyloric ring, even if cancer invasion was confined to the mucosa or submucosa, was more likely to invade the duodenum. The present study reveals that the elevated type of EGC is associated with more extensive duodenal invasion when the tumor size is > 60 mm, thus highlighting the importance of identification of duodenal invasion in these cases. We also reveal that sufficient duodenal resection with a cancer-free distal surgical margin should be performed in cases of duodenal invasion.

Key words: Duodenal invasion; Early gastric cancer; Gastrectomy; Superficial spreading type; Tumor differentiation

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INTRODUCTION

Early gastric cancer (EGC), which is defined as a lesion confined to the mucosa or the submucosa, regardless of the presence of lymph node metastasis, has a good prognosis with surgical treatment. However, a small number of patients experience recurrence of EGC after resection. Sano *et al*[1] have reported that, in a study of 1475 patients with EGC treated with surgery, 1.4% experienced disease recurrence. The incidence of recurrence of EGC was shown to be significantly higher in the patient group with submucosal, node-positive and undifferentiated tumors. Furthermore, some rare cases show distant metastasis, such as in liver, lung, or bone, even though the depth of cancer invasion is confined to the mucosa[2]. Sufficient resection margins are necessary to prevent recurrence of EGC, because inadequate resection that does not maintain surgical margins free of cancer can lead to disease recurrence. Duodenal invasion by gastric cancer is encountered in 11.9%-23.8% of all patients with cancer in the gastric antrum[3-5]. However, EGC with duodenal invasion is rare amongst cases of advanced gastric cancer[6]. There have been very few case reports of this type of cancer. Since the literature on this subject consists mostly of isolated case reports, the clinicopathological features of EGC with duodenal invasion remain unclear. We attempted to elucidate the clinicopathological features of patients with EGC extended to the duodenum, and discuss the possible mechanisms underlying this rare condition and practical surgical strategies.

PATIENTS AND CLINICOPATHOLOGICAL PRESENTATION

We reviewed 41 patients who underwent surgical resection for EGC with duodenal invasion between

Table 1 Clinicopathological data for 41 cases of EGC with duodenal invasion

Authors	Year	Age (yr)	Gender	Location	Type	Size (mm)	Depth of invasion	Lymph node metastasis	Histological type	Distance of duodenal invasion (mm)	Preoperative diagnosis
Ishii	1975	50	M	Circ	Depressed	32 × 25	m	-	Intestinal	7	ND
		47	M	Less	Elevated	30 × 15	sm	-	Intestinal	5	ND
Kuwayama[15]	1976	72	M	Ant-Less	Depressed	40 × 35	m	-	Diffuse	4	Impossible
Uchida[13]	1979	50	M	Circ	Depressed	32 × 25	m	-	Diffuse	7	ND
		61	M	ND	Mixed	35 × 21	sm	-	Intestinal	2	ND
		47	M	ND	Elevated	30 × 15	sm	-	Intestinal	5	ND
Kuwata[18]	1981	ND	ND	Less	Depressed	ND	sm	-	Diffuse	5	Impossible
		ND	ND	Gre	Elevated	ND	sm	-	Intestinal	6	Impossible
		ND	ND	Less	Elevated	ND	sm	-	Intestinal	2	Impossible
		ND	ND	Post	Mixed	ND	sm	-	Intestinal	1	Impossible
		ND	ND	Less	Mixed	30 × 15	sm	-	Intestinal	2	Impossible
		ND	ND	Less	Depressed	35 × 21	sm	-	Intestinal	1	Impossible
		67	M	Less	Mixed	30 × 15	sm	-	Intestinal	5	Impossible
Kato[17]	1993	63	F	Circ	Elevated	68 × 38	m	-	Intestinal	16	Possible
Nakazawa[9]	1994	58	M	Ant-Gre	Depressed	10 × 10	sm	-	Intestinal	3	Impossible
Boku[27]	1996	73	F	Circ	Elevated	70	sm	-	Intestinal	35	Possible
Ito[14]	1996	76	F	Less	Mixed	45 × 35	sm	-	Intestinal	25	Possible
Matsumoto[16]	2000	ND	ND	Less	Elevated	25 × 9	m	ND	Diffuse	3	Impossible
		61	M	Gre-Post	Elevated	25 × 10	sm	ND	Diffuse	10	Possible
		ND	ND	ND	Elevated	30 × 13	m	ND	Intestinal	3	Impossible
		ND	ND	ND	Elevated	65 × 23	m	ND	Intestinal	3	Impossible
		ND	ND	ND	Superficial	45 × 45	sm	ND	Intestinal	5	Impossible
Nogueira[12]	2000	ND	ND	ND	ND	ND	m	-	Diffuse	3	ND
		ND	ND	ND	ND	ND	sm	-	Intestinal	7	ND
Yasuda[23]	2000	59	F	Circ	Depressed	72 × 15	m	-	Diffuse	11	Impossible
Nakayama[37]	2001	59	F	Circ	Mixed	85 × 75	sm	-	Intestinal	38	Possible
Koufuji[29]	2003	77	M	Circ	Mixed	70 × 50	sm	-	Intestinal	2	ND
		65	M	Circ	Elevated	90 × 55	sm	-	Intestinal	2	ND
		66	F	Circ	Depressed	120 × 98	m	-	Diffuse	2	ND
		70	F	Circ	Depressed	130 × 102	m	-	Diffuse	8	ND
		58	M	Less	Depressed	55 × 24	sm	+	Diffuse	3	ND
		81	F	Gre	Depressed	30 × 20	sm	+	Intestinal	2	ND
		44	M	Less	Depressed	52 × 30	sm	-	Intestinal	5	ND
		57	F	Circ	Depressed	57 × 33	m	-	Diffuse	3	ND
		68	F	Gre	Depressed	40 × 38	sm	+	Diffuse	5	ND
		58	F	Circ	Depressed	80 × 65	sm	+	Diffuse	2	ND
Ishikawa[24]	2005	72	M	Less-Post	Elevated	68 × 37	m	-	Intestinal	20	Possible
Matsuda[6]	2007	79	F	Circ	Elevated	30 × 15	sm	-	Intestinal	12	Possible
Our case	2008	49	M	Less	Depressed	30 × 12	m	-	Intestinal	1	Impossible
		63	M	Less	Elevated	35 × 15	m	-	Intestinal	3	Impossible
		84	F	Circ	Elevated	85 × 80	m	-	Intestinal	38	Possible

F: Female; M: Male; ND: Not described; Circ: Circumferential; Less: Lesser curvature; Gre: Greater curvature; Ant: Anterior; Post: Posterior; Intestinal: Papillary and tubular adenocarcinomas; m: Mucosa; sm: Submucosa; Diffuse: Poorly differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma.

1975 and 2008. Thirty-eight cases were identified in the available literature using a Medline search and Japan Centra Revuo Medicina by use of the keywords "early gastric cancer" and "duodenal invasion". Additional articles were obtained from references within the papers identified by the searches. Three cases were patients treated in our hospital. Data on age, gender, tumor location, tumor type, tumor size, depth of invasion, lymph node metastasis, histological type, and preoperative diagnosis of duodenal invasion for each patient were obtained. The clinicopathological features of the 41 reported cases are listed in Table 1. Of the 41 patients analyzed, the mean age of patients was 63.2 years (range, 44-84 years), and there was a slight male predominance, with a male-to-female ratio of 16:13. The average diameter of tumors was 51.6 mm (range, 10-130 mm). The average distance of duodenal invasion was 7.9 mm (range, 1.0-38 mm). The case with the maximal distance of duodenal invasion was one of our cases. All patients had undergone curative tumor resection. There was no lymph node metastasis in cases in which the tumor was confined to the mucosa, whereas of the 25 patients in which the tumor had invaded the submucosa, four had lymph node metastasis. There was no lymphatic or venous invasion or distant metastasis.

The Mann-Whitney *U* test was used to assess correlations among the mean values for each group. The Pearson χ^2 test was applied to qualitative variables. All values are expressed as mean ± SD. $P < 0.05$ was considered significant.

EFFECT OF TUMOR INVASION DISTANCE

Table 2 shows the results of univariate analysis of the distance of duodenal invasion from the pyloric ring in relation to eight selected variables: age, gender, gross appearance, tumor size, depth of invasion, histological type, lymph node metastasis, and preoperative diagnosis

Table 2 Clinicopathological characteristics of EGC with duodenal invasion

Characteristics	No. of patients	Length of duodenal invasion (mm)	*P* value
Age (yr)			0.276
< 60	13	7.2	
> 60	16	11.7	
Gender			0.029
Male	16	5.3	
Female	13	15.2	
Gross appearance			0.046
Depressed	16	4.3	
Elevated	15	10.9	
Tumor size (mm)			0.049
< 60	23	5.3	
> 60	12	14.8	
Depth of invasion			0.836
Mucosa	16	8.3	
Submucosa	25	7.6	
Histological type			0.088
Intestinal	28	9.1	
Diffuse	13	5.1	
Lymph node metastasis			0.006
Negative	32	8.9	
Positive	4	3	
Preoperative diagnosis of duodenal invasion			0.001
Possible	8	24.3	
Impossible	16	3.6	

of duodenal invasion. The distance of duodenal invasion by EGC was 4.5 mm for depressed type tumors, 11.4 mm for elevated type tumors, 5.3 mm for tumors with a diameter < 60 mm, and 14.8 mm for tumors with a diameter > 60 mm. These results revealed a positive correlation between more extensive duodenal invasion and elevated type tumors with a size > 60 mm.

In advanced gastric cancer, the rate of metastasis to the lymph nodes was high when the distance of duodenal invasion was > 10 mm[7]. By comparison, we found lymph node metastasis in only four cases of EGC, and in each of these, invasion had reached the submucosa and the distance of duodenal invasion was < 10 mm. This result suggests that there is a strong positive correlation between the incidence of lymph node metastasis and submucosal invasion, regardless of the distance of duodenal invasion.

PREOPERATIVE DIAGNOSIS OF EGC WITH DUODENAL INVASION

Generally, preoperative diagnosis of malignant invasion to the duodenum is difficult[8,9], because spread of gastric cancer to the duodenum is often infiltrative and invades directly through the submucosal or subserosal layer[10-12]. Most of these cases are advanced gastric cancer[13]. In EGC, gastroenteroscopic examination is a reliable technique for identifying the area of cancer infiltration[14]. It is necessary to accurately define the tumor margin in order to determine the resection line. However, it is occasionally difficult to accurately determine the margin of the tumor in the vicinity of the pyloric ring by endoscopy[15-17]. This is because the pyloric ring is a narrow lumen, making it difficult to observe the tumor, and it can be deformed by ulcers, mucosal atrophy, and metaplastic changes. Moreover, pyloric movement caused by strong peristalsis and reflux of bile prevent the satisfactory observation of the lesion on the pyloric ring[15].

Duodenal invasion by EGC was diagnosed preoperatively by esophagogastroduodenoscopy (EGD) or barium meal examination in only eight cases (Table 1). The mean distance of duodenal invasion was 24.3 mm in the group in which a preoperative diagnosis was possible, whereas it was 3.6 mm in the group in which a preoperative diagnosis was not possible. There was a significant difference between the two groups (Table 2). In these cases, the distance of duodenal invasion was greater for elevated or mixed type tumors > 10 mm in diameter. Of the nine cases in which the distance of duodenal invasion was > 10 mm, there was only one case in which a preoperative diagnosis of duodenal invasion was not possible. By comparison, no case could be diagnosed preoperatively where the distance of duodenal invasion was < 10 mm. These results suggest that a preoperative diagnosis of duodenal invasion is related to tumor type and size. Kuwata *et al*[18] have reported that radiological diagnosis of duodenal invasion is more useful in the elevated type than in the depressed type of tumor, and that the compression method gives a more accurate diagnosis than the double-contrast method. Furthermore, despite extensive preoperative examination, determination of the tumor margin is often not possible in patients with a superficial spreading type of gastric cancer[19-22]. Thus, a satisfactorily precise diagnostic approach to assess the extent of tumor invasion has not been established.

MECHANISMS OF DUODENAL INVASION BY EGC

The border between the stomach and the duodenum is not clinically obvious. Brunner's glands can be considered as the start of the duodenum for the clinicopathological assessment of duodenal invasion by gastric cancer[3,13]. When gastric cancer directly invades the mucosal layer, the Brunner's glands remain intact, even when surrounded by cancer cells[3]. For this reason, it is thought that Brunner's glands prevent direct cancer invasion from the gastric mucosa to the duodenal mucosa. In a study of 141 patients with gastric carcinoma with duodenal invasion, there was only one case of intramucosal carcinoma[3]. In the case of a lesion caused by an ulcer, it is speculated that destruction of the mucosal structure of the duodenum by an ulcer located in the pylorus allowed gastric cancer to invade the duodenum[23]. In another case in which endoscopic mucosal resection (EMR) had been performed previously for gastric cancer in the area of the pyloric ring, it is thought that destruction of the gastroduodenal mucosal microanatomy by EMR allowed carcinoma cells to invade the duodenal mucosa[24].

The superficial spreading type of EGC is characterized by wide and superficial spreading activity of the

Table 3 Clinicopathological characteristics of EGC with duodenal invasion for superficial spreading and small-sized types

Characteristics	Superficial spreading type	Small-sized type	*P* value
Number of cases (%)	10 (27.0)	27 (73.0)	
Age (yr)	68.7 ± 8.1	60.4 ± 11.2	0.031
Gender			0.048
Male	3	13	
Female	7	6	
Gross appearance			0.281
Depressed	3	12	
Elevated	5	8	
Depth of invasion			0.614
Mucosa	5	11	
Submucosa	5	16	
Histological type			0.847
Intestinal	7	18	
Diffuse	3	9	
Lymph node metastasis			0.773
Negative	9	19	
Positive	1	3	
Length of duodenal invasion (mm)	16.3	5.4	0.044
Preoperative diagnosis of duodenal invasion			0.003
Possible	5	3	
Impossible	0	12	

cancer compared with a more limited depth of vertical invasion[25]. According to Yasui *et al*[26], EGC is classified as a superficial spreading type of tumor when the product of the longest diameter of the tumor and the diameter perpendicular to it is > 25 cm^2. Our study has revealed that gastric cancer with duodenal invasion is most often the superficial spreading type. Relations between the superficial spreading tumor and duodenal invasion of EGC may refer to multiple occurrence of cancer[27]. Previous authors have reported that the superficial spreading type accounted for 5.46%-11.0% of all EGC[19-22], whereas it accounted for 27.0% of EGC cases with duodenal invasion (Table 3). Duodenal invasion was more extensive in superficial spreading cancer lesions (16.3 mm) than in small-sized cancer lesions (5.4 mm). In both of these groups, there was no significant difference in the gross appearance, depth of invasion, histological type, or the incidence of lymph node metastasis. Taken together, these results suggest that the superficial spreading type of gastric cancer adjacent to the pyloric ring may have the potential to invade the duodenum.

STRATEGY FOR SURGICAL TREATMENT OF EGC WITH DUODENAL INVASION

The outcome of surgical treatment for EGC is generally considered to be satisfactory[1,28]. If EGC is treated with the appropriate surgical strategy, the outcome of treatment is excellent, even in patients with duodenal invasion[29]. However, Kakeji *et al*[30] analyzed 95 patients with duodenal invasion by gastric cancer, including advanced cases, and found that tumor spread into the duodenum was limited to within 2 cm in 76% of the patients and to within 3 cm in 81% of the patients. Therefore, for patients with advanced gastric cancer with duodenal invasion, gastrectomy with resection of 3-4 cm of the duodenum and sufficient lymph node dissection is recommended.

Recent advances in endoscopic and laparoscopic surgery now offer a better quality of life to patients with EGC[31]. Although the 5-year survival rate for EGC is ⩾ 90%[32], complete surgical extirpation of gastric cancer with a sufficient resection margin from the tumor, and removal of metastatic lymph nodes, is necessary for good prognosis in all EGC cases, including those with duodenal invasion[1,29,31,32]. Previous reports have revealed that the prognosis of gastric cancer patients is affected mostly by depth of invasion, followed by lymph node metastasis and tumor location[33,34]. Tumor size in gastric cancer is a reliable prognostic factor that might be a suitable candidate for use in the staging system[35]. However, tumor size is not an independent prognostic factor[36]. Tumor diameter > 3.5 cm has been identified as an independent factor for the occurrence of lymph node metastasis[33]. Our review revealed that many cases of EGC with duodenal invasion had larger tumors, with an average diameter of 51.6 mm, than cases without duodenal invasion. Among the cases of EGC with duodenal invasion, there was no cancer recurrence because suitable surgical resection had been performed. It is necessary for surgeons to identify a suitable resection line for the distal margin for preoperative diagnosis of duodenal invasion. In cases in which there is further extension of the tumor toward the duodenum, it may be necessary to determine a resection line using intraoperative EGD[37].

The indistinct tumor margins characteristic of superficial spreading tumors in EGC can lead to discrepancies in tumor area between surgical findings and pathological diagnosis[20,22]. Kasakura *et al*[19] have reported that, despite extensive preoperative examination, determination of the tumor margin was not possible in 26 of 59 patients with superficial spreading cancer. Furthermore, the number of metastatic lymph nodes was greater than with the common tumor type[21]. Accordingly, gastrectomy with extensive lymph node dissection with wide and sufficient surgical margin seems to be a most appropriate treatment for the superficial spreading type of EGC, including those cases with duodenal invasion. Based on these findings, treatment of superficial spreading type EGC, in which the distal margin is near the pyloric ring, should focus on attaining a satisfactory margin from the tumor.

CONCLUSION

Gastric cancer located adjacent to the pyloric ring, even if cancer invasion is confined to the mucosal or submucosal layer, has the potential for duodenal invasion, and surgeons should be aware of this possibility. The present study indicates that EGC of the elevated type with a tumor size > 60 mm correlates positively with more extensive duodenal invasion. Our findings highlight the importance of identification of duodenal invasion by pre- and intra-operative closed observation, and reveal that the resection line in cases of duodenal invasion should be performed with a cancer-free margin.

REFERENCES

1 **Sano T**, Sasako M, Kinoshita T, Maruyama K. Recurrence of early gastric cancer. Follow-up of 1475 patients and review of the Japanese literature. *Cancer* 1993; **72**: 3174-3178
2 **Kobayashi M**, Okabayashi T, Sano T, Araki K. Metastatic bone cancer as a recurrence of early gastric cancer -- characteristics and possible mechanisms. *World J Gastroenterol* 2005; **11**: 5587-5591
3 **Kakeji Y**, Tsujitani S, Baba H, Moriguchi S, Mori M, Maehara Y, Kamegawa T, Sugimachi K. Clinicopathologic features and prognostic significance of duodenal invasion in patients with distal gastric carcinoma. *Cancer* 1991; **68**: 380-384
4 **Perng DS**, Jan CM, Wang WM, Chen LT, Liu CS, Huang TJ, Chen CY. Clinicopathologic study of gastric carcinoma with duodenal invasion. *Kaohsiung J Med Sci* 1996; **12**: 461-465
5 **Castleman B**. Extension of gastric carcinoma into the duodenum. *Ann Surg* 1936; **103**: 348-352
6 **Matsuda A**, Kato S, Furuya M, Shimizu Y, Okino T, Sasaki J, Tajiri T. Multiple early gastric cancer with duodenal invasion. *World J Surg Oncol* 2007; **5**: 125
7 **Yamamoto K**. A clinicopathological study of gastric cancer with duodenal invasion. *Jpn J Gastroenterol Surg* 1993; **26**: 2293-2301
8 **Koehler RE**, Hanelin LG, Laing FC, Montgomery CK, Margulis AR. Invasion of the duodenum by carcinoma of the stomach. *AJR Am J Roentgenol* 1977; **128**: 201-205
9 **Nakazawa O**, Hirayama Y, Kure T, Matsumoto S, Terada S, Koda K, Takayanagi N, Ezoe A, Ikeda J, Hishiyama H, Ando M. Invasion of the duodenal bulb by early gastric carcinoma, report of a case. *Med J Asahikawa RCH* 1994; **8**: 80-83
10 **Ohta J**, Kodama I, Takamiya H, Mizutani K, Yano S, Aoyagi K, Koufuji K, Takeda J, Shirouzu K. A clinicopathological study of distal advanced gastric carcinoma with duodenal invasion. *Kurume Med J* 1996; **43**: 189-198
11 **Zinninger MM**, Collins WT. Extension of carcinoma of the stomach into the duodenum and esophagus. *Ann Surg* 1949; **130**: 557-566
12 **Nogueira AM**, Silva AC, Paiva EB, Carvalho SP, Salles PG. [Distal gastric carcinoma with duodenal invasion. Histopathologic study and review of the literature] *Arq Gastroenterol* 2000; **37**: 168-173
13 **Uchida Y**, Nogawa T, Yamashita M, Hashimoto S, Fujii R, Azekura K, Hashimoto Y, Ishikawa Y, Kotake Y, Ino M, Hidaka S, Kitasato S, Ooe H, Shibata O, Ishii T, Shimoyama T, Miura T, Tsuji Y, Sekine I. Clinicopathological studies on carcinoma invading in stomach and duodenum. *Jpn J Gastroenterol Surg* 1979; **12**: 891-900
14 **Ito T**, Hara A, Yoshioka M, Kodama H, Tokui M, Yamakawa H, Nagata H, Yamataka K, Takizawa K, Ishii T, Ishikawa Y. A case of early gastric cancer with white-like duodenal invasion. *Gastroenterol Endosc* 1996; **38**: 2889-2893
15 **Kuwayama H**, Sato Y, Okazaki M, Iwaki K, Gomi K, Hayashi T, Koizumi H, Honda T. A case of duodenal infiltration of early gastric cancer. *Prog of Dig Endosc* 1976; **9**: 173-175
16 **Matsumoto M**, Azuma M, Shimoda W, Tanaka N, Okawa T, Yamaguchi N, Ito I, Sasaki K, Miyachi K, Nanba N, Takada E, Sunagawa M. A study of early gastric cancer in the vicinity of the pylorus ring or invading the duodenum. *Jpn J Surg Assoc* 2000; **61**: 22-26
17 **Kato T**, Yoshimura H, Itoh Y, Nishiwaki H, Takeuchi K, Hukuura T, Takeuchi T, Ishikura N, Ota M, Yamamoto T, Tada T. Early gastric carcinoma with continuous extension to the duodenal bulb: report of a case. *Stomach and Intestine* 1993; **28**: 195-201
18 **Kuwata H**, Katsumata T, Saigenji K, Okabe H, Hiki Y, Ohida M, Atari H, Mieno H. Histological study on the extension of early gastric cancer into the duodenum. *Prog of Dig Endosc* 1981; **18**: 124-127
19 **Kasakura Y**, Fujii M, Mochizuki F, Imai S, Kanamori N, Suzuki T. Clinicopathological features of the superficial spreading type of early gastric cancer. *Gastric Cancer* 1999; **2**: 129-135
20 **Imai M**, Kondo Y, Osawa S, Nishida Y, Okada K, Ishizu H, Masuko H, Hata T, Uemura K, Kina M, Honda S, Ishiyama G, Takahashi T, Hino A. Clinicopathological characteristics of superficial spreading type early gastric cancer. *J Surg Oncol* 2003; **83**: 94-98
21 **Kunisaki C**, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono H, Shimada H. Surgical outcome in superficially spreading early gastric cancer. *Oncology* 2005; **68**: 52-57
22 **Kitamura K**, Yamaguchi T, Okamoto K, Nishida T, Takahashi T. Superficial spreading type of early gastric cancer. *Br J Cancer* 1996; **74**: 1834-1837
23 **Yasuda M**, Kaji M, Aoki R, Nakamoto J, Sakashita O. Takeuchi Y, Fuke K, Takada J, Oguro T, Yamanoi A, Torisu R. A case of intramucosal signet-ring cell carcinoma in the gastropylorus with duodenal invasion. *Gastroenterol Endosc* 2000; **42**: 834-839
24 **Ishikawa M**, Kitayama J, Fujii S, Ishigami H, Kaizaki S, Nagawa H. Recurrent intramucosal gastric carcinoma with extensive invasion to duodenal mucosa after endoscopic mucosal resection: a case report. *Am Surg* 2005; **71**: 366-368
25 **Golden R**, Stout AP. Superficial spreading carcinoma of the stomach. *Am J Roentgenol Radium Ther* 1948; **59**: 157-167
26 **Yasui A**, Hirase Y, Miyake M, Kidokoro T, Murakami T. Pathology of superficial spreading type of gastric cancer. *Stomach and Intestine* 1973; **8**: 1305-1310
27 **Boku T**, Nakane Y, Okusa T, Tanaka K, Hioki K. Superficial extended early gastroduodenal cancer of the pyloric ring, report of a case. *Stomach and Intestine* 1996; **31**: 655-660
28 **Itoh H**, Oohata Y, Nakamura K, Nagata T, Mibu R, Nakayama F. Complete ten-year postgastrectomy follow-up of early gastric cancer. *Am J Surg* 1989; **158**: 14-16
29 **Koufuji K**, Takeda J, Aoyagi K, Yano S, Miyagi M, Koga A, Shirouzu K. Clinicopathological study of early gastric cancers with duodenal invasion. *Jpn J Cancer Clin* 2003; **49**: 211-213
30 **Kakeji Y**, Korenaga D, Baba H, Watanabe A, Tsujitani S, Maehara Y, Sugimachi K. Surgical treatment of patients with gastric carcinoma and duodenal invasion. *J Surg Oncol* 1995; **59**: 215-219
31 **Shimada S**, Yagi Y, Shiomori K, Honmyo U, Hayashi N, Matsuo A, Marutsuka T, Ogawa M. Characterization of early gastric cancer and proposal of the optimal therapeutic strategy. *Surgery* 2001; **129**: 714-719
32 **Nakajima T**. Gastric cancer treatment guidelines in Japan. *Gastric Cancer* 2002; **5**: 1-5
33 **Okabayashi T**, Kobayashi M, Nishimori I, Sugimoto T, Namikawa T, Onishi S, Hanazaki K. Clinicopathological features and medical management of early gastric cancer. *Am J Surg* 2008; **195**: 229-232
34 **Yokota T**, Ishiyama S, Saito T, Teshima S, Narushima Y, Murata K, Iwamoto K, Yashima R, Yamauchi H, Kikuchi S. Lymph node metastasis as a significant prognostic factor in gastric cancer: a multiple logistic regression analysis. *Scand J Gastroenterol* 2004; **39**: 380-384
35 **Kunisaki C**, Makino H, Takagawa R, Oshima T, Nagano Y, Kosaka T, Ono HA, Otsuka Y, Akiyama H, Ichikawa Y, Shimada H. Tumor diameter as a prognostic factor in patients with gastric cancer. *Ann Surg Oncol* 2008; **15**: 1959-1967
36 **Yokota T**, Ishiyama S, Saito T, Teshima S, Yamada Y, Iwamoto K, Takahashi M, Murata K, Yamauchi H. Is tumor size a prognostic indicator for gastric carcinoma? *Anticancer Res* 2002; **22**: 3673-3677
37 **Nakayama Y**, Kadowaki K, Hirata K, Higure A, Nagata N, Ito H. A case report of early gastric cancer with duodenal extension. *Jpn J Gastroenterol Surg* 2004; **37**: 506-511

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TOPIC HIGHLIGHT

Yusuf Bayraktar, Professor, *Series Editor*

Non-classical phenotypes of autoimmune hepatitis and advances in diagnosis and treatment

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Abstract

Non-classical manifestations of autoimmune hepatitis can delay diagnosis and treatment. Our aims were to describe the clinical phenotypes that can confound the diagnosis, detail scoring systems that can ensure their recognition, and outline advances in treatment that can improve their outcome. Prime source and review articles in English were selected through Medline from 1970-2008 and assimilated into personal libraries spanning 32 years. Acute severe or asymptomatic presentations and atypical histological findings, including centrilobular zone 3 necrosis and concurrent bile duct changes, are compatible with the diagnosis. Cholangiographic abnormalities may be present in children and adults with the disease, and autoimmune hepatitis must be considered in patients without autoantibodies or with antimitochondrial antibodies and no other cholestatic features. Asymptomatic patients frequently become symptomatic; mild disease can progress; and there are no confident indices that justify withholding treatment. Two diagnostic scoring systems with complementary virtues have been developed to evaluate patients with confusing features. Normal liver tests and tissue constitute the optimal end point of treatment, and the first relapse is an indication for long-term azathioprine therapy. Cyclosporine, tacrolimus and mycophenolate mofetil are promising salvage therapies, and budesonide with azathioprine may be a superior frontline treatment. We conclude that the non-classical phenotypes of autoimmune hepatitis can be recognized promptly, diagnosed accurately, and treated effectively.

Key words: Non-classical phenotypes; Scoring systems; Treatment strategies

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INTRODUCTION

Autoimmune hepatitis was initially perceived as a self-perpetuating, inflammatory liver disease in young amenorrheic women with hirsutism, acne and cirrhosis[1-3]. The validity of this classical phenotype was subsequently strengthened by technological advances that excluded a viral cause for the condition[4] and by studies that implicated perturbations of the immune system in its pathogenesis[5-8]. The clinical phenotype expanded as the concept of autoimmunity was applied broadly to liver diseases of unknown cause and as the requirement for 6 mo of disease activity was eliminated from its definition[9,10]. Autoimmune hepatitis still lacks an etiological agent and disease-specific laboratory test, but its designation now applies to patient populations far more diverse and numerous than the original patients with "lupoid hepatitis"[11].

Autoimmune hepatitis must be considered in all individuals with acute and chronic hepatitis of undetermined cause and with graft dysfunction after liver transplantation[11]. Acute[12-15], acute severe[16-19],

and asymptomatic[20-22] forms have been described; progression to cirrhosis may be indolent and unsuspected[23-25]; transitions between active and inactive disease may occur spontaneously[26,27]; concurrent immune diseases may obscure the diagnosis[28,29]; serological markers may be variably expressed and absent at presentation[30-32]; histological findings may include centrilobular zone 3 necrosis[33-37] or concurrent biliary changes[38-42]; and different ethnic groups may have non-classical clinical phenotypes[43-47]. The identification of autoimmune hepatitis in diverse clinical situations is critical since prompt institution of corticosteroid therapy can be life-saving[48-50].

Corticosteroids alone or a lower dose in combination with azathioprine induce clinical, laboratory and histological improvement in 80% of patients within 3 years[51,52]. Ten- and twenty-year survivals exceed 80%[24], and hepatic fibrosis is reduced or prevented[53-56]. These therapeutic successes are counterbalanced against adverse outcomes that justify the continued pursuit of new drugs and regimens. Nine percent of treated patients deteriorate despite compliance with corticosteroid schedules (treatment failure)[57,58]; 13% develop treatment-related side effects that compel premature withdrawal of medication (drug toxicity)[59]; and 9% improve but not to a degree to justify discontinuation of the medication (incomplete response)[59]. Furthermore, 50%-86% of patients who enter remission relapse after drug withdrawal and require re-treatment[27,51,60-64].

The diagnostic criteria of autoimmune hepatitis have been codified by an international panel[9,10], and scoring systems can establish the diagnosis in difficult cases[9,10,65,66]. The re-definition of treatment goals[67-70] and the revision of current treatment strategies[71-75] promise to improve results. The emergence of powerful immunosuppressive agents, mainly from the transplantation arena, promises to strengthen the treatment repertoire[76,77], and the clarification of critical pathogenic pathways make site-specific molecular interventions feasible[76,78,79]. The clinical spectrum of autoimmune hepatitis has expanded, but the diagnostic instruments and therapeutic options have also improved.

The objectives of this review are to describe the non-classical clinical phenotypes of autoimmune hepatitis, detail the diagnostic instruments that can ensure their recognition, and introduce the evolving treatment strategies. Classical syndromes are the cornerstones of clinical practice, but variations from the classical are its realities. The changing spectrum of autoimmune hepatitis and its treatment underscores the importance of the disease and the vigor of the investigative effort that it has generated.

NON-CLASSICAL CLINICAL PHENOTYPES

Autoimmune hepatitis is defined as a self-perpetuating inflammation of the liver of unknown cause that is characterized by interface hepatitis on histological examination, hypergammaglobulinemia, and autoantibodies[80]. The diagnosis has great latitude since no features are disease-specific. The phenotypes that satisfy the definition of autoimmune hepatitis but are outside the boundaries of classical disease have acute severe presentations, few or no symptoms, atypical histological findings, absent or variant serological markers, concurrent cholangiographic changes, male gender, and non-Caucasian backgrounds.

Table 1 Non-classical phenotypes of autoimmune hepatitis

Non-classical phenotype	Salient features
Acute severe disease	Corticosteroids effective in 36%-100%[49] Protracted treatment can be complicated by infection[49] High mortality if no better within 2 wk of therapy[85] MELD score ⩾ 12 identifies 97% of treatment failures[58]
Asymptomatic mild hepatitis	Common (25%-34%) but unstable state[20-22,87-89] Symptoms develop in 26%-70%[20,21] Progression possible if untreated[20-22,87] Improves quickly with therapy[22]
Atypical histological features	Centrilobular necrosis is an early acute form[18,33-37,92] Transition to interface hepatitis possible[35] Coincidental biliary changes lack cholestatic profile[41] Fatty changes may co-exist[58,94]
Absent or variant serological markers	Seronegativity possible in 13%[31] Other features and treatment outcome similar[31,32,100] Non-standard autoantibodies possible[101-104] Conventional autoantibodies may be expressed later[30] Screen for celiac disease[105-108]
Concurrent cholangiographic changes	Abnormal cholangiograms in 44% with CUC[116] Poor outcome if biliary changes and CUC[121-124] MRC abnormalities in 8% adults without CUC[117] MRC abnormalities may be associated with fibrosis[119]
Male gender	0.2-0.5 cases/100 000 per year[127,128] Low frequency of concurrent immune diseases[130-132] No diversity of HLA DRB1*04 alleles[130-132] Better survival than women[136]
Non-Caucasian	Cholestatic features may be common[45,141-143] Male predominance possible[47] Socioeconomic factors important[137,138,146,147]

MELD: Model of End Stage Liver Disease; MRC: Magnetic resonance cholangiography; CUC: Chronic ulcerative colitis; HLA: Human leukocyte antigen.

Acute severe presentations

The diagnosis of autoimmune hepatitis is no longer restricted by a time requirement for disease activity[9,10]. An acute severe or fulminant presentation can reflect *de novo* inflammation[12-15] or the spontaneous exacerbation of a previously unsuspected chronic disease[81]. An acute or abrupt onset occurs in 40% of patients with autoimmune hepatitis[14,81,82], whereas an acute severe presentation is rare[83] (Table 1).

The acute form can be differentiated from the chronic form by laboratory features [higher serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, total serum bilirubin concentration, and serum γ-glutamyl transpeptidase level] and by histological findings (more frequent centrilobular zone 3 necrosis with plasmacytic infiltration and bile duct injury), but its recognition in individual cases relies mainly on an awareness that acute severe autoimmune hepatitis is possible[84].

Corticosteroid therapy suppresses inflammatory

activity in 36%-100% of patients with acute severe presentations[49], whereas delay in treatment can result in a poor outcome[51,83] (Table 1). Immediate survival and the need for urgent transplantation depend on the rapidity and nature of the response to corticosteroids[50,85,86]. Failure to improve at least one laboratory abnormality reflective of liver inflammation or function, especially a pre-treatment hyperbilirubinemia, within 2 wk indicates that liver transplantation should be considered[85]. Relentless pursuit of an unachievable benefit from corticosteroid therapy can be complicated by infection and the lost opportunity for a successful transplantation[49].

The Model of End Stage Liver Disease (MELD) can be used to identify individuals with autoimmune hepatitis who are likely to fail corticosteroid therapy and require liver transplantation[58] (Table 1). MELD scores $\geqslant$ 12 points at presentation have a sensitivity of 97% and specificity of 68% for treatment failure, and patients with such scores warrant close scrutiny and preparedness for liver transplantation. A MELD score $\geqslant$ 12 points at presentation captures all problematic patients, but it does not preclude their salvation through prompt and vigorous corticosteroid treatment.

Asymptomatic mild presentations

Autoimmune hepatitis is asymptomatic in 25%-34% of patients at presentation[20-22], and retrospective analyses have estimated that 25%-85% of individuals have mild disease[20-22,87-89] (Table 1). These presentations contrast with those described in the classical treatment trials in which selection criteria focused on severe symptomatic and immediately life-threatening disease[51,90,91]. Treatment guidelines have been promulgated for the individuals with severe disease, but they remain arbitrary and inconsistent for those with mild disease[80]. These difficulties reflect in part the lack of a codified definition of mild autoimmune hepatitis and uncertainty about its natural history.

Untreated mild autoimmune hepatitis has a better outcome than severe disease, but it does not have a benign prognosis (Table 1). Cirrhosis develops in 49% of untreated patients within 15 years[87]; liver failure and hepatocellular carcinoma are possible[22]; asymptomatic patients become symptomatic in 26%-70% of instances[20,21]; and 10-year mortality exceeds 10%[21,22]. Spontaneous resolution is possible, but untreated patients with mild autoimmune hepatitis improve less commonly (12% *vs* 63%, $P = 0.006$) and more slowly than treated patients, and they have a lower 10-year survival (67% *vs* 98%, $P = 0.01$)[22].

A "safe" subset of patients with non-aggressive autoimmune hepatitis who require no therapy cannot be reliably identified, and the clinical threshold for starting corticosteroid therapy cannot be so high that all patients with mild or asymptomatic disease are excluded (Table 1). Mild autoimmune hepatitis can improve spontaneously, and this prospect may dampen therapeutic zeal, especially if measured against the possibility of serious treatment-related complications[22]. A dictum to do no harm, however, that focuses more concern on the treatment than the disease may be incorrect.

The aggressive potential of mild autoimmune hepatitis at presentation, the inability to predict outcome by clinical parameters, the expected rapidity of the treatment response, and the safety of current treatment regimens favor a proactive management strategy[22]. Until randomized clinical trials are performed comparing treatment against no treatment, the management strategy in patients with mild disease should lean toward conventional therapy. Mild asymptomatic autoimmune hepatitis is a non-classical phenotype, but it should not be regarded or managed as a different disease.

Atypical histological features

The histological hallmark of autoimmune hepatitis is interface hepatitis, but other histological findings are compatible with the disease[9,10] (Table 1). Centrilobular zone 3 necrosis (Figure 1) is probably an early form of autoimmune hepatitis that is detected mainly in patients with an acute onset[18,33-37,92]. Successive liver tissue examinations have disclosed transition of the centrilobular zone 3 pattern of necrosis to that of typical interface hepatitis during the course of the disease[35]. This non-classical finding may suggest an acute viral or toxic injury, but the diagnosis of autoimmune hepatitis should not be discounted.

Concurrent biliary changes, including isolated destructive cholangitis (Figure 2), may also be found in patients with otherwise classical autoimmune hepatitis[38-42] (Table 1). These patients do not have a cholestatic clinical or laboratory profile, and successive tissue examinations have not disclosed persistence or progression of the biliary injury[41]. The biliary changes probably reflect collateral damage associated with an exuberant inflammatory process rather than a transition state to a cholestatic disease or variant syndrome. The biliary changes should not alter the diagnosis or the treatment strategy.

Fatty changes (Figure 3) may also be present at accession or after corticosteroid therapy[58,93,94] (Table 1). Non-alcoholic fatty liver disease (NAFLD) is a common finding in the general population, and it may be associated with autoantibodies and hypergammaglobulinemia[94-97]. Both conditions can co-exist, and corticosteroid therapy can ameliorate the autoimmune hepatitis and intensify the NAFLD[58,94]. The presence of coincidental fatty change should not discourage the diagnosis or treatment of autoimmune hepatitis, but it compels an accurate diagnosis. Worsening of the laboratory indices during therapy justifies liver tissue examination and reassessment of the treatment strategy[58,94]. Progressive fatty change can be a cause of treatment failure[58].

Absent or atypical serological markers

Antinuclear antibodies (ANA), smooth muscle antibodies (SMA), and antibodies to liver kidney microsome type 1 (anti-LKM1) are the classical serological markers of autoimmune hepatitis[98,99]. These antibodies are not pathogenic or disease-specific, and their expression can vary in individual cases and in different geographical

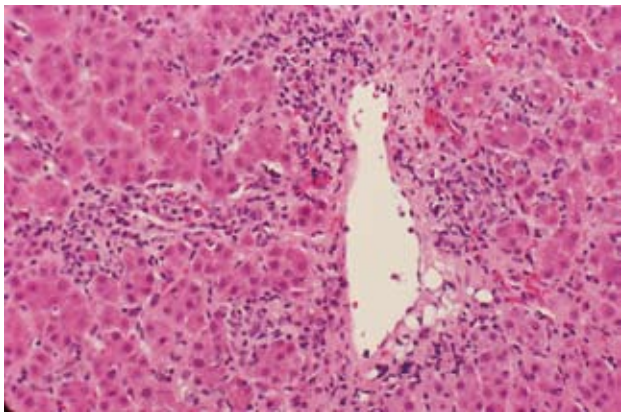

Figure 1 Centrilobular zone 3 necrosis. Inflammation and hepatocyte drop out are present around a terminal hepatic venule in conjunction with hepatic plate thickening, architectural disorganization, and rosette formation. Centrilobular (perivenular) zone 3 necrosis can be an early acute form of autoimmune hepatitis that can transform to interface hepatitis (HE, × 200).

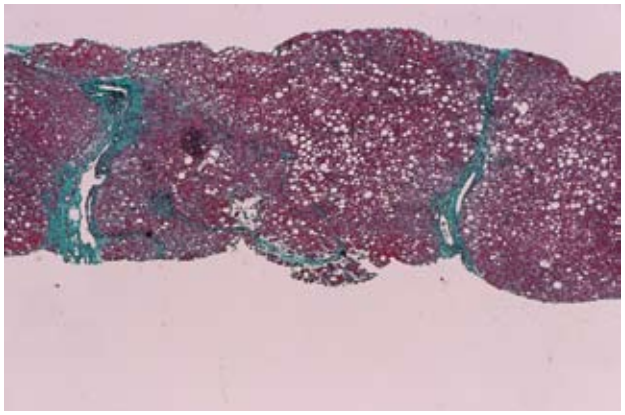

Figure 3 Steatosis. Macrovesicular steatosis is the predominant histological feature after corticosteroid treatment. Fatty changes may be present before or during corticosteroid treatment and perpetuate or extend the laboratory indices of liver inflammation (Trichrome stain, × 40).

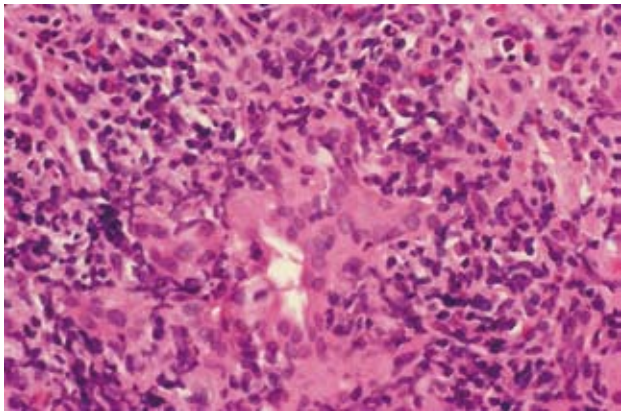

Figure 2 Concurrent pleomorphic cholangitis. Lymphocytes and histiocytes surround, infiltrate and damage an interlobular bile duct. Bile duct injury in the absence of cholestatic clinical and laboratory manifestations may represent collateral injury that is transient (HE, × 400).

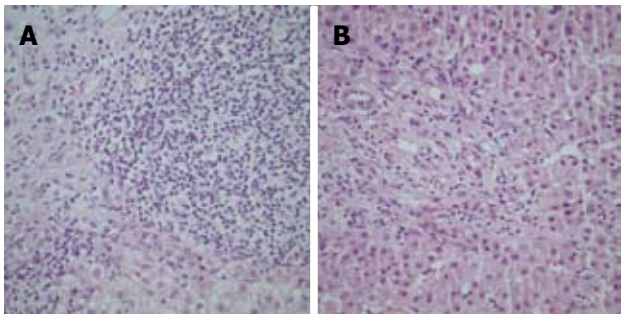

Figure 4 Histological features of a Turkish patient with the "overlap syndrome" (autoimmune hepatitis and primary biliary cirrhosis) characterized by heavy portal infiltration with lymphocytes and plasma cells (A) and bile ductular proliferation and ductopenia (B) (HE, × 200).

regions and ethnic groups. Thirteen percent of white North American adults with classical features of autoimmune hepatitis lack ANA, SMA, and anti-LKM1[31] (Table 1).

Seronegative patients with autoimmune hepatitis have a non-classical phenotype, and they constitute an "autoantibody-negative autoimmune hepatitis"[31,32,100]. These patients are indistinguishable from those with classical disease, including their HLA profiles, and they also respond to corticosteroid therapy[31,32,100] (Table 1). Twenty percent may express non-standard autoantibodies, such as antibodies to soluble liver antigen (anti-SLA)[101,102] or atypical perinuclear anti-neutrophil cytoplasmic antibodies (atypical pANCA)[103,104], and others may express SMA, ANA or both later in their course[30]. Some corticosteroid-responsive patients remain seronegative throughout their disease, and they may await discovery of their signature autoantibody[31,32,100]. All such patients must be screened for celiac disease by testing for immunoglobulin A antibodies to tissue transglutaminase or endomysium[105-108].

Antimitochondrial antibodies (AMA) can be present in 8%-35% of patients with otherwise classical autoimmune hepatitis, and they define another non-classical serological phenotype[108-112]. These coincidental AMA are not associated with cholestatic features, histological findings of biliary injury, or different response to corticosteroid therapy[110-113]. They may persist for as long as 27 years in the absence of primary biliary cirrhosis (PBC)[111]; they may disappear spontaneously; or they may appear late in the course of the disease without apparent clinical relevance[112]. Severe inflammatory activity may result in modification of the mitochondrial antigens through oxidative stress and facilitate the production of AMA which in turn can disappear when the inflammatory stress subsides[114]. AMA in the absence of cholestatic laboratory or histological features should not dissuade the diagnosis of autoimmune hepatitis or compel a different treatment strategy. The "serological overlap" with PBC does not constitute a hybrid disease or pathological process in transition.

Concurrent cholangiographic changes

Concurrent cholangiographic changes have been described in children[115] and adults[116,117] with autoimmune hepatitis, and these findings constitute another non-classical clinical phenotype (Table 1). The

emergence of magnetic resonance cholangiography (MRC) as a safe, effective and non-invasive mechanism by which to assess the biliary system[118] has indicated that cholangiographic changes that resemble primary sclerosing cholangitis (PSC) occur in 8% of adults with autoimmune hepatitis[117]. These changes occur predominately in women who typically lack inflammatory bowel disease, and they are associated with histological features that reflect increased lobular activity rather than biliary injury[117].

The nature and significance of the biliary changes by MRC remain unclear since most adults with these changes respond to corticosteroid therapy[117]. The possibility of a disease process other than typical PSC or an unusual but nonspecific biliary distortion induced by fibrosis cannot be discounted (Table 1). Recent prospective studies have indicated that while adults with autoimmune hepatitis have a high frequency of intrahepatic biliary changes by MRC (24%), the occurrence of PSC is rare (2%)[119]. Furthermore, the frequency of biliary changes by MRC in adults with autoimmune hepatitis is similar to that in patients with cirrhosis of a non-biliary and non-autoimmune nature. Hepatic fibrosis rather than the nature of the liver disease may be the most important parameter independently associated with the biliary changes[119].

Cholangiographic abnormalities by endoscopic or intrahepatic cholangiography are present in 44% of adults with autoimmune hepatitis and inflammatory bowel disease[116,120], and patients with these changes are typically refractory to corticosteroid therapy[121-124] (Table 1). This is the non-classical phenotype that has immediate clinical relevance, and its discovery impacts on the diagnosis, treatment, and outcome. Biliary studies should be performed mainly in adult patients with inflammatory bowel disease or recalcitrance to corticosteroid therapy[121,125]. Not all biliary changes have independent pathological significance or clinical importance[126].

Male gender

Autoimmune hepatitis does occur in white northern European men[127,128], and its development in this gender constitutes another non-classical phenotype (Table 1). Women with autoimmune hepatitis outnumber men with the disease by more than three-fold[129], and estimates of the incidence of autoimmune hepatitis in northern European men ranges from 0.2-0.5 cases per 100 000 persons per year[127,128]. The existence of an important clinical distinction between men and women with autoimmune hepatitis is still unsettled, but clearly the experiences over the decades have not identified a striking difference between the genders.

White North American women with autoimmune hepatitis are distinguished from men with autoimmune hepatitis and the same ethnicity by having higher frequencies of concurrent immune diseases (34% *vs* 17%, $P = 0.05$) and HLA DRB1*04 (49% *vs* 24%, $P = 0.007$)[130-132] (Table 1). Women with HLA DRB1*04 also have higher frequencies of concurrent immune diseases than women without HLA DRB1*04 (52% *vs* 22%, $P < 0.000001$) as do men with HLA DRB1*04 compared to men without HLA DRB1*04 (26% *vs* 4%, $P = 0.002$)[129,132]. These findings suggest that the clinical phenotype is driven by the genetic predisposition of the host as well as the gender[129,133].

Retrospective surveys have suggested gender-based differences in disease behavior and treatment outcome, but results have been discrepant[134-136]. Differences in age at presentation (39 years *vs* 49 years, $P = 0.06$) and the frequency of relapse after drug withdrawal (71% *vs* 55%, $P = 0.06$) have not reliably distinguished men from women with autoimmune hepatitis. The higher frequency of HLA A1-B8-DRB1*03 in men who relapse (50% *vs* 23%, $P = 0.003$) and greater mortality in women than men ($P = 0.02$) have been contrasting features in some experiences, and these findings require further examination[135,136] (Table 1).

The principal clinical concern related to gender is that the diagnosis of autoimmune hepatitis might be overlooked in men. Gender may be a surrogate marker that signifies different antigenic exposures, hormonal effects on immune responsiveness, chromosomal imbalances that favor loss of self-tolerance, and genetic predispositions for immunocyte activation[132]. The diagnosis of autoimmune hepatitis in men should trigger the same treatment strategies and monitoring schedules as in women.

Non-Caucasians

Racial background can affect the clinical phenotype of autoimmune hepatitis, and diagnostic criteria developed mainly in white northern European and North American populations may not apply in different ethnic groups and geographical regions (Table 1). Black North American patients have cirrhosis at presentation more commonly than white North American patients (85% *vs* 38%)[43,137,138]. Japanese patients typically have mild, late onset disease[139]. South American patients are younger than white North American counterparts, and they have more severe laboratory abnormalities at presentation[140]. Alaskan natives have a higher frequency of acute icteric disease, asymptomatic illness, and advanced fibrosis at accession than non-native patients[44]. Aboriginal North Americans have disproportionately high frequencies of immune-mediated disorders, cholestatic features, and advanced disease at presentation[141-143]. African, Asian and Arab patients have a higher frequency of biliary changes on histological examination than white northern European patients[45], and patients from Somalia are frequently men with rapidly progressive disease[47] (Table 1).

The variations in clinical phenotype suggest that genetic background and geographical location affect occurrence and behavior of the disease[129,133]. Indigenous etiological agents or population-dependent genetic factors may modulate susceptibility to autoimmune hepatitis, determine targets of the immune response, and affect the vigor of the inflammatory reaction[144,145]. Socioeconomic status, healthcare access, and quality of care are other factors that must be analyzed when assessing discrepancies

in disease occurrence and outcome among different racial groups[137,138,146,147] (Table 1).

TURKISH PERSPECTIVE

The importance of recognizing the diverse manifestations of autoimmune hepatitis in different regions and ethnic groups is illustrated by the appearance and behavior of the disease in Turkey. Autoimmune hepatitis in this region has a character that aligns with the disease of white northern Europeans and North Americans, but it can be difficult to recognize if only the western phenotype is considered.

Autoimmune hepatitis is a relatively rare disorder in Turkey when compared with chronic viral hepatitis, but it is still the most common autoimmune liver disease[148,149]. Its high prevalence in women is not unusual, but its 9:1 female-to-male ratio[148,149] exceeds the female propensity (3:1 female-to-male ratio) in North America[129]. The age of occurrence in adults (age ranges, 18-59 years; mean age, 42 years) is as broad as elsewhere[150], but there are many patients with signs of hepatitis who are negative for viral markers and the conventional autoantibodies[149]. These patients are typically designated as having "cryptogenic chronic hepatitis", but they respond well to treatment with corticosteroids and azathioprine. Other liver diseases must be carefully excluded, especially in men and those who lack the conventional autoantibodies, and the diagnosis must be supported by the demonstration of compatible liver enzyme abnormalities, serum immunoglobulin G (IgG) elevation, and histological findings. The presence of periportal lymphoplasmacytic infiltration in liver tissue is an important clue to the diagnosis, and all patients in whom there is a suspicion of autoimmune hepatitis should undergo liver tissue examination.

As in other regions, the features of autoimmune hepatitis may be intermixed with those typical of other liver diseases, especially the cholestatic disorders ("overlap syndromes"), and the diagnosis must be secured by expert histological interpretation and cholangiographic studies (Figure 4)[148,151,152]. In Turkey, as elsewhere, liver tissue examination is the most important tool in directing the diagnosis, and a second examination of the liver tissue after institution of treatment provides a comparison that can support or change the original impression.

Concurrent immune diseases, such as autoimmune thyroiditis[153], celiac disease[154], Sjogren syndrome[155], autoimmune diabetes[155], and various rheumatic conditions[156], can accompany the autoimmune hepatitis of Turkey, but unlike the disease in other regions, the liver disease in Turkey can frequently be linked to different triggers, including indigenous infections [prolonged hepatitis A virus (HAV) infection[157] and brucellosis[158]] and medicinal agents (Echinacea[159], doxycycline[158], estrogen[160], cyproterone acetate[160], and ornidazole[161]). A genetic basis for the liver disease and its immune manifestations has not been well studied in Turkey, but the classical HLA phenotype, A1-B8-DRB1*03, of western countries does not appear to be an important susceptibility factor in this area[162].

Corticosteroid therapy remains the mainstay of treatment in Turkey, but azathioprine, ursodeoxycholic acid and budesonide have been added to the list of available and effective drugs. Combination therapy is the preferred regimen, and budesonide is gaining favor over prednisolone in combination with azathioprine. An example of tailoring the treatment strategy to the population base is the practice of maintaining individuals in remission on low dose prednisolone (4 mg on alternate days) either alone or in combination with azathioprine long-term. By recognizing the regional variations in the clinical phenotype and tailoring therapy to suit the prevalent disease behavior, autoimmune hepatitis in different regions and ethnic groups can be diagnosed promptly and treated successfully in a cost-effective, low risk manner.

NEW DIAGNOSTIC INSTRUMENTS

New diagnostic instruments have evolved that have the flexibility to accommodate atypical features of autoimmune hepatitis and the sensitivity and specificity to ensure accurate diagnosis of the non-classical phenotypes. A diagnostic scoring system that was promulgated mainly as a research tool in 1993[9] was revised in 1997[10] to exclude cholestatic syndromes. A simplified diagnostic scoring system was added in 2008 to ease clinical application[65], and both systems can now be exploited to strengthen the diagnosis in difficult cases[66].

Original revised diagnostic scoring system

The revised original diagnostic scoring system developed by the International Autoimmune Hepatitis Group (IAIHG) evaluates 13 clinical components and renders 27 possible grades[10] (Table 2). It is a comprehensive template that grades each component of the disease, including gender, laboratory manifestations of liver inflammation and cholestasis, the conventional autoantibodies, viral markers, epidemiological risk factors such as drug or alcohol exposures, HLA phenotype, concurrent immune diseases, novel autoantibodies, and individual histological features. It also grades the treatment response, and a score can be rendered before and after treatment.

A pre-treatment score of 10 points or higher or a post-treatment score of 12 points or higher indicates the likelihood of autoimmune hepatitis[10] (Table 2). No single test or finding defeats or ensures the diagnosis of autoimmune hepatitis if other components are sufficiently strong to outweigh it. The Receiver Operating Characteristic (ROC) curve for the revised original scoring system shows that the minimum pre-treatment score of 10 points has a sensitivity of 100% and a specificity of 73% for autoimmune hepatitis. A pre-treatment score of 15 points or higher has a specificity of greater than 90% for autoimmune hepatitis[66].

The principal virtues of the revised original scoring

Table 2 Revised original pre-treatment scoring system[10]

Variable	Result	Points	Variable	Result	Points
Gender	Female	+2	HLA	DR3 or DR4	+1
AP:AST (or ALT) ratio	> 3	-2	Immune disease	Thyroiditis, colitis, others	+2
	< 1.5	+2			
γ-globulin or IgG level above normal	> 2.0	+3	Other markers	Anti-SLA, actin, LC1, pANCA	+2
	1.5-2.0	+2			
	1.0-1.5	+1			
	< 1.0	0			
ANA, SMA, or anti-LKM1 titers	> 1:80	+3	Histological features	Interface hepatitis	+3
	1:80	+2		Plasmacytic	+1
	1:40	+1		Rosettes	+1
	< 1:40	0		None of above	-5
				Biliary changes	-3
				Other features	-3
AMA	Positive	-4	Treatment response	Complete	+2
				Relapse	+3
Viral markers	Positive	-3			
	Negative	+3			
Drugs	Yes	-4	Pretreatment aggregate score		
	No	+1	Definite diagnosis		> 15
			Probable diagnosis		10-15
Alcohol	< 25 g/d	+2	Post-treatment aggregate score		
	> 60 g/d	-2	Definite diagnosis		> 17
			Probable diagnosis		12-17

AP: AST (or ALT) ratio: Ratio of alkaline phosphatase level to aspartate or alanine aminotransferase level; Anti-SLA: Antibodies to soluble liver antigen; Anti-LC1: Antibodies to liver cytosol type 1; pANCA: Perinuclear anti-neutrophil cytoplasmic antibodies; IgG: Immunoglobulin G; ANA: Antinuclear antibodies; SMA: Smooth muscle antibodies; Anti-LKM1: Antibodies to liver/kidney type 1; AMA: Antimitochondrial antibodies; HLA: Human leukocyte antigen.

Table 3 Simplified scoring system of the International Autoimmune Hepatitis Group[65]

Variable	Result	Points
Autoantibodies		
ANA or SMA	≥ 1:40	+1
ANA or SMA	≥ 1:80	+2
Antibodies to liver kidney microsome type 1	≥ 1:40	+2
Antibodies to soluble liver antigen	Positive	+2
Immunoglobulin level		
Immunoglobulin G	> UNL	+1
	> 1.1 ULN	+2
Histological findings		
Morphological features	Compatible	+1
	Typical	+2
Viral disease		
Absence of viral hepatitis	No viral markers	+2
Pretreatment aggregate score		
Definite diagnosis		≥ 7
Probable diagnosis		6

ULN: Upper limit of normal.

system are that it ensures the systematic assessment of all key features of the disease and it is not compromised by a missing or atypical feature[66]. The revised original diagnostic scoring system is most useful in evaluating patients with few or atypical findings of autoimmune hepatitis, including the variant syndromes, because of its comprehensive nature. It quantifies the strength of the diagnosis, and it is a valuable research tool that ensures comparable study populations within clinical trials.

Simplified diagnostic scoring system

The simplified diagnostic scoring system eases clinical application by evaluating only 4 clinical components, and it has been validated in diverse ethnic groups and liver diseases[65] (Table 3). The simplified scoring system is based on the presence and level of autoantibody expression by indirect immunofluorescence, serum IgG concentration, compatible or typical histological features, and the absence of viral markers. It does not grade treatment response.

The ROC curve for the simplified scoring system shows that a minimum score of 6 points has a sensitivity and specificity of 90% for the diagnosis of autoimmune hepatitis[66] (Table 3). Scores of 7 points or higher are nearly 100% specific for the diagnosis of autoimmune hepatitis with only a small decrease in sensitivity. The virtues of the simplified scoring system are the ease of its clinical application, and its combined high sensitivity and specificity for the diagnosis[66]. It is especially useful in excluding autoimmune hepatitis in patients with other distinct conditions who have confusing concurrent immune features. The revised original scoring system has greater sensitivity for the diagnosis, whereas the simplified system has superior specificity and accuracy.

NEW TREATMENT STRATEGIES

New treatment strategies for autoimmune hepatitis are evolving as current regimens are being used more effectively and new drugs are being exploited in selected situations. The non-classical phenotypes of autoimmune hepatitis are managed in the same fashion as the classical phenotypes, and they benefit similarly from these advances.

Table 4 Therapeutic advances in autoimmune hepatitis

Advance	Nature	Attribute
Improved current therapy	Initial therapy until resolution of liver tests and tissue	Prevention of relapse after drug withdrawal[70]
	Long-term azathioprine therapy after relapse or incomplete response	Prevention of disease progression[71,73,125]
	Pretreatment vaccination for viruses	Protection against morbidity of concurrent viral infection[74]
New drugs	Calcineurin inhibitors (cyclosporine, tacrolimus)	Salvage therapy[150-157]
	Purine antagonists (6-mercaptopurine, mycophenolate)	Salvage therapy[161-166]
	Budesonide (combined with azathioprine)	Effective and safe front line therapy[75]
Potential molecular interventions	Synthetic blocking peptides	Block autoantigen presentation[186,187]
	Cytokine manipulations	Promote anti-inflammatory effects[188]
	T cell vaccination	Eliminate cytotoxic liver-infiltrating clone[189]
	Oral tolerance (high or low dose regimen)	Reduce immune response (low dose) or induce anergy (high dose)[190,191]
	Mesenchymal stem cells (human bone marrow-derived)	Replace damaged hepatocytes[200]

Improvements in current treatment strategies

The ideal end point of initial corticosteroid therapy has now been defined[67-70]; the treatment adjustments after relapse and incomplete response have been formalized[71-73]; and vaccination against hepatitis A (HAV) and hepatitis B (HBV) viruses prior to therapy has been proposed[74]. These improvements constitute advances in the current treatment regimens (Table 4).

Corticosteroid therapy should be continued until the clinical, laboratory and histological features of autoimmune hepatitis have fully resolved (Table 4)[67-70]. Relapse after drug withdrawal is the most common management problem in autoimmune hepatitis, and this occurrence can be reduced by continuing treatment until liver tests and tissue are normal[59,70,163]. Patients who sustain remission after treatment withdrawal have better laboratory indices and liver tissue examinations at the time of drug withdrawal than patients who relapse, and treatment until complete resolution of the inflammatory features is the ideal end point of therapy.

Sixty percent of patients who achieve an ideal treatment end point still relapse after drug withdrawal, and 40% of treated patients are unable to achieve full resolution of their disease[70]. The relentless pursuit of an ideal but unachievable treatment end point in these individuals can result in drug-related side effects[59,62]. Patients with relapse after drug withdrawal, incomplete response to conventional treatment, and drug intolerances must be managed differently[125].

Repeated relapse and re-treatment is associated with a progressive increase in the cumulative frequencies of cirrhosis, requirement for liver transplantation, and death from hepatic failure[64]. The preferred management strategy after the first relapse is to institute treatment with long-term fixed dose azathioprine (Table 4)[71,73]. Prednisone and azathioprine are re-started until clinical and laboratory resolution is achieved. The dose of azathioprine is then increased to 2 mg/kg daily as the dose of prednisone is withdrawn. Azathioprine is then continued indefinitely as a chronic maintenance therapy. Eighty percent of patients are able to sustain remission in this fashion over a 10 year period of observation. Patients who improve during treatment but not to a degree to satisfy remission criteria (incomplete response) can also be managed by this regimen[125].

Vaccination against HAV and HBV is an important adjunct to conventional treatment (Table 4). Susceptibility to infections with HAV (51%) and HBV (86%) is high in patients with autoimmune liver disease, and the incidence of these infections is 1.3-1.4 per 1000 person-years[74]. Vaccination frequencies are only 11% for HBV and 13% for HAV in these patients, and the response to the HBV vaccine is poor or absent in most individuals vaccinated during immunosuppressive therapy[74]. These observations suggest that pre-treatment vaccination for HAV and HBV is under-utilized in autoimmune liver disease and that outcomes can be improved by early vaccination to prevent viral super-infection and mortality.

Advances in pharmacological options

Treatment options have increased in autoimmune hepatitis as new drugs with targeted immunosuppressive actions have been used empirically[76,164] and a third generation corticosteroid has been evaluated by randomized clinical trial[75]. None of these treatments has been incorporated into standard management algorithms, but they constitute an evolving armamentarium that promises to improve outcomes by either interrupting critical pathogenic pathways or eliminating intolerances to the current medications.

The calcineurin inhibitors (cyclosporine[165-169] and tacrolimus[170-172]) have been used as frontline and salvage therapies in children[173-175] and adults[165-172] with autoimmune hepatitis, and these multiple small clinical experiences have supported their efficacy and tolerance (Table 4). Additional clinical trials are necessary to determine their target population, dosing schedule, and safety profile.

The purine antagonists (6-mercaptopurine[176] and mycophenolate mofetil[177-181]) have also been effective in some patients refractory to conventional corticosteroid regimens (Table 4). 6-mercaptopurine has reduced disease activity in patients unresponsive to azathioprine, and should be considered as a salvage therapy[176]. Intolerances to azathioprine based on thiopurine methyltransferase deficiency contraindicate its use, and the drug should not be administered to patients with

azathioprine-related side effects[182].

Mycophenolate mofetil is independent of the thiopurine methyltransferase metabolic pathway, and several small experiences have indicated that it can be effective in problematic patients[177-181] (Table 4). Improvement occurs in 39%-84% of patients who tolerate the drug, but the intention to treat is thwarted in 34%-78% of patients because of drug intolerances (nausea, vomiting, pancreatitis, rash, alopecia, deep venous thrombosis, diarrhea and failure to normalize liver tests)[180,183,184]. Mycophenolate mofetil is another promising alternative drug in the treatment of autoimmune hepatitis, but only a minority of problematic patients may reap its benefits[183-185].

Budesonide is a third generation corticosteroid that has been used empirically as frontline[186-188] and salvage[189] therapy in autoimmune hepatitis (Table 4). Its high first-pass clearance by the liver and its breakdown to inactive metabolites promised to improve efficacy and safety compared to conventional corticosteroid regimens[190]. Its advantage, however, was never fully realized until it was evaluated by randomized clinical trial in 203 treatment-naïve patients with autoimmune hepatitis[75]. Budesonide in combination with azathioprine has been found to be superior to prednisolone and azathioprine in normalizing the serum ALT level (47% *vs* 18%, $P < 0.00001$) and reducing the frequency of steroid-related side effects (28% *vs* 53%, $P = 0.0001$) after 6 mo of treatment[75]. The frequency of histological improvement and the durability of the results are unknown, but the findings suggest that budesonide may be an alternative, more effective, and safer frontline regimen than a prednisone-based schedule. Budesonide has not been effective as a salvage therapy in patients with severe disease on long-standing corticosteroid treatment[189], and corticosteroid-induced side effects are still possible, especially in patients who have been treated previously with prednisone[189] or who have cirrhosis[189,191].

Various other drugs (cyclophosphamide[192], methotrexate[193], rapamycin[194], rituximab[195], intravenous immunoglobulin[196], deflazacort[197], and ursodeoxycholic acid[198]) have been proposed for use in autoimmune hepatitis, and their number reflects the need for better salvage therapies in the treatment of autoimmune hepatitis (Table 4). Prospective and scientifically rigorous collaborative studies are needed to expand the therapeutic repertoire and comprehensive analyses are required to demonstrate that these incremental improvements in outcome are cost-effective[199,200].

Site-specific molecular inventions, including antigen-blocking synthetic peptides[201,202], cytokine manipulations[203], T cell vaccination[204], and oral tolerance regimens[205,206], become feasible when the critical pathogenic mechanisms of the disease are clarified[207-209], and confident animal models of the human disease are developed[210-214] (Table 4). Mesenchymal stem cells from human bone marrow that can differentiate into functional hepatocytes have the potential to rescue individuals from liver failure, reduce reliance on whole organ transplantation, and obviate the complications of whole organ rejection and drug toxicity[215] (Table 4). The treatment options for autoimmune hepatitis are already plentiful and effective, but the drive for further improvement must be continuous and vigorous.

CONCLUSION

Autoimmune hepatitis can have acute severe or asymptomatic presentations, centrilobular zone 3 necrosis, concurrent bile duct damage or non-alcoholic fatty changes on histological examination, absent or atypical serological markers, cholangiographic abnormalities, and variable clinical phenotypes related to gender or ethnicity. These non-classical manifestations do not alter the management strategy, but they require prompt recognition and confident diagnosis. The revised original diagnostic scoring system of the IAIHG is useful in evaluating patients with few or atypical findings of autoimmune hepatitis because of its comprehensive nature. The simplified diagnostic scoring system is useful in excluding autoimmune hepatitis in patients with other distinct conditions who have confusing concurrent immune features because of its high specificity. Current treatment regimens have been improved by pursuing resolution of liver tests and liver tissue prior to drug withdrawal, instituting long-term azathioprine therapy after the first relapse, and vaccinating against HAV and HBV prior to treatment. Cyclosporine, tacrolimus, 6-mercaptopurine, and mycophenolate mofetil are promising salvage therapies, whereas budesonide in combination with azathioprine may be a superior frontline therapy to prednisone and azathioprine.

REFERENCES

1 **Bongiovanni AM**, Eisenmenger WJ. Adrenal cortical metabolism in chronic liver disease. *J Clin Endocrinol Metab* 1951; **11**: 152-172

2 **Bearn AG**, Kunkel HG, Slater RJ. The problem of chronic liver disease in young women. *Am J Med* 1956; **21**: 3-15

3 **Cowling DC**, Mackay IR, Taft LI. Lupoid hepatitis. *Lancet* 1956; **271**: 1323-1326

4 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB, Taswell HF, Homburger HA. Evidence against hepatitis viruses as important causes of severe autoimmune hepatitis in the United States. *J Hepatol* 1993; **18**: 342-352

5 **Taft LI**, Mackay IR, Cowling DC. Autoclasia: a perpetuacting mechanism in hepatitis. *Gastroenterology* 1960; **38**: 563-566

6 **Cochrane AM**, Moussouros A, Thomsom AD, Eddleston AL, Wiiliams R. Antibody-dependent cell-mediated (K cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. *Lancet* 1976; **1**: 441-444

7 **Hodgson HJ**, Wands JR, Isselbacher KJ. Alteration in suppressor cell activity in chronic active hepatitis. *Proc Natl Acad Sci USA* 1978; **75**: 1549-1553

8 **Frazer IH**, Mackay IR. T lymphocyte subpopulations defined by two sets of monoclonal antibodies in chronic active hepatitis and systemic lupus erythematosus. *Clin Exp Immunol* 1982; **50**: 107-114

9 **Johnson PJ**, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology* 1993; **18**: 998-1005

10 **Alvarez F**, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WG, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston AL, Fainboim L,

Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RN, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929-938
11 **Czaja AJ**. Diverse manifestations and evolving treatments of autoimmune hepatitis. *Minerva Gastroenterol Dietol* 2005; **51**: 313-333
12 **Crapper RM**, Bhathal PS, Mackay IR, Frazer IH. 'Acute' autoimmune hepatitis. *Digestion* 1986; **34**: 216-225
13 **Amontree JS**, Stuart TD, Bredfeldt JE. Autoimmune chronic active hepatitis masquerading as acute hepatitis. *J Clin Gastroenterol* 1989; **11**: 303-307
14 **Nikias GA**, Batts KP, Czaja AJ. The nature and prognostic implications of autoimmune hepatitis with an acute presentation. *J Hepatol* 1994; **21**: 866-871
15 **Kanda T**, Yokosuka O, Hirasawa Y, Imazeki F, Nagao K, Suzuki Y, Saisho H. Acute-onset autoimmune hepatitis resembling acute hepatitis: a case report and review of reported cases. *Hepatogastroenterology* 2005; **52**: 1233-1235
16 **Maggiore G**, Porta G, Bernard O, Hadchouel M, Alvarez F, Homberg JC, Alagille D. Autoimmune hepatitis with initial presentation as acute hepatic failure in young children. *J Pediatr* 1990; **116**: 280-282
17 **Herzog D**, Rasquin-Weber AM, Debray D, Alvarez F. Subfulminant hepatic failure in autoimmune hepatitis type 1: an unusual form of presentation. *J Hepatol* 1997; **27**: 578-582
18 **Kessler WR**, Cummings OW, Eckert G, Chalasani N, Lumeng L, Kwo PY. Fulminant hepatic failure as the initial presentation of acute autoimmune hepatitis. *Clin Gastroenterol Hepatol* 2004; **2**: 625-631
19 **Miyake Y**, Iwasaki Y, Terada R, Onishi T, Okamoto R, Sakai N, Sakaguchi K, Shiratori Y. Clinical characteristics of fulminant-type autoimmune hepatitis: an analysis of eleven cases. *Aliment Pharmacol Ther* 2006; **23**: 1347-1353
20 **Kogan J**, Safadi R, Ashur Y, Shouval D, Ilan Y. Prognosis of symptomatic versus asymptomatic autoimmune hepatitis: a study of 68 patients. *J Clin Gastroenterol* 2002; **35**: 75-81
21 **Feld JJ**, Dinh H, Arenovich T, Marcus VA, Wanless IR, Heathcote EJ. Autoimmune hepatitis: effect of symptoms and cirrhosis on natural history and outcome. *Hepatology* 2005; **42**: 53-62
22 **Czaja AJ**. Features and consequences of untreated type 1 autoimmune hepatitis. *Liver Int* 2008; Epub ahead of print
23 **Davis GL**, Czaja AJ, Ludwig J. Development and prognosis of histologic cirrhosis in corticosteroid-treated hepatitis B surface antigen-negative chronic active hepatitis. *Gastroenterology* 1984; **87**: 1222-1227
24 **Roberts SK**, Therneau TM, Czaja AJ. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. *Gastroenterology* 1996; **110**: 848-857
25 **Czaja AJ**, Carpenter HA. Progressive fibrosis during corticosteroid therapy of autoimmune hepatitis. *Hepatology* 2004; **39**: 1631-1638
26 **Schalm SW**, Korman MG, Summerskill WH, Czaja AJ, Baggenstoss AH. Severe chronic active liver disease. Prognostic significance of initial morphologic patterns. *Am J Dig Dis* 1977; **22**: 973-980
27 **Czaja AJ**, Ludwig J, Baggenstoss AH, Wolf A. Corticosteroid-treated chronic active hepatitis in remission: uncertain prognosis of chronic persistent hepatitis. *N Engl J Med* 1981; **304**: 5-9
28 **Bittencourt PL**, Farias AQ, Porta G, Cançado EL, Miura I, Pugliese R, Kalil J, Goldberg AC, Carrilho FJ. Frequency of concurrent autoimmune disorders in patients with autoimmune hepatitis: effect of age, gender, and genetic background. *J Clin Gastroenterol* 2008; **42**: 300-305
29 **Czaja AJ**. Clinical features, differential diagnosis and treatment of autoimmune hepatitis in the elderly. *Drugs Aging* 2008; **25**: 219-239
30 **Czaja AJ**. Behavior and significance of autoantibodies in type 1 autoimmune hepatitis. *J Hepatol* 1999; **30**: 394-401
31 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB, Homburger HA. The nature and prognosis of severe cryptogenic chronic active hepatitis. *Gastroenterology* 1993; **104**: 1755-1761
32 **Gassert DJ**, Garcia H, Tanaka K, Reinus JF. Corticosteroid-responsive cryptogenic chronic hepatitis: evidence for seronegative autoimmune hepatitis. *Dig Dis Sci* 2007; **52**: 2433-2437
33 **Pratt DS**, Fawaz KA, Rabson A, Dellelis R, Kaplan MM. A novel histological lesion in glucocorticoid-responsive chronic hepatitis. *Gastroenterology* 1997; **113**: 664-668
34 **Singh R**, Nair S, Farr G, Mason A, Perrillo R. Acute autoimmune hepatitis presenting with centrizonal liver disease: case report and review of the literature. *Am J Gastroenterol* 2002; **97**: 2670-2673
35 **Okano N**, Yamamoto K, Sakaguchi K, Miyake Y, Shimada N, Hakoda T, Terada R, Baba S, Suzuki T, Tsuji T. Clinicopathological features of acute-onset autoimmune hepatitis. *Hepatol Res* 2003; **25**: 263-270
36 **Hofer H**, Oesterreicher C, Wrba F, Ferenci P, Penner E. Centrilobular necrosis in autoimmune hepatitis: a histological feature associated with acute clinical presentation. *J Clin Pathol* 2006; **59**: 246-249
37 **Zen Y**, Notsumata K, Tanaka N, Nakanuma Y. Hepatic centrilobular zonal necrosis with positive antinuclear antibody: a unique subtype or early disease of autoimmune hepatitis? *Hum Pathol* 2007; **38**: 1669-1675
38 **Ludwig J,** Czaja AJ, Dickson ER, LaRusso NF, Wiesner RH. Manifestations of nonsuppurative cholangitis in chronic hepatobiliary diseases: morphologic spectrum, clinical correlations and terminology. *Liver* 1984; **4**: 105-116
39 **Ben-Ari Z**, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. *Hepatology* 1993; **18**: 10-15
40 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB. Autoimmune cholangitis within the spectrum of autoimmune liver disease. *Hepatology* 2000; **31**: 1231-1238
41 **Czaja AJ**, Carpenter HA. Autoimmune hepatitis with incidental histologic features of bile duct injury. *Hepatology* 2001; **34**: 659-665
42 **Czaja AJ**, Muratori P, Muratori L, Carpenter HA, Bianchi FB. Diagnostic and therapeutic implications of bile duct injury in autoimmune hepatitis. *Liver Int* 2004; **24**: 322-329
43 **Lim KN**, Casanova RL, Boyer TD, Bruno CJ. Autoimmune hepatitis in African Americans: presenting features and response to therapy. *Am J Gastroenterol* 2001; **96**: 3390-3394
44 **Hurlburt KJ**, McMahon BJ, Deubner H, Hsu-Trawinski B, Williams JL, Kowdley KV. Prevalence of autoimmune liver disease in Alaska Natives. *Am J Gastroenterol* 2002; **97**: 2402-2407
45 **Zolfino T**, Heneghan MA, Norris S, Harrison PM, Portmann BC, McFarlane IG. Characteristics of autoimmune hepatitis in patients who are not of European Caucasoid ethnic origin. *Gut* 2002; **50**: 713-717
46 **Gohar S**, Desai D, Joshi A, Bhaduri A, Deshpande R, Balkrishna C, Chawla M, Rodrigues C, Joshi VR. Autoimmune hepatitis: a study of 50 patients. *Indian J Gastroenterol* 2003; **22**: 140-142
47 **D'Souza R**, Sinnott P, Glynn MJ, Sabin CA, Foster GR. An unusual form of autoimmune hepatitis in young Somalian men. *Liver Int* 2005; **25**: 325-330
48 **Viruet EJ**, Torres EA. Steroid therapy in fulminant hepatic failure secondary to autoimmune hepatitis. *P R Health Sci J* 1998; **17**: 297-300
49 **Ichai P**, Duclos-Vallée JC, Guettier C, Hamida SB, Antonini T, Delvart V, Saliba F, Azoulay D, Castaing D, Samuel D. Usefulness of corticosteroids for the treatment of severe and fulminant forms of autoimmune hepatitis. *Liver Transpl* 2007; **13**: 996-1003
50 **Czaja AJ**. Corticosteroids or not in severe acute or fulminant autoimmune hepatitis: therapeutic brinksmanship and the point beyond salvation. *Liver Transpl* 2007; **13**: 953-955

51 **Soloway RD**, Summerskill WH, Baggenstoss AH, Geall MG, Gitnićk GL, Elveback IR, Schoenfield LJ. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology* 1972; **63**: 820-833

52 **Summerskill WH**, Korman MG, Ammon HV, Baggenstoss AH. Prednisone for chronic active liver disease: dose titration, standard dose, and combination with azathioprine compared. *Gut* 1975; **16**: 876-883

53 **Dufour JF**, DeLellis R, Kaplan MM. Reversibility of hepatic fibrosis in autoimmune hepatitis. *Ann Intern Med* 1997; **127**: 981-985

54 **Cotler SJ**, Jakate S, Jensen DM. Resolution of cirrhosis in autoimmune hepatitis with corticosteroid therapy. *J Clin Gastroenterol* 2001; **32**: 428-430

55 **Czaja AJ**, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. *J Hepatol* 2004; **40**: 646-652

56 **Mohamadnejad M**, Malekzadeh R, Nasseri-Moghaddam S, Hagh-Azali S, Rakhshani N, Tavangar SM, Sedaghat M, Alimohamadi SM. Impact of immunosuppressive treatment on liver fibrosis in autoimmune hepatitis. *Dig Dis Sci* 2005; **50**: 547-551

57 **Schalm SW**, Ammon HV, Summerskill WH. Failure of customary treatment in chronic active liver disease: causes and management. *Ann Clin Res* 1976; **8**: 221-227

58 **Montano-Loza AJ**, Carpenter HA, Czaja AJ. Features associated with treatment failure in type 1 autoimmune hepatitis and predictive value of the model of end-stage liver disease. *Hepatology* 2007; **46**: 1138-1145

59 **Czaja AJ**, Davis GL, Ludwig J, Taswell HF. Complete resolution of inflammatory activity following corticosteroid treatment of HBsAg-negative chronic active hepatitis. *Hepatology* 1984; **4**: 622-627

60 **Czaja AJ**, Ammon HV, Summerskill WH. Clinical features and prognosis of severe chronic active liver disease (CALD) after corticosteroid-induced remission. *Gastroenterology* 1980; **78**: 518-523

61 **Hegarty JE**, Nouri Aria KT, Portmann B, Eddleston AL, Williams R. Relapse following treatment withdrawal in patients with autoimmune chronic active hepatitis. *Hepatology* 1983; **3**: 685-689

62 **Czaja AJ**, Beaver SJ, Shiels MT. Sustained remission after corticosteroid therapy of severe hepatitis B surface antigen-negative chronic active hepatitis. *Gastroenterology* 1987; **92**: 215-219

63 **Czaja AJ**, Menon KV, Carpenter HA. Sustained remission after corticosteroid therapy for type 1 autoimmune hepatitis: a retrospective analysis. *Hepatology* 2002; **35**: 890-897

64 **Montano-Loza AJ**, Carpenter HA, Czaja AJ. Consequences of treatment withdrawal in type 1 autoimmune hepatitis. *Liver Int* 2007; **27**: 507-515

65 **Hennes EM**, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48**: 169-176

66 **Czaja AJ**. Performance parameters of the diagnostic scoring systems for autoimmune hepatitis. *Hepatology* 2008; **48**: 1540-1548

67 **Kanzler S**, Gerken G, Löhr H, Galle PR, Meyer zum Büschenfelde KH, Lohse AW. Duration of immunosuppressive therapy in autoimmune hepatitis. *J Hepatol* 2001; **34**: 354-355

68 **Verma S**, Gunuwan B, Mendler M, Govindrajan S, Redeker A. Factors predicting relapse and poor outcome in type I autoimmune hepatitis: role of cirrhosis development, patterns of transaminases during remission and plasma cell activity in the liver biopsy. *Am J Gastroenterol* 2004; **99**: 1510-1516

69 **Miyake Y**, Iwasaki Y, Terada R, Takagi S, Okamaoto R, Ikeda H, Sakai N, Makino Y, Kobashi H, Takaguchi K, Sakaguchi K, Shiratori Y. Persistent normalization of serum alanine aminotransferase levels improves the prognosis of type 1 autoimmune hepatitis. *J Hepatol* 2005; **43**: 951-957

70 **Montano-Loza AJ**, Carpenter HA, Czaja AJ. Improving the end point of corticosteroid therapy in type 1 autoimmune hepatitis to reduce the frequency of relapse. *Am J Gastroenterol* 2007; **102**: 1005-1012

71 **Stellon AJ**, Keating JJ, Johnson PJ, McFarlane IG, Williams R. Maintenance of remission in autoimmune chronic active hepatitis with azathioprine after corticosteroid withdrawal. *Hepatology* 1988; **8**: 781-784

72 **Czaja AJ**. Low-dose corticosteroid therapy after multiple relapses of severe HBsAg-negative chronic active hepatitis. *Hepatology* 1990; **11**: 1044-1049

73 **Johnson PJ**, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. *N Engl J Med* 1995; **333**: 958-963

74 **Wörns MA**, Teufel A, Kanzler S, Shrestha A, Victor A, Otto G, Lohse AW, Galle PR, Höhler T. Incidence of HAV and HBV infections and vaccination rates in patients with autoimmune liver diseases. *Am J Gastroenterol* 2008; **103**: 138-146

75 **Manns MP**, Bahr MJ, Woynarowski M, Kreisel W, Oren R, Günther R, Hultcrantz R, Proels M, Rust C, Spengler U, Szalay F. Budesonide 3 mg TID is superior to prednisolone in combination with azathioprine in the treatment of autoimmune hepatitis (abstract). *J Hepatol* 2008; **48** suppl 2: S369-S370

76 **Vierling JM**, Flores PA. Evolving new therapies of autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 825-850, ix

77 **Montano Loza AJ**, Czaja AJ. Current therapy for autoimmune hepatitis. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 202-214

78 **Czaja AJ**. Autoimmune hepatitis. Part A: pathogenesis. *Expert Rev Gastroenterol Hepatol* 2007; **1**: 113-128

79 **Czaja AJ**. Evolving concepts in the diagnosis, pathogenesis and treatment of autoimmune hepatitis. *Minerva Gastroenterol Dietol* 2007; **53**: 43-78

80 **Czaja AJ**, Freese DK. Diagnosis and treatment of autoimmune hepatitis. *Hepatology* 2002; **36**: 479-497

81 **Burgart LJ**, Batts KP, Ludwig J, Nikias GA, Czaja AJ. Recent-onset autoimmune hepatitis. Biopsy findings and clinical correlations. *Am J Surg Pathol* 1995; **19**: 699-708

82 **Czaja AJ**, Davis GL, Ludwig J, Baggenstoss AH, Taswell HF. Autoimmune features as determinants of prognosis in steroid-treated chronic active hepatitis of uncertain etiology. *Gastroenterology* 1983; **85**: 713-717

83 **Davis GL**, Czaja AJ, Baggenstoss AH, Taswell HF. Prognostic and therapeutic implications of extreme serum aminotransferase elevation in chronic active hepatitis. *Mayo Clin Proc* 1982; **57**: 303-309

84 **Iwai M**, Jo M, Ishii M, Mori T, Harada Y. Comparison of clinical features and liver histology in acute and chronic autoimmune hepatitis. *Hepatol Res* 2008; **38**: 784-789

85 **Czaja AJ**, Rakela J, Ludwig J. Features reflective of early prognosis in corticosteroid-treated severe autoimmune chronic active hepatitis. *Gastroenterology* 1988; **95**: 448-453

86 **Tan P**, Marotta P, Ghent C, Adams P. Early treatment response predicts the need for liver transplantation in autoimmune hepatitis. *Liver Int* 2005; **25**: 728-733

87 **De Groote J**, Fevery J, Lepoutre L. Long-term follow-up of chronic active hepatitis of moderate severity. *Gut* 1978; **19**: 510-513

88 **Koretz RL**, Lewin KJ, Higgins J, Fagen ND, Gitnick GL. Chronic active hepatitis. Who meets treatment criteria? *Dig Dis Sci* 1980; **25**: 695-699

89 **Hodges JR**, Millward-Sadler GH, Wright R. Chronic active hepatitis: the spectrum of disease. *Lancet* 1982; **1**: 550-552

90 **Cook GC**, Mulligan R, Sherlock S. Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Q J Med* 1971; **40**: 159-185

91 **Murray-Lyon IM**, Stern RB, Williams R. Controlled trial

of prednisone and azathioprine in active chronic hepatitis. *Lancet* 1973; **1**: 735-737
92 **Misdraji J**, Thiim M, Graeme-Cook FM. Autoimmune hepatitis with centrilobular necrosis. *Am J Surg Pathol* 2004; **28**: 471-478
93 **Czaja AJ**, Carpenter HA. Sensitivity, specificity, and predictability of biopsy interpretations in chronic hepatitis. *Gastroenterology* 1993; **105**: 1824-1832
94 **Adams LA**, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2004; **99**: 1316-1320
95 **Tajiri K**, Takenawa H, Yamaoka K, Yamane M, Marumo F, Sato C. Nonalcoholic steatohepatitis masquerading as autoimmune hepatitis. *J Clin Gastroenterol* 1997; **25**: 538-540
96 **Loria P**, Lonardo A, Leonardi F, Fontana C, Carulli L, Verrone AM, Borsatti A, Bertolotti M, Cassani F, Bagni A, Muratori P, Ganazzi D, Bianchi FB, Carulli N. Non-organ-specific autoantibodies in nonalcoholic fatty liver disease: prevalence and correlates. *Dig Dis Sci* 2003; **48**: 2173-2181
97 **Czaja AJ**, Carpenter HA. Optimizing diagnosis from the medical liver biopsy. *Clin Gastroenterol Hepatol* 2007; **5**: 898-907
98 **Czaja AJ**. Autoantibodies in autoimmune liver disease. *Adv Clin Chem* 2005; **40**: 127-164
99 **Czaja AJ**. Autoimmune hepatitis. Part B: diagnosis. *Expert Rev Gastroenterol Hepatol* 2007; **1**: 129-143
100 **Czaja AJ**, Hay JE, Rakela J. Clinical features and prognostic implications of severe corticosteroid-treated cryptogenic chronic active hepatitis. *Mayo Clin Proc* 1990; **65**: 23-30
101 **Baeres M**, Herkel J, Czaja AJ, Wies I, Kanzler S, Cancado EL, Porta G, Nishioka M, Simon T, Daehnrich C, Schlumberger W, Galle PR, Lohse AW. Establishment of standardised SLA/LP immunoassays: specificity for autoimmune hepatitis, worldwide occurrence, and clinical characteristics. *Gut* 2002; **51**: 259-264
102 **Czaja AJ**, Shums Z, Norman GL. Frequency and significance of antibodies to soluble liver antigen/liver pancreas in variant autoimmune hepatitis. *Autoimmunity* 2002; **35**: 475-483
103 **Targan SR**, Landers C, Vidrich A, Czaja AJ. High-titer antineutrophil cytoplasmic antibodies in type-1 autoimmune hepatitis. *Gastroenterology* 1995; **108**: 1159-1166
104 **Zauli D**, Ghetti S, Grassi A, Descovich C, Cassani F, Ballardini G, Muratori L, Bianchi FB. Anti-neutrophil cytoplasmic antibodies in type 1 and 2 autoimmune hepatitis. *Hepatology* 1997; **25**: 1105-1107
105 **Volta U**, De Franceschi L, Molinaro N, Cassani F, Muratori L, Lenzi M, Bianchi FB, Czaja AJ. Frequency and significance of anti-gliadin and anti-endomysial antibodies in autoimmune hepatitis. *Dig Dis Sci* 1998; **43**: 2190-2195
106 **Kaukinen K**, Halme L, Collin P, Färkkilä M, Mäki M, Vehmanen P, Partanen J, Höckerstedt K. Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. *Gastroenterology* 2002; **122**: 881-888
107 **Abdo A**, Meddings J, Swain M. Liver abnormalities in celiac disease. *Clin Gastroenterol Hepatol* 2004; **2**: 107-112
108 **Kenny RP**, Czaja AJ, Ludwig J, Dickson ER. Frequency and significance of antimitochondrial antibodies in severe chronic active hepatitis. *Dig Dis Sci* 1986; **31**: 705-711
109 **Muratori P**, Muratori L, Gershwin ME, Czaja AJ, Pappas G, MacCariello S, Granito A, Cassani F, Loria P, Lenzi M, Bianchi FB. 'True' antimitochondrial antibody-negative primary biliary cirrhosis, low sensitivity of the routine assays, or both? *Clin Exp Immunol* 2004; **135**: 154-158
110 **Nezu S**, Tanaka A, Yasui H, Imamura M, Nakajima H, Ishida H, Takahashi S. Presence of antimitochondrial autoantibodies in patients with autoimmune hepatitis. *J Gastroenterol Hepatol* 2006; **21**: 1448-1454
111 **O'Brien C**, Joshi S, Feld JJ, Guindi M, Dienes HP, Heathcote EJ. Long-term follow-up of antimitochondrial antibody-positive autoimmune hepatitis. *Hepatology* 2008; **48**: 550-556
112 **Montano-Loza AJ**, Carpenter HA, Czaja AJ. Frequency, behavior, and prognostic implications of antimitochondrial antibodies in type 1 autoimmune hepatitis. *J Clin Gastroenterol* 2008; **42**: 1047-1053
113 **Mishima S**, Omagari K, Ohba K, Kadokawa Y, Masuda J, Mishima R, Kinoshita H, Hayashida K, Isomoto H, Shikuwa S, Mizuta Y, Kohno S. Clinical implications of antimitochondrial antibodies in type 1 autoimmune hepatitis: a longitudinal study. *Hepatogastroenterology* 2008; **55**: 221-227
114 **Leung PS**, Rossaro L, Davis PA, Park O, Tanaka A, Kikuchi K, Miyakawa H, Norman GL, Lee W, Gershwin ME. Antimitochondrial antibodies in acute liver failure: implications for primary biliary cirrhosis. *Hepatology* 2007; **46**: 1436-1442
115 **Gregorio GV**, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, Mieli-Vergani G. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. *Hepatology* 2001; **33**: 544-553
116 **Perdigoto R**, Carpenter HA, Czaja AJ. Frequency and significance of chronic ulcerative colitis in severe corticosteroid-treated autoimmune hepatitis. *J Hepatol* 1992; **14**: 325-331
117 **Abdalian R**, Dhar P, Jhaveri K, Haider M, Guindi M, Heathcote EJ. Prevalence of sclerosing cholangitis in adults with autoimmune hepatitis: evaluating the role of routine magnetic resonance imaging. *Hepatology* 2008; **47**: 949-957
118 **Sahni VA**, Mortele KJ. Magnetic resonance cholangiopancreatography: current use and future applications. *Clin Gastroenterol Hepatol* 2008; **6**: 967-977
119 **Lewin M**, Vilgrain V, Ozenne V, Lemoine M, Wendum D, Paradis V, Ziol M, Beaugrand M, Poupon R, Valla D, Chazouilleres O, Corpechot C. MRI abnormalities of bile ducts in adults with autoimmune hepatitis: myth or reality? Results of a controlled prospective study (abstract). *Hepatology* (Suppl) 2008; **48**: 421A
120 **Gohlke F**, Lohse AW, Dienes HP, Löhr H, Märker-Hermann E, Gerken G, Meyer zum Büschenfelde KH. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. *J Hepatol* 1996; **24**: 699-705
121 **Czaja AJ**. Frequency and nature of the variant syndromes of autoimmune liver disease. *Hepatology* 1998; **28**: 360-365
122 **McNair AN**, Moloney M, Portmann BC, Williams R, McFarlane IG. Autoimmune hepatitis overlapping with primary sclerosing cholangitis in five cases. *Am J Gastroenterol* 1998; **93**: 777-784
123 **Floreani A**, Rizzotto ER, Ferrara F, Carderi I, Caroli D, Blasone L, Baldo V. Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. *Am J Gastroenterol* 2005; **100**: 1516-1522
124 **Al-Chalabi T**, Portmann BC, Bernal W, McFarlane IG, Heneghan MA. Autoimmune hepatitis overlap syndromes: an evaluation of treatment response, long-term outcome and survival. *Aliment Pharmacol Ther* 2008; **28**: 209-220
125 **Czaja AJ**. Treatment strategies in autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 799-824
126 **Carpenter HA**, Czaja AJ. The role of histologic evaluation in the diagnosis and management of autoimmune hepatitis and its variants. *Clin Liver Dis* 2002; **6**: 685-705
127 **Boberg KM**, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand J Gastroenterol* 1998; **33**: 99-103
128 **Werner M**, Prytz H, Ohlsson B, Almer S, Björnsson E, Bergquist A, Wallerstedt S, Sandberg-Gertzén H, Hultcrantz R, Sangfelt P, Weiland O, Danielsson A. Epidemiology and the initial presentation of autoimmune hepatitis in Sweden: a nationwide study. *Scand J Gastroenterol* 2008; **43**: 1232-1240
129 **Czaja AJ**, dos Santos RM, Porto A, Santrach PJ, Moore SB. Immune phenotype of chronic liver disease. *Dig Dis Sci* 1998; **43**: 2149-2155

130 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB. Significance of HLA DR4 in type 1 autoimmune hepatitis. *Gastroenterology* 1993; **105**: 1502-1507

131 **Czaja AJ**, Strettell MD, Thomson LJ, Santrach PJ, Moore SB, Donaldson PT, Williams R. Associations between alleles of the major histocompatibility complex and type 1 autoimmune hepatitis. *Hepatology* 1997; **25**: 317-323

132 **Czaja AJ**, Donaldson PT. Gender effects and synergisms with histocompatibility leukocyte antigens in type 1 autoimmune hepatitis. *Am J Gastroenterol* 2002; **97**: 2051-2057

133 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB. Genetic predispositions for the immunological features of chronic active hepatitis. *Hepatology* 1993; **18**: 816-822

134 **Tong MJ**, Hsieh J, Blatt LM. Demographics, treatment and a predictive model of survival in a population of chronic auto- immune hepatitis patients (abstract). *Hepatology* 1998; **28**: 549A

135 **Heneghan MA**, Kosar Y, McDougall NI, Portmann B, McFarlane IG, Harrison PM. Characteristics and outcome of a large male cohort with autoimmune hepatitis: Male sex as a favorable prognostic indicator: Treat-ment response and outcome (abstract). *Gastroenterology* 2000; **118**: A1009

136 **Al-Chalabi T**, Underhill JA, Portmann BC, McFarlane IG, Heneghan MA. Impact of gender on the long-term outcome and survival of patients with autoimmune hepatitis. *J Hepatol* 2008; **48**: 140-147

137 **Verma S**, Torbenson M, Thuluvath PJ. The impact of ethnicity on the natural history of autoimmune hepatitis. *Hepatology* 2007; **46**: 1828-1835

138 **Nguyen GC**, Thuluvath PJ. Racial disparity in liver disease: Biological, cultural, or socioeconomic factors. *Hepatology* 2008; **47**: 1058-1066

139 **Nakamura K**, Yoneda M, Yokohama S, Tamori K, Sato Y, Aso K, Aoshima M, Hasegawa T, Makino I. Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis. *J Gastroenterol Hepatol* 1998; **13**: 490-495

140 **Czaja AJ**, Souto EO, Bittencourt PL, Cancado EL, Porta G, Goldberg AC, Donaldson PT. Clinical distinctions and pathogenic implications of type 1 autoimmune hepatitis in Brazil and the United States. *J Hepatol* 2002; **37**: 302-308

141 **Chung HV**, Riley M, Ho JK, Leung B, Jevon GP, Arbour LT, Barker C, Schreiber R, Yoshida EM. Retrospective review of pediatric and adult autoimmune hepatitis in two quaternary care centres in British Columbia: increased prevalence seen in British Columbia's First Nations community. *Can J Gastroenterol* 2007; **21**: 565-568

142 **Minuk GY**, Liu S, Kaita K, Wong S, Renner E, Rempel J, Uhanova J. Autoimmune hepatitis in a North American Aboriginal/First Nations population. *Can J Gastroenterol* 2008; **22**: 829-834

143 **Scott JD**, Garland N. Chronic liver disease in Aboriginal North Americans. *World J Gastroenterol* 2008; **14**: 4607-4615

144 **Donaldson PT**, Czaja AJ. Genetic effects on susceptibility, clinical expression, and treatment outcome of type 1 autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 707-725

145 **Czaja AJ**. Genetic factors affecting the occurrence, clinical phenotype, and outcome of autoimmune hepatitis. *Clin Gastroenterol Hepatol* 2008; **6**: 379-388

146 **Siegel AB**, McBride RB, El-Serag HB, Hershman DL, Brown RS Jr, Renz JF, Emond J, Neugut AI. Racial disparities in utilization of liver transplantation for hepatocellular carcinoma in the United States, 1998-2002. *Am J Gastroenterol* 2008; **103**: 120-127

147 **Flores YN**, Yee HF Jr, Leng M, Escarce JJ, Bastani R, Salmerón J, Morales LS. Risk factors for chronic liver disease in Blacks, Mexican Americans, and Whites in the United States: results from NHANES IV, 1999-2004. *Am J Gastroenterol* 2008; **103**: 2231-2238

148 **Günsar F**, Akarca US, Ersöz G, Karasu Z, Yüce G, Batur Y. Clinical and biochemical features and therapy responses in primary biliary cirrhosis and primary biliary cirrhosis-autoimmune hepatitis overlap syndrome. *Hepatogastroenterology* 2002; **49**: 1195-1200

149 **Kaymakoglu S**, Cakaloglu Y, Demir K, Türkoglu S, Badur S, Gürel S, Beşişik F, Cevikbaş U, Okten A. Is severe cryptogenic chronic hepatitis similar to autoimmune hepatitis? *J Hepatol* 1998; **28**: 78-83

150 **Balaban YH**, Batman F, Us D, Hascelik G, Bayraktar Y. Serum hepatocyte growth factor in autoimmune and hepatitis B-associated liver diseases. *Indian J Gastroenterol* 2007; **26**: 192-193

151 **Usta Y**, Gurakan F, Akcoren Z, Ozen S. An overlap syndrome involving autoimmune hepatitis and systemic lupus erythematosus in childhood. *World J Gastroenterol* 2007; **13**: 2764-2767

152 **Kayacetin E**, Köklü S, Temuçin T. Overlap syndrome of primary biliary cirrhosis and autoimmune hepatitis with unusual initial presentation as fulminant hepatic failure. *Dig Liver Dis* 2004; **36**: 419-422

153 **Cindoruk M**, Yetkin I, Karakan T, Kandilci U. The prevalence of autoimmune hepatitis in Hashimoto's thyroiditis in a Turkish population. *Acta Gastroenterol Belg* 2002; **65**: 143-145

154 **Arslan N**, Büyükgebiz B, Oztürk Y, Ozer E. The prevalence of liver function abnormalities in pediatric celiac disease patients and its relation with intestinal biopsy findings. *Acta Gastroenterol Belg* 2005; **68**: 424-427

155 **Kocaman O**, Aygun C, Gurbuz Y, Cefle A, Konduk T, Senturk O, Celebi A, Hulagu S. Burst of autoimmunity with the emergence of primary Sjogren syndrome, cholestatic autoimmune hepatitis and latent autoimmune diabetes of adults (LADA). *South Med J* 2006; **99**: 1014-1015

156 **Soy M**, Guldiken S, Arikan E, Altun BU, Tugrul A. Frequency of rheumatic diseases in patients with autoimmune thyroid disease. *Rheumatol Int* 2007; **27**: 575-577

157 **Tabak F**, Ozdemir F, Tabak O, Erer B, Tahan V, Ozaras R. Autoimmune hepatitis induced by the prolonged hepatitis A virus infection. *Ann Hepatol* 2008; **7**: 177-179

158 **Selimoglu MA**, Ertekin V. Autoimmune hepatitis triggered by Brucella infection or doxycycline or both. *Int J Clin Pract* 2003; **57**: 639-641

159 **Kocaman O**, Hulagu S, Senturk O. Echinacea-induced severe acute hepatitis with features of cholestatic autoimmune hepatitis. *Eur J Intern Med* 2008; **19**: 148

160 **Kacar S**, Akdogan M, Koşar Y, Parlak E, Sasmaz N, Oguz P, Aydog G. Estrogen and cyproterone acetate combination-induced autoimmune hepatitis. *J Clin Gastroenterol* 2002; **35**: 98-100

161 **Koşar Y**, Saşmaz N, Oguz P, Kacar S, Erden E, Parlak E, Akdogan M. Ornidazole-induced autoimmune hepatitis. *Eur J Gastroenterol Hepatol* 2001; **13**: 737-739

162 **Kosar Y**, Kacar S, Sasmaz N, Oguz P, Turhan N, Parlak E, Heneghan MA, McFarlane IG. Type 1 autoimmune hepatitis in Turkish patients: absence of association with HLA B8. *J Clin Gastroenterol* 2002; **35**: 185-190

163 **Czaja AJ**, Carpenter HA. Histological features associated with relapse after corticosteroid withdrawal in type 1 autoimmune hepatitis. *Liver Int* 2003; **23**: 116-123

164 **Heneghan MA**, McFarlane IG. Current and novel immunosuppressive therapy for autoimmune hepatitis. *Hepatology* 2002; **35**: 7-13

165 **Mistilis SP**, Vickers CR, Darroch MH, McCarthy SW. Cyclosporin, a new treatment for autoimmune chronic active hepatitis. *Med J Aust* 1985; **143**: 463-465

166 **Hyams JS**, Ballow M, Leichtner AM. Cyclosporine treatment of autoimmune chronic active hepatitis. *Gastroenterology* 1987; **93**: 890-893

167 **Jackson LD**, Song E. Cyclosporin in the treatment of corticosteroid resistant autoimmune chronic active hepatitis. *Gut* 1995; **36**: 459-461

168 **Fernandes NF**, Redeker AG, Vierling JM, Villamil FG, Fong TL. Cyclosporine therapy in patients with steroid resistant autoimmune hepatitis. *Am J Gastroenterol* 1999; **94**: 241-248

169 **Malekzadeh R**, Nasseri-Moghaddam S, Kaviani MJ, Taheri

H, Kamalian N, Sotoudeh M. Cyclosporin A is a promising alternative to corticosteroids in autoimmune hepatitis. *Dig Dis Sci* 2001; **46**: 1321-1327
170 **Van Thiel DH**, Wright H, Carroll P, Abu-Elmagd K, Rodriguez-Rilo H, McMichael J, Irish W, Starzl TE. Tacrolimus: a potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. *Am J Gastroenterol* 1995; **90**: 771-776
171 **Aqel BA**, Machicao V, Rosser B, Satyanarayana R, Harnois DM, Dickson RC. Efficacy of tacrolimus in the treatment of steroid refractory autoimmune hepatitis. *J Clin Gastroenterol* 2004; **38**: 805-809
172 **Larsen FS**, Vainer B, Eefsen M, Bjerring PN, Adel Hansen B. Low-dose tacrolimus ameliorates liver inflammation and fibrosis in steroid refractory autoimmune hepatitis. *World J Gastroenterol* 2007; **13**: 3232-3236
173 **Alvarez F**, Ciocca M, Cañero-Velasco C, Ramonet M, de Davila MT, Cuarterolo M, Gonzalez T, Jara-Vega P, Camarena C, Brochu P, Drut R, Alvarez E. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. *J Hepatol* 1999; **30**: 222-227
174 **Debray D**, Maggiore G, Girardet JP, Mallet E, Bernard O. Efficacy of cyclosporin A in children with type 2 autoimmune hepatitis. *J Pediatr* 1999; **135**: 111-114
175 **Cuarterolo M**, Ciocca M, Velasco CC, Ramonet M, González T, López S, Garsd A, Alvarez F. Follow-up of children with autoimmune hepatitis treated with cyclosporine. *J Pediatr Gastroenterol Nutr* 2006; **43**: 635-639
176 **Pratt DS**, Flavin DP, Kaplan MM. The successful treatment of autoimmune hepatitis with 6-mercaptopurine after failure with azathioprine. *Gastroenterology* 1996; **110**: 271-274
177 **Richardson PD**, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. *J Hepatol* 2000; **33**: 371-375
178 **Devlin SM**, Swain MG, Urbanski SJ, Burak KW. Mycophenolate mofetil for the treatment of autoimmune hepatitis in patients refractory to standard therapy. *Can J Gastroenterol* 2004; **18**: 321-326
179 **Chatur N**, Ramji A, Bain VG, Ma MM, Marotta PJ, Ghent CN, Lilly LB, Heathcote EJ, Deschenes M, Lee SS, Steinbrecher UP, Yoshida EM. Transplant immunosuppressive agents in non-transplant chronic autoimmune hepatitis: the Canadian association for the study of liver (CASL) experience with mycophenolate mofetil and tacrolimus. *Liver Int* 2005; **25**: 723-727
180 **Czaja AJ**, Carpenter HA. Empiric therapy of autoimmune hepatitis with mycophenolate mofetil: comparison with conventional treatment for refractory disease. *J Clin Gastroenterol* 2005; **39**: 819-825
181 **Inductivo-Yu I**, Adams A, Gish RG, Wakil A, Bzowej NH, Frederick RT, Bonacini M. Mycophenolate mofetil in autoimmune hepatitis patients not responsive or intolerant to standard immunosuppressive therapy. *Clin Gastroenterol Hepatol* 2007; **5**: 799-802
182 **Czaja AJ**. Safety issues in the management of autoimmune hepatitis. *Expert Opin Drug Saf* 2008; **7**: 319-333
183 **Hlivko JT**, Shiffman ML, Stravitz RT, Luketic VA, Sanyal AJ, Fuchs M, Sterling RK. A single center review of the use of mycophenolate mofetil in the treatment of autoimmune hepatitis. *Clin Gastroenterol Hepatol* 2008; **6**: 1036-1040
184 **Hennes EM**, Oo YH, Schramm C, Denzer U, Buggisch P, Wiegard C, Kanzler S, Schuchmann M, Boecher W, Galle PR, Adams DH, Lohse AW. Mycophenolate mofetil as second line therapy in autoimmune hepatitis? *Am J Gastroenterol* 2008; **103**: 3063-3070
185 **Oo YH**, Neuberger J. Use of mycophenolate in the treatment of autoimmune hepatitis. *Liver Int* 2005; **25**: 687-691
186 **Danielsson A**, Prytz H. Oral budesonide for treatment of autoimmune chronic active hepatitis. *Aliment Pharmacol Ther* 1994; **8**: 585-590
187 **Wiegand J**, Schüler A, Kanzler S, Lohse A, Beuers U, Kreisel W, Spengler U, Koletzko S, Jansen PL, Hochhaus G, Möllmann HW, Pröls M, Manns MP. Budesonide in previously untreated autoimmune hepatitis. *Liver Int* 2005; **25**: 927-934
188 **Zandieh I**, Krygier D, Wong V, Howard J, Worobetz L, Minuk G, Witt-Sullivan H, Yoshida EM. The use of budesonide in the treatment of autoimmune hepatitis in Canada. *Can J Gastroenterol* 2008; **22**: 388-392
189 **Czaja AJ**, Lindor KD. Failure of budesonide in a pilot study of treatment-dependent autoimmune hepatitis. *Gastroenterology* 2000; **119**: 1312-1316
190 **Clissold SP**, Heel RC. Budesonide. A preliminary review of its pharmacodynamic properties and therapeutic efficacy in asthma and rhinitis. *Drugs* 1984; **28**: 485-518
191 **Geier A**, Gartung C, Dietrich CG, Wasmuth HE, Reinartz P, Matern S. Side effects of budesonide in liver cirrhosis due to chronic autoimmune hepatitis: influence of hepatic metabolism versus portosystemic shunts on a patient complicated with HCC. *World J Gastroenterol* 2003; **9**: 2681-2685
192 **Kanzler S**, Gerken G, Dienes HP, Meyer zum Büschenfelde KH, Lohse AW. Cyclophosphamide as alternative immunosuppressive therapy for autoimmune hepatitis--report of three cases. *Z Gastroenterol* 1997; **35**: 571-578
193 **Burak KW**, Urbanski SJ, Swain MG. Successful treatment of refractory type 1 autoimmune hepatitis with methotrexate. *J Hepatol* 1998; **29**: 990-993
194 **Kerkar N**, Dugan C, Rumbo C, Morotti RA, Gondolesi G, Shneider BL, Emre S. Rapamycin successfully treats post-transplant autoimmune hepatitis. *Am J Transplant* 2005; **5**: 1085-1089
195 **Santos ES**, Arosemena LR, Raez LE, O'Brien C, Regev A. Successful treatment of autoimmune hepatitis and idiopathic thrombocytopenic purpura with the monoclonal antibody, rituximab: case report and review of literature. *Liver Int* 2006; **26**: 625-629
196 **Carmassi F**, Morale M, Puccetti R, Pistelli F, Palla R, Bevilacqua G, Viacava P, Antonelli A, Mariani G. Efficacy of intravenous immunoglobulin therapy in a case of autoimmune-mediated chronic active hepatitis. *Clin Exp Rheumatol* 1992; **10**: 13-17
197 **Rebollo Bernárdez J**, Cifuentes Mimoso C, Piñar Moreno A, Caunedo Alvarez A, Salas Herrero E, Jiménez-Sáenz M, Herrerías Gutiérrez J. Deflazacort for long-term maintenance of remission in type I autoimmune hepatitis. *Rev Esp Enferm Dig* 1999; **91**: 630-638
198 **Czaja AJ**, Carpenter HA, Lindor KD. Ursodeoxycholic acid as adjunctive therapy for problematic type 1 autoimmune hepatitis: a randomized placebo-controlled treatment trial. *Hepatology* 1999; **30**: 1381-1386
199 **Czaja AJ**, Bianchi FB, Carpenter HA, Krawitt EL, Lohse AW, Manns MP, McFarlane IG, Mieli-Vergani G, Toda G, Vergani D, Vierling J, Zeniya M. Treatment challenges and investigational opportunities in autoimmune hepatitis. *Hepatology* 2005; **41**: 207-215
200 **Heneghan MA**, Al-Chalabi T, McFarlane IG. Cost-effectiveness of pharmacotherapy for autoimmune hepatitis. *Expert Opin Pharmacother* 2006; **7**: 145-156
201 **Fridkis-Hareli M**, Rosloniec EF, Fugger L, Strominger JL. Synthetic amino acid copolymers that bind to HLA-DR proteins and inhibit type II collagen-reactive T cell clones. *Proc Natl Acad Sci USA* 1998; **95**: 12528-12531
202 **Fridkis-Hareli M**, Rosloniec EF, Fugger L, Strominger JL. Synthetic peptides that inhibit binding of the collagen type II 261-273 epitope to rheumatoid arthritis-associated HLA-DR1 and -DR4 molecules and collagen-specific T-cell responses. *Hum Immunol* 2000; **61**: 640-650
203 **Boetticher NC**, Peine CJ, Kwo P, Abrams GA, Patel T, Aqel B, Boardman L, Gores GJ, Harmsen WS, McClain CJ, Kamath PS, Shah VH. A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. *Gastroenterology* 2008; **135**: 1953-1960

204 **Lohse AW**, Dienes HP, Meyer zum Büschenfelde KH. Suppression of murine experimental autoimmune hepatitis by T-cell vaccination or immunosuppression. *Hepatology* 1998; **27**: 1536-1543
205 **Wardrop RM 3rd**, Whitacre CC. Oral tolerance in the treatment of inflammatory autoimmune diseases. *Inflamm Res* 1999; **48**: 106-119
206 **Nagler A**, Pines M, Abadi U, Pappo O, Zeira M, Rabbani E, Engelhardt D, Ohana M, Chowdhury NR, Chowdhury JR, Ilan Y. Oral tolerization ameliorates liver disorders associated with chronic graft versus host disease in mice. *Hepatology* 2000; **31**: 641-648
207 **Czaja AJ**. Understanding the pathogenesis of autoimmune hepatitis. *Am J Gastroenterol* 2001; **96**: 1224-1231
208 **Manns MP**, Vogel A. Autoimmune hepatitis, from mechanisms to therapy. *Hepatology* 2006; **43**: S132-S144
209 **Lapierre P**, Béland K, Alvarez F. Pathogenesis of autoimmune hepatitis: from break of tolerance to immune-mediated hepatocyte apoptosis. *Transl Res* 2007; **149**: 107-113
210 **Jaeckel E**. Animal models of autoimmune hepatitis. *Semin Liver Dis* 2002; **22**: 325-338
211 **Christen U**, Holdener M, Hintermann E. Animal models for autoimmune hepatitis. *Autoimmun Rev* 2007; **6**: 306-311
212 **Bowen DG**. Of mice and molecular mimicry: modeling autoimmune hepatitis. *Hepatology* 2008; **48**: 1013-1015
213 **Lapierre P**, Djilali-Saiah I, Vitozzi S, Alvarez F. A murine model of type 2 autoimmune hepatitis: Xenoimmunization with human antigens. *Hepatology* 2004; **39**: 1066-1074
214 **Holdener M**, Hintermann E, Bayer M, Rhode A, Rodrigo E, Hintereder G, Johnson EF, Gonzalez FJ, Pfeilschifter J, Manns MP, Herrath MG, Christen U. Breaking tolerance to the natural human liver autoantigen cytochrome P450 2D6 by virus infection. *J Exp Med* 2008; **205**: 1409-1422
215 **Kuo TK**, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, Yang VW, Lee OK. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. *Gastroenterology* 2008; **134**: 2111-2121, 2121.e1-2121.e3

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Signal transduction mechanism of TRB3 in rats with non-alcoholic fatty liver disease

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Abstract

AIM: To evaluate the possible role of Tribble 3 (TRB3) in a rat model of non-alcoholic fatty liver disease (NAFLD) and its signal transduction mechanism.

METHODS: Thirty Sprague-Dawley rats were randomized into three groups: normal control group, non-alcoholic fatty liver group A (fed on a high-fat diet for 8 wk) and group B (fed on a high-fat diet for 16 wk). To determine the degree of hepatic steatosis in rats of each group, livers were stained with hematoxylin and eosin, and evaluated; real-time fluorescent quantitative reverse transcriptase-polymerase chain reaction was performed to measure the expression levels of TRB3 mRNA; and Western blotting analysis was done to determine the expression levels of protein kinase B (Akt) and phosphorylated protein kinase B (p-Akt-Thr308, p-Akt-Ser473).

RESULTS: Hepatic steatosis was evident in both NAFLD groups: mild to moderate hepatic steatosis occurred in group A, mainly as mild steatosis. Moderate to severe hepatic steatosis occurred in group B, mainly as severe steatosis. The expression level of TRB3 mRNA in group B was significantly higher than in the control group (122.28 ± 95.37 *vs* 3.06 ± 2.33, $P = 0.001$) and group A (122.28 ± 95.37 *vs* 5.77 ± 4.20, $P = 0.001$). There was no significant difference in the expression levels of Akt (1.03 ± 0.53 *vs* 1.12 ± 0.77, $P = 0.729$) and p-Akt-Thr308 (0.82 ± 0.45 *vs* 0.92 ± 0.38, $P = 0.592$) between group A and the control group. The expression level of Akt and p-Akt-Thr308 in group B was significantly lower than in group A (Akt 0.41 ± 0.16 *vs* 1.12 ± 0.77, $P = 0.008$; p-Akt-Thr308 0.47 ± 0.19 *vs* 0.82 ± 0.45, $P = 0.036$) and the control group (Akt 0.41 ± 0.16 *vs* 1.03 ± 0.53, $P = 0.018$; p-Akt-Thr308 0.47 ± 0.19 *vs* 0.92 ± 0.38, $P = 0.010$). The expression level of p-Akt-Ser473 in group A was significantly higher than in group B (1.48 ± 0.50 *vs* 0.81 ± 0.39, $P = 0.041$) as well as the control group (1.48 ± 0.50 *vs* 0.45 ± 0.26, $P = 0.003$).

CONCLUSION: TRB3 blocks insulin signaling by inhibiting Akt activation, which contributes to insulin resistance. It may be an important factor in the occurrence and development of NAFLD.

Key words: Non-alcoholic fatty liver disease; Rat; Tribble 3; Protein Kinase B; Insulin resistance

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INTRODUCTION

Three pseudo kinases of the Tribble family[1,2] have been recognized recently, which include Tribble 1 (TRB1), TRB2 and TRB3. Different from the typical kinase domain structure, tribbles lack a conventional ATP binding site and activity domain of protein kinases. Thus, no kinase activity by tribbles has been detected. They are classified as members of the family of pseudo-kinases[3]. TRB3 is a mammalian homolog[4] of *Drosphila* tribbles and is also called a neuronal cell death-inducible putative protein kinase gene in rodents. TRB3 is located

on the 20p13 region of the human chromosome[5]. Its full-length translated region in mRNA is 1074 bp, its protein product is made of 358 amino acids and it is a kind of nucleoprotein.

Studies indicate that TRB3 is involved in many biological processes, including insulin resistance (IR), blocking of insulin signaling pathway[6], endoplasmic reticulum stress responses[7] and the regulation of cell growth and differentiation[8,9]. Du *et al*[6] have found that the expression of hepatic TRB3 increased in a rat model of diabetes. It inhibits the activation of the Akt/PKB signaling pathway by insulin, resulting in IR. TRB3 inhibits the phosphorylation of Thr-308 and Ser-473 by binding with them, thus inhibiting the activity of Akt. Then, the insulin signaling pathway is blocked.

Research by Chitturi *et al*[10] indicates that IR exits in about 98% of patients with non-alcoholic fatty liver disease (NAFLD). IR is possibly of key importance in inducing NAFLD. Therefore, any factor related to IR may play an important role in the development of NAFLD. Therefore, TRB3 may not only be a cause of IR, but also an important factor in the occurrence and development of NAFLD. Rat models of NAFLD have been developed by feeding them a high-fat diet. The objective was to study the expression of TRB3 mRNA using reverse transcriptase-polymerase chain reaction (RT-PCR) in rat models of NAFLD, and to evaluate the role of TRB3, using Western blotting analysis, in the occurrence and development of NAFLD.

MATERIALS AND METHODS

Animals

Thirty healthy Sprague-Dawley rats weighing 210-260 g (15 male and 15 female) were purchased from Shanghai Slac Laboratory Animal Co. Ltd., Chinese Academy of Sciences. The rats were fed normal food for 1 wk.

Reagents

The materials for the high-fat-diet rat models and the reagents for pathological tests were all purchased from Shanghai Lanji Technology Development Co., Ltd.; Trizol and SYBR Green I were purchased from Invitrogen. Akt (A444) antibody, p-Akt (S473) antibody and p-Akt (T308) antibody were obtained from Bioworld. Actin and horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (H + L) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). PVDF membranes were supplied by Millipore. Diaminobenzidine was purchased from Sigma. DEPC-treated Water, TBE and loading buffer were obtained from Shanghai Gene and Biotech Co., Ltd. Taq enzyme and random primers were supplied by Takara Biotechnology (Dalian) Co., Ltd. RNase inhibitor, dNTP, Moloney murine leukemia virus and TEMED were procured from Promega. DTT, SDS, Tris, Glycin, N, N'-Methylene bisacrylamide and acrylamide were obtained from Amresco. BSA was acquired from the Huamei Biotech Company (packaged separately after being imported). Protein markers were purchased by the Shanghai Institute of Biochemistry, Chinese Academy of Sciences. Methyl alcohol was purchased from the Sinopharm Reagent Group (Shanghai, China).

Apparatus

Applied Biosystems 7500 Real-Time PCR System, Vertical Electrophoresis Tank, Electrophoresis Apparatus PAC3000 and Semi-Dry Transfer Unit were purchased from Bio-Rad; high-speed freezing centrifuge, Centrifuge 5417R, was purchased from Eppendorf; Gel Imaging System (GIS)-2008 was purchased from Shanghai Tanon Science & Technology Co., Ltd.; electronic balance BP310P was from Sartorius; pipettes were from Gilson; glass homogenizer was from Ningbo Scientz Scientific Instruments Research Institute; UV-VIS Spectro Photometer Unico UV-2000 was from Unico (Shanghai) Instruments Co., Ltd.; ultrapure water system from Millipore; and ultrasonic cell disruption system Soniprep150 from SANYO.

Animals

The rats were divided into three groups according to random number tables. The control group (n = 10) was fed on a normal diet; NAFLD groups (n = 20) were fed on high-fat diet, which was prepared by adding 10% lard and 2% cholesterol to the normal diet. Group A (n = 10) was fed on high-fat diet for 8 wk and group B (n = 10) was fed on high-fat diet for 16 wk. All the rats lived in an air-conditioned room at room temperature at 18-23℃ and 60% humidity. All the rats were fed *ad libitum* and had free access to water.

Histopathology

According to the schedule of the experiment, the rats were anesthetized with 2.5% pentobarbital sodium solution (1.5 mL/kg), injected into the abdominal cavity after an overnight fasting. They were sacrificed after blood samples were taken from the inferior vena cava, and the livers were removed immediately. Serum and liver paraffin embedded tissue sections were prepared according to routine methods. The liver specimens were fixed in a neutral formalin solution. The tissue sections were hematoxylin and eosin (HE), and the HE-stained sections were examined under a light microscope for the evaluation of hepatic steatosis.

Measurements for observation indexes

Fluorescent quantitative RT-PCR for measurement of TRB3 mRNA expression levels: RNA extraction and cDNA synthesis: The Trizol method was used to extract total RNA from tissues and an UV-VIS Spectrophotometer was used to determine the purity and concentration. Two micrograms of total RNA was reverse transcribed into cDNA.

Real-time fluorescent quantitative PCR: The SYBR

Table 1 Expression levels of TRB3 mRNA, Akt, p-Akt-Thr308, p-Akt-Ser473

Group	Case	TRB3 mRNA (10^{-5})	Akt	p-Akt-Thr308	p-Akt-Ser473
Control	10	3.06 ± 2.33	1.03 ± 0.53	0.92 ± 0.38	0.45 ± 0.26
A	10	5.77 ± 4.20	1.12 ± 0.77	0.82 ± 0.45	1.48 ± 0.50[b]
B	10	122.28 ± 95.37[b]	0.41 ± 0.16[b]	0.47 ± 0.19[b]	0.81 ± 0.39[c]

[b]$P < 0.01$ *vs* control group/group A; [c]$P < 0.05$ *vs* group A.

Green I dye method was adopted. GADPH and TRB3 were amplified by reverse transcription. After gel electrophoresis of amplified products, a fully-automatic Gel Imaging System was used to analyze mRNA expression to compare the intensity between the groups. All the results were normalized to GADPH. The GADPH primers were used as follows: upstream primer: 5'-ACCACAGTCCATGCCATCAC-3', downstream primer: 5'-TCCACCACCCTGTTGCTGTA-3'. The length of the amplified product was 440 bp. The TRB3 primers were used as follows: upstream primer: 5'-TCA TCTTGCGCGACCTCAA-3', downstream primer: 5'-TCCACCACCCTGTTGCTGTA-3'. The length of the amplified product was 296 bp. Thirty-six cycles of pre-degeneration at 95℃ for 2 min, degeneration at 95℃ for 10 s, annealing at 50℃ for 10 s, and extension at 72℃ for 45 s were used for all experiments.

Western blotting analysis for the expression levels of total Akt and phosphorylated Akt (p-Akt-Thr308, p-Akt-Ser473): Equal samples of tissue were prepared and put into protein extracts to be ground as plasm form. Then the plasm was high-speed centrifuged under freezing conditions for protein extraction. The protein concentration was determined according to the fixed steps. The protein samples (30 μL) were subjected to SDS-PAGE electrophoresis, transferred to PVDF membranes, and shaken on a rotary shaker at room temperature for 2 h. After that, a TBST buffer solution was used to wash the membrane three times. Then, Akt-related antibodies (Akt1, p-Akt-Thr308, p-Akt-Ser473) were incubated at 4℃ overnight under constant shaking on a rotary shaker. After three washes with TBST buffer solution, HRP-labeled goat anti-rabbit IgG (H + L) was incubated at room temperature while shaking on a rotary shaker for 2 h. NBT/BCIP reagent was applied for color development. The membrane was rinsed with deionized water. All the procedures were repeated three times. β-actin was chosen as an internal control. The GIS was used for data analysis, and statistical analysis was used to detect differences between samples and the internal control.

Statistical analysis

SPSS11 software was used for the statistical analysis. All the statistical data were expressed as mean ± SD for single factor analysis of variance and paired comparisons were performed by the least-square deconvolution method. Two-tailed tests ($\alpha = 0.05$) were used for statistical treatment. $P < 0.05$ was considered a significant difference.

RESULTS

Histopathological changes

A high-fat diet causes obvious hepatic steatosis in rats, which was evident for both model groups. Mild to moderate hepatic steatosis occurred in group A, mainly as mild steatosis. Moderate to severe hepatic steatosis occurred in model group B, mainly as severe steatosis. Mild hepatic steatosis tissue was defined as hepatic steatosis that accounted for 30%-50% of the total liver cells in the microscopic field; for moderate hepatic steatosis, liver cells with hepatic steatosis accounted for 50%-75% of the total liver cells; and for severe hepatic steatosis tissues, liver cells with hepatic steatosis accounted for over 75% of the total liver cells. In portal areas, severe inflammation featured infiltration of large numbers of diffuse lymphocytes and neutrophils, destroyed limiting plates and hepatic lobules were infiltrated by inflammatory cells that surrounded liver cells (Figure 1).

Fluorescent quantitative RT-PCR for measurement of TRB3 mRNA expression levels

We adopted real-time fluorescent quantitative RT-PCR methods to measure the expression levels of TRB3 mRNA in rats (Figure 2A-C). The relation ($r = 0.99$) between amplified results of PCR and Ct value of standard samples is shown in Figure 2C. From the melting curve, no primer dimer formation was detected during the PCR reaction (Figure 2B). We found that the expression level of TRB3 mRNA in group B was significantly higher than in the control group (122.28 ± 95.37 *vs* 3.06 ± 2.33, $P = 0.001$) and group A (122.28 ± 95.37 *vs* 5.77 ± 4.20, $P = 0.001$). There was no significant difference (5.77 ± 4.20 *vs* 3.06 ± 2.33, $P = 0.914$) in the expression levels of TRB3 between group A and the control group (Figure 3, Table 1). All these data indicate that a simple hepatic steatosis pathomorphism showed no significant difference between group A and the control group for the expression of TRB3 mRNA. As the time to set up the model increased, the degree of hepatic steatosis was raised. When the model deteriorated to show evidence of a fatty hepatitis pathomorphism, the expression of TRB3 mRNA was significantly higher.

Western blotting analysis for the expression levels of total Akt and phosphorylation Akt (p-Akt-Thr308, p-Akt-Ser473)

The protein bands of Akt p-Akt-Thr308 and p-Akt-

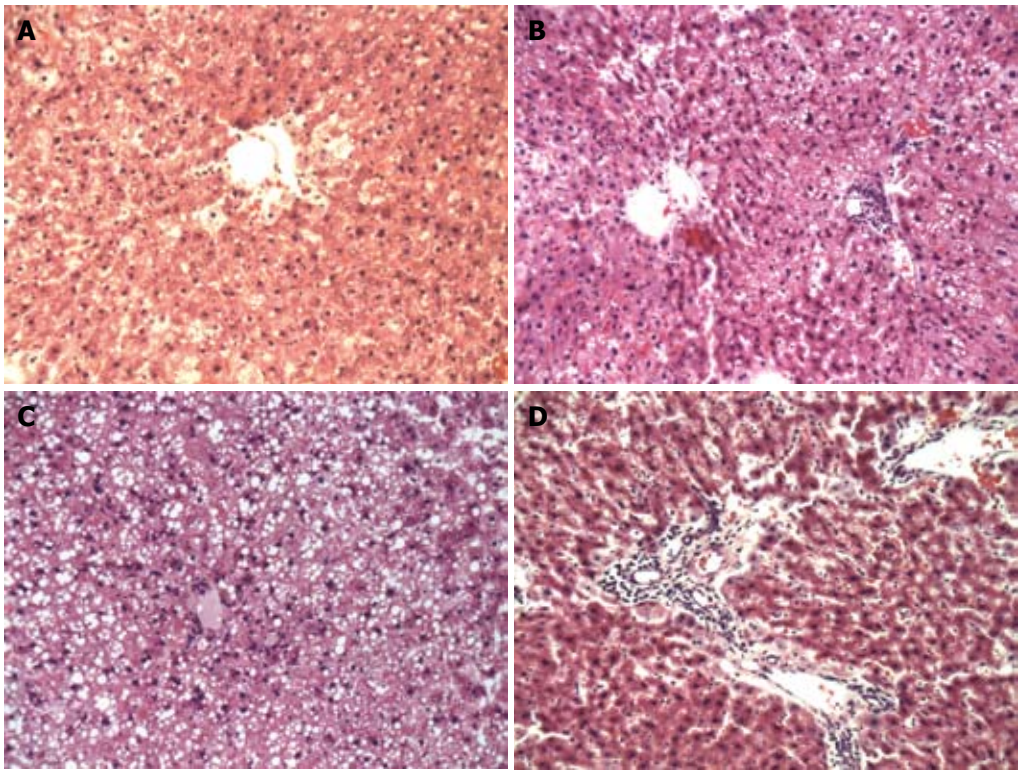

Figure 1 The degree of hepatic steatosis in each group. A: In group A, mild hepatic steatosis was observed on histological examination (HE, × 200); B: In group A, moderate hepatic steatosis was observed (HE, × 200); C: In group B, hepatocytes showed severe hepatic steatosis. Liver cells with hepatic steatosis accounted for over 75% of the total liver cells (HE, × 200); D: In group B, hepatocytes showed severe steatosis along with portal inflammation (HE, × 200).

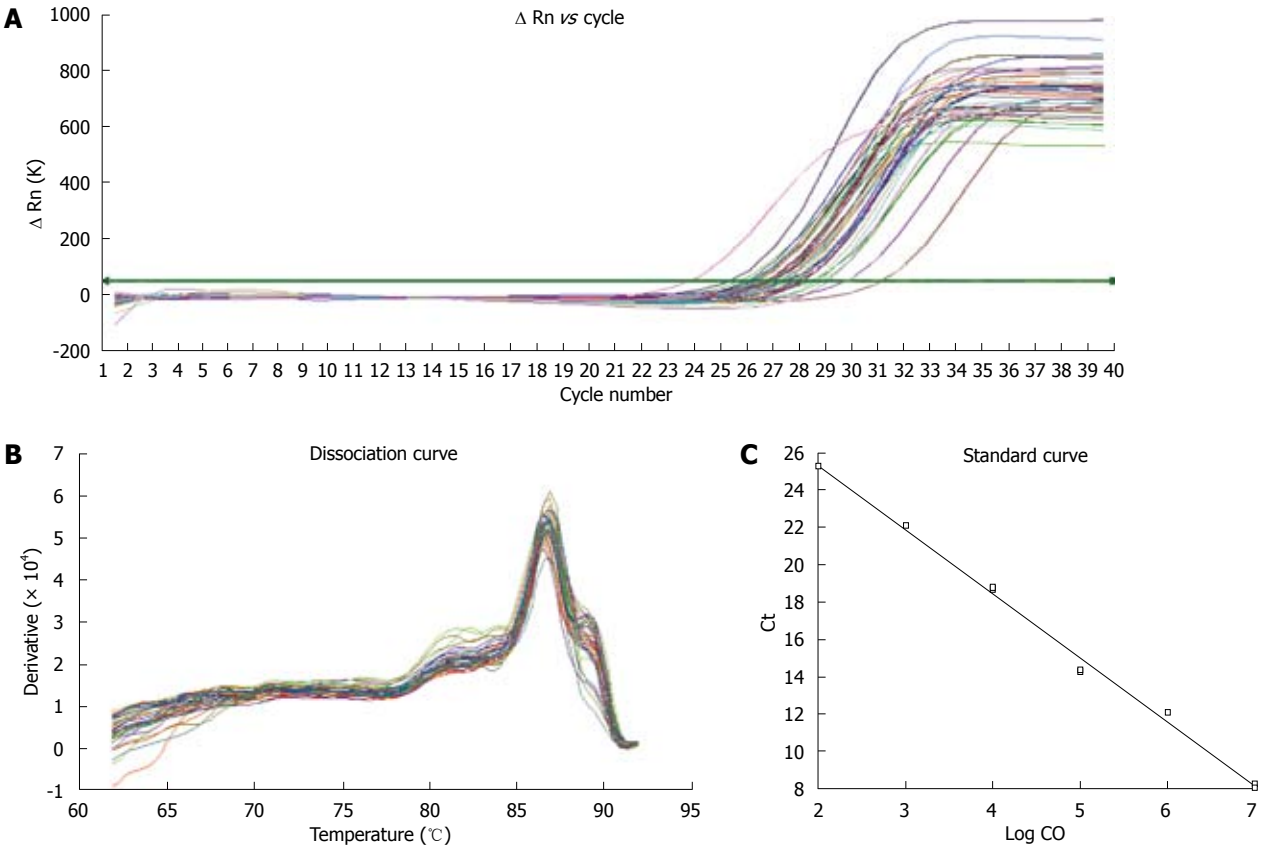

Figure 2 Fluorescent quantitative PCR for measurement of TRB3 mRNA expression levels. Real-time PCR was used for absolute quantification analysis. A: Amplification curve with the threshold value set in the exponential growth phase; B: Melting curve showing no primer dimer in PCR reaction; C: Standard curve for the relation (r = 0.99) between amplified results of PCR and Ct value of standard samples was in accordance with the requirements of real-time PCR. The intensities of fluorescent signals indicated the variation of product concentrations.

Ser473 are shown for each group. There was no significant difference in the expression levels of Akt (1.03 ± 0.53 *vs* 1.12 ± 0.77, P = 0.729) and p-Akt-Thr308 (0.82 ± 0.45 *vs* 0.92 ± 0.38, P = 0.592) between group A and

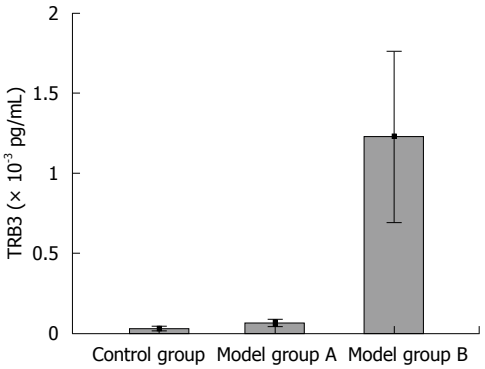

Figure 3 Comparison of TRB3 expression levels among the three groups. The TRB3 mRNA expression level in group B was significantly ($P < 0.01$) higher than in the control group and group A. There was no significant difference ($P > 0.05$) between group A and the control group.

the control group. However, the expression levels of Akt and p-Akt-Thr308 in group B was significantly lower than in group A (Akt 0.41 ± 0.16 *vs* 1.12 ± 0.77, $P = 0.008$; p-Akt-Thr308 0.47 ± 0.19 *vs* 0.82 ± 0.45, $P = 0.036$) and the control group (Akt 0.41 ± 0.16 *vs* 1.03 ± 0.53, $P = 0.018$; p-Akt-Thr308 0.47 ± 0.19 *vs* 0.92 ± 0.38, $P = 0.010$). The expression level of p-Akt-Ser473 in group A was significantly higher than in group B (1.48 ± 0.50 *vs* 0.81 ± 0.39, $P = 0.041$) as well as the control group (1.48 ± 0.50 *vs* 0.45 ± 0.26, $P = 0.003$) (Table 1). All these data indicate that a simple hepatic steatosis pathomorphism does not produce a significant difference between the model group and the control group in the expression of total Akt. As the rats were exposed to longer periods of a high-fat diet, the degree of hepatic steatosis was increased. When the rats deteriorated to a fatty hepatitis pathomorphism, the expression of total Akt, p-Akt-Thr308 and p-Akt-Ser473 was significantly lower than the simple fatty liver disease modeled by group A and the control group.

DISCUSSION

NAFLD has increased in recently years, and is one of the major causes for cryptogenic cirrhosis[11,12]. The pathogenesis of NAFLD is complicated. There is an interaction between genetic susceptibility and multiple metabolic disorders involved in the disease. The pathophysiologic basis of the condition is mainly insulin resistance and oxidative stress. No perfect theory exists for all its clinical manifestations[13,14]. At present, IR along with hepatocyte fatty degeneration is believed to be a key factor in the occurrence and development of fatty liver disease[15,16]. Research indicates[10] that IR exits in about 98% of patients with NAFLD. IR is probably of key importance for the induction of NAFLD. Therefore, any factor related to IR may play an important role in NAFLD.

Studies indicate that TRB3 is involved in many biological processes, including IR and blocking of the insulin signaling pathway[6,17]. In this study, we generated a rat model of NAFLD using a high-fat diet. Group A modeled simple fatty liver, and we continued to feed the rats with the high-fat diet. Simple hepatic steatosis produced no significant difference between the model group and the control group in the expression of TRB3 mRNA. As the rats were exposed to longer periods with the high-fat diet, the extent of hepatic steatosis was raised. When the condition of the rats deteriorated to a fatty hepatitis pathomorphism (group B), the expression of TRB3 mRNA became significantly higher. This result indicates that TRB3 is involved in the occurrence and development of NAFLD.

On the basis of recent research, through this study, we made further efforts to discover the possible mechanism of TRB3 involved in the occurrence and development of NAFLD. One study has shown that IR is related to the insulin signaling pathway phosphoinositide 3-kinase/protein kinase B (PI3-K/PKB)[18]. Akt is a key protein[19] in the PI3-K insulin signaling pathway. Two of its sites need to be phosphorylated[20,21] for its normal physiological function. One site is Thr-308, located in the kinase domain, and the other one is Ser-473, located in hydrophobic motif of the regulatory domain. After the binding of insulin and its receptors in the cell membrane, the upstream signaling proteins in this pathway are activated step by step. Thr-308 and Ser-473[22,23] phosphorylation sites for Akt are activated, and then endocytose from membrane to cytoplasm, which starts a cascade of reactions of the downstream related substrate proteins. Through this process, insulin contributes to glycogen synthesis, glucose transport, glycolysis and the inhibition of gluconeogenesis[24]. The quantity of Akt decreases and its activity changes in rats with IR[25]. Ijuin *et al*[26] have found that TRB3 may inhibit the signal transduction of insulin-activated PI3-K in CHO cells, which suggests that TRB3 affects insulin signal transduction and inhibits uptake and utilization of glucose by cells. Du *et al*[6] and Matsushima *et al*[27] have found that in the hepatic cells of TRB3 transgenic rats, TRB3 inhibited the phosphorylation and activation of Thr-308 and Ser-473 of Akt, but did not affect the protein expression of Akt. Therefore, TRB3 may decrease glucose tolerance and cause blood glucose elevation. The phosphorylation of substrate proteins like glycogen synthase kinase-3β by Akt was inhibited, and glycogen synthesis and the function of insulin on glucose metabolism was also lowered. TRB3 plays an important role in IR. TRB3 gene knockouts may increase the sensitivity of hepatic cells to insulin stimulation. The activity of Akt may be enhanced, and blood glucose levels may be lower[28]. The above mentioned studies indicate that TRB3 could block the insulin signaling pathway through inhibiting Akt activation[6]. Since TRB3 inhibits the phosphorylation of Thr-308 and Ser-473 by binding with them, it inhibits the activity of Akt. As a result, the insulin signaling pathway is blocked. Our results also support this hypothesis. When the pathomorphism was simple hepatic steatosis, there was no significant difference between the model group and control group in the expression of TRB3

mRNA. The expression level of total Akt did not change much either. As the degree of hepatic steatosis was raised and deteriorated to fatty hepatitis, the expression of total Akt, p-Akt-Thr308 and p-Akt-Ser473 was significantly lower than that in the simple fatty liver model group.

The data for p-Akt-Ser473 in Table 1 show that in mild steatosis (group A), expression levels are much greater than control while in severe steatosis (group B), levels go back to the control value. We assume that the complex regulation of active molecules *in vivo* may lead to another pathway. Balendran *et al*[29] have shown that 3-phosphoinositide-dependent protein kinase-1, Akt and protein-kinase-C-related kinase-2 interact with each other after the phosphorylation of Thr308, which can be converted into 3-phosphoinositide-dependent protein kinase-2 (PDK2) and modify Ser473. Kroner *et al*[30] have found that the existence of PDK2 can be proven by the complex relationship between Thr308 and Ser473, which is phosphorylated independently. A study by Ferguson *et al*[31] has shown that Akt can be activated in a PI3-K-independent pathway. Therefore, this interesting phenomenon is worthy of further study.

In conclusion, TRB3 can block the insulin signaling pathway by inhibiting the activation of Akt[32,33], and contributing to IR. Therefore, TRB3 may be an important factor in the occurrence and development of NAFLD. This study provides an experimental basis for future studies about the role of TRB3 in NAFLD. The control of the expression level of TRB3 in liver may become a new target for NAFLD therapy.

COMMENTS

Background

Tribble 3 (TRB3) is involved in many biological processes, including insulin resistance (IR), blocking of the insulin signaling pathway, endoplasmic reticulum stress responses, and the regulation of cell growth and differentiation. Any factor related to IR will play an important role in the development of non-alcoholic fatty liver disease (NAFLD). Therefore, the authors of this study investigated the relationship between TRB3 and IR, and aimed to establish its importance in the occurrence and development of NAFLD.

Research frontiers

The study is the first to evaluate the role of TRB3 with IR in NAFLD. The potential effect of TRB3 is likely to block the insulin signaling pathway through inhibiting Akt activation. TRB3 may play an important role in the occurrence and development of NAFLD.

Innovations and breakthroughs

This study explained one of the possible mechanisms of IR, which could produce a potentially facilitative effect on the occurrence and development of NAFLD.

Applications

This study provides an experimental basis for future studies on the role of TRB3 in NAFLD. The control of the expression level of TRB3 in liver may become a new target for therapy for NAFLD.

Peer review

In the present study, the authors tested the effect of TRB3 in NAFLD in rats, and found a facilitative effect in the occurrence and development of NAFLD.

REFERENCES

1 **Mata J**, Curado S, Ephrussi A, Rørth P. Tribbles coordinates mitosis and morphogenesis in Drosophila by regulating string/CDC25 proteolysis. *Cell* 2000; **101**: 511-522

2 **Seher TC**, Leptin M. Tribbles, a cell-cycle brake that coordinates proliferation and morphogenesis during Drosophila gastrulation. *Curr Biol* 2000; **10**: 623-629

3 **Boudeau J**, Miranda-Saavedra D, Barton GJ, Alessi DR. Emerging roles of pseudokinases. *Trends Cell Biol* 2006; **16**: 443-452

4 **Grosshans J**, Wieschaus E. A genetic link between morphogenesis and cell division during formation of the ventral furrow in Drosophila. *Cell* 2000; **101**: 523-531

5 **Bowers AJ**, Scully S, Boylan JF. SKIP3, a novel Drosophila tribbles ortholog, is overexpressed in human tumors and is regulated by hypoxia. *Oncogene* 2003; **22**: 2823-2835

6 **Du K**, Herzig S, Kulkarni RN, Montminy M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science* 2003; **300**: 1574-1577

7 **Selim E**, Frkanec JT, Cunard R. Fibrates upregulate TRB3 in lymphocytes independent of PPAR alpha by augmenting CCAAT/enhancer-binding protein beta (C/EBP beta) expression. *Mol Immunol* 2007; **44**: 1218-1229

8 **Hegedus Z**, Czibula A, Kiss-Toth E. Tribbles: novel regulators of cell function; evolutionary aspects. *Cell Mol Life Sci* 2006; **63**: 1632-1641

9 **Sung HY**, Francis SE, Crossman DC, Kiss-Toth E. Regulation of expression and signalling modulator function of mammalian tribbles is cell-type specific. *Immunol Lett* 2006; **104**: 171-177

10 **Chitturi S**, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001; **21**: 27-41

11 **Jansen PL**. Nonalcoholic steatohepatitis. *Neth J Med* 2004; **62**: 217-224

12 **Kallwitz ER**, McLachlan A, Cotler SJ. Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease. *World J Gastroenterol* 2008; **14**: 22-28

13 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845

14 **Adams LA**, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. *CMAJ* 2005; **172**: 899-905

15 **Choudhury J**, Sanyal AJ. Insulin resistance in NASH. *Front Biosci* 2005; **10**: 1520-1533

16 **Duvnjak M**, Lerotić I, Barsić N, Tomasić V, Virović Jukić L, Velagić V. Pathogenesis and management issues for non-alcoholic fatty liver disease. *World J Gastroenterol* 2007; **13**: 4539-4550

17 **Ding J**, Kato S, Du K. PI3K activates negative and positive signals to regulate TRB3 expression in hepatic cells. *Exp Cell Res* 2008; **314**: 1566-1574

18 **Whiteman EL**, Cho H, Birnbaum MJ. Role of Akt/protein kinase B in metabolism. *Trends Endocrinol Metab* 2002; **13**: 444-451

19 **Taniguchi CM**, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006; **7**: 85-96

20 **Nicholson KM**, Anderson NG. The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* 2002; **14**: 381-395

21 **Neri LM**, Borgatti P, Capitani S, Martelli AM. The nuclear phosphoinositide 3-kinase/AKT pathway: a new second messenger system. *Biochim Biophys Acta* 2002; **1584**: 73-80

22 **Cantley LC**. The phosphoinositide 3-kinase pathway. *Science* 2002; **296**: 1655-1657

23 **Hanada M**, Feng J, Hemmings BA. Structure, regulation and function of PKB/AKT--a major therapeutic target. *Biochim Biophys Acta* 2004; **1697**: 3-16

24 **Asano T**, Fujishiro M, Kushiyama A, Nakatsu Y, Yoneda M, Kamata H, Sakoda H. Role of phosphatidylinositol 3-kinase activation on insulin action and its alteration in diabetic conditions. *Biol Pharm Bull* 2007; **30**: 1610-1616

25 **Kim YB**, Peroni OD, Franke TF, Kahn BB. Divergent

regulation of Akt1 and Akt2 isoforms in insulin target tissues of obese Zucker rats. *Diabetes* 2000; **49**: 847-856
26 **Ijuin T**, Takenawa T. SKIP negatively regulates insulin-induced GLUT4 translocation and membrane ruffle formation. *Mol Cell Biol* 2003; **23**: 1209-1220
27 **Matsushima R**, Harada N, Webster NJ, Tsutsumi YM, Nakaya Y. Effect of TRB3 on insulin and nutrient-stimulated hepatic p70 S6 kinase activity. *J Biol Chem* 2006; **281**: 29719-29729
28 **He L**, Marecki JC, Serrero G, Simmen FA, Ronis MJ, Badger TM. Dose-dependent effects of alcohol on insulin signaling: partial explanation for biphasic alcohol impact on human health. *Mol Endocrinol* 2007; **21**: 2541-2550
29 **Balendran A**, Casamayor A, Deak M, Paterson A, Gaffney P, Currie R, Downes CP, Alessi DR. PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2. *Curr Biol* 1999; **9**: 393-404
30 **Kroner C**, Eybrechts K, Akkerman JW. Dual regulation of platelet protein kinase B. *J Biol Chem* 2000; **275**: 27790-27798
31 **Ferguson KM**, Kavran JM, Sankaran VG, Fournier E, Isakoff SJ, Skolnik EY, Lemmon MA. Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains. *Mol Cell* 2000; **6**: 373-384
32 **Bi XP**, Tan HW, Xing SS, Wang ZH, Tang MX, Zhang Y, Zhang W. Overexpression of TRB3 gene in adipose tissue of rats with high fructose-induced metabolic syndrome. *Endocr J* 2008; **55**: 747-752
33 **He L**, Simmen FA, Mehendale HM, Ronis MJ, Badger TM. Chronic ethanol intake impairs insulin signaling in rats by disrupting Akt association with the cell membrane. Role of TRB3 in inhibition of Akt/protein kinase B activation. *J Biol Chem* 2006; **281**: 11126-11134

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BRIEF ARTICLES

Non-steroidal anti-inflammatory drugs and statins in relation to colorectal cancer risk

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Abstract

AIM: To investigate the association between individual or combined use of non-steroidal anti-inflammatory drugs (NSAIDs) or statins and colorectal cancer risk.

METHODS: In a population-based case-control study in women, we examined the association between NSAIDs and statin use and the risk of colorectal cancers. We further investigated whether the use of statins modifies the protective effect of NSAIDs. Female cases (n = 669) of colorectal cancer aged 50-74 years were identified from a statewide registry in Wisconsin during 1999-2001. Community control women (n = 1375) were randomly selected from lists of licensed drivers and Medicare beneficiaries. Medication use and risk factor information were gathered during a structured telephone interview. A multivariable logistic regression model was used to calculate odds ratio (OR) and 95% confidence interval (CI).

RESULTS: Overall, NSAIDs users had a 30% reduction in risk of colorectal cancer (95% CI: 0.56-0.88). Statin use was not associated with colorectal cancer risk (OR = 1.17, 95% CI: 0.74-1.85), regardless of structural type (lipophilic or hydrophilic), duration of use, or recency. There was no evidence of an interaction between NSAIDs and statins and colorectal cancer risk (P-interaction = 0.28).

CONCLUSION: Although our results confirm the inverse association between NSAIDs use and colorectal cancer risk, they do not support a risk reduction in statin users, or an interaction effect of combined NSAIDs and statin use.

Key words: Non-steroidal anti-inflammatory drugs; Statin; Colorectal cancer; Cancer prevention; Chemoprevention

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INTRODUCTION

There is strong evidence for a reduced risk of colorectal cancer in regular users of non-steroidal anti-inflammatory drugs (NSAIDs)[1,2] and some promising but inconsistent observational data regarding a role of statins in this risk[3-11]. An interaction between the use of NSAIDs and statin on the risk of colorectal cancer is suggested by both *in vivo* and *in vitro* studies[5,12,13].

NSAIDs induce apoptosis in colon cancer cells[14,15]. By blocking cyclooxygenase enzymes, they also inhibit prostaglandin production, which is known to promote tumor angiogenesis and cell proliferation[1]. Statins have anti-neoplastic effects through both HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) dependent and independent processes[13,16,17]. Inhibition of the prenylation of cell signaling proteins, as well as the anti-inflammatory and anti-oxidative properties of statins, are thought to be responsible for their anti-cancer effects[16]. Augmentation of sulindac or celecoxib induced apoptosis by Lovastatin in colon cancer cell lines and

the increased activation of caspase-3, a pro-apoptotic protein, in combined statin and NSAIDs use[12,18], suggest a synergistic anti-cancer effect. These observations have also been supported by some observational data[5].

The purpose of this study was to investigate the effects of NSAIDs and statin use in relation to colorectal cancer in a population-based case-control study in women. We also investigated whether the use of statins modified the relationship between NSAIDs and statins.

MATERIALS AND METHODS

Female cases (n = 669) of colorectal cancer aged 50-74 years were identified from the Wisconsin cancer reporting system, the statewide tumor registry, during 1999-2001. Registry reports included stage, histology and limited treatment information. Of the 1038 eligible cases, 170 (16.4%) were deceased, 19 (1.8%) were not contacted due physicians' disapproval, 22 (2.1%) could not be located and 154 (14.8%) declined to participate, resulting in a 65% response rate. We also excluded four cases with unreliable interviews. Community control (n = 1375) women were randomly selected to match the age distribution of cases from two sampling frames: lists of licensed drivers (age < 65 years) and Medicare beneficiaries (age ⩾ 65 years). Women were ineligible as controls if they reported a history of colorectal cancer. The response rate for controls was 79%.

Structured telephone interviews were conducted to obtain information regarding medication use, including NSAIDs and statins, and other factors (Table 1). We considered the most commonly used statins that were approved by the Food and Drug Administration from 1995 through 2000. Having ever used NSAIDs or statins was confined to subjects who reported using the medications for at least 30 d. We defined the duration of each period of NSAIDs or statin use. Use of these preparations within one year before the reference year was considered as current use. We categorized statins according to whether they were lipophilic (simvastatin, lovastatin and fluvastatin) or hydrophilic (pravastatin), as it has been suggested that the anti-cancer activity of statins might be limited to the ones with lipophilic structure[16].

Odds ratios (OR) and 95% confidence intervals (CI) were calculated from multivariable logistic regression models to estimate the associations between NSAIDs and statins with the risk of colorectal cancer. We also evaluated possible interaction between NSAIDs and statin use by including a cross-product term of "ever use" of these medications in the regression model. We adjusted for the potential confounding factors (Table 1) by including them in the multivariate models.

RESULTS

Overall, 657 cases of colorectal cancer and 1342 controls were included in the analysis (Table 1). The prevalence of regular NSAIDs use in the sample was 33% (20% aspirin and 13% non-aspirin, 26% current users). The prevalence of statin use was 7% (6% current users, 5% lipophylic and 2% hydrophylic) (Table 2).

Table 1 Characteristics of women with colorectal cancer and controls n (%)

Characteristic	Cases (n = 657)	Controls (n = 1342)[1]
Education		
No high school diploma	95 (14.8)	119 (11.8)
High school diploma	312 (48.7)	632 (50.0)
Some college	143 (22.3)	312 (20.7)
College degree	91 (14.2)	257 (17.6)
Type of postmenopausal hormone therapy		
Never	417 (65.2)	696 (55.7)
Estrogen only	73 (11.4)	145 (10.9)
Estrogen and progestin only	41 (6.4)	133 (7.2)
Other combination	109 (17.0)	344 (26.2)
Family history of colorectal cancer		
No	492 (80.9)	1060 (87.2)
Yes	116 (19.1)	171 (12.8)
Body mass index (kg/m^2)		
< 25	273 (42.7)	538 (40.9)
25-30	206 (32.2)	465 (36.9)
⩾ 30	160 (25.0)	314 (22.1)
History of colorectal cancer endoscopic screening (colonoscopy/ sigmoidoscopy)		
No	429 (67.3)	816 (61.5)
Yes	208 (32.6)	455 (38.5)
Smoking history (pack-years)		
Never	311 (48.7)	677 (54.7)
< 10	101 (15.8)	208 (15.1)
10-20	53 (8.3)	127 (7.4)
⩾ 20	174 (27.2)	305 (22.9)

[1]Control percentages were age-adjusted to the cases age distribution. In this table, percentages are based on excluding unknowns in that category.

Those who had ever used NSAIDs had a 30% decrease in colorectal cancer risk (OR = 0.70; 95% CI: 0.56-0.88) compared to those who had never used NSAIDs. The risk reduction was statistically significant in current users but not in former users and there was no trend for increasing duration (P = 0.75).

Having ever used statins was not associated with colorectal cancer risk (OR = 1.17; 95% CI: 0.74-1.85) regardless of the type of statin (lipophilic or hydrophilic). Neither long term (> 3 years) nor current statin use were associated with risk.

Having ever used both NSAIDs and statins was not associated with colorectal cancer risk (OR = 0.96; 95% CI: 0.49-1.78). The association between NSAIDs use and colorectal cancer risk was not modified by use of statins (P-interaction = 0.28) (data not shown).

DISCUSSION

Our finding of a 30% reduced risk of colorectal cancer with NSAIDs use is consistent with the current evidence. The observed colorectal cancer risk reductions range from 20% to 40%, possibly due to the heterogeneity of study designs[1].

In contrast to our findings on NSAIDs use, we did not observe an association between statin use and colorectal cancer risk. This association has been examined in secondary analyses of randomized controlled trials that

Table 2 Multivariable OR of colorectal cancer associated with statin and NSAIDs use

	Cases *n* (%)	Controls *n* (%)	OR[1]	95% CI[1]	OR[2]	95% CI[2]
NSAIDS						
Never	462 (71.9)	837 (63.6)	1.00	Reference	1.00	Reference
Ever	181 (28.1)	480 (36.4)	0.69	0.55-0.86	0.70	0.56-0.88
Former	41 (6.4)	109 (8.3)	0.74	0.50-1.11	0.77	0.51-1.15
Current	140 (21.8)	371 (28.2)	0.68	0.53-0.86	0.68	0.53-0.88
Duration (yr)						
< 1	8 (1.2)	25 (1.9)	0.71	0.30-1.65	0.71	0.30-1.69
1-4	85 (13.2)	233 (17.7)	0.70	0.52-0.93	0.70	0.52-0.94
⩾ 5	88 (13.7)	222 (16.9)	0.68	0.51-0.92	0.71	0.52-0.96
Statins						
Never use	453 (92.6)	1114 (93.2)	1.00	Reference	1.00	Reference
Ever use	36 (7.4)	81 (6.8)	1.03	0.66-1.60	1.17	0.74-1.85
Former	4 (0.8)	9 (0.8)	1.63	0.49-5.44	1.93	0.56-6.06
Current	32 (6.5)	72 (6.0)	0.97	0.60-1.55	1.09	0.67-1.78
Duration (yr)						
< 3	17 (3.5)	41 (3.4)	0.96	0.51-1.80	1.07	0.56-2.03
⩾ 3	19 (3.9)	40 (3.3)	1.10	0.60-2.00	1.27	0.68-2.38
Type						
Lipophilic use	30 (6.1)	63 (5.3)	1.04	0.64-1.70	1.20	0.72-2.00
Hydrophilic use	7 (1.4)	20 (1.7)	1.06	0.43-2.63	1.10	0.44-2.77

[1]Adjusted for age and reference year. [2]Adjusted for age, reference year, education, post menopausal hormone use, first degree family history of colorectal cancers, body mass index, history of colorectal cancer endoscopic screening, and smoking.

did not show a risk reduction among users[19]. The small number of colorectal cancer cases should be considered while interpreting these trial results as they were designed to measure cardiovascular outcomes. Observational studies have also produced inconsistent findings. While two case-control studies[5,10] reported risk reduction in long term statin users, other studies[3,4,6,9,10] did not show such an inverse association[20-25]. In a large case-controlled study[10] of 1953 cases and 2015 controls, a 50% reduction in colorectal cancer risk (OR = 0.53; 95% CI: 0.38-0.74) was observed in long term statin users (more than 5 years).The difference between databases from which the cases and controls were selected might have influenced the results. In their study, all the incident cases from northern Israel were included, while controls were recruited from a health maintenance organization, possibly making them more likely to have a healthier life style. In another population-based case-controlled study conducted in Germany[5] (537 cases and 612 controls), a 35% risk reduction (OR = 0.65; 95% CI: 0.43-0.99) was observed among statin users. However, after adjustment for NSAIDs use, the estimate did not remain statistically significant.

We also did not find any combined effect for NSAIDs and statins. To our knowledge, only two other population-based studies[3,5] have looked at the combined effect of NSAIDs and statins on colorectal cancer risk. While one[5] suggested a stronger risk reduction in combined users than we hypothesized, neither found evidence of a statistically significant interaction between NSAIDs and statin use (*P* interactions = 0.37 and 0.21, respectively).

Statin use was uncommon in our study subjects, which may have limited our ability to detect a true reduced risk. However, in another study from our group[26], with a similar design and population, a significant reduction in breast cancer risk was observed only among regular users of fluvastatin, which also had low prevalence of use. Statins are relatively new medications, therefore examining outcomes like adenomatous polyps as an intermediate step in colorectal cancer development might be a reasonable approach to evaluate both individual and combined effect of statins on colorectal cancer risk. Our study was restricted to women, but there are no reported gender effects on the association of drugs with colorectal cancer risk. The availability of detailed information, control for potential confounding factors, and reliable exposure measurements are the major strengths of our study.

In conclusion, these results support the inverse association between NSAIDs use and colorectal cancer risk in women, especially in current users. We did not detect an association between colorectal cancer risk and statin use, regardless of type (lipophilic *vs* hydrophilic), recency or duration of use. Further, there was no interaction effect of combined NSAIDs and statin use.

COMMENTS

Background

The use of non-steroidal anti-inflammatory drugs (NSAIDs) such as Aspirin is known to be inversely associated with risk of developing colorectal cancer. Some studies have suggested such an association with the use of the commonly used lipid lowering drugs, statins. There is also some experimental data suggesting a synergistic effect for these two popular drug families against colorectal cancer risk.

Research frontiers

While NSAIDs have some possible protective effect against colorectal cancer, they are not yet approved for routine use for this purpose, mainly because of their potentially fatal side effect, bleeding. Finding another protective agent that works synergistically with NSAIDs, allowing a decreased NSAIDs dose, could lower the incidence of the side effect whilst preserving the desired effect; cancer prevention. The promising evidence indicating such an effect for statins is exciting, because these drugs are a hot topic for different preventive strategies, especially in cardiovascular diseases.

Applications

The study results confirm the previously known inverse association between NSAIDs use and colorectal cancer risk.

Peer review

This is a retrospective case-controlled study investigating if NSAIDs or/and statins have chemopreventive effects in women with regard to colorectal cancer (CRC). It is well known that regular users of NSAIDs are at less risk of developing gastrointestinal cancers, including CRC. This paper supports this hypothesis.

REFERENCES

1 **Chan A**. NSAIDs (including aspirin): Role in prevention of colorectal cancer. Waltham, MA: UpToDate, 2007

2 **Bertagnolli MM**, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenstein RB, Wittes J, Corle D, Hess TM, Woloj GM, Boisserie F, Anderson WF, Viner JL, Bagheri D, Burn J, Chung DC, Dewar T, Foley TR, Hoffman N, Macrae F, Pruitt RE, Saltzman JR, Salzberg B, Sylwestrowicz T, Gordon GB, Hawk ET. Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med* 2006; **355**: 873-884

3 **Coogan PF**, Smith J, Rosenberg L. Statin use and risk of colorectal cancer. *J Natl Cancer Inst* 2007; **99**: 32-40

4 **Friis S**, Poulsen AH, Johnsen SP, McLaughlin JK, Fryzek JP, Dalton SO, Sørensen HT, Olsen JH. Cancer risk among statin users: a population-based cohort study. *Int J Cancer* 2005; **114**: 643-647

5 **Hoffmeister M**, Chang-Claude J, Brenner H. Individual and joint use of statins and low-dose aspirin and risk of colorectal cancer: a population-based case-control study. *Int J Cancer* 2007; **121**: 1325-1330

6 **Jacobs EJ**, Rodriguez C, Brady KA, Connell CJ, Thun MJ, Calle EE. Cholesterol-lowering drugs and colorectal cancer incidence in a large United States cohort. *J Natl Cancer Inst* 2006; **98**: 69-72

7 **Welch HG**. Statins and the risk of colorectal cancer. *N Engl J Med* 2005; **353**: 952-954; author reply 952-954

8 **Sacks FM**, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996; **335**: 1001-1009

9 **Vinogradova Y**, Hippisley-Cox J, Coupland C, Logan RF. Risk of colorectal cancer in patients prescribed statins, nonsteroidal anti-inflammatory drugs, and cyclooxygenase-2 inhibitors: nested case-control study. *Gastroenterology* 2007; **133**: 393-402

10 **Poynter JN**, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, Low M, Greenson JK, Rennert G. Statins and the risk of colorectal cancer. *N Engl J Med* 2005; **352**: 2184-2192

11 **Strandberg TE**, Pyörälä K, Cook TJ, Wilhelmsen L, Faergeman O, Thorgeirsson G, Pedersen TR, Kjekshus J. Mortality and incidence of cancer during 10-year follow-up of the Scandinavian Simvastatin Survival Study (4S). *Lancet* 2004; **364**: 771-777

12 **Agarwal B**, Bhendwal S, Halmos B, Moss SF, Ramey WG, Holt PR. Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. *Clin Cancer Res* 1999; **5**: 2223-2229

13 **Swamy MV**, Patlolla JM, Steele VE, Kopelovich L, Reddy BS, Rao CV. Chemoprevention of familial adenomatous polyposis by low doses of atorvastatin and celecoxib given individually and in combination to APCMin mice. *Cancer Res* 2006; **66**: 7370-7377

14 **Shureiqi I**, Chen D, Lee JJ, Yang P, Newman RA, Brenner DE, Lotan R, Fischer SM, Lippman SM. 15-LOX-1: a novel molecular target of nonsteroidal anti-inflammatory drug-induced apoptosis in colorectal cancer cells. *J Natl Cancer Inst* 2000; **92**: 1136-1142

15 **Yang W**, Velcich A, Mariadason J, Nicholas C, Corner G, Houston M, Edelmann W, Kucherlapati R, Holt PR, Augenlicht LH. p21(WAF1/cip1) is an important determinant of intestinal cell response to sulindac in vitro and in vivo. *Cancer Res* 2001; **61**: 6297-6302

16 **Demierre MF**, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer* 2005; **5**: 930-942

17 **Reddy BS**, Wang CX, Kong AN, Khor TO, Zheng X, Steele VE, Kopelovich L, Rao CV. Prevention of azoxymethane-induced colon cancer by combination of low doses of atorvastatin, aspirin, and celecoxib in F 344 rats. *Cancer Res* 2006; **66**: 4542-4546

18 **Swamy MV**, Cooma I, Reddy BS, Rao CV. Lamin B, caspase-3 activity, and apoptosis induction by a combination of HMG-CoA reductase inhibitor and COX-2 inhibitors: a novel approach in developing effective chemopreventive regimens. *Int J Oncol* 2002; **20**: 753-759

19 **Dale KM**, Coleman CI, Henyan NN, Kluger J, White CM. Statins and cancer risk: a meta-analysis. *JAMA* 2006; **295**: 74-80

20 **The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group.** Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med* 1998; **339**: 1349-1357

21 **Blais L**, Desgagné A, LeLorier J. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors and the risk of cancer: a nested case-control study. *Arch Intern Med* 2000; **160**: 2363-2368

22 **Friis S**, Sørensen HT, McLaughlin JK, Johnsen SP, Blot WJ, Olsen JH. A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin. *Br J Cancer* 2003; **88**: 684-688

23 **Graaf MR**, Beiderbeck AB, Egberts AC, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. *J Clin Oncol* 2004; **22**: 2388-2394

24 **Kaye JA**, Jick H. Statin use and cancer risk in the General Practice Research Database. *Br J Cancer* 2004; **90**: 635-637

25 **Setoguchi S**, Glynn RJ, Avorn J, Mogun H, Schneeweiss S. Statins and the risk of lung, breast, and colorectal cancer in the elderly. *Circulation* 2007; **115**: 27-33

26 **Pocobelli G**, Newcomb PA, Trentham-Dietz A, Titus-Ernstoff L, Hampton JM, Egan KM. Statin use and risk of breast cancer. *Cancer* 2008; **112**: 27-33

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BRIEF ARTICLES

Study of the patency of different peritoneal drains used prophylactically in bariatric surgery

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Abstract

AIM: To compare the performance of different types of abdominal drains used in bariatric surgery.

METHODS: A vertical banded Roux-en-Y gastric bypass was performed in 33 morbidly obese patients. Drainage of the peritoneal cavity was performed in each case using three different types of drain selected in a randomized manner: a latex tubular drain, a Watterman tubulolaminar drain, and a silicone channeled drain. Drain permeability, contamination of the drained fluid, ease of handling, and patient discomfort were evaluated postoperatively over a period of 7 d.

RESULTS: The patients with the silicone channeled drain had larger volumes of drainage compared to patients with tubular and tubulolaminar drains between the third and seventh postoperative days. In addition, a lower incidence of discomfort and of contamination with bacteria of a more pathogenic profile was observed in the patients with the silicone channeled drain.

CONCLUSION: The silicone channeled drain was more comfortable and had less chance of occlusion, which is important in the detection of delayed dehiscence.

Key words: Bariatric surgery; Contamination; Drains; Gastric leak; Patency; Peritonitis

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INTRODUCTION

Most of the immediate complications occurring after bariatric surgery are due to technical errors that may go unrecognized[1]. Among them, intraoperative bleeding and dehiscence of anastomoses, although infrequent, are the most feared complications. Dehiscence occurs at a frequency of 1% to 4.4% of cases, resulting in significant morbidity and eventually even death. The early detection of this complication could reduce morbidity and mortality[2-4].

Resources for the early diagnosis of dehiscence during the postoperative period are limited. The clinical signs and symptoms are difficult to interpret and imaging exams, when they can be performed, may yield false results due to excess body weight.

Over the last three decades, efforts have been made to investigate the effectiveness of prophylactic drainage of the peritoneal cavity in controlled randomized clinical trials[5-8]. Although there are no evidence-based data justifying the use of drains in various situations, including bariatric surgery, most services routinely use them for the early identification of fistulae and their treatment[9].

Different types of drains are available but the search is ongoing for the ideal model. A closed-system model of a silicone drain was recently produced, with multiple channels in its intra-abdominal portion and vacuum

aspiration (Blake®-Ethicon), which has been used in various operations including bariatric surgery[10-16].

The objective of the present study was to assess the patency of three different types of abdominal drains used in bariatric surgery.

MATERIALS AND METHODS

During the period from January to September 2007, 33 morbidly obese patients were selected for surgical treatment by banded Roux-en-Y gastric bypass. The patients were divided into three groups according to the type of drain employed in the peritoneal cavity. The study was approved by the Research Ethics Committee of the hospital and all patients gave written informed consent to participate.

The type of drain used was selected at random: Group 1, closed-system latex tubular drain with multiple holes and without aspiration; Group 2, Watterman drain consisting of two No. 16 Levin catheters with multiple holes wrapped with a No. 4 Penrose tube (open system) (Figure 1); Group 3, silicone drain with multiple channels (Blake®Ethicon) 24 Fr connected to a 300 mL J-Vac® reservoir (Ethicon) under continuous vacuum. All drains were left in place for seven days after surgery.

Before removal of the drain, the patient received 120 mL of a methylene blue solution by the oral route in order to test for the presence of possible anastomosis dehiscence of staple lines. No radiological test was applied. For the evaluation of drain permeability, the daily output of each drain was recorded over a postoperative period of seven days.

Microbiological and antimicrobial analysis of the intraperitoneal end of the drains was performed on the seventh postoperative day during the interruption of drainage. In order to obtain peritoneal fluid the drains were punctured in their external portion. The end of each drain located in the peritoneal cavity was also sent for analysis. Both procedures were carried out under rigorous asepsis.

Subjective evaluation of the comfort of each drain was performed using a questionnaire which was completed by the patient on the day of drain removal. The information obtained referred to pain at the drain site and to pain during drain removal (graded from 0 to 5), ease of handling and discomfort with the presence of odors.

Groups were compared by one way analysis of variance (ANOVA) and then paired for application of the Tukey post-test. The level of significance was set at 5%.

RESULTS

All patients who underwent surgery were evaluated. Mean patient age, weight and BMI were 37.1 years, 138.20 kg and 51.42 kg/m^2, respectively. The characteristics of the groups studied were similar (Table 1).

All patients had a favorable postoperative course without major complications. There was no extravasation of methylene blue during the tests carried out on the seventh postoperative day. However, the intraperitoneal end of the Watterman drain in a group 2 patient was stained blue at the time of removal. A No. 16 Levin catheter was immediately introduced in this patient in order to maintain patency. No significant drainage occurred on subsequent days and the patient's course was favorable.

Table 1 Individual characteristics of the experimental groups

	Group 1-Latex	Group 2-Watterman	Group 3-Blake
Age (yr)	35.45 ± 7.56	36.18 ± 10.68	39.81 ± 9.52
Gender: M/F	3/8	3/8	1/10
Weight (kg)	138.48 ± 17.58	135.98 ± 19.86	140.30 ± 24.58

Data are reported as mean ± SD.

Table 2 Volume of liquid collected daily with each type of drain

Postoperative days	Group 1 Latex	Group 2 Watterman	Group 3 Blake	*P*
Day 1	146 ± 57.5	190 ± 178.6	150 ± 79	0.656
Day 2	89 ± 74.1	96.7 ± 68.8	168.2 ± 107.6	0.091
Day 3	29.4 ± 27.7	57.3 ± 53	107.5 ± 79.2	0.016
Day 4	25.3 ± 16.6	34.8 ± 39.3	106.2 ± 106.5	0.021
Day 5	28.3 ± 40.7	34.1 ± 30.4	123.5 ± 105.3	0.005
Day 6	26.8 ± 40.6	21.3 ± 17	88.5 ± 51.8	0.001
Day 7	26.9 ± 36	19.5 ± 18.6	89.7 ± 76	0.007

Results in milliliters and represented by mean ± SD. *P* value obtained by ANOVA.

Table 3 Paired comparison between the drains, regarding the drained volumes

Postoperative days	Blake *vs* Watterman	Blake *vs* Latex	Latex *vs* Watterman
Day 1	$P > 0.05$	$P > 0.05$	$P > 0.05$
Day 2	$P > 0.05$	$P > 0.05$	$P > 0.05$
Day 3	$P > 0.05$	$P < 0.05$	$P > 0.05$
Day 4	$P < 0.05$	$P < 0.05$	$P > 0.05$
Day 5	$P < 0.05$	$P < 0.05$	$P > 0.05$
Day 6	$P < 0.01$	$P < 0.01$	$P > 0.05$
Day 7	$P < 0.05$	$P < 0.05$	$P > 0.05$

P value obtained by the Tukey post-test.

Drain output

No difference in collected fluid volume was observed up to the second postoperative day. Starting on the third day, the silicone channeled drain showed significantly greater drainage compared to the others (Table 2) and this difference persisted up to the 7th postoperative day. No difference in collected volume was observed between the tubular latex drain and the tubulolaminar (Watterman) drain (Table 3).

Microbiological analysis

Microbiological evaluation of the fluid from the peritoneal cavity collected through the various drains revealed that nine patients with the silicone channel drain had a positive culture, with the bacteria most frequently detected being *Staphylococcus* spp., *Proteus* spp. and *Klebsiella* spp.; for the

Table 4 Microbiology of the fluid drained from the peritoneal cavity and from a part of the intraperitoneal segment of the drain

	Group 1-Latex	Group 2-Watterman	Group 3-Blake
Patient 1	*Pseudomonas aeruginosa*	*Enterobacter cloacae*[1]	*Staphylococcus aureus*
	Pseudomonas aeruginosa	*Escherichia coli*[1]	*Staphylococcus aureus*
Patient 2	*Enterobacter aerogenes*	*Proteus mirabilis*	*Proteus mirabilis*
	Enterobacter aerogenes	*Proteus mirabilis*	*Pseudomonas aeruginosa + Morganella morgani*
Patient 3	*Klebsiella pneumoniae + Staphylococcus simulans*	*Serratia marcescens*	*Staphylococcus aureus + Proteus mirabilis + Enterobacter cloacae*
	Staphylococcus aureus + Klebsiella pneumoniae + Morganella morgani + Proteus mirabilis	*Serratia marcescens*	*Serratia marcescens + Enterococcus faecalis*
Patient 4	*Serratia marcescens*	*Pseudomonas aeruginosa*	*Proteus mirabilis + Klebsiella pneumoniae + Enterococcus faecalis*
	Serratia marcescens	*Pseudomonas aeruginosa*	*Staphylococcus aureus + Proteus mirabilis*
Patient 5	*Escherichia coli + Proteus mirabilis*	*Enterobacter cloacae*	*Proteus mirabilis*
	Pseudomonas aeruginosa	*Klebsiella pneumoniae*	*Proteus mirabilis*
Patient 6	*Citrobacter koseri*	*Proteus vulgaris*	*Klebsiella pneumoniae + Proteus mirabilis*
	Proteus mirabilis + Citrobacter koseri	*Proteus vulgaris*	*Klebsiella pneumoniae + Proteus mirabilis*
Patient 7	*Staphylococcus aureus + Proteus mirabilis*	*Pseudomonas aeruginosa + Klebsiella pneumoniae*	-
	Staphylococcus aureus + Proteus mirabilis	*Pseudomonas aeruginosa*	-
Patient 8	*Proteus mirabilis*	*Enterococcus faecalis + Staphylococcus aureus*	-
	Proteus mirabilis + Serratia marcescens	*Enterococcus faecalis + Staphylococcus aureus*	-
Patient 9	*Enterobacter cloacae*	*Escherichia coli*	*Staphylococcus epidermidis*
	Enterobacter cloacae	*Escherichia coli*	*Staphylococcus aureus*
Patient 10	*Escherichia coli + Enterococcus faecalis*	*Enterococcus faecalis*	*Staphylococcus simulans*
	Morganella morganii	*Enterococcus faecalis*	*Klebsiella pneumoniae*
Patient 11	*Enterobacter cloacae*	*Proteus mirabilis + Kebsiella pneumoniae*	*Staphylococcus epidermidis*
	Enterobacter cloacae	*Proteus mirabilis*	*Staphylococcus epidermidis*

[1]Patient with a methylene blue test that was positive for dehiscence.

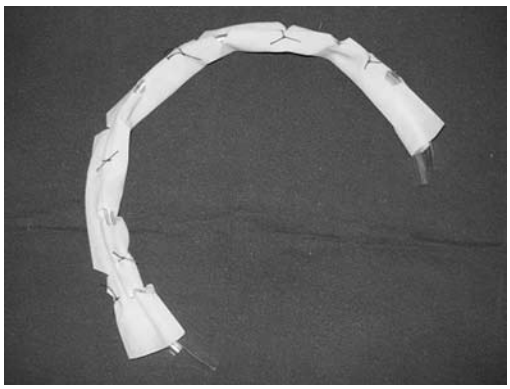

Figure 1 Watterman drain.

latex tubular drain all cultures were positive and the most frequent bacteria were *Enterobacter* spp., *Enterococcus* spp., *Proteus* spp. and *Pseudomonas* spp.; all the cultures of the tubulolaminar Watterman drains were also positive and the most frequent bacteria were *Serratia* spp., *Morganella* spp., *Proteus* spp. and *Enterobacter* spp..

Microbiological evaluation of the drain end located in the peritoneal cavity showed a similar frequency of culture positivity and similar bacterial species identified in the peritoneal fluid for all drains (Table 4).

Subjective evaluation

Drain handling and emptying of the collecting bag were considered easy for all drain types. The tubular latex drain was considered to be the most painful and the silicone channeled drain was considered to present fewer unpleasant odors (Table 5).

Table 5 Subjective evaluation of the ease of handling and comfort of the abdominal drains

	Blake	Watterman	Latex
Ease of emptying the collecting bag			
Very easy	7	7	9
Easy	3	4	2
Difficult	1	0	0
Very difficult	0	0	0
Odor during the dressings			
None	9	1	5
Bad	2	3	3
Very bad	0	7	3
Pain at the drain site (pain scale)			
0 (no pain)	6	5	2
1	2	3	3
2	2	2	0
3	3	1	3
4	0	0	1
5 (very intense pain)	0	0	2
Pain during drain removal (pain scale)			
0 (no pain)	7	4	3
1	2	6	2
2	2	1	1
3	0	0	2
4	0	0	1
5 (very intense pain)	0	0	2

DISCUSSION

Drainage of body cavities has been practiced in medicine for a long time. During the last three decades, surgeons have made efforts to investigate the value of prophylactic drainage after abdominal surgery in controlled randomized clinical trials[8,9]. The utility of closed suction drains after gastrointestinal procedures

has long been debated. Although there is some data against the use of prophylactic drains, bariatric surgeons often use them for a variety of reasons: as an early alert to the presence of leakage and hemorrhage, and as a resource for the treatment of these complications[16].

It was not the subject of this work to study the benefits or disadvantages of the presence of drains or how often they are used. For this type of study, a greater number of patients must be evaluated. Although there is a lack of consensus regarding prophylactic drainage in gastric surgery[9], at our institution, we always use tubular closed drains without suction in gastrointestinal procedures and, in accordance with many bariatric centers, prophylactic drains are routinely used in bariatric surgery. On the other hand, the effectiveness of a tubular closed drain without suction is very low for prolonged postoperative periods and may impair the diagnosis and treatment of fistulae after bariatric surgery, especially those with delayed occurrence[5]. In an experimental study, the tubular drain was found to be obstructed early, 24 to 48 h after its introduction, due to envelopment by the omentum and penetration of omental fringes into the draining orifices. Contamination around the drain has also been observed, causing washing for relief of obstruction to be risky[6].

Early studies have demonstrated that the persistence of serous drainage after obstruction of drains placed in the peritoneal cavity originates from a reaction by the organism to the presence of a foreign body, in this case the drain itself[7]. The migration of bacteria into the peritoneal cavity through the drain has also been reported[6,7].

In general, tubular closed drains tend to result in lower infection rates compared to laminar open catheters. On the other hand, laminar open drains are less frequently obstructed[11]. Thus, it is pertinent to look for an alternative way of keeping drains permeable for a prolonged period of time in order to facilitate the diagnosis and management of fistulae after bariatric surgery, especially those occurring in a delayed manner.

In the present study, the performance of the latex tubular drain without suction was similar to that of the Watterman model, which functions as a tubulolaminar drain, also without suction. There was no difference in terms of drained volume, culture positivity or diversity of the bacterial species isolated. Subjective evaluation revealed that the tubulolaminar drain had an unpleasant odor when dressings were changed compared with the tubular drain, which was more painful when handled.

The silicone channeled closed drain with vacuum and without multiple perforations had some advantages over the two more traditional models, such as a lower incidence of obstruction and pain at the site of insertion, as well as easy handling, and represents a more recent alternative that deserves to be evaluated in view of the additional costs[12].

In the present study, a persistently greater volume of daily drainage was observed with the silicone channeled closed drain, suggesting lower obstruction rates. A lower incidence of pain and fewer unpleasant odors were also recorded. Bacterial contamination by the retrograde route occurred in 81% of cases, however, the bacteria most frequently identified had a less pathogenic profile compared to the other two types of drain.

Thus, we can conclude that the silicone drain with multiple channels has a more prolonged permeability, and is recommended as an alternative for drainage of the peritoneal cavity after bariatric surgery. This recommendation is made in view of the fact that dehiscence can manifest in a delayed manner, as we experienced a patient with staple line dehiscence on the seventh postoperative day.

COMMENTS

Background

With the current increase in bariatric surgery, some complications such as, intraoperative bleeding and dehiscence of anastomoses, although infrequent, are matters of concern. The resources for the early diagnosis of these complications are limited. Drainage of the peritoneal cavity may result in the early identification and treatment of fistulae. Different types of drains are available but the search is ongoing for the ideal model.

Research frontiers

A closed-system model of a silicone drain, with multiple channels in its intra-abdominal portion (Blake®Ethicon) was recently produced. This silicone channeled closed drain with vacuum had some advantages over the two more traditional models, such as a lower incidence of obstruction and pain at the site of insertion, as well as easy handling.

Innovations and breakthroughs

This silicone drain (Blake®Ethicon) is being used by a great number of surgeons around the world and for a wide variety of surgical procedures such as cardiothoracic surgery, transplantation and bariatric surgery

Applications

This study suggests that the silicone drain is a good alternative for drainage of the peritoneal cavity after bariatric surgery, if the surgeon decides to drain it.

Terminology

Bariatric surgery is carried out in severely obese patients with the objective of reducing body weight and the comorbidity related to obesity. Dehiscence is any rupture or opening of surgical sutures. Drains are a device by which a channel or open area may be established for the exit of fluids or purulent material from a cavity, wound, or infected area.

Peer review

The authors compared the performance of different types of abdominal drains used during bariatric surgery. This article is interesting and well written.

REFERENCES

1 **Serafini F**, Anderson W, Ghassemi P, Poklepovic J, Murr MM. The utility of contrast studies and drains in the management of patients after Roux-en-Y gastric bypass. *Obes Surg* 2002; **12**: 34-38
2 **Ovnat A**, Peiser J, Solomon H, Charuzi I. Early detection and treatment of a leaking gastrojejunostomy following gastric bypass. *Isr J Med Sci* 1986; **22**: 556-558
3 **Sims TL**, Mullican MA, Hamilton EC, Provost DA, Jones DB. Routine upper gastrointestinal Gastrografin swallow after laparoscopic Roux-en-Y gastric bypass. *Obes Surg* 2003; **13**: 66-72
4 **Schauer PR**, Ikramuddin S, Gourash W, Ramanathan R, Luketich J. Outcomes after laparoscopic Roux-en-Y gastric bypass for morbid obesity. *Ann Surg* 2000; **232**: 515-529
5 **Robinson JO**. Surgical drainage: an historical perspective. *Br J Surg* 1986; **73**: 422-426
6 **Agrama HM**, Blackwood JM, Brown CS, Machiedo GW, Rush BF. Functional longevity of intraperioneal drains: an experimental evaluation. *Am J Surg* 1976; **132**: 418-421

7 **Yates JL**. An experimental study of the local effects of peritoneal drainage. *Surg Gynecol Obstet* 1905; **1**: 473-492
8 **Nora PF**, Vanecko RM, Bransfield JJ. Prophylactic abdominal drains. *Arch Surg* 1972; **105**: 173-176
9 **Petrowsky H**, Demartines N, Rousson V, Clavien PA. Evidence-based value of prophylactic drainage in gastrointestinal surgery: a systematic review and meta-analyses. *Ann Surg* 2004; **240**: 1074-1084; discussion 1084-1085
10 **Ernst R**, Wiemer C, Rembs E, Friemann J, Theile A, Schäfer K, Zumtobel V. [Local effects and changes in wound drainage in the free peritoneal cavity] *Langenbecks Arch Chir* 1997; **382**: 380-392
11 **Raves JJ**, Slifkin M, Diamond DL. A bacteriologic study comparing closed suction and simple conduit drainage. *Am J Surg* 1984; **148**: 618-620
12 **Gundry SR**, Shattuck OH, Razzouk AJ, del Rio MJ, Sardari FF, Bailey LL. Facile minimally invasive cardiac surgery via ministernotomy. *Ann Thorac Surg* 1998; **65**: 1100-1104
13 **Angelini GD**, Penny WJ, el-Ghamary F, West RR, Butchart EG, Armistead SH, Breckenridge IM, Henderson AH. The incidence and significance of early pericardial effusion after open heart surgery. *Eur J Cardiothorac Surg* 1987; **1**: 165-168
14 **Porter KA**, O'Connor S, Rimm E, Lopez M. Electrocautery as a factor in seroma formation following mastectomy. *Am J Surg* 1998; **176**: 8-11
15 **Mehran A**, Szomstein S, Zundel N, Rosenthal R. Management of acute bleeding after laparoscopic Roux-en-Y gastric bypass. *Obes Surg* 2003; **13**: 842-847
16 **Dallal RM**, Bailey L, Nahmias N. Back to basics--clinical diagnosis in bariatric surgery. Routine drains and upper GI series are unnecessary. *Surg Endosc* 2007; **21**: 2268-2271

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Celecoxib enhances the detoxification of diethylnitrosamine in rat liver cancer

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Abstract

AIM: To study the effect of celecoxib (CXB) on diethylnitrosamine activation through the regulation of cytochrome P450 in a hepatocarcinogenesis model.

METHODS: Six-week-old male Sprague-Dawley rats were randomly divided into five groups, a non-treated group (NT), a diethylnitrosamine-treated group (DEN), a DEN+CXB-treated group (DEN+CXB), and CXB 8 d-treated and CXB 32 d-treated groups. The effects of celecoxib on the enzymatic activities of CYP1A1, 2A, 2B1/2, and 2E1 were assessed in hepatic microsomes 24 h after DEN administration. Changes in CYP1A1 and CYP2B1/2 protein expression were also evaluated. The rate of DEN metabolism was measured by the production of the deethylation metabolite acetaldehyde, and the denitrosation metabolite nitrite.

RESULTS: DEN+CXB administration produced a significant increase in the enzymatic activities of CYP2B1/2 and 1A1, whereas it did not change the activities of CYP2A and 2E1, compared to that of the DEN group. CXB treatment for eight days did not produce a significant effect on enzymatic activity when compared to the NT group; however, when it was administered for prolonged times (CXB 32 d group), the enzymatic activities were increased in a similar pattern to those in the DEN+CXB group. The observed increase in the enzymatic activities in the DEN+CXB group was accompanied by an increase in the CYP2B1/2 protein levels; no changes were observed in the levels of CYP1A1. *In vitro*, CXB increased the denitrosation of DEN, a pathway of metabolic detoxification. The addition of SKF-525A, a preferential inhibitor of CYP2B, abrogated the denitrosation of DEN.

CONCLUSION: These results suggest that the mechanism of action of CXB involves enhancement of the detoxification of DEN by an increasing denitrosation *via* CYP2B1/2.

Key words: Hepatocarcinogenesis; Chemoprevention; Diethylnitrosamine; Denitrosation; Celecoxib; Cytochromes P450

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common tumors, with about one million new cases per

year worldwide. Despite progress in early diagnosis and novel therapies, the overall survival of HCC patients has not been significantly improved over the last three decades. Therefore, preventive strategies are of paramount importance and need to be actively explored in order to reduce the incidence of this disease[1].

Numerous epidemiological studies have demonstrated that long-term use of cyclooxygenase-2 (COX-2) specific inhibitors, such as celecoxib (CXB), are associated with a reduced incidence of several types of cancer[2]. Studies in rodents have shown that CXB inhibits the development of chemically induced cancers, including colon, skin, urinary bladder, and breast[3-6]. The proposed mechanisms for the effects of CXB in these models include inhibition of cell proliferation, reduction of angiogenesis and induction of apoptosis[7]. Recently, we have demonstrated that CXB acts as a chemopreventive agent against the development of preneoplastic lesions induced by diethylnitrosamine (DEN), 2-acetylaminofluorene and partial hepatectomy in the modified resistant hepatocyte (MRH) model[8]. However, the exact mechanism of action by which CXB decreases liver preneoplastic lesions remains unclear, because there was no evidence of apoptosis or of changes in COX-2 expression or PGE2 production after CXB treatment. The observed reduction in proliferation markers was not sufficient to explain the reduced number of preneoplastic lesions, thus other mechanisms must be involved in the CXB effect, probably during the initial stages of hepatocarcinogenesis.

In the MRH model, DEN bioactivation is required to produce preneoplastic lesions and subsequently HCC[9]. The metabolic activation of DEN occurs during the first hours after administration, *via* cytochrome P450 (CYP)-dependent α-hydroxylation, which results in an ethylating agent capable of forming DNA adducts. The CYP1A1/2, 2B, 2A1/2 and 2E1 subfamilies are the major enzymes involved in the bioactivation of DEN[9-11]. In addition to the activation reaction, a denitrosation reaction may also occur, which results in nitrite production. Nitrite formation is an alternative pathway for the formation of an alkylating intermediate, and represents a carcinogen detoxification pathway[12-14]. These two pathways of DEN metabolism could occur in parallel, and although some studies suggest that both pathways are catalyzed by the same CYP enzyme, the participation of distinct isoforms must be considered. In the absence of CYP inducers, the predominant reaction is activation; nevertheless, when specific isoforms are induced, the two mechanisms compete with each other, favoring the DEN detoxification pathway[9,14].

Since there is no information in the literature about CYP regulation by CXB as a chemopreventive mechanism, the aim of this study was to determine the effect of CXB on DEN activation by affecting CYP regulation in the MRH model. These data demonstrate that the preferential modulation of CYP2B1/2 by CXB enhances DEN detoxification, which therefore blocks the initiation of the hepatocarcinogenic process.

MATERIALS AND METHODS

Materials

DEN was purchased from Sigma Chemical Co. (St. Louis, MO). Ethoxy- and pentoxy-resorufin were purchased from Molecular Probes, Inc. (Eugene, OR). Electrophoresis reagents were purchased from Bio-Rad (Hercules, CA). The monoclonal anti-rat CYP1A1 antibody was purchased from Oxford Biochemicals Research, Inc. (Oxford, MI). The monoclonal anti-rat CYP2B1/2 antibody was kindly provided by Dr. Colin Jefcoate (University of Wisconsin-Madison, Dept. of Pharmacology, Madison, WI). The horseradish peroxidase-conjugated goat anti-mouse IgG antibody was acquired from Pierce Protein Research Products (Rockford, IL).

Experimental diet

CXB was extracted from the commercial drug Celebrex® (Pfizer, Mexico City, Mexico). The identity and purity of the molecule was above 99%, as determined by nuclear magnetic resonance analysis in the Department of Chemistry at CINVESTAV (Mexico City, Mexico). Diet 5001 containing 1500 ppm of CXB was prepared by Purina Test Diet (Richmond, IN).

Animals

Six-week-old male Sprague-Dawley rats were purchased from Harlan Industries (Mexico City, Mexico). Rats were fed ad libitum and housed in a controlled environment with a 12 h light/dark cycle, 50% relative humidity and a temperature of 21 ± 2℃. All experiments were performed according to the guidelines established by the Institutional Animal Care Committee in agreement with Mexican Official Norm NOM-062-ZOO-1999.

Experimental procedure

After acclimation, the rats were separated into five treatment groups (Figure 1). In the non-treated (NT) group, rats were fed with 5001 basal diet and sacrificed eight days after the beginning of the experiment; the DEN and DEN+CXB groups received a single intraperitoneal dose of DEN (200 mg/kg) and were sacrificed 24 h later. The DEN group was fed the basal diet. The DEN+CXB group was pretreated with CXB from seven days before DEN administration until sacrifice. The CXB 8 d and CXB 32 d groups were treated only with CXB for the indicated times. Animals were sacrificed by cervical dislocation and the livers were then removed and processed to obtain microsomes, as described by Mayer *et al*[15].

Enzymatic activities

Alkoxyresorufin metabolism assays: Microsomal *O*-dealkylation of 7-ethoxy-(EROD, CYP1A1) and 7-pentoxy-resorufin (PROD, CYP2B1/2) were measured fluorometrically at 37℃ using 530 and 585 nm excitation and emission wavelengths, respectively[16,17].

***p*-Nitrophenol hydroxylase (PNPH) assay:** The

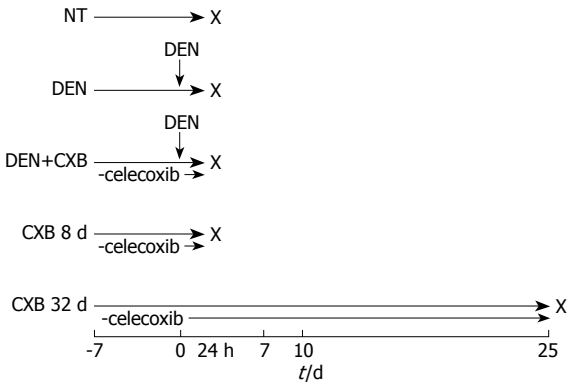

Figure 1 Schematic representation of CXB administration in the hepatocarcinogenesis model. In the NT group, rats were maintained on a basal diet. DEN and DEN+CXB groups were treated with DEN. The CXB diet was given from 7 d before DEN administration until sacrifice, indicated with X ($n = 4$).

activity of CYP2E1 was measured by the formation of 4-nitrocatechol, which was determined spectrophotometrically at 546 nm[18].

7α-Testosterone hydroxylation: The activity of CYP2A1 was determined in microsomal suspensions obtained from treated and control rats as previously described[19]. Protein concentration was determined by Lowry's method[20] using bovine serum albumin as a standard.

Immunoblotting

Microsomal proteins (15 and 30 μg/lane for CYP2B1/2 and CYP1A1, respectively) were separated by 10% SDS-PAGE. Proteins were blotted onto PVDF membranes. These membranes were blocked overnight at 4℃ with 100 mmol/L glycine, 1% BSA and 5% non-fat dry milk in a PBS-1% Triton X-100 solution. Then, membranes were challenged with anti-rat CYP1A1 or 2B1/2 antibodies for 1 h at room temperature, followed by incubation with a horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The specific protein bands were visualized by chemiluminescence (Santa Cruz Biotechnology, Inc.) and exposure to radiographic film. Densitometric analysis of bands was carried out using Sigma Gel software (Jandel Scientific, San Rafael, CA).

In vitro biotransformation of DEN by rat hepatic microsomes

The rate of DEN metabolism in control and CXB-treated rat hepatic microsomes was measured by the production of both a deethylation metabolite, acetaldehyde, and a denitrosation metabolite, nitrite, as previously described[12,14,21]. The enzymatic assay was performed in a final volume of 1 mL TMP buffer (50 mmol/L Tris-HCl, 10 mmol/L $MgCl_2$, 150 mmol/L KCl, pH 7.0) containing 0.5 mg rat hepatic microsomal protein, 1.2 mmol/L NADPH and 50 mmol/L DEN. Reactions were initiated by adding DEN and incubating at 37℃ for 30 min, and were then stopped by adding 0.1 mL 25% $ZnSO_4$ and 0.1 mL saturated $Ba(OH)_2$ in an ice bath. Samples were vortexed and centrifuged at 5000 *g* for 10 min. One-hundred microliter aliquots of supernatant were used for nitrite measurements using a specific colorimetric assay kit (Cayman Chemical Co., Ann Arbor, MI), according to the manufacturer's instructions. Acetaldehyde production was determined by HPLC in a Waters Liquid Chromatography model 600 using an Xterra C18 phase reverse column (3.9 mm × 150 mm), as previously described[21]. As a control for CYP2B1/2-specificity, these assays were carried out in the presence of 50 mmol/L SKF-525A.

Statistical analysis

Data are presented as mean ± SD. Analysis of variance and the Bonferroni test were used to assess statistical differences among the tested groups, and the level of significance was set at $P < 0.05$. All statistical analyses were performed using SigmaStat software version 3.1 (Systat Software, Inc., Point Richmond, CA).

RESULTS

CXB modulates the enzymatic activity of CYPs

To determine whether the chemopreventive effect of CXB is associated with changes in the enzymatic activities of some CYPs, the activities of CYP1A1, CYP2A1, CYP2B1/2 and CYP2E1 were determined 24 h after DEN administration (Table 1). Eight days of CXB treatment did not produce a significant effect on any of the evaluated enzyme activities. DEN treatment significantly decreased the CYP1A1 and CYP2A1 activities by 66% and 58%, respectively, whereas CYP2B1/2 and CYP2E1 activities were increased 3.6- and 2.5-fold, respectively, in comparison to the NT group. When CXB was administered in combination with DEN (DEN+CXB group), the CYP1A1 activity was increased 3.5-fold and the CYP2B1/2 activity was increased 9-fold over the DEN group. Compared with the NT group, the increase in CYP2B1/2 activity was 33-fold, while no significant changes were observed for CYP1A1 activity. On the other hand, the DEN+CXB treatment had no influence on the activities of CYP2E1 and CYP2A1 (Table 1). Interestingly, the prolonged treatment with CXB (CXB 32 d group) produced an increase in the majority of the enzymatic activities analyzed: CYP1A1, CYP2B1/2 and CYP2E1. These results suggest that pretreatment with CXB in combination with the administration of DEN elicited a preferential induction of CYP1A1 and 2B1/2 enzymatic activities, with the 2B1/2 isoforms induced to a greater degree.

Celecoxib induces the expression of CYP2B1/2 but not CYP1A1

To determine whether the increases observed in the enzymatic activities of CYP1A1 and 2B1/2 were related to increases in protein levels, these isoforms were analyzed by immunoblotting. In the DEN-treated group, there were no significant differences in the protein expression of CYP1A1 and CYP2B1/2 compared to the NT group. The pretreatment with CXB in the DEN+CXB group significantly increased CYP2B1/2 protein expression (2.5-fold), but it had no significant effect on CYP1A1 protein expression compared to the DEN group (Figure 2).

Table 1 Effect of celecoxib on hepatic microsomal enzyme activities in a hepatocarcinogenesis assay

Treatment	Alkoxyresorufin *O*-dealkylation activity (pmol resorufin/min per mg protein)		Testosterone hydroxylase activity (pmol of product/min per mg protein)	*p*-Nitrophenol hydroxylase activity (nmol 4-nitrocatechol/min per mg protein)
	EROD (CYP1A1)	PROD (CYP2B1/2)	7α-OHT (CYP2A1/2)	PNPH (CYP2E1)
NT	13.1 ± 0.5	2.0 ± 0.5	129.2 ± 18.9	0.38 ± 0.07
CXB 8 d	13.3 ± 4.3	4.2 ± 2.2	ND	0.53 ± 0.16
DEN	4.5 ± 1.3[a]	7.3 ± 3.2[a]	54.2 ± 19.2[a]	0.97 ± 0.14[a]
DEN+CXB	15.9 ± 4.8[b]	65.9 ± 35.4[a,b]	66.0 ± 5.9[a]	0.80 ± 0.25[a]
CXB 32 d	54.5 ± 11.3[a]	114.2 ± 23.2[a]	151.5 ± 29.5	1.40 ± 0.44[a]

Male rats were treated with a single dose of DEN (200 mg/kg) *ip* and sacrificed 24 h after administration (DEN and DEN+CXB groups). The CXB diet was provided 7 d before DEN treatment and until sacrifice (8 d). The CXB 8 d and CXB 32 d groups received only a CXB-containing diet. [a]Significantly different from the NT group; [b]From the DEN group, according to the Bonferroni test ($P < 0.05$). Values shown are the mean ± SD from $n = 4$. ND: Not determined.

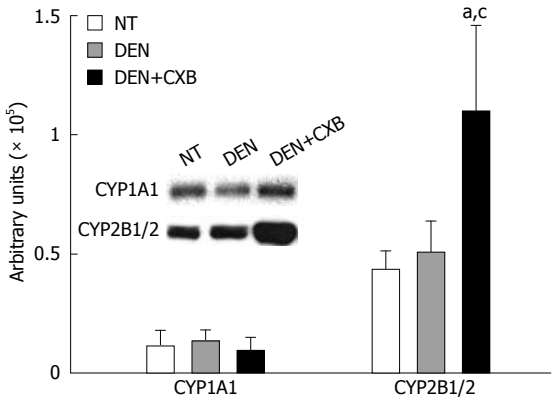

Figure 2 Immunodetection of hepatic microsomal CYP isoforms from DEN- and/or CXB-treated rats. Microsomal proteins (15 and 30 μg/lane for CYP2B1/2 and CYP1A1, respectively) were separated by SDS-PAGE and tested for different CYPs using specific anti-rat CYP antibodies. The bands in the inset box correspond to CYP2B1/2 and CYP1A1 protein detected in the NT, DEN and DEN+CXB groups. The graphic represents the densitometric analysis of the CYP amounts in the experimental groups. [a]$P < 0.05$, *vs* NT group; [c]$P < 0.05$ *vs* DEN group, Bonferroni test. $n = 4$ for all groups.

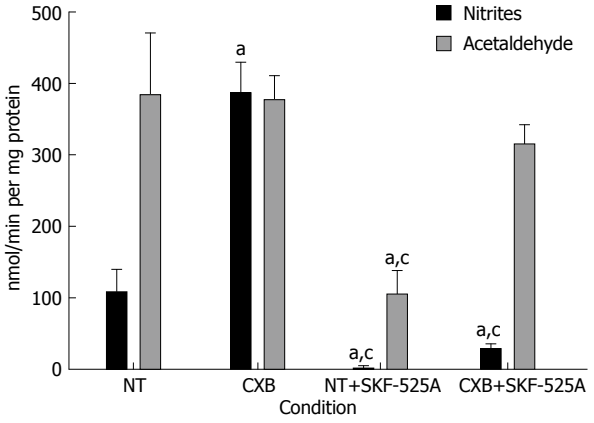

Figure 3 *In vitro* metabolism of DEN in non-treated and CXB-treated rat hepatic microsomes. The denitrosation rate was measured by the production of nitrites, and the deethylation rate was measured by the production of acetaldehyde in the presence or absence of SKF-525A. Values are presented as mean ± SD. [a]$P < 0.05$, *vs* NT group; [c]$P < 0.05$ *vs* CXB group. Bonferroni test, $n = 4$ for all groups.

In summary, these results show that CXB differentially affects these two isoforms; the increase in the protein expression of CYP2B1/2 suggests that the regulation might be at the transcriptional level, while in the case of CYP1A1, CXB seems to regulate only the enzymatic activity.

CXB favors detoxification by denitrosation of DEN

To explore whether regulation of the CYP isoforms by CXB induces the detoxification pathway of DEN as a chemopreventive mechanism, nitrite and acetaldehyde yields were measured in the microsomes of non-treated and CXB-treated rats. We used rat microsomes treated with CXB for 32 d, where the pattern of induction of enzymatic activities was similar to the pattern observed in the DEN+CXB group (with preferential induction of the 2B1/2 isoform), because the eight days of CXB treatment did not produce significant changes in the enzymatic activities.

Microsomes of non-treated rats showed a production of acetaldehyde that was 3.5-fold higher than that of nitrites, which suggests that the predominant route for the DEN metabolism is deethylation, leading to the bioactivation of the carcinogen.

Microsomes obtained from CXB-treated rats showed a 3.6-fold increase in the rate of denitrosation of DEN, while there was no effect on DEN deethylation. This result indicates an induction of the detoxification pathway of the carcinogen. To confirm whether this effect resulted from induction of the enzymatic activity of CYP2B1/2 by CXB, we included SKF-525A in the assay, a CYP inhibitor that acts preferentially on this isoform. Inhibition of CYP2B1/2 resulted in a 98% reduction in nitrite production in the microsomes isolated from non-treated animals, and a 95% reduction in the microsomes of CXB-treated rats, suggesting that CYP2B1/2 was involved in the denitrosation of DEN under basal conditions (NT animals) and CXB-induced conditions. The deethylation rate in non-treated rat microsomes decreased 73%, and no statistically significant changes were observed in the rat microsomes treated with CXB. One possible explanation for this result is that CYP2E1 and 1A1 are minimally affected by SKF-525A and could be responsible for the acetaldehyde production under these conditions (Figure 3).

DISCUSSION

CXB has shown to have anticancer effects in several

experimental models, including the MRH model, where it showed a striking chemopreventive activity by inhibiting liver preneoplastic lesions in rats[8]. Although that study demonstrated a reduction in proliferation markers and in the nuclear translocation of NF-κB, the exact mechanism of action remains unclear[8].

Altered expression of CYP genes is a common feature in hepatic preneoplastic and neoplastic lesions induced by various carcinogens, including DEN[22]. Therefore, DEN metabolism *via* hepatic microsomal CYPs provides molecular targets for chemoprevention. This study demonstrates that the chemopreventive effect of CXB in the modified resistant hepatocyte model is mediated by changes in DEN metabolism *via* CYP regulation. CXB treatment for 8 d did not induce significant changes in enzymatic activities; however, when it was administered for 32 d or in combination with DEN, CXB strongly enhanced the enzymatic activity of CYP2B1/2. Moreover, CXB treatment increased the nitrite levels, which have been proposed to result from the DEN detoxification pathway[12,13]. This finding supports the explanation that the chemopreventive activity of CXB is carried out by reducing carcinogen-induced DNA damage, thus preventing the initiation of hepatocarcinogenesis. We propose that the effect of CXB is due to the preferential induction of CYP2B1/2. This hypothesis is reinforced by *in vitro* results, where the increase in DEN denitrosation elicited by CXB was inhibited by the addition of SKF-525A, an inhibitor of several CYP isoforms including 2B1/2B2, 3A1/2 and 2A, whereas CYP2E1 and 1A are less affected[23]. The deethylation rate was not affected by the inhibitor, suggesting that CYP2E1, and possibly CYP1A, could be the main isoforms involved in this pathway.

According to a previous report, the enzymatic activity of CYP2E1 increased with DEN treatment[24]. This is congruent with the participation of this isoform in DEN metabolism[10,24]. However, CXB did not have any effect on this increase, suggesting that there is no contribution of CYP2E1 to the chemoprotective effect of CXB. On the other hand, CXB reversed the effect of DEN on CYP1A1-specific EROD activity. A decrease in the CYP1A1 enzyme activity in preneoplastic lesions induced by DEN has been previously reported[25]. Induction of CYP1A1 has been related to chemoprevention[26]; thus, the induction of this isoform by CXB could explain its chemopreventive effect, but comparing the levels of enzymatic activity induced reveals that its participation is probably minor compared to CYP2B1/2. Additionally, CYP2A was not affected by CXB, and considering that it is partially affected by SKF-525A, this isoform could be involved in the deethylation reaction of DEN, although to a lesser extent than CYP2E1.

This is the first report that describes the effect of CXB on hepatic CYP regulation. Other chemoprotectors have been shown to act in a similar way. For example, among their multiple effects, diallyl sulfide, indole-3-carbinol, *d*-limonene and bicyclol induced the enzymatic activity of several CYP isoforms, including CYP2B1/2[21,26]. In particular, the effect of bicyclol on CYP2B1 was associated with an increase in the denitrosation rate of DEN[21]. In that case, bicyclol reduced the Km values for denitrosation below the values of deethylation, which may be attributed to the induction of specific CYPs. According to these results on DEN metabolism, the chemopreventive CXB effect in the MRH model could be similar to that of bicyclol[21], mediated mainly by the 2B1/2 isoform. Isoforms of the 1A, 2A, 2B and 2E CYP families share a broader overlap in substrate selectivity. In addition, a single enzyme can bind a variety of substrates, multiple substrates, and/or generate multiple products from a single substrate, which makes it difficult to discriminate between these possibilities in *in vivo* systems[27,28]. Further studies are required to clarify the mechanism by which CXB induces the denitrosation of DEN, and whether this is generated simply by the preferential induction of isoforms or by other effects.

In summary, the modulation of several hepatic CYPs by CXB modifies the bioactivation of DEN, favoring detoxification *via* denitrosation. This pathway may constitute an additional mechanism of action to explain the chemoprotective effects of CXB at the initiation stage in this hepatocarcinogenesis model.

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COMMENTS

Background

Celecoxib, a non-steroidal antiinflammatory drug, is associated with a reduced incidence of several types of cancer, including hepatocellular carcinoma. Study of the mechanism of action has been possible by means of animal models. In the modified resistant hepatocyte model, celecoxib has shown a chemoprotector effect in the development of liver preneoplastic lesions; however, the action mechanism has not been defined completely.

Research frontiers

Diethylnitrosamine bioactivation is a crucial event in the initiation stage of the modified resistant hepatocyte model, a process dependent on hepatic cytochrome P450 (CYP). Therefore, modulation of liver CYP provides molecular targets for chemoprevention. This study demonstrates that the chemopreventive effect of celecoxib is mediated by changes in diethylnitrosamine metabolism *via* CYP regulation.

Innovations and breakthroughs

Recent investigations have demonstrated several mechanisms through which celecoxib exerts its chemoprotector effect. However, this is the first report that describes the capacity of celecoxib to modulate liver CYP expression and explains how the preferential induction of CYP2B1/2 activates the detoxification pathway by increasing nitrite formation. These effects represent an additional mechanism to elucidate the chemopreventive activity of celecoxib.

Applications

This study contributes to the understanding of the mode of action of celecoxib, which may represent a future strategy for therapeutic intervention in the treatment of patients with a high risk of suffering hepatocellular carcinoma.

Terminology

CYP is hepatic microsomal protein involved in the phase I metabolism. Celecoxib is a nonsteroidal anti-inflammatory drug that specifically inhibits cyclooxygenase-2. Diethylnitrosamine is a carcinogen initiator used in the modified resistant hepatocyte model.

Peer review

The authors examined the capability of celecoxib to modulate CYP as part of its chemopreventive mechanism in the modified resistant hepatocyte model. The results suggest that celecoxib favors the diethylnitrosamine detoxification and contribute to clarifying the chemopreventive mechanism in the chemical hepatocarcinogenesis of rat.

REFERENCES

1 **Blum HE**. Hepatocellular carcinoma: therapy and prevention. *World J Gastroenterol* 2005; **11**: 7391-7400
2 **Rüegg C**, Zaric J, Stupp R. Non steroidal anti-inflammatory drugs and COX-2 inhibitors as anti-cancer therapeutics: hypes, hopes and reality. *Ann Med* 2003; **35**: 476-487
3 **Kawamori T**, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res* 1998; **58**: 409-412
4 **Pentland AP**, Schoggins JW, Scott GA, Khan KN, Han R. Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis* 1999; **20**: 1939-1944
5 **Grubbs CJ**, Lubet RA, Koki AT, Leahy KM, Masferrer JL, Steele VE, Kelloff GJ, Hill DL, Seibert K. Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. *Cancer Res* 2000; **60**: 5599-5602
6 **Harris RE**, Alshafie GA, Abou-Issa H, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. *Cancer Res* 2000; **60**: 2101-2103
7 **Kern MA**, Schubert D, Sahi D, Schöneweiss MM, Moll I, Haugg AM, Dienes HP, Breuhahn K, Schirmacher P. Proapoptotic and antiproliferative potential of selective cyclooxygenase-2 inhibitors in human liver tumor cells. *Hepatology* 2002; **36**: 885-894
8 **Márquez-Rosado L**, Trejo-Solís MC, García-Cuéllar CM, Villa-Treviño S. Celecoxib, a cyclooxygenase-2 inhibitor, prevents induction of liver preneoplastic lesions in rats. *J Hepatol* 2005; **43**: 653-660
9 **Verna L**, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. *Pharmacol Ther* 1996; **71**: 57-81
10 **Bellec G**, Goasduff T, Dreano Y, Menez JF, Berthou F. Effect of the length of alkyl chain on the cytochrome P450 dependent metabolism of N-diakylnitrosamines. *Cancer Lett* 1996; **100**: 115-123
11 **Yamazaki H**, Inui Y, Yun CH, Guengerich FP, Shimada T. Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of N-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. *Carcinogenesis* 1992; **13**: 1789-1794
12 **Janzowski C**, Pool BL, Preussmann R, Eisenbrand G. Fluoro-substituted N-nitrosamines. 2. Metabolism of N-nitrosodiethylamine and of fluorinated analogs in liver microsomal fractions. *Carcinogenesis* 1982; **3**: 155-159
13 **Appel KE**, Rühl CS, Hildebrandt AG. Metabolic inactivation of N-nitrosamines by cytochrome P-450 in vitro and in vivo. *IARC Sci Publ* 1984; 443-451
14 **Wade D**, Yang CS, Metral CJ, Roman JM, Hrabie JA, Riggs CW, Anjo T, Keefer LK, Mico BA. Deuterium isotope effect on denitrosation and demethylation of N-nitrosodimethylamine by rat liver microsomes. *Cancer Res* 1987; **47**: 3373-3377
15 **Mayer RT**, Netter KJ, Heubel F, Hahnemann B, Buchheister A, Mayer GK, Burke MD. 7-Alkoxyquinolines: new fluorescent substrates for cytochrome P450 monooxygenases. *Biochem Pharmacol* 1990; **40**: 1645-1655
16 **Burke MD**, Thompson S, Elcombe CR, Halpert J, Haaparanta T, Mayer RT. Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* 1985; **34**: 3337-3345
17 **Lubet RA**, Mayer RT, Cameron JW, Nims RW, Burke MD, Wolff T, Guengerich FP. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* 1985; **238**: 43-48
18 **Reinke LA**, Moyer MJ. p-Nitrophenol hydroxylation. A microsomal oxidation which is highly inducible by ethanol. *Drug Metab Dispos* 1985; **13**: 548-552
19 **Sierra-Santoyo A**, Hernández M, Albores A, Cebrián ME. DDT increases hepatic testosterone metabolism in rats. *Arch Toxicol* 2005; **79**: 7-12
20 **Lowry OH**, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275
21 **Zhu B**, Liu GT, Wu RS, Strada SJ. Chemoprevention of bicyclol against hepatic preneoplastic lesions. *Cancer Biol Ther* 2006; **5**: 1665-1673
22 **Roomi MW**, Ho RK, Sarma DS, Farber E. A common biochemical pattern in preneoplastic hepatocyte nodules generated in four different models in the rat. *Cancer Res* 1985; **45**: 564-571
23 **Ono S**, Hatanaka T, Hotta H, Satoh T, Gonzalez FJ, Tsutsui M. Specificity of substrate and inhibitor probes for cytochrome P450s: evaluation of in vitro metabolism using cDNA-expressed human P450s and human liver microsomes. *Xenobiotica* 1996; **26**: 681-693
24 **Liu LL**, Gong LK, Qi XM, Cai Y, Wang H, Wu XF, Xiao Y, Ren J. Altered expression of cytochrome P450 and possible correlation with preneoplastic changes in early stage of rat hepatocarcinogenesis. *Acta Pharmacol Sin* 2005; **26**: 737-744
25 **Buchmann A**, Schwarz M, Schmitt R, Wolf CR, Oesch F, Kunz W. Development of cytochrome P-450-altered preneoplastic and neoplastic lesions during nitrosamine-induced hepatocarcinogenesis in the rat. *Cancer Res* 1987; **47**: 2911-2918
26 **Guengerich FP**. Influence of nutrients and other dietary materials on cytochrome P-450 enzymes. *Am J Clin Nutr* 1995; **61**: 651S-658S
27 **Harrelson JP**, Henne KR, Alonso DO, Nelson SD. A comparison of substrate dynamics in human CYP2E1 and CYP2A6. *Biochem Biophys Res Commun* 2007; **352**: 843-849
28 **Atkins WM**. Non-Michaelis-Menten kinetics in cytochrome P450-catalyzed reactions. *Annu Rev Pharmacol Toxicol* 2005; **45**: 291-310

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Efficacy of the revised Vienna Classification for diagnosing colorectal epithelial neoplasias

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Abstract

AIM: To prospectively investigate the efficacy of the revised Vienna Classification for diagnosing colorectal epithelial neoplastic lesions in cold biopsy specimens.

METHODS: Patients were selected for inclusion if they had colorectal epithelial lesions that were not considered suitable for direct endoscopic resection. These included colorectal polyps ⩾ 10 mm and lesions suspected of being carcinomas capable of invading the colorectal submucosa or beyond, including strictures, based on the cold biopsies obtained from each lesion prior to resection. We investigated the relationship between diagnoses based on cold biopsy samples using the revised Vienna Classification and resected specimens of the same lesions, and the therapeutic implications of diagnoses made using the revised Vienna Classification. The same cold biopsy specimens were also examined using the Japanese Group Classification guidelines, and compared with the resected specimens of the same lesions for reference.

RESULTS: A total of 179 lesions were identified. The sensitivity, specificity, positive and negative predictive values of the revised Vienna Classification for distinguishing between intramucosal lesions and submucosal invasive carcinomas in cold biopsy specimens was 22.2%, 100%, 100%, and 71.4%, respectively, and for distinguishing between intramucosal lesions and those invading the submucosa or beyond was 59.7%, 100%, 100%, and 37.6%, respectively. The sensitivity, specificity, positive and negative predictive values of the Japanese Group Classification for distinguishing between intramucosal lesions and submucosal invasive carcinomas in cold biopsy specimens was 83.3%, 91.4%, 83.3%, and 91.4%, respectively, and for distinguishing between intramucosal lesions and those invading the submucosa or beyond was 95.1%, 91.4%, 97.9%, and 82.1%, respectively. A total of 137 of 144 carcinomas that had invaded the submucosa or beyond and three high-grade intraepithelial neoplasias were diagnosed as "carcinoma" using the Japanese Group Classification system.

CONCLUSION: The revised Vienna Classification for cold biopsy specimens has high positive predictive value in the diagnosis of colorectal carcinoma invasive to the submucosa or beyond.

Key words: Biopsy; Cancer; Colonoscopy; Colorectal epithelial neoplasia; Revised Vienna Classification

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INTRODUCTION

Considerable discrepancies have been reported between diagnoses of colorectal epithelial neoplastic lesions made by Western and Japanese pathologists from endoscopic cold biopsies and resected specimens of the same

lesions[1,2]. Japanese pathologists have distinguished five groups of lesions within the spectrum of colorectal epithelial neoplasia for cold biopsy specimens [Japanese Group Classification (JGC)], namely: normal or benign changes (inflammation/hyperplasia) without atypia [Group 1 (G1)]; non-neoplastic lesions with atypia resulting from inflammation, hyperplasia or regeneration [Group 2 (G2)]; neoplastic lesions with low-grade atypia, including adenomas with mild or moderate atypia and lesions difficult to diagnose as neoplastic or non-neoplastic [Group 3 (G3)]; neoplastic lesions strongly suspected of carcinoma, including adenomas with severe atypia [Group 4 (G4)]; and definite carcinoma [Group 5 (G5)], irrespective of intramucosal or submucosal invasion[3,4]. This different criterion for the diagnosis of "colorectal carcinoma" may be the reason why there are fewer discrepancies between diagnoses from cold biopsies and resected specimens by Japanese pathologists.

In the clinical setting, cold biopsies are required to facilitate management decisions for large and/or advanced lesions. The therapeutic implications of resecting adenomatous polyps equal to or larger than 10 mm ($\geqslant$ 10 mm) should be considered carefully because these polyps are at risk of becoming submucosal invasive carcinomas[5]. Compared to the endoscopic diagnosis of colorectal polyps including submucosal invasive carcinomas, the endoscopic diagnosis of more advanced colorectal carcinomas rarely presents a problem and can be referred for surgical resection[6]. However, histopathologic confirmation of these lesions from cold biopsy specimens should always be sought. Discrepancies between diagnoses based on cold biopsies and resected specimens of the same lesions are more likely to occur for these large and/or advanced lesions because cold biopsy-based diagnoses are subject to the limitations of superficiality and sampling errors[3]. In contrast, direct endoscopic resection (ER) without prior cold biopsy of small (< 10 mm) colorectal polyps is feasible and histopathologic examination of completely resected lesions enables adequate diagnosis and appropriate treatment, therefore, cold biopsies for small polyps are not mandatory.

Diagnostic discrepancies do not matter to patients if Western and Japanese physicians understand the implications of their respective pathology reports and apply management strategies that are appropriate to the needs of their patients[7]. However, continued attempts to unify Western and Japanese reporting systems are desirable because merging the terminologies of these systems will help codify the advantages of each into a language that is universally understood[8].

To overcome the differences between the conventional Western criteria and the JGC, the Vienna Classification attempted to combine the basic concepts of the conventional Western criteria, which emphasizes that invasion is an indicator of metastatic potential, with the strong points of the JGC, which values consistency between diagnoses of cold biopsy and resected specimens[2,9]. In the revised Vienna Classification (rVC), histopathologic diagnoses are classified into five categories according to neoplastic severity and depth of invasion. This classification also distinguishes between epithelial neoplastic lesions limited to the mucosa and those invading the submucosa[2].

To examine the efficacy of the rVC for diagnosing colorectal polyps $\geqslant$ 10 mm, and colorectal lesions suspected of being carcinomas invasive to the submucosa or beyond, including strictures, we prospectively compared the diagnoses from cold biopsy specimens using the rVC guidelines with the diagnoses from resected specimens of the same lesions using the World Health Organization (WHO) classification[10]. We investigated the value of the rVC system for distinguishing intramucosal lesions from those capable of invading the submucosa or beyond, with special reference to distinguishing between intramucosal lesions and submucosal invasive carcinomas because of the different therapeutic implications among these lesions. In addition, the same cold biopsy specimens were examined using the JGC guidelines and the resulting diagnoses compared to those obtained from the resected specimens of the same lesions, graded according to the WHO classification.

MATERIALS AND METHODS

Patients

In total, 5465 colonoscopies, sigmoidoscopies or proctoscopies were performed prospectively on 3719 patients at the Toho University Ohashi Medical Center, Tokyo, Japan, between January 2001 and December 2003. The study was approved by the Toho University Ohashi Hospital ethics committee. Signed informed consent was obtained from all participating patients. This study was performed in accordance with the Helsinki Declaration.

Inclusion/exclusion criteria

Patients were selected for inclusion in this study if they had colorectal epithelial lesions that were not considered suitable for direct ER. These included colorectal polyps $\geqslant$ 10 mm and lesions suspected of being carcinomas capable of invading the colorectal submucosa or beyond, including strictures, based on the cold biopsies obtained from each lesion prior to resection. The histopathologic diagnosis of each cold biopsy specimen was compared with the final histopathologic diagnosis of each resected lesion. Exclusion criteria included: no epithelial lesions; polyps < 10 mm; polyps $\geqslant$ 10 mm and lesions suspected of being carcinomas invasive to the submucosa or beyond, including strictures, but with no cold biopsy specimens; the inability to compare the histopathologic diagnosis of cold biopsy specimens with the final histopathologic diagnosis of the resected lesion; carcinoid tumors; familial adenomatous polyposis; inflammatory bowel disease; local recurrence after resection for epithelial neoplastic lesions; and the inability to give informed consent.

Endoscopic evaluation

All lesions were diagnosed macroscopically using

conventional colonoscopes (CF-200I, 230I, or 240I; Olympus Co, Ltd, Tokyo, Japan) by endoscopists who had performed more than 500 colonoscopic procedures by direct visualization. If necessary, the lesions were then delineated using 0.1% indigo carmine solution. Polyps and early colorectal carcinomas were classified as Ⅰp (pedunculated type), Ⅰsp (semipedunculated type), Ⅰs (sessile type), Ⅱa (superficial elevated type), Ⅱb (superficial flat type), or Ⅱc (superficial depressed type) according to the criteria outlined by the Japanese Society for Cancer of the Colon and Rectum[4]. Early colorectal carcinoma was defined as carcinoma with invasion limited to the mucosa or submucosa, regardless of the presence or absence of lymph node metastases[1,4]. Lesions that had become invasive carcinomas and had advanced into the muscularis propria or beyond were classified as exophytic/fungating, endophytic/ulcerative, diffusely infiltrative/linitis plastica, or annular according to the WHO classification[10].

Measurements of lesions and tissue sampling

The size of each lesion was estimated *in situ* by using a fully opened standard biopsy forcep (8 mm) (FB-24Q-1; Olympus) adjacent to the lesion, and measured after resection. The cold biopsies were performed using the same forceps (FB-24Q-1; Olympus). The number of cold biopsy specimens and the areas biopsied were dependent on the discretion of each endoscopist; if possible, specimens were obtained from different areas, and included the edges and the center of the lesion.

Treatment modality

Treatment modality was dependent on the size of the lesion, the endoscopic assessment of the depth of invasion and the degree of stricture, and on factors such as the patient's age and morbidity. This was also aided by the histopathologic diagnoses from cold biopsy specimens according to the JGC as routinely practiced.

Histopathologic evaluation

The cold biopsy specimens were fixed with 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The only clinical information available to the examining pathologists was that the specimen in question represented a biopsy/biopsies of a colorectal epithelial lesion. All cold biopsy specimen slides were examined independently by two experienced pathologists, and all discrepancies were resolved by a conjoint review of the slides in question. Histopathologic type and grade was evaluated according to the WHO classification[10]. Histopathologic diagnosis of each cold biopsy specimen was made using both the rVC and JGC guidelines[2-4]. If more than one cold biopsy specimen was taken, the most advanced diagnosis was taken as the final diagnosis of the lesion. After resection, tissue samples of the entire lesion were cut from resected specimens that had been fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The histopathologic diagnoses of resected specimens were made for each lesion using the WHO classification[10]. The relationship between the diagnoses of cold biopsy specimens using the rVC and JGC guidelines, and the depth of invasion in resected specimens of the same lesions was investigated.

Statistical analysis

The sensitivity and specificity, and positive and negative predictive values were all calculated with 95% confidence intervals (CI)[11]. The varying proportion of categorical variables between two groups (i.e. intramucosal lesions *versus* submucosal invasive carcinomas, and intramucosal lesions *versus* those invading the submucosa or beyond) was tested by Fisher's exact test. Statistical significance was defined as $P < 0.05$.

RESULTS

Clinicopathologic data

One patient with subserosal invasive transverse colon carcinoma with three cold biopsy specimens was excluded from the analysis because all three specimens showed necrotic tissue only. There were 171 patients (93 men, 78 women; mean age, 66.9 years; range, 33-93) with 179 lesions. A single lesion was found in 165 (96.5%) cases with five (2.9%) and one (0.6%) patients having two or four lesions, respectively. Ten lesions were located in the cecum (5.6%), 34 in the ascending colon (19.0%), 25 in the transverse colon (14.0%), 11 in the descending colon (6.1%), 46 in the sigmoid colon (25.7%), and 53 in the rectum (29.6%). Eight lesions were classified as Ⅰp (4.5%), seven as Ⅰsp (3.9%), 20 as Ⅰs (11.2%), 13 as Ⅱa (7.3%), six as Ⅱa + Ⅱc (3.4%), seven as exophytic/fungating (3.9%), 63 as endophytic/ulcerative (35.2%), and 55 as annular (30.7%). The lesions ranged from 10 to 180 mm in diameter (mean, 46.8 mm). No carcinomas < 10 mm invading the submucosa or beyond were found. Ileocecal resection ($n = 7$), right hemicolectomy ($n = 40$), partial resection of the transverse colon ($n = 6$), left hemicolectomy ($n = 5$), partial resection of the descending colon ($n = 3$), sigmoidectomy ($n = 32$), anterior resection ($n = 37$), abdominoperineal resection ($n = 7$), subtotal colectomy ($n = 4$), Hartmann's procedure ($n = 3$), transsacral resection ($n = 1$), transanal resection ($n = 4$), and ER ($n = 30$) procedures were performed.

Histopathologic diagnoses of cold biopsy specimens from 179 lesions

A total of 404 cold biopsy specimens were obtained from 179 lesions, ranging from one to six specimens per lesion (mean, 2.3). Five inadequate specimens [exudative material (2); granulation tissue (2); necrotic tissue (1)] were excluded; therefore, 399 cold biopsy specimens were included in the analysis. The histopathologic type and grade of each cold biopsy specimen was classified as follows: four non-neoplastic lesions; one indefinite neoplastic lesion; 31 low-grade intraepithelial neoplasias; 55 high-grade intraepithelial neoplasias; 69 well-differentiated adenocarcinomas; 16 moderately differentiated adenocarcinomas; and three poorly differentiated adenocarcinomas.

Table 1 Relationship between the histopathologic diagnoses of cold biopsy specimens using the revised Vienna Classification and the depth of invasion in resected specimens of the same lesions

Invasion depth[1]	The revised Vienna Classification								Total (%)
	C1	C2	C3	C4.1	C4.2	C4.3	C4.4	C5	
Non-N	2	0	0	0	0	0	0	0	2 (1.1)
LGIN	0	0	12	0	0	0	0	0	12 (6.7)
HGIN	0	0	16	2	1	0	2	0	21 (11.7)
Submucosa	0	0	2	1	0	3	8	4	18 (10.1)
MP or beyond	2	1	1	0	6	4	30	82	126 (70.4)
Total (%)	4 (2.2)	1 (0.6)	31 (17.3)		57 (31.8)			86 (48.0)	179 (100)

C: Category; Non-N: Non-neoplastic; LGIN: Low-grade intraepithelial neoplasia; HGIN: High-grade intraepithelial neoplasia; MP: Muscularis propria. [1]The histopathologic diagnoses of resected specimens were made using the World Health Organization classification. The comparison of two groups (intramucosal lesions (i.e. Non-N, LGIN and HGIN) *versus* submucosal invasive carcinomas) tested by Fisher's exact test showed $P = 0.01$. The comparison of two groups (intramucosal lesions *versus* those that had invaded the submucosa or beyond) tested by Fisher's exact test showed $P < 0.001$.

Table 2 Relationship between the histopathologic diagnoses of cold biopsy specimens using the Japanese Group Classification and the depth of invasion in resected specimens of the same lesions

Invasion depth[1]	The Japanese Group Classification					Total (%)
	G1	G2	G3	G4	G5	
Non-N	2	0	0	0	0	2 (1.1)
LGIN	0	0	12	0	0	12 (6.7)
HGIN	0	0	16	2	3	21 (11.7)
Submucosa	0	0	2	1	15	18 (10.1)
MP or beyond	2	1	1	0	122	126 (70.4)
Total (%)	4 (2.2)	1 (0.6)	31 (17.3)	3 (1.7)	140 (78.2)	179 (100)

G: Group. [1]The histopathologic diagnoses of resected specimens were made using the World Health Organization classification. The comparison of two groups [intramucosal lesions (i.e. Non-N, LGIN and HGIN) *versus* submucosal invasive carcinomas] tested by Fisher's exact test showed $P < 0.0001$. The comparison of two groups (intramucosal lesions *versus* those that had invaded the submucosa or beyond) tested by Fisher's exact test showed $P < 0.0001$.

Relationship between the diagnoses of cold biopsy specimens made under the rVC guidelines and the depth of invasion in resected specimens

The histopathologic diagnoses of 399 cold biopsy specimens made using the rVC guidelines were as follows: 51 for C1; one for C2; 50 for C3; four for C4.1; 14 for C4.2; 28 for C4.3; 98 for C4.4; and 153 for C5. The final rVC diagnoses for the 179 lesions included four C1 lesions, one C2 lesion, 31 C3 lesions, 57 C4 lesions, and 86 C5 lesions. Table 1 shows the relationship between the final histopathologic diagnoses of the cold biopsy specimens using the rVC criteria and the depth of invasion in resected specimens of the same lesions. The resected specimens were diagnosed as follows: 35 intramucosal lesions (two non-neoplastic lesions; 12 low-grade intraepithelial neoplasias; 21 high-grade intraepithelial neoplasias); 18 submucosal lesions; and 126 lesions in the muscularis propria or beyond. The sensitivity of the rVC system to distinguish intramucosal lesions from submucosal invasive carcinomas was 22.2% (95% CI, 3.0%-41.4%), with a positive predictive value of 100%. Specificity and negative predictive value were 100% and 71.4% (95% CI, 58.8%-84.1%), respectively. The comparison of two groups (intramucosal lesions *versus* submucosal invasive carcinomas) tested by Fisher's exact test showed $P = 0.01$. The sensitivity of the rVC system to distinguish intramucosal lesions from lesions invasive to the submucosa or beyond was 59.7% (95% CI, 51.7%-67.7%), with a positive predictive value of 100%. Specificity and negative predictive value were 100% and 37.6% (95% CI, 27.7%-47.4%), respectively. The comparison of two groups (intramucosal lesions *versus* those that had invaded the submucosa or beyond) tested by Fisher's exact test showed $P < 0.001$.

Relationship between the diagnoses of cold biopsy specimens made under the JGC guidelines and the depth of invasion in resected specimens

Histopathologic diagnoses of 399 cold biopsy specimens made using the JGC criteria were as follows: 51 specimens in G1; one in G2; 50 in G3; four in G4; and 293 in G5. The final diagnoses for the 179 lesions using the JGC guidelines were as follows: four G1 lesions; one G2 lesion; 31 G3 lesions; three G4 lesions; and 140 G5 lesions. Table 2 shows the relationship between the final histopathologic diagnoses of the cold biopsy specimens using the JGC guidelines and the depth of invasion in resected specimens of the same lesions. The histopathologic diagnoses made for the 179 resected specimens are described in the section above. The sensitivity of the JGC system to distinguish intramucosal lesions from submucosal invasive carcinomas was 83.3% (95% CI, 66.1%-100%), with a positive predictive value of 83.3% (95% CI, 66.1%-100%). Specificity and negative predictive value were 91.4% (95% CI, 82.2%-100%) and 91.4% (95% CI, 82.2%-100%), respectively. The comparison of two groups (intramucosal lesions *versus* submucosal invasive carcinomas) tested by Fisher's exact test showed $P < 0.0001$. The sensitivity of

the JGC system to distinguish intramucosal lesions from lesions invasive to the submucosa or beyond was 95.1% (95% CI, 91.6%-98.7%), with a positive predictive value of 97.9% (95% CI, 95.5%-100%). Specificity and negative predictive value were 91.4% (95% CI, 82.2%-100%) and 82.1% (95% CI, 70.0%-94.1%), respectively. The comparison of two groups (intramucosal lesions *versus* those that had invaded the submucosa or beyond) tested by Fisher's exact test showed $P < 0.0001$. Three high-grade intraepithelial neoplasias and 137 of 144 carcinomas that had invaded the submucosa or beyond were diagnosed as "carcinoma" (G5) under the JGC guidelines.

DISCUSSION

From a therapeutic point of view, the most important histopathologic distinction in cold biopsy specimens taken from colorectal epithelial neoplastic lesions is whether there is evidence of invasion into the submucosa (or beyond). Histopathologic confirmation of lesions using cold biopsy specimens are ideal for predicting the therapeutic implications of colorectal epithelial neoplasia that cannot be treated by direct ER. We have shown that the rVC system had a high positive predictive value (100%) in diagnosing submucosal invasive carcinomas and carcinomas that had invaded the muscularis propria or beyond from cold biopsy specimens. These results may provide both patients and physicians with valuable information that will facilitate management decisions.

In cases of colorectal polyps, Livstone *et al*[12] reported 13 discrepancies (26%) between the diagnoses from single fractional biopsies and the final diagnoses of colonic lesions in 42 patients with 50 colonic polyps (0.8 to 4.5 cm in diameter). Of these discrepancies, four carcinomas invasive to the submucosa or beyond were found; adenomatous epithelium was detected in the fractional biopsies from two cases and normal colonic epithelium in the other two cases[12]. Pugliese *et al*[13] reported that among 53 patients with 59 colorectal polyps ($\geqslant$ 5 mm), seven cases had carcinomas that had invaded the submucosa or beyond, and four of these had been underestimated from the cold biopsy specimens. Gondal *et al*[14] reported that among 442 patients with a total of 532 colorectal adenomas ($\geqslant$ 2 mm) biopsied by flexible sigmoidoscopy and removed by colonoscopy, the assessment of the intraepithelial neoplasia status was changed in 51 adenomas (10%), and 38 (7%) of these had been underestimated from the cold biopsy diagnoses compared with the diagnoses based on polypectomy samples. Of these lesions, 389 (73%) were < 10 mm in diameter. In addition, four carcinomas invading the submucosa or beyond had been underestimated as being low-grade or high-grade intraepithelial neoplasias[14].

These observations suggest that cold biopsy-based diagnoses underestimate histopathologic diagnoses of the resected lesions in some cases of colorectal epithelial neoplastic lesions. In our study, the rVC system underestimated the distinction between intramucosal lesions and submucosal invasive carcinomas in 26.4% (14/53) of lesions. The sensitivity of the rVC system for distinguishing between intramucosal lesions and submucosal invasive carcinomas was poor (22.2%). Therefore, histopathologic examination of completely resected lesions was essential for the adequate diagnosis and appropriate treatment of the colorectal polyps including submucosal invasive carcinomas[15].

Overall, the rVC system had a high specificity (100%) for the histopathologic diagnoses of carcinomas invasive to the colorectal submucosa or beyond, whereas the sensitivity was poor (59.7%). The rVC system underestimated the distinction between intramucosal lesions and lesions that invaded the submucosa or beyond in 32.4% (58/179) of lesions. The poor sensitivity and high underestimation rate of the rVC system was caused by the high prevalence (80.4%) of submucosal or beyond invasive colorectal carcinomas in our cohort, and because the pathologists used invasion of the submucosa or beyond as an obligatory criterion for the diagnosis of carcinoma.

Direct ER without prior cold biopsy of small (< 10 mm) lesions is usually feasible and histopathologic examination of completely resected lesions enables adequate diagnosis and appropriate treatment. Therefore, cold biopsies for small lesions are not needed and our cases did not include these lesions. Under the JGC criteria, 137 of 144 carcinomas that invaded the submucosa or beyond were diagnosed as "carcinoma" (i.e. G5). The diagnostic criteria for colorectal carcinoma according to the JGC guidelines appear to attach more importance on nuclear features and glandular structures, and the presence of evident invasion into the submucosal layer is not considered mandatory[1]. Therefore, although the cold biopsy forceps were usually capable of sampling intramucosal lesions only, the diagnosis of "carcinoma" was possible under the JGC guidelines. For the same reason, distinguishing between intramucosal lesions and those invasive to the submucosa or beyond, or overestimating intramucosal lesions as those invasive to the submucosa or beyond was not a problem under the rVC guidelines, whereas three high-grade intraepithelial neoplasias were diagnosed as "carcinomas" using the JGC system.

Lesions can be diagnosed as low-grade dysplasia in the West and as carcinomas in Japan due to the differences in interpreting nuclear and structural features[1]. Japanese pathologists consider these features as clues for the diagnosis of carcinoma, but Western pathologists either do not take these features into consideration (such as rounded nuclei and variable shape of glands) or do not attach similar importance to these features with regard to the severity of dysplasia (such as marked hyperchromatism of nuclei and enlarged prominent nucleoli)[1]. These different histopathologic interpretations of the nuclear and structural features of lesions between Western and Japanese pathologists require further investigation.

The use of the rVC guidelines for cold biopsy specimens has a high positive predictive value in diagnosing carcinomas invasive to the colorectal

submucosa or beyond. However, it is of limited value in predicting the depth of invasion assigned to the resected specimens, especially for the diagnosis of submucosal invasive carcinomas. This should be supplemented by endoscopic assessment of the depth of invasion.

COMMENTS

Background

Large differences have been found between Western and Japanese pathologists in their diagnosis of colorectal epithelial neoplastic lesions. To overcome the differences between the conventional Western and the Japanese criteria, the Vienna Classification attempted to combine the basic concepts of the conventional Western criteria, which emphasizes that invasion is an indicator of metastatic potential, with the strong points of the Japanese criteria, which values consistency between diagnoses from cold biopsies and resected specimens. In the revised Vienna Classification, histopathologic diagnoses are classified into five categories according to neoplastic severity and depth of invasion. However, the efficacy of the revised Vienna Classification for diagnosing colorectal epithelial neoplastic lesions has not been reported.

Research frontiers

Diagnostic discrepancies do not matter to patients if Western and Japanese physicians understand the implications of their respective pathology reports and apply management strategies that are appropriate to the needs of their patients. However, continued attempts to unify Western and Japanese reporting systems are desirable because merging the terminologies of these systems will help codify the advantages of each into a language that is universally understood.

Innovations and breakthroughs

This is the first report investigating the efficacy of the revised Vienna Classification for diagnosing colorectal epithelial neoplastic lesions in cold biopsy specimens.

Applications

The revised Vienna Classification of colorectal epithelial neoplastic lesions seeks to be more closely in tune patient management, however, it should be emphasized that cold biopsy-based diagnoses are subject to the limitations of superficiality and sampling errors.

Peer review

The authors prospectively investigated the efficacy of the revised Vienna Classification for diagnosing colorectal epithelial neoplastic lesions in cold biopsy specimens. The studies are well done, and the manuscript is well written.

REFERENCES

1 **Schlemper RJ**, Itabashi M, Kato Y, Lewin KJ, Riddell RH, Shimoda T, Sipponen P, Stolte M, Watanabe H. Differences in the diagnostic criteria used by Japanese and Western pathologists to diagnose colorectal carcinoma. *Cancer* 1998; **82**: 60-69

2 **Schlemper RJ**, Kato Y, Stolte M. Diagnostic criteria for gastrointestinal carcinomas in Japan and Western countries: proposal for a new classification system of gastrointestinal epithelial neoplasia. *J Gastroenterol Hepatol* 2000; **15** Suppl: G49-G57

3 **Dixon MF**. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002; **51**: 130-131

4 **Japanese Society for Cancer of the Colon and Rectum**. Response assessment of nonsurgical treatment for colorectal carcinoma. Japanese classification of colorectal carcinoma. 1st English ed. Tokyo: Kanehara & Co Ltd, 1997: 77-82

5 **Muto T**, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. *Cancer* 1975; **36**: 2251-2270

6 **Williams CB**, Talbot IC. Polyps and tumors of the colon. In: Sivak MV Jr, ed. Gastroenterologic Endoscopy. 2nd ed. Philadelphia: WB Saunders, 2000: 1351-1378

7 **Jass JR**. Discrepancies between East and West. *Cancer* 2000; **88**: 969-970

8 **Willis J**, Riddell RH. Biology versus terminology: East meets West in surgical pathology. *Gastrointest Endosc* 2003; **57**: 369-376

9 **Schlemper RJ**, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Fléjou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, Yamabe H. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; **47**: 251-255

10 **Hamilton SR**, Aaltonen LA. World Health Organization classification of tumours: Pathology and genetics of tumours of the digestive system. Lyon: IARC Press, 2000

11 **Straus SE**, Richardson WS, Glasziou P, Haynes RB. Evidence-based medicine: how to practice and teach EBM. 3rd ed. Edinburgh, New York: Elsevier Churchill Livingstone, 2005

12 **Livstone EM**, Troncale FJ, Sheahan DG. Value of a single forceps biopsy of colonic polyps. *Gastroenterology* 1977; **73**: 1296-1298

13 **Pugliese V**, Gatteschi B, Aste H, Nicolò G, Munizzi F, Giacchero A, Bruzzi P. Value of multiple forceps biopsies in assessing the malignant potential of colonic polyps. *Tumori* 1981; **67**: 57-62

14 **Gondal G**, Grotmol T, Hofstad B, Bretthauer M, Eide TJ, Hoff G. Biopsy of colorectal polyps is not adequate for grading of neoplasia. *Endoscopy* 2005; **37**: 1193-1197

15 **Tominaga K**, Nakanishi Y, Nimura S, Yoshimura K, Sakai Y, Shimoda T. Predictive histopathologic factors for lymph node metastasis in patients with nonpedunculated submucosal invasive colorectal carcinoma. *Dis Colon Rectum* 2005; **48**: 92-100

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Sclerosing cholangitis associated with autoimmune pancreatitis differs from primary sclerosing cholangitis

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Abstract

AIM: To clarify the characteristic features of biliary lesions in patients with autoimmune pancreatitis (AIP) and compare them with those of primary sclerosing cholangitis (PSC).

METHODS: The clinicopathological characteristics of 34 patients with sclerosing cholangitis (SC) associated with AIP were compared with those of 4 patients with PSC.

RESULTS: SC with AIP occurred predominantly in elderly men. Obstructive jaundice was the most frequent initial symptom in SC with AIP. Only SC patients with AIP had elevated serum IgG4 levels, and sclerosing diseases were more frequent in these patients. SC patients with AIP responded well to steroid therapy. Segmental stenosis of the lower bile duct was observed only in SC patients with AIP, but a beaded and pruned-tree appearance was detected only in PSC patients. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall and the periportal area, as well as in the pancreas, of SC patients with AIP.

CONCLUSION: SC with AIP is distinctly different from PSC. The two diseases can be discriminated based on cholangiopancreatographic findings and serum IgG4 levels.

Key words: Autoimmune pancreatitis; IgG4; Primary sclerosing cholangitis; Sclerosing cholangitis

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INTRODUCTION

Autoimmune pancreatitis (AIP) is a unique form of pancreatitis in which autoimmune mechanisms are suspected of being involved in the pathogenesis. AIP has many clinical, radiological, serological and histopathological characteristics: (1) elderly male preponderance; (2) initial symptom is frequently painless obstructive jaundice; (3) occasional association with impaired pancreatic endocrine or exocrine function, and various extrapancreatic lesions; (4) favorable response to steroid therapy; (5) radiological findings of irregular narrowing of the main pancreatic duct and enlargement of the pancreas; (6) serological findings of elevated serum γ globulin, IgG, or IgG4 levels, along with the presence of some autoantibodies; and (7) histopathological findings of dense infiltration of T lymphocytes and IgG4-positive plasma cells with fibrosis and obliterative phlebitis in the pancreas[1-3]. Bile duct stenosis occurs frequently with AIP, and the major initial symptom in AIP patients is obstructive jaundice. The lower portion of the common bile duct is frequently stenotic. However, when AIP patients develop stenosis in the intrahepatic bile duct, the cholangiographic appearance is similar to that of primary sclerosing cholangitis (PSC)[4,5]. PSC is a progressive disease involving the intra- and extra-hepatic bile ducts. Despite therapy, PSC sometimes leads to liver cirrhosis. However, since AIP patients respond well to steroid therapy, it is necessary to discriminate between sclerosing cholangitis (SC) associated with AIP and PSC. This study aimed

to clarify the characteristic features of biliary lesions in AIP patients and compare them with those of PSC.

MATERIALS AND METHODS

Study patients

Over a 27-year-period, 43 patients (36 male and 7 female, average age 66.4 years) at Tokyo Metropolitan Komagome Hospital were diagnosed with AIP based on the following clinicopathological criteria: irregular narrowing of the main pancreatic duct on endoscopic retrograde pancreatography (n = 43), pancreatic enlargement on ultrasonography (US) or computed tomography (CT) (n = 42), presence of autoantibodies (n = 22), elevated serum IgG4 level in excess of 135 mg/dL (n = 31), characteristic histological findings in the pancreas (n = 12), and responsiveness to steroid therapy (n = 32). In the 43 AIP patients, 34 had SC (lower bile duct in 34, and intrahepatic bile duct in 4). During the same time, 4 patients were diagnosed with PSC according to appropriate criteria[6].

Methods

The stenotic portion of the bile duct was examined by endoscopic retrograde cholangiopancreatography and/or magnetic resonance cholangiopancreatography, and wall thickening of the bile duct in which stenosis was not obvious on cholangiography was assessed on CT and US. Two experienced gastroenterologists retrospectively reviewed these imaging findings without information on the patients. Extrapancreatic lesions, including sclerosing sialadenitis, sclerosing cholecystitis, and retroperitoneal fibrosis, were evaluated on physical examination, CT, and US. Serum IgG4 levels were measured in 30 AIP patients and 2 PSC patients. Histological examination and immunostaining with anti-IgG4 antibody were performed on specimens of the extrahepatic bile duct (6 AIP patients and 1 PSC patient) and liver (3 AIP and 2 PSC patients).

Statistical analysis

Statistical differences between the two groups were analyzed first by the Kruskal-Wallis H-test, followed by Mann-Whitney's U-test if significant. Other analyses were performed using Fisher's exact test. In all tests, $P < 0.05$ was considered statistically significant.

RESULTS

Clinical features

Men were significantly more commonly affected by SC with AIP than by PSC. Patients' age at diagnosis was significantly older in those with SC with AIP. Among the initial symptoms, obstructive jaundice was the most frequently observed in SC with AIP. Elevated serum IgG4 levels were frequent in SC patients with AIP, but not in the 2 PSC patients examined. Sclerosing diseases were frequently associated with SC with AIP. Ulcerative colitis was present in only 2 young PSC patients (Table 1). Thirty-two SC patients with AIP were treated with steroid therapy, and all of them showed a good response. All PSC patients were treated with ursodeoxycholic acid, and 1 patient underwent steroid therapy for associated ulcerative colitis. Cholangiographic findings progressed gradually in three PSC patients, and one patient ultimately required liver transplantation. All SC patients with AIP had a favorable outcome without liver failure.

Table 1 Clinical differences between sclerosing cholangitis with autoimmune pancreatitis and primary sclerosing cholangitis

	SC with AIP	PSC	P value
Average age (yr)	63.8	39.2	< 0.01
Male/Female	29/5	1/3	< 0.05
Obstructive jaundice +/-	30/4	0/4	< 0.01
Elevated serum IgG4 +/-	26/30	0/2	
Associated sclerosing disease +/-	20/14	0/4	< 0.05
Associated ulcerative colitis +/-	0/34	2/2	< 0.01

SC with AIP: Sclerosing cholangitis with autoimmune pancreatitis; PSC: Primary sclerosing cholangitis.

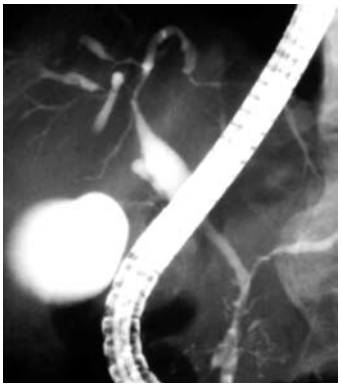

Figure 1 Endoscopic retrograde cholangiography of a patient with autoimmune pancreatitis showing a relatively long stricture of the hepatic hilar bile duct.

Cholangiopancreatographic findings

On pancreatography, narrowing of the main pancreatic duct was detected in all SC patients with AIP, but no abnormal findings were detected in any of the PSC patients. On cholangiography, the intrahepatic bile duct was involved in all PSC patients, but was involved in only four SC patients with AIP (Figure 1). Segmental stenosis of the lower bile duct was observed in all SC patients with AIP, but was not detected in any of the PSC patients. Extensive involvement of the bile duct, showing widespread wall thickening of the middle and upper bile duct where stenosis was not obvious on cholangiography, was detected only in 14 SC patients with AIP, although there was no significant difference between the two groups. A diffusely distributed, beaded and pruned-tree appearance and diverticular formation were detected only in PSC patients (Figure 2 and Table 2). A long stricture was detected in the hepatic hilar region in all 4 SC patients with AIP involving the intrahepatic bile duct.

Histological and immunohistochemical findings

In PSC, the hilar bile duct displayed diffuse fibrosis with moderate lymphoplasmacytic infiltration. The liver of PSC patients showed features of biliary cirrhosis, and fibro-obliterative lesions characterized by onion skin-like periductal fibrosis with predominantly lymphocytic infiltration were observed around the intrahepatic bile

Table 2 Cholangiopancreatographic differences between sclerosing cholangitis with autoimmune pancreatitis and primary sclerosing cholangitis

	SC with AIP	PSC	*P* value
Narrowing of the main pancreatic duct +/-	34/0	0/4	< 0.01
Stenosis of the intrahepatic bile duct +/-	4/30	4/0	< 0.01
Stenosis of the lower bile duct +/-	34/0	0/4	< 0.01
Extensive bile duct wall thickening	14/20	0/4	NS
Beaded appearance	0/34	2/2	< 0.01
Pruned-tree appearance	0/34	3/1	< 0.01
Diverticular formation	0/34	2/2	< 0.01

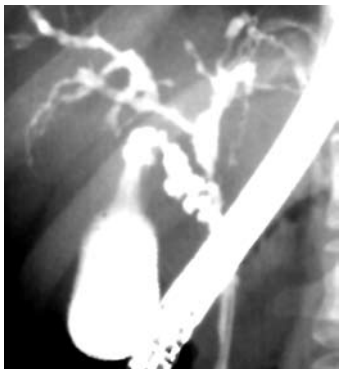

Figure 2 Endoscopic retrograde cholangiography of a patient with primary sclerosing cholangitis showing beaded and pruned-tree appearance.

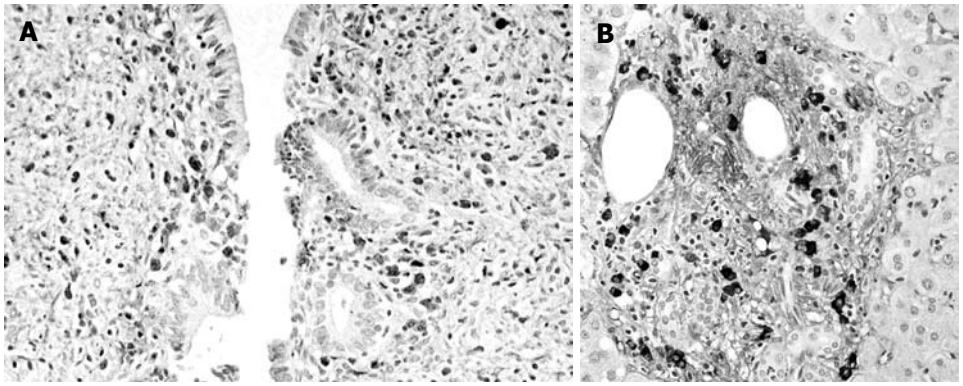

Figure 3 IgG4-immunostaining of the bile duct (A) and liver (B) of a patient with autoimmune pancreatitis. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall (A) and the periportal area of the liver (B).

duct. However, infiltration of IgG4-positive plasma cells was not detected in the bile duct or liver.

The histological findings of SC associated with AIP included transmural fibrosis and dense lymphoplasmacytic infiltration of the bile duct wall, along with lymphoplasmacytic infiltration and fibrosis in the periportal area of the liver. Compared with PSC, lymphoplasmacytic infiltration was denser, the degree of fibrosis was less severe, and the onion skin-like appearance was not observed. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall (Figure 3A) and the periportal area (Figure 3B), as well as in the pancreas, of patients with AIP.

DISCUSSION

SC is a heterogeneous disease that may be associated with choledocholithiasis, biliary tumor, or infection. SC of unknown origin is called PSC. PSC is progressive despite conservative therapy and involves the intra- and extrahepatic bile ducts, resulting in liver cirrhosis. The effect of steroid therapy is questionable, and liver transplantation currently provides the greatest hope for a possible cure. It occurs among patients in their 30 and 40 s and is frequently associated with inflammatory bowel disease[7,8]. Pancreatograms are not abnormal in most cases[9].

However, an analysis of 192 PSC patients in Japan found that their characteristics differed from those in Western counties, with regard to age distribution and the incidence of complications[10]. In that analysis, the patients were predominantly men, and two peaks in age distribution at diagnosis (20-30 years and 50-70 years) were identified. Compared to younger patients, those aged 40 years or older displayed a lower incidence of associated ulcerative colitis, whereas the incidence of chronic pancreatitis was higher.

SC is frequently associated with AIP, and occurs predominantly in elderly men. The major initial symptom of SC with AIP is obstructive jaundice, which differs from PSC. The most prominent feature on cholangiography for SC with AIP was stenosis of the lower bile duct. When stenosis is found in the intrahepatic or the hilar hepatic bile duct, the cholangiographic appearance is very similar to that of PSC[4,5]. However, a long stricture was detected in the hepatic hilar region in SC patients with AIP, instead of the beaded and pruned-tree appearance that is frequently observed in PSC. Widespread wall thickening of the middle and upper bile ducts was also detected only in SC patients with AIP.

SC patients with AIP responded dramatically well to steroid therapy and showed a favorable outcome[5,9,11]. Histologically, dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall and the periportal area of SC patients with AIP, but it was not detected in PSC patients. SC with AIP is sometimes associated with sclerosing diseases such as sclerosing sialadenitis, sclerosing cholecystitis, or retroperitoneal fibrosis, and these salivary, gallbladder, and retroperitoneal lesions show similar histological findings to those in the bile duct and pancreas. Furthermore, abundant infiltration of IgG4-positive plasma cells is detected in various organs of AIP patients[12,13]. Therefore, we proposed a new clinicopathological entity, an IgG4-related sclerosing disease, which is histopathologically characterized by extensive IgG4-positive plasma cell and T lymphocyte infiltration of various organs. We also suspect that AIP and SC with AIP is a pancreatic and bile duct lesion of this systemic disease[13-15]. Based on the above findings, SC with AIP should be differentiated from

PSC. In particular, since SC with AIP responds well to steroid therapy, discrimination between the two diseases is necessary before making therapeutic decisions. Clinically, serum IgG4 levels and cholangiopancreatographic findings are useful in differentiating between the two diseases.

Considering the predominance of elderly men, the infrequent association with inflammatory bowel disease, and the frequent association with chronic pancreatitis, many older patients diagnosed with PSC in Japan may actually have SC with AIP.

In conclusion, since SC with AIP is induced by different mechanisms to those in PSC, the condition should be differentiated from PSC. The two diseases can be discriminated based on their cholangiopancreatographic findings and serum IgG4 levels.

COMMENTS

Background

When patients with autoimmune pancreatitis (AIP) develop stenosis in the intrahepatic bile duct, the cholangiographic appearance is similar to that of primary sclerosing cholangitis (PSC). PSC is a progressive disease involving the intra- and extrahepatic bile ducts.

Innovations and breakthroughs

Sclerosing cholangitis with AIP is distinctly different from PSC. Only SC patients with AIP had elevated serum IgG4 levels and responded well to steroid therapy. Segmental stenosis of the lower bile duct was observed only in SC patients with AIP, but a beaded and pruned-tree appearance was detected only in PSC patients. Dense infiltration of IgG4-positive plasma cells was detected in the bile duct wall and the periportal area of SC patients with AIP.

Applications

Sclerosing cholangitis with AIP responds well to steroid therapy. The differential diagnosis between sclerosing cholangitis with AIP and PSC is important to ensure optimal patient treatment.

Peer review

The authors described the characteristic features of biliary lesions in autoimmune pancreatitis patients. The paper is well presented and the result are interesting.

REFERENCES

1 **Okazaki K**, Uchida K, Chiba T. Recent concept of autoimmune-related pancreatitis. *J Gastroenterol* 2001; **36**: 293-302

2 **Kim KP**, Kim MH, Song MH, Lee SS, Seo DW, Lee SK. Autoimmune chronic pancreatitis. *Am J Gastroenterol* 2004; **99**: 1605-1616

3 **Lara LP**, Chari ST. Autoimmune pancreatitis. *Curr Gastroenterol Rep* 2005; **7**: 101-106

4 **Nakazawa T**, Ohara H, Yamada T, Ando H, Sano H, Kajino S, Hashimoto T, Nakamura S, Ando T, Nomura T, Joh T, Itoh M. Atypical primary sclerosing cholangitis cases associated with unusual pancreatitis. *Hepatogastroenterology* 2001; **48**: 625-630

5 **Kamisawa T**, Egawa N, Tsuruta K, Okamoto A, Funata N. Primary sclerosing cholangitis may be overestimated in Japan. *J Gastroenterol* 2005; **40**: 318-319

6 **Linder K**, LaRusso NF. Primary sclerosing cholangitis. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Disease of the liver. 9th ed. Philadelphia: JB Lippincott, 2003: 673-684

7 **LaRusso NF**, Wiesner RH, Ludwig J, MacCarty RL. Current concepts. Primary sclerosing cholangitis. *N Engl J Med* 1984; **310**: 899-903

8 **Wiesner RH**, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, Fleming TR, Fisher LD, Beaver SJ, LaRusso NF. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology* 1989; **10**: 430-436

9 **Nakazawa T**, Ohara H, Sano H, Ando T, Aoki S, Kobayashi S, Okamoto T, Nomura T, Joh T, Itoh M. Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis. *Pancreas* 2005; **30**: 20-25

10 **Takikawa H**, Manabe T. Primary sclerosing cholangitis in Japan--analysis of 192 cases. *J Gastroenterol* 1997; **32**: 134-137

11 **Kamisawa T**, Egawa N, Nakajima H, Tsuruta K, Okamoto A. Morphological changes after steroid therapy in autoimmune pancreatitis. *Scand J Gastroenterol* 2004; **39**: 1154-1158

12 **Kamisawa T**, Funata N, Hayashi Y, Tsuruta K, Okamoto A, Amemiya K, Egawa N, Nakajima H. Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis. *Gut* 2003; **52**: 683-687

13 **Kamisawa T**, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K, Okamoto A, Egawa N, Nakajima H. A new clinicopathological entity of IgG4-related autoimmune disease. *J Gastroenterol* 2003; **38**: 982-984

14 **Kamisawa T**, Nakajima H, Egawa N, Funata N, Tsuruta K, Okamoto A. IgG4-related sclerosing disease incorporating sclerosing pancreatitis, cholangitis, sialadenitis and retroperitoneal fibrosis with lymphadenopathy. *Pancreatology* 2006; **6**: 132-137

15 **Kamisawa T**, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol* 2006; **41**: 613-625

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Endoscopic ultrasonography does not differentiate neoplastic from non-neoplastic small gallbladder polyps

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Abstract

AIM: To assess the ability of endoscopic ultrasonography (EUS) to differentiate neoplastic from non-neoplastic polypoid lesions of the gallbladder (PLGs).

METHODS: The uses of EUS and transabdominal ultrasonography (US) were retrospectively analyzed in 94 surgical cases of gallbladder polyps less than 20 mm in diameter.

RESULTS: The prevalence of neoplastic lesions with a diameter of 5-10 mm was 17.2% (10/58); 11-15 mm, 15.4% (4/26), and 16-20 mm, 50% (5/10). The overall diagnostic accuracies of EUS and US for small PLGs were 80.9% and 63.9% ($P < 0.05$), respectively. EUS correctly distinguished 12 (63.2%) of 19 neoplastic PLGs but was less accurate for polyps less than 1.0 cm (4/10, 40%) than for polyps greater than 1.0 cm (8/9, 88.9%) ($P = 0.02$).

CONCLUSION: Although EUS was more accurate than US, its accuracy for differentiating neoplastic from non-neoplastic PLGs less than 1.0 cm was low. Thus, EUS alone is not sufficient for determining a treatment strategy for PLGs of less than 1.0 cm.

Key words: Endoscopic ultrasonography; Neoplastic lesion; Polypoid gallbladder lesion

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INTRODUCTION

Polypoid lesions of the gallbladder (GB) are increasingly detected by ultrasonography (US). Indeed, 4%-7% of healthy individuals have been reported to have polyps of the GB[1,2]. The significance of these polypoid lesions is poorly understood, and the appropriate management of these lesions is controversial. Although most GB polyps are benign, some early carcinomas of the GB share the same appearance as benign polyps. Currently, GB polyps larger than 1 cm should be surgically removed because of the increased risk of malignancy[3]. On the other hand, patients with smaller polyps usually require repeated US and follow-up. Distinguishing between non-neoplastic, neoplastic, and potentially malignant lesions is a major diagnostic dilemma, and the therapeutic options for these lesions remain controversial.

Endoscopic ultrasonography (EUS) is considered to be superior to conventional US for imaging GB lesions, because EUS can provide high-resolution images of small lesions with higher ultrasound frequencies (7.5-12 MHz *vs* 3.5-5 MHz)[4,5]. The improved accuracy of EUS in imaging small GB lesions has been previously reported in a surgical series[5-7]. However, polyps with a maximum diameter of less than 10-15 mm are difficult to differentially diagnose in many cases. The present study assesses the predictive value of EUS in the differential diagnosis of small polypoid lesions (maximum diameter, $\leqslant$ 20 mm) of the GB in a surgical series.

MATERIALS AND METHODS

Patients

Between 1996 and 2006, 365 patients underwent EUS for small (maximum diameter, $\leqslant$ 20 mm) polypoid lesions

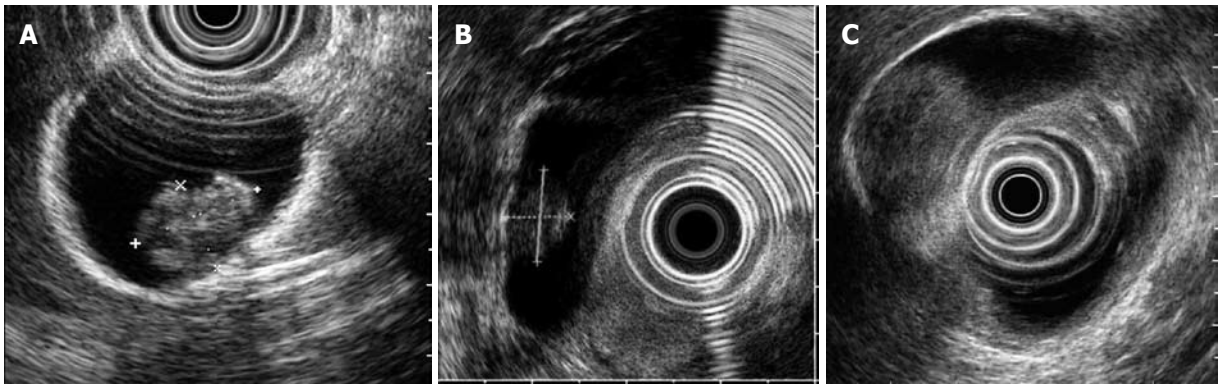

Figure 1 Polypoid lesions of gallbladder. A: Cholesterol polyp of the gallbladder. EUS shows a 13-mm-diameter, granular-surfaced, pedunculated mass with an internal echo pattern characterized by an aggregation of echogenic spots. Histological examination of the surgical specimen showed a cholesterol polyp; B: Adenoma of the gallbladder. EUS shows a 10-mm-diameter, homogeneously isoechoic, pedunculated mass. The histological diagnosis was tubulovillous adenoma with focal high-grade dysplasia; C: Adenocarcinoma of the gallbladder. EUS shows a 19-mm-diameter, smooth-surfaced, heterogeneously echogenic, pedunculated mass. Histological examination of the surgical specimen showed adenocarcinoma.

of the GB detected by transabdominal US. Of these 365 patients, 94 patients who underwent laparoscopic cholecystectomy for GB polyps were enrolled. US was performed as an abdominal screening test for asymptomatic patients or as a detailed examination for patients suspected of having a gastrointestinal disorder because of clinical symptoms. When US revealed polypoid lesions inside the GB, the patient then underwent EUS. In principle, EUS was indicated for polypoid lesions exceeding 5 mm or for potentially neoplastic polyps. Patients with localized adenomyomatosis or diffuse wall-thickening lesions resulting from inflammation were excluded from the study.

Methods

All patients with suspected neoplastic lesions based on EUS underwent surgery. Generally, surgery was not indicated for patients with a EUS diagnosis of non-neoplastic lesions, except for symptomatic cases or patients undergoing combined operations for other abdominal diseases. In our surgical series, the EUS diagnosis was compared with the histopathological diagnosis. Based on the pathological evaluation of specimens obtained upon cholecystectomy, the GB polyps were assigned into two groups: neoplastic (adenoma and carcinoma) and non-neoplastic (cholesterol, inflammatory, and fibrous). In patients with multiple polyps, the size of the largest polyp was measured.

Demographics and EUS findings were prospectively collected at the time of the procedure and were analyzed retrospectively. EUS was performed by one of the authors with knowledge of the ultrasonographic findings. In all cases, the differential diagnosis of polypoid lesions of the GB by EUS and US was made according to the criteria outlined below[5,6].

Cholesterol polyps (Figure 1A) are pedunculated lesions with a granular surface. The internal echo is hyperechoic to isoechoic with a tiny, spotty echo pattern. Relatively large polyps, those greater than 10 mm in diameter, may not give the typical image but may have a spotty echo area. Localized adenomyomatosis is imaged as a sessile echogenic mass containing multiple microcysts or with a comet tail artifact. Neoplastic polyps (adenoma, Figure 1B or carcinoma, Figure 1C) are pedunculated or sessile masses without echogenic spots, multiple microcysts, or comet tail artifacts; the internal echo is hypoechoic to isoechoic and almost homogeneous.

Transabdominal US was performed using a real-time scanner with a 3.5-MHz curved array transducer (SSD-2000; Aloka, Tokyo, Japan). EUS was performed using an echoendoscope with a 7.5-MHz or 12-MHz radial sector scan transducer (GF-UM2, UM3, UM20; Olympus Co., Tokyo, Japan). The GB was visualized from the duodenum and gastric antrum. For sedation, 5 mg of midazolam were administered intravenously.

Statistical analysis

The results were analyzed by Fisher's exact probability test or the Wilcoxon test, as appropriate. Differences were considered significant at $P < 0.05$.

RESULTS

Patient characteristics

Of the 94 patients, 19 had neoplastic lesions and 75 had non-neoplastic lesions. The mean age of the patients with non-neoplastic polyps was 50 ± 12.5 years, and that of patients with neoplastic polyps was 51 ± 11.3 years. Most of the non-neoplastic polyps were cholesterol polyps (56/75, 74.7%). Seventeen polypoid lesions were adenomyomatosis, and two polyps were inflammatory polyps. Adenocarcinoma was found in two patients; and adenomas, in 17. Two of the 17 adenomas contained focal high-grade dysplasia. The prevalence of neoplastic lesions with a diameter of 5-10 mm was 17.2% (10/58); 11-15 mm, 15.4% (4/26), and 16-20 mm, 50% (5/10) (Table 1). The average size of non-neoplastic polyps was 9.8 ± 2.8 mm (5-18). Among neoplastic polyps, the average size of an adenoma without high grade dysplasia, adenoma with high grade dysplasia, and adenocarcinoma were 9.9 ± 3.6 mm (6-17), 12.0 mm (7 and 17), and 19.0 mm (13 and 25), respectively. The average size

Table 1 Histological diagnosis and size of polypoid gallbladder lesions in the surgical series (*n*)

Size (mm)	Cholesterol	Adenomyomatosis	Inflammatory	Adenoma	Cancer	Total
5-10	39	9	0	10	0	58
11-15	14	7	1	3	1	26
16-20	3	1	1	4	1	10

Table 2 EUS and US diagnosis of polypoid gallbladder lesions in the surgical series

	Pathologic diagnosis (*n*)			
	Cholesterol polyp	Adenomyomatosis	Inflammatory polyp	Neoplastic lesions
EUS diagnosis				
Cholesterol	47	4	0	7
Adenomayomatosis	0	11	1	0
Neoplastic lesion	9	2	1	12
US diagnosis				
Cholesterol	41	8	0	10
Adenomayomatosis	0	4	1	0
Neoplastic lesion	15	5	1	9

Table 3 EUS and US diagnosis according to the size of the polypoid gallbladder lesion

	Pathology (*n*)					
	Cholesterol		Adenomyomatosis		Neoplastic lesion	
Size (mm)	5-10	11-20	5-10	11-20	5-10	11-20
EUS						
Cholesterol	33	14	0	0	6	3
Adenomyomatosis	6	0	4	7	1	1
Inflammatory	0	0	0	1	0	1
Neoplastic lesions	4	1	0	0	4	8
US						
Cholesterol	32	9	0	0	7	8
Adenomyomatosis	7	1	1	3	1	4
Inflammatory	0	0	0	1	0	1
Neoplastic lesions	8	2	0	0	2	7

Table 4 Differential diagnosis between neoplastic and benign polyps by EUS and US

	Diagnosis by postoperative histological examination	
	Neoplastic polyp	Non-neoplastic polyp
Diagnosis by EUS		
Neoplastic polyps	12	12
Non-neoplastic polyps	6	64
Diagnosis by US		
Neoplastic polyps	9	21
Non-neoplastic polyps	10	54

of neoplastic polyps including adenoma with high grade dysplasia and carcinoma tended to be larger than neoplastic polyps without high grade dysplasia and non-neoplastic polyps.

Differential diagnosis by EUS and US

Differential diagnosis by EUS and US was successful in 70 (74.5%) and 54 (57.4%) of 94 patients, respectively; the difference between these rates was significant (P = 0.014). When the results of EUS were assessed according to the pathological results (Table 2), cholesterol polyps were correctly identified in 47 of 56 patients (83.9%). The unsuccessful diagnoses included nine cases that were misjudged as adenoma. Adenomyomatosis was correctly identified in 11 of 17 patients (64.7%). Among the six misdiagnosed cases, four were cholesterol polyps and two were neoplastic polyps. Neoplastic polyps were correctly identified in 12 of 19 patients (63.2%). The unsuccessful diagnoses included seven cases misjudged as cholesterol polyps. Five of the seven cases were less than 1.0 cm in size, and another of the cases was 17 mm in size before surgery. EUS showed a homogeneously isoechoic, pedunculated mass, and abdominal CT showed an enhanced polypoid mass of the GB in the arterial phase. Therefore, we diagnosed it as an early cancer. However, this polyp was confirmed to be a cholesterol polyp after cholecystectomy (Figure 2). Of the 19 neoplastic polyps, two were adenocarcinoma, with diameters of 10 and 19 mm, respectively.

Table 3 shows the EUS results categorized according to the size of the polypoid GB lesion (< 10 mm *vs* 10-20 mm). Of the 58 cases with a diameter less than 10 mm, EUS correctly distinguished 84.6% (33/39) of the cholesterol polyps, 36.4% (4/11) of the adenomyomatosis, and 50.0% (4/8) of the neoplastic lesions. Of the 36

Table 5 Sensitivity, specificity, and accuracy of EUS and US diagnoses for neoplastic lesions according to the size of the polypoid gallbladder lesion (%)

	Sensitivity	Specificity	PPV	NPV	Accuracy
EUS					
Overall	66.7	84.2	50.0	91.4	80.9
5-10 mm	44.4	86.0	36.4	89.6	79.7
11-20 mm	88.9	81.5	61.5	95.7	83.3
US					
Overall	47.4	72.0	30.0	84.4	67.0
5-10 mm	20.0	83.3	20.0	83.3	72.4
11-20 mm	77.8	51.9	35.0	87.5	63.9

PPV: Positive predictive value; NPV: Negative predictive value.

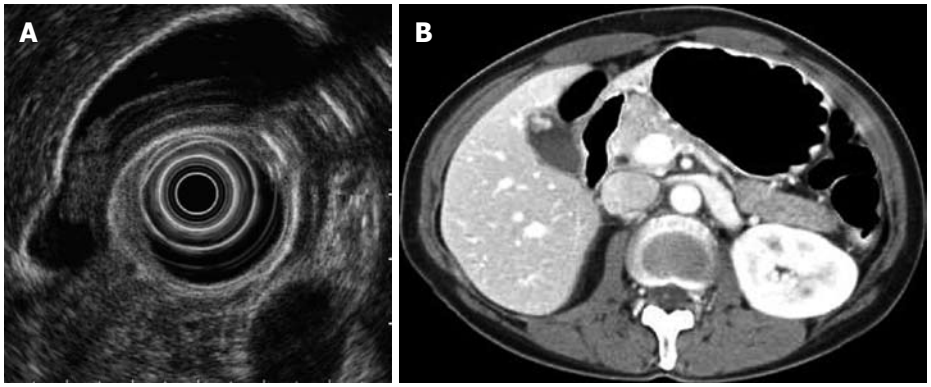

Figure 2 Misjudged case diagnosed as adenoma or carcinoma before surgery. A: EUS shows a 17.5-mm-diameter, homogeneously isoechoic, pedunculated mass; B: Abdominal CT shows an enhanced polypoid mass of the gallbladder in arterial phase. Histological examination of the surgical specimen showed a cholesterol polyp.

cases with polyps greater than 10 mm in diameter, EUS correctly distinguished 82.4% (14/17) of the cholesterol polyps, 87.5% (7/8) of the adenomyomatosis, and 88.9% (8/9) of the neoplastic lesions. The accuracy of EUS in diagnosing neoplastic lesions tended to be lower for polyps greater than 10 mm (79.7%) than for polyps less than 10 mm (83.3%) ($P = 0.12$). There was no significant difference between EUS and US in the diagnosis of cholesterol polyps.

Table 4 summarizes the results of differential diagnoses between neoplastic and benign polyps assessed by EUS and US. The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for EUS (US) in the diagnosis of neoplastic lesions were 66.7% (47.5%), 84.2% (72.0%), 50.0% (30.0%), 91.4% (84.4%), and 80.9% (67.0%), respectively (Table 5). When the results for relatively smaller polyps (diameter, < 10 mm) and for larger polyps (diameter, > 10 mm) were considered separately, the sensitivity, specificity, PPV, NPV, and accuracy for US were all lower than the values for EUS in both groups. The values for EUS in polyps less than 1.0 cm in diameter were lower than those in polyps greater than 1.0 cm in diameter.

DISCUSSION

Owing to the widespread use of conventional US, an increasing number of polypoid lesions of the GB are being identified. However, it is difficult to make differential diagnoses of polypoid GB lesions by US, CT, and magnetic resonance imaging. In general, factors that increase the probability that a GB polyp will be malignant include age greater than 50 years, a solitary lesion, a polyp greater than 1.0 cm in size, the presence of gallstones, a sessile lesion, and a rapid change in lesion size on serial ultrasonography[8,9]. All of these factors should be taken into consideration when advising patients with a polypoid lesion of the GB (PLG). The correct surgical management of PLGs is controversial. Although it is widely agreed that patients with symptomatic PLGs should be offered cholecystectomy, preferably by the laparoscopic route, the best treatment for an asymptomatic patient is not clear. In cases with a high probability of a malignant lesion, such as a PLG larger than 2 cm, open surgery is preferred to reduce the risk of tumor seeding associated with laparoscopic surgery. For asymptomatic PLGs smaller than 1 cm, follow-up US every 6 to 12 mo is necessary to exclude a rapidly growing malignant tumor[3].

There are a number of reports suggesting that sessile lesions smaller than 1.0 cm have an increased incidence of malignancy compared with those with a stalk[10]. In this study, 10 of 19 (52.6%) neoplastic polyps were pedunculated lesions smaller than 1 cm. Sugiyama *et al*[11] reported that approximately 30% of polyps with a diameter of 11-15 mm were cholesterol polyps and that about 40% of neoplastic polyps were 6-10 mm in diameter. Kubota *et al*[12] found that 57% of cholesterol polyps, 75% of adenomas, and 13% of neoplastic polyps were less than 10 mm in diameter. Thus, for polyps less than 10 mm in diameter, criteria other than size, along with an aggressive work-up, are needed to discriminate between neoplastic and non-neoplastic polyps.

EUS is considered to be superior to conventional US for imaging GB lesions, because EUS can provide high-resolution images of small lesions at higher ultrasound frequencies (7.5-12 MHz *vs* 3.5-5 MHz). Many

studies have investigated the relationship between the neoplastic nature of GB polyps and their morphological characteristics such as the number of polyps, the polyp shape, the diameter of the largest polyp, the echo level and internal echo pattern, and the polyp margin[12-14]. Among these variables, size is the most significant predictor of neoplastic polyps. However, the accuracy of EUS in identifying neoplastic lesions among polyps smaller than 10 mm in our study was only 44.4%, which was significantly lower than the identification rate among polyps greater than 1.0 cm (88.9%, $P < 0.05$).

To overcome the limitation of EUS in the differential diagnosis of neoplastic and non-neoplastic polypoid lesions less than 10-15 mm or less than 20 mm in size, an EUS scoring system has been adopted[7,15]. According to this system, the sensitivity, specificity, and accuracy of the risk for a neoplastic polyp were 81%, 86%, and 83.7%, respectively, for polyps with an EUS score of 6 or greater, whereas the sensitivity, specificity, and accuracy using a 10 mm cut-off diameter were 60%, 64%, and 62.7%, respectively[7]. Based on the EUS scoring system of Sadamoto *et al*[15], the sensitivity, specificity, and accuracy of the risk for neoplasia in polyps with scores of 12 or higher were 77.8%, 82.7%, and 82.9%, respectively. The EUS scoring system will be useful for differentiating between neoplastic and non-neoplastic polyps of the GB, however, the EUS variables used to calculate the score differ between the different EUS scoring systems.

The accuracy of EUS results tend to be lower for polyps smaller than 1 cm than for polyps greater than 1 cm in size. In our study, seven of 11 (63.6%) polyps less than 1.0 cm in size that were determined to be neoplastic by EUS before surgery were confirmed after surgery to be non-neoplastic lesions, including six cholesterol polyps and one adenomyomatosis. Using US, only two of 10 cases were determined to be neoplastic polyps after surgery. Thus, despite its higher accuracy compared with conventional US, EUS could not differentiate malignant from benign polyps smaller than 1.0 cm. No carcinoma was found in polyps less than 1.0 cm in size, but the prevalence of adenoma was 17.2% in our study.

Although EUS was more accurate than US, its accuracy for differentiating malignant from benign PLGs of less than 1.0 cm was low. EUS could not differentiate malignant lesions from benign polyps less than 1.0 cm in size, because such small polyps do not often show findings typical of cholesterol polyps, localized types of adenomyomatosis, or neoplastic lesions. Thus, EUS alone is not sufficient for determining a treatment strategy for PLGs of less than 1.0 cm. Polyps less than 1.0 cm in diameter without typical EUS or US findings should be followed-up by US at intervals of 6-12 mo. Changes in the size or structure of polypoid lesions should prompt reinvestigation with EUS and lead physicians to consider cholecystectomy.

COMMENTS

Background

The distinction between non-neoplastic, neoplastic and potentially malignant lesions is a major diagnostic dilemma and the therapeutic options for small polypoid lesions of the gallbladder remain controversial. Although endoscopic ultrasonography (EUS) was more accurate than ultrasonography (US), its accuracy for differentiating malignant from benign polypoid gallbladder lesions (PLGs) of less than 1.0 cm was low.

Research frontiers

Among many variables, size is the most significant predictor of neoplastic polyps. Although EUS was more accurate than US, its accuracy for differentiating malignant from benign PLGs of less than 1.0 cm was low. Thus, EUS alone is not sufficient for determining a treatment strategy for PLGs of less than 1.0 cm.

Innovations and breakthroughs

Many studies have investigated the relationship between the neoplastic nature of gallbladder (GB) polyps and their morphological characteristics such as the number of polyps, the polyp shape, the diameter of the largest polyp, the echo level and internal echo pattern, and the polyp margin. Among these variables, size is the most significant predictor of neoplastic polyps. However, the accuracy of EUS results tends to be lower for polyps smaller than 1 cm than for polyps greater than 1 cm in size. To overcome the limitation of EUS in the differential diagnosis of neoplastic from benign PLGs, an EUS scoring system has been adopted. The EUS scoring system will be useful for differentiating between neoplastic and non-neoplastic polyps of the GB, however, the EUS variables used to calculate the score differ between the different EUS scoring systems. Thus, EUS alone is not sufficient for determining a treatment strategy for PLGs of less than 1.0 cm.

Applications

The study results suggest that EUS alone is not sufficient for determining a treatment strategy for PLGs of less than 1.0 cm. Thus, new diagnostic criteria of EUS or tools are needed to distinguish between non-neoplastic, neoplastic, and potentially malignant lesions in small PLGs.

Terminology

Neoplastic gallbladder polyps: A neoplastic polyp has the properties of a neoplasm including adenoma and carcinoma. A non-neoplastic polyp does not have the properties of a neoplasm and includes cholesterol, inflammatory and fibrous polyps.

Peer review

Although retrospective, this study involves a large series of patients and describes the role of EUS well. The results suggest that EUS alone is not sufficient for determining a treatment strategy for PLGs of less than 1.0 cm and a new diagnostic approach is needed.

REFERENCES

1 **Segawa K**, Arisawa T, Niwa Y, Suzuki T, Tsukamoto Y, Goto H, Hamajima E, Shimodaira M, Ohmiya N. Prevalence of gallbladder polyps among apparently healthy Japanese: ultrasonographic study. *Am J Gastroenterol* 1992; **87**: 630-633

2 **Chen CY**, Lu CL, Chang FY, Lee SD. Risk factors for gallbladder polyps in the Chinese population. *Am J Gastroenterol* 1997; **92**: 2066-2068

3 **Lee KF**, Wong J, Li JC, Lai PB. Polypoid lesions of the gallbladder. *Am J Surg* 2004; **188**: 186-190

4 **Muguruma N**, Okamura S, Ichikawa S, Tsujigami K, Suzuki M, Tadatsu M, Kusaka Y, Okita Y, Yano M, Ito S. Endoscopic sonography in the diagnosis of gallbladder wall lesions in patients with gallstones. *J Clin Ultrasound* 2001; **29**: 395-400

5 **Azuma T**, Yoshikawa T, Araida T, Takasaki K. Differential diagnosis of polypoid lesions of the gallbladder by endoscopic ultrasonography. *Am J Surg* 2001; **181**: 65-70

6 **Sugiyama M**, Xie XY, Atomi Y, Saito M. Differential diagnosis of small polypoid lesions of the gallbladder: the value of endoscopic ultrasonography. *Ann Surg* 1999; **229**: 498-504

7 **Choi WB**, Lee SK, Kim MH, Seo DW, Kim HJ, Kim DI, Park ET, Yoo KS, Lim BC, Myung SJ, Park HJ, Min YI. A new strategy to predict the neoplastic polyps of the gallbladder based on a scoring system using EUS. *Gastrointest Endosc* 2000; **52**: 372-379

8 **Koga A**, Watanabe K, Fukuyama T, Takiguchi S, Nakayama F. Diagnosis and operative indications for polypoid lesions of the gallbladder. *Arch Surg* 1988; **123**: 26-29

9 **Mainprize KS**, Gould SW, Gilbert JM. Surgical management of polypoid lesions of the gallbladder. *Br J Surg* 2000; **87**: 414-417
10 **Ishikawa O**, Ohhigashi H, Imaoka S, Nakaizumi A, Kitamura T, Sasaki Y, Shibata T, Wada A, Iwanaga T. The difference in malignancy between pedunculated and sessile polypoid lesions of the gallbladder. *Am J Gastroenterol* 1989; **84**: 1386-1390
11 **Sugiyama M**, Atomi Y, Kuroda A, Muto T, Wada N. Large cholesterol polyps of the gallbladder: diagnosis by means of US and endoscopic US. *Radiology* 1995; **196**: 493-497
12 **Kubota K**, Bandai Y, Noie T, Ishizaki Y, Teruya M, Makuuchi M. How should polypoid lesions of the gallbladder be treated in the era of laparoscopic cholecystectomy? *Surgery* 1995; **117**: 481-487
13 **Yang HL**, Sun YG, Wang Z. Polypoid lesions of the gallbladder: diagnosis and indications for surgery. *Br J Surg* 1992; **79**: 227-229
14 **Collett JA**, Allan RB, Chisholm RJ, Wilson IR, Burt MJ, Chapman BA. Gallbladder polyps: prospective study. *J Ultrasound Med* 1998; **17**: 207-211
15 **Sadamoto Y**, Oda S, Tanaka M, Harada N, Kubo H, Eguchi T, Nawata H. A useful approach to the differential diagnosis of small polypoid lesions of the gallbladder, utilizing an endoscopic ultrasound scoring system. *Endoscopy* 2002; **34**: 959-965

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BRIEF ARTICLES

Mucin gene expression in bile of patients with and without gallstone disease, collected by endoscopic retrograde cholangiography

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Abstract

AIM: To investigate the pattern of mucin expression and concentration in bile obtained during endoscopic retrograde cholangiography (ERC) in relation to gallstone disease.

METHODS: Bile samples obtained at ERC from 29 consecutive patients, 17 with and 12 without gallstone disease were evaluated for mucin content by gel filtration on a Sepharose CL-4B column. Dot blot analysis for bile mucin apoproteins was performed with antibodies to Mucin 1 (MUC1), MUC2, MUC3, MUC5AC, MUC5B and MUC6. Staining intensity score (0-3) was used as a measure of antigen expression.

RESULTS: MUC1, MUC2, MUC3, MUC5AC, MUC5B and MUC6 were demonstrated in 34.4%, 34.4%, 51.7%, 51.7%, 55.1% and 27.5% of bile samples, respectively. The staining intensity scores were 0.62 ± 0.94, 0.58 ± 0.90, 0.79 ± 0.97, 1.06 ± 1.22, 1.20 ± 1.26 and 0.41 ± 0.73, respectively. Mean mucin concentration measured in bile by the Sepharose CL-4B method was 22.8 ± 24.0 mg/mL (range 3.4-89.0 mg/mL). Mean protein concentration was 8.1 ± 4.8 mg/mL (range 1.7-23.2 mg/mL).

CONCLUSION: High levels of MUC3, MUC5AC and MUC5B are expressed in bile aspirated during ERC examination. A specific pattern of mucin gene expression or change in mucin concentration was not found in gallstone disease.

INTRODUCTION

Mucins are high-molecular-weight glycoproteins containing oligosaccharide side-chains attached to serine or threonine residues of the apomucin backbone by O-glycosidic linkages[1-4]. Several mucin (MUC) genes located on different chromosomes have been sequenced and cloned[5-14]. These genes encode apoproteins with specific tandem repeats of amino acids. Antibodies have been developed against the tandem repeats, enabling the identification of specific mucins by immunohistochemistry.

Mucins can be divided into two classes: gel-forming and membrane-associated. Bile mucin has two main domains: one rich in serine, threonine and proline, which contains the majority of the covalently-bound carbohydrates; and another, nonglycosylated domain, enriched in serine, glutamic acid, glutamine and glycine, which binds hydrophobic ligands such as bilirubin. An increased expression of gel-forming mucin, such as MUC5AC and MUC2, was found in patients with hepatolithiasis[15]. Although bile-duct mucin production has been extensively studied in malignant diseases[16-22], little is known about mucin synthesis and expression in cholelithiasis, choledocholithiasis and cholangitis.

The aim of the present study was to examine mucin concentration and specific expression in bile samples of patients undergoing endoscopic retrograde cholangiography (ERC) for the evaluation

of symptomatic bile duct disease, and to investigate the possible association between mucin expression and the clinical states of gallstone disease.

MATERIALS AND METHODS

Sampling

Twenty-nine patients who underwent ERC due to symptomatic bile duct disease were included in the study. Background data and results for ultrasound examinations and liver function tests were obtained from the files. Bile was collected by aspiration, as completely as possible, after the papilla was cannulated and before proceeding to any other procedures, such as papillotomy or choledochal stone removal. The Institutional Review Board (Ethical Committee) of Rabin Medical Center approved the study.

Bile analysis

To determine mucin concentration in the bile, we used the gel-filtration technique, as previously described[23,24]. Briefly, after centrifugation to remove debris, samples of bile were subjected to gel filtration on Sepharose CL-4B columns (1 × 40 cm). We used the closed-column system of Pharmacia Biotech (Cambridge, MA, USA): peristaltic pump, P-1; columns and adapters, C10; fraction collector, Redifrac and Ultraspec 1000; VV/visible spectrophotometer; and chart reader, 80-2109-03. Samples of 2 mL were applied to the columns and eluted with 10 mmol/L Tris-HCl buffer at pH 8.0. Fractions of 1 mL were collected, and optical density was determined at a wavelength of 280 nm. Findings were correlated with a standard curve of readings of mucin purified from porcine stomach (1% bound sialic acid), purchased from Sigma (St. Louis, MO, USA). The amount of protein was estimated by the Laury method.

Dot blot analysis

Samples were subjected to dot blot analysis on nitrocellulose membranes. Membranes were incubated with monoclonal antibodies to Mucin 1 (MUC1), MUC2, MUC3, MUC5AC, MUC5B and MUC6 (all mouse), followed by incubation with anti-mouse and IgG labeled with biotin. Antibody binding was detected with streptavidin-horseradish peroxide and chemiluminescent reagents (EZ-ECL, Beit-Haemek, Israel). Monoclonal antibodies were purchased from Neomarkers (Fremont, CA, USA). Staining intensity was scored (0-3) as a measure of antigen expression.

Statistical analysis

All results are expressed as mean ± SD. The analyses included descriptive statistics, χ^2 test, Student's *t*-test, and linear regression analysis. $P < 0.05$ was considered significant.

RESULTS

Study population

The study group consisted of 13 men and 16 women aged 64.5 ± 16.8 years (Table 1). A gallstone disease was diagnosed in 17 patients and excluded in 12 patients. The indications for ERC, abdominal ultrasound results, and liver function test results before ERC are presented in Table 1.

Table 1 Demographic and clinical data (n = 29)

Clinical parameter	n (%) or mean ± SD
Age	
mean ± SD (yr)	64.5 ± 16.8
Range	24-86
Sex	
Men	13 (44.8)
Women	16 (55.2)
Main indication for ERC	
Cholestasis/jaundice	16 (55.2)
Choledocholithiasis/dilated CBD	5 (17.2)
Cholangitis	3 (10.3)
SOL of papilla	3 (10.3)
Unresolved pancreatitis	2 (6.9)
Abdominal ultrasound results	
Dilated CBD	15 (51.7)
Cholelithiasis	12 (41.4)
Dilated intrahepatic ducts	10 (34.5)
Choledocholithiasis	3 (10.3)
Pancreatitis	1 (3.4)
CBD width (mm)	
mean ± SD	8.7 ± 3.7
Range	6-18
Liver function tests, mean ± SD (range)	
Total bilirubin (mg/dL)	4.5 ± 6.9 (0.3-32)
Direct bilirubin (mg/dL)	3.0 ± 4.9 (0.1-23)
Alanine aminotransferase (U/L)	206.5 ± 243.3 (12-895)
Aspartate aminotransferase (U/L)	156.4 ± 185.6 (15-812)
Gamma glutamyl transpeptidase (U/L)	369.4 ± 370.4 (13-1337)
Alkaline phosphatase (U/L)	291.0 ± 371.4 (56-1893)

ERC: Endoscopic retrograde cholangiography; CBD: Common bile duct; SOL: Space-occupying lesion.

ERC results

The ERC findings are shown in Table 2. Linear regression analysis revealed a positive correlation between the mean common bile duct (CBD) width measured on abdominal ultrasound and ERC. There was also a positive correlation between ultrasound findings of cholelithiasis and ERC findings of dilated CBD; between the presence of a clinical syndrome of cholangitis and ultrasound findings of pancreatitis; and between increased concentrations of serum direct bilirubin and ERC findings of CBD stricture. A wider CBD was demonstrated in patients with evidence of choledocholithiasis on ERC (10.90 ± 4.97 mm) than in patients without CBD stones (7.44 ± 1.98 mm). Information from the ultrasound studies and ERC results was used to stratify the patients into a group with gallstone related disease, and a group without gallstone disease.

Mucin concentration in bile

Mean ± SD mucin concentration in bile, measured by the Sepharose CL-4B method, was 22.8 ± 24.0 mg/mL (range 3.4-89.0 mg/mL). Mean protein concentration was 8.1 ± 4.8 mg/mL (range 1.7-23.2 mg/mL). Mucin concentration in bile was not significantly different between men and women (24.68 ± 27.29 mg/mL *vs* 21.38 ± 21.96 mg/mL), patients younger or older than 70

Table 2 Results of ERC (n = 29)

Clinical parameter	n (%) or mean ± SD
Diagnosis	
CBD width (mm)	9.4 ± 4.0
Range	6-18
Choledocholithiasis	11 (37.9)
Pigmented stones	4 (13.8)
Cholelithiasis	5 (17.2)
Enlarged papilla	7 (24.1)
Dilated CBD	15 (51.7)
CBD stricture	4 (13.8)
Intrahepatic ducts dilation & stricture	3 (10.3)
Torn papilla	3 (10.3)
Bile leakage	1 (3.4)
Mirizzi syndrome	1 (3.4)
Treatment	
Sphincterotomy	16 (55.2)
Biopsy of the papilla	5 (17.2)
Stent insertion	2 (6.9)
Cholecystostomy	1 (3.4)

years (18.87 ± 15.72 mg/mL *vs* 26.59 ± 30.07 mg/mL), and patients with or without choledocholithiasis (22.46 ± 24.94 mg/mL *vs* 23.11 ± 24.29 mg/mL).

Mucin expression in bile

The expression of the mucin genes examined by dot blot analysis is shown in Table 3. Linear regression analysis revealed a positive correlation between MUC5AC and MUC5B expression [MUC5B = 0.273 + (0.874 × MUC5AC); R = 0.845]. There was also a positive correlation between MUC1 expression and papillary enlargement on ERC. The correlation between the expressions of the different MUC genes in bile is shown in Table 4.

Comparison of patients with and without gallstone disease

Summarizing the clinical and imaging data allowed the patients to be stratified into a group with diagnosed gallstone disease (n = 17), and a group with no evidence of gallstone disease (n = 12). There were no significant differences in gender, age, laboratory results, ultrasound finding, indication and results of ERC, except in the presence of gallstone disease (Table 5). Mucin concentration in bile was similar in both groups (21.68 ± 7.87 mg/mL *vs* 24.54 ± 24.10 mg/mL, P = 0.759), as was mucin gene expression (Table 5).

Table 3 Mucin gene expression in bile collected in ERC

Mucin gene	Score mean ± SD (range)	Cases (%)
MUC1	0.62 ± 0.94 (0-3)	34.4
MUC2	0.58 ± 0.90 (0-3)	34.4
MUC3	0.79 ± 0.97 (0-3)	51.7
MUC5AC	1.06 ± 1.22 (0-3)	51.7
MUC5B	1.20 ± 1.26 (0-3)	55.1
MUC6	0.41 ± 0.73 (0-2)	27.5

Table 4 Correlation between the expression of the different mucin genes in bile collected by ERC

Mucin gene	Correlation with mucin gene	P value
MUC1	MUC2	0.0001
	MUC3	0.0001
	MUC5AC	0.0125
	MUC5B	0.049
	MUC6	0.0001
MUC2	MUC3	0.0001
	MUC5AC	0.0080
	MUC5B	0.0001
	MUC6	0.0001
MUC3	MUC5AC	0.0031
	MUC6	0.0003

DISCUSSION

Different mucin genes are expressed in bile, and the role of each is unclear. Bile mucin is derived from pure hepatic bile, gallbladder-concentrated bile, and mucin secreted by the bile duct epithelium. Ko *et al*[25] found that in patients with biliary sludge, mucin concentration was higher in bile collected by ERC than in gallbladder bile. They concluded that the biochemical composition of hepatic bile is modified during residence in the gallbladder, contributing to sludge formation, and that hepatic bile samples are therefore inappropriate for microscopic detection of microlithiasis. However, although the mucin concentration in hepatic bile in the present study was similar to that reported by Ko *et al*[25] (22.8 ± 24.0 mg/mL *vs* 20 ± 30 mg/mL), the concentration of mucin in gallbladder bile in our previous study was 17.5 ± 16.4 mg/mL[26], close to that of hepatic bile and much lower than the 450 ± 290 mg/mL found by Ko *et al*[25]. Thus, our studies do not support the assumption of Ko *et al*[25], and this controversy requires further investigation.

We demonstrated a higher expression of two secretory mucin proteins, MUC5AC and MUC5B, and the membrane-bound protein, MUC3. MUC5AC and MUC5B are both gel-forming mucins that may increase the viscosity of bile in cases of symptomatic bile duct disease. Since we could not find a change in mucin concentration or in these specific genes expressions in bile derived from patients with or without gallstone disease, our findings do not support a role for MUC5AC or MUC5B in the etiopathogenesis of gallstones.

Zen and coworkers described a lipopolysaccharide-induced increase in MUC2 and MUC5AC expression in cultured murine biliary epithelial cells, which was mediated by tumor necrosis factor alpha[27]. They concluded that since lipopolysaccharide is a bacterial component, bacterial infection may be involved in the altered mucin secretion in the intrahepatic biliary tree and, thereby, in the lithogenesis of hepatolithiasis. Wandenhaute and coworkers noted a strong mRNA expression of MUC5B, MUC3 and MUC6, and a weak expression of MUC1, MUC2 and MUC5AC, in biliary epithelial cells[28]. Lee and Liu found that MUC3 and MUC5B were the main mucin genes expressed in the biliary epithelium of stone-containing intrahepatic bile ducts and normal controls[29]. Mucin gene expression

Table 5 Comparison between patients with (n = 17) and without (n = 12) gallstones

	Gallstones diseas n (%)	No evidence for gallstones n (%)	P value
Age, mean ± SD (years)	61.35 ± 20.13	69.00 ± 9.58	0.234
Sex (men)	8 (47.1)	5 (41.7)	0.927
Main indication for ERC			
Jaundice	7 (41.1)	9 (75)	0.153
Dilated CBD	4 (23.5)	1 (8.0)	0.554
Cholangitis	3 (20.0)	0	0.288
SOL of papilla	1 (6.0)	2 (17.0)	0.737
Unresolved pancreatitis	2 (12.0)	0	0.288
Abdominal ultrasound results			
Dilated CBD	10 (59.0)	5 (42)	0.599
Cholelithiasis	12 (70.6)	0	< 0.0001
Dilated intrahepatic ducts	6 (40)	4 (30)	0.873
Choledocholithiasis	3 (17.6)	0	0.360
Pancreatitis	1 (5.9)	0	0.861
CBD width (mm), mean ± SD	9.65 ± 4.39	7.50 ± 2.24	0.132
Liver function tests, mean ± SD			
Total bilirubin (mg/dL)	5.12 ± 7.83	3.68 ± 5.52	0.589
Direct bilirubin (mg/dL)	3.38 ± 5.53	2.55 ± 4.03	0.662
Alanine aminotransferase (U/L)	245.41 ± 270.76	151.58 ± 196.14	0.315
Aspartate aminotransferase (U/L)	167.29 ± 150.54	141.08 ± 233.11	0.715
Gamma glutamyl transpeptidase (U/L)	443.47 ± 388.13	264.50 ± 331.14	0.206
Alkaline phosphatase (U/L)	352.88 ± 449.95	185.08 ± 174.44	0.323
ERC diagnosis			
CBD width (mm), mean ± SD	9.59 ± 3.99	9.17 ± 4.37	0.790
Choledocholithiasis	11 (64.7)	0	0.002
Pigmented stones	4 (24.0)	0	0.198
Cholelithiasis	5 (29.0)	0	0.122
Enlarged papilla	3 (16.0)	4 (33.0)	0.533
Dilated CBD	10 (60.0)	5 (40.0)	0.494
CBD stricture	1 (10.0)	3 (30.0)	0.376
Torn papilla	3 (17.6)	0	0.360
Treatment			
Sphincterotomy	12 (71.0)	4 (33.0)	0.099
Biopsy of the papilla	1 (10.0)	4 (33.0)	0.288
Mucin gene score, mean ± SD			
Mucin concentration (mg/mL)	21.68 ± 7.87	24.54 ± 24.1	0.759
Protein concentration (mg/mL)	7.87 ± 4.53	8.61 ± 5.48	0.694
MUC1	0.59 ± 0.87	0.66 ± 1.07	0.848
MUC2	0.53 ± 0.80	0.66 ± 1.07	0.711
MUC3	0.88 ± 0.93	0.66 ± 1.07	0.560
MUC5AC	0.47 ± 0.80	0.33 ± 0.65	0.621
MUC5B	1.23 ± 1.25	0.83 ± 1.19	0.394
MUC6	1.29 ± 1.26	1.08 ± 1.31	0.667

was altered in dysplastic preneoplastic cells.

The main weakness of our study is the absence of healthy controls. We could not compare mucin concentration and gene expression in the cholestatic situation with that of normal bile collected in ERC, since ERC is usually performed with therapeutic intent in symptomatic patients.

In the present study, we observed a positive correlation between MUC1 expression in bile and the expression of all the other mucin genes examined. Wang and coworkers reported a similar result in mice[30]. They described a positive correlation between MUC1 and MUC5AC expression, indicating a gene-gene interaction that might affect the accumulation of mucin gel and cholesterol gallstone formation.

In summary, we could not demonstrate a change in mucin secretion and expression between patients with and without gallstone disease, or support the role of mucin in the etiopathogenesis of biliary sludge or stone formation.

COMMENTS

Background

Secretory mucins are gel-forming and may increase bile viscosity. The biochemical composition of hepatic bile is modified during residence in the gallbladder, contributing to sludge formation. An increased expression of gel-forming mucin, such as MUC5AC and MUC2, was found in patients with hepatolithiasis. Little is known about mucin synthesis and expression in cholelithiasis, choledocholithiasis and cholangitis.

Innovations and breakthroughs

High levels of MUC3, MUC5AC and MUC5B are expressed in bile aspirated during endoscopic retrograde cholangiography examination. A specific pattern of mucin gene expression or change in mucin concentration was not found in gallstone disease.

Applications

Expression of other mucin genes or changes in concentration should be investigated in gallstone disease. The role of mucin synthesis and secretion in gallstone formation is still unknown.

Peer review

The manuscript by Vilkin *et al* describes the analysis of certain members of the Mucin gene family in the bile of patients with and without gallstone disease. The authors demonstrate the presence of Mucin 1 (MUC1), MUC2, MUC3, MUC5AC, MUC5B and MUC6 in the bile of all patients, but there was no correlation to

the presence of gall stones. While this manuscript contains negative data, with no conclusive outcomes, the data is nevertheless important as it disproves a currently regarded theory about the role of mucins in gall stone formation.

REFERENCES

1 **Neutra MR**, Forster JF. Gastrointestinal mucus: synthesis, secretion and function. In: Johnson DF, ed. Physiology of the gastrointestinal tract. 2nd ed. New York: Raven Press, 1987: 975-1009
2 **Gum JR**, Byrd JC, Hicks JW, Toribara NW, Lamport DT, Kim YS. Molecular cloning of human intestinal mucin cDNAs. Sequence analysis and evidence for genetic polymorphism. *J Biol Chem* 1989; **264**: 6480-6487
3 **Gum JR**, Hicks JW, Swallow DM, Lagace RL, Byrd JC, Lamport DT, Siddiki B, Kim YS. Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. *Biochem Biophys Res Commun* 1990; **171**: 407-415
4 **Kim YS**. Mucin glycoprotein alterations in the gastrointestinal and metastasis. *Eur J Gastroenterol Hepatol* 1993; **5**: 219-225
5 **Abe M**, Kufe D. Characterization of cis-acting elements regulating transcription of the human DF3 breast carcinoma-associated antigen (MUC1) gene. *Proc Natl Acad Sci USA* 1993; **90**: 282-286
6 **Porchet N**, Nguyen VC, Dufosse J, Audie JP, Guyonnet-Duperat V, Gross MS, Denis C, Degand P, Bernheim A, Aubert JP. Molecular cloning and chromosomal localization of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. *Biochem Biophys Res Commun* 1991; **175**: 414-422
7 **Meezaman D**, Charles P, Daskal E, Polymeropoulos MH, Martin BM, Rose MC. Cloning and analysis of cDNA encoding a major airway glycoprotein, human tracheobronchial mucin (MUC5). *J Biol Chem* 1994; **269**: 12932-12939
8 **Toribara NW**, Roberton AM, Ho SB, Kuo WL, Gum E, Hicks JW, Gum JR Jr, Byrd JC, Siddiki B, Kim YS. Human gastric mucin. Identification of a unique species by expression cloning. *J Biol Chem* 1993; **268**: 5879-5885
9 **Bobek LA**, Tsai H, Biesbrock AR, Levine MJ. Molecular cloning, sequence, and specificity of expression of the gene encoding the low molecular weight human salivary mucin (MUC7). *J Biol Chem* 1993; **268**: 20563-20569
10 **D'Cruz OJ**, Dunn TS, Pichan P, Hass GG Jr, Sachdev GP. Antigenic cross-reactivity of human tracheal mucin with human sperm and trophoblasts correlates with the expression of mucin 8 gene messenger ribonucleic acid in reproductive tract tissues. *Fertil Steril* 1996; **66**: 316-326
11 **Lapensée L**, Paquette Y, Bleau G. Allelic polymorphism and chromosomal localization of the human oviductin gene (MUC9). *Fertil Steril* 1997; **68**: 702-708
12 **Williams SJ**, Wreschner DH, Tran M, Eyre HJ, Sutherland GR, McGuckin MA. Muc13, a novel human cell surface mucin expressed by epithelial and hemopoietic cells. *J Biol Chem* 2001; **276**: 18327-18336
13 **Yin BW**, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. *J Biol Chem* 2001; **276**: 27371-27375
14 **Gum JR Jr**, Crawley SC, Hicks JW, Szymkowski DE, Kim YS. MUC17, a novel membrane-tethered mucin. *Biochem Biophys Res Commun* 2002; **291**: 466-475
15 **Sasaki M**, Nakanuma Y, Kim YS. Expression of apomucins in the intrahepatic biliary tree in hepatolithiasis differs from that in normal liver and extrahepatic biliary obstruction. *Hepatology* 1998; **27**: 54-61
16 **Goto M**, Shibahara H, Tamada S, Hamada T, Oda K, Nagino M, Nagasaka T, Imai K, Nimura Y, Yonezawa S. Aberrant expression of pyloric gland-type mucin in mucin-producing bile duct carcinomas: a clear difference between the core peptide and the carbohydrate moiety. *Pathol Int* 2005; **55**: 464-470
17 **Yamamoto K**, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, Hamanaka Y, Ikematsu Y, Imai K, Oka M, Hinoda Y. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. *Anticancer Res* 2005; **25**: 3575-3579
18 **Sasaki M**, Nakanuma Y, Ho SB, Kim YS. Cholangiocarcinomas arising in cirrhosis and combined hepatocellular-cholangiocellular carcinomas share apomucin profiles. *Am J Clin Pathol* 1998; **109**: 302-308
19 **Amaya S**, Sasaki M, Watanabe Y, Tsui WM, Tsuneyama K, Harada K, Nakanuma Y. Expression of MUC1 and MUC2 and carbohydrate antigen Tn change during malignant transformation of biliary papillomatosis. *Histopathology* 2001; **38**: 550-560
20 **Wongkham S**, Sheehan JK, Boonla C, Patrakitkomjorn S, Howard M, Kirkham S, Sripa B, Wongkham C, Bhudhisawasdi V. Serum MUC5AC mucin as a potential marker for cholangiocarcinoma. *Cancer Lett* 2003; **195**: 93-99
21 **Boonla C**, Wongkham S, Sheehan JK, Wongkham C, Bhudhisawasdi V, Tepsiri N, Pairojkul C. Prognostic value of serum MUC5AC mucin in patients with cholangiocarcinoma. *Cancer* 2003; **98**: 1438-1443
22 **Ishikawa A**, Sasaki M, Ohira S, Ohta T, Oda K, Nimura Y, Chen MF, Jan YY, Yeh TS, Nakanuma Y. Aberrant expression of CDX2 is closely related to the intestinal metaplasia and MUC2 expression in intraductal papillary neoplasm of the liver in hepatolithiasis. *Lab Invest* 2004; **84**: 629-638
23 **Morgenstern S**, Koren R, Moss SF, Fraser G, Okon E, Niv Y. Does Helicobacter pylori affect gastric mucin expression? Relationship between gastric antral mucin expression and H. pylori colonization. *Eur J Gastroenterol Hepatol* 2001; **13**: 19-23
24 **Niv Y**, Hardy B, Koren R, Rodiomov G, Fraser GM. Association between gastric acid and mucin secretion in dyspeptic patients. *Digestion* 2002; **65**: 141-148
25 **Ko CW**, Schulte SJ, Lee SP. Biliary sludge is formed by modification of hepatic bile by the gallbladder mucosa. *Clin Gastroenterol Hepatol* 2005; **3**: 672-678
26 **Vilkin A**, Nudelman I, Morgenstern S, Geller A, Bar Dayan Y, Levi Z, Rodionov G, Hardy B, Konikoff F, Gobbic D, Niv Y. Gallbladder inflammation is associated with increase in mucin expression and pigmented stone formation. *Dig Dis Sci* 2007; **52**: 1613-1620
27 **Zen Y**, Harada K, Sasaki M, Tsuneyama K, Katayanagi K, Yamamoto Y, Nakanuma Y. Lipopolysaccharide induces overexpression of MUC2 and MUC5AC in cultured biliary epithelial cells: possible key phenomenon of hepatolithiasis. *Am J Pathol* 2002; **161**: 1475-1484
28 **Vandenhaute B**, Buisine MP, Debailleul V, Clément B, Moniaux N, Dieu MC, Degand P, Porchet N, Aubert JP. Mucin gene expression in biliary epithelial cells. *J Hepatol* 1997; **27**: 1057-1066
29 **Lee KT**, Liu TS. Altered mucin gene expression in stone-containing intrahepatic bile ducts and cholangiocarcinomas. *Dig Dis Sci* 2001; **46**: 2166-2172
30 **Wang HH**, Afdhal NH, Gendler SJ, Wang DQ. Targeted disruption of the murine mucin gene 1 decreases susceptibility to cholesterol gallstone formation. *J Lipid Res* 2004; **45**: 438-447

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BRIEF ARTICLES

Determination of correlation of Adjusted Blood Requirement Index with outcome in patients presenting with acute variceal bleeding

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Abstract

AIM: To determine the correlation of Adjusted Blood Requirement Index (ABRI) with the 7th day outcome in patients presenting with acute variceal bleeding.

METHODS: All patients presenting with acute variceal hemorrhage (AVH) were included. Patients with previous band ligation, sclerotherapy, gastrointestinal or hepatic malignancies were excluded. Patients were managed as per standard protocol for AVH with terlipressin and band ligation. ABRI scores were calculated using the formula outcome of alive or expired up to the 7th day after treatment. The correlation between ABRI and mortality was estimated and a receiver operative characteristic (ROC) curve was plotted.

RESULTS: A total of 113 patients (76 male; 37 female) were included. On assessment, 18 were in Child's Pugh Class A, 82 in Class B and 13 were in Class C. The median number of blood units transfused ± inter-quartile range was 3.0 ± 2.0. The median ± inter-quartile range for ABRI was 1.3 ± 1.1. The ROC curve of ABRI for expiry showed a significantly large area of 0.848 ($P < 0.0001$; 95% CI: 0.75-0.95). A significant correlation of log transformation of ABRI with an outcome of mortality was present ($P < 0.0001$).

CONCLUSION: ABRI correlates strongly with mortality.

INTRODUCTION

Chronic liver diseases and cirrhosis are now being recognized as an important cause of morbidity and mortality worldwide. Acute variceal hemorrhage (AVH) secondary to cirrhosis is to date the most important cause of mortality in cirrhosis[1]. In Pakistan, hepatitis B and C are the most important causes of cirrhosis[2]. The frequency of varices is very high in cirrhotic patients, nearly 40% of patients with compensated cirrhosis and 60% with decompensated cirrhosis have varices[3]. Due to recent advancements, mortality from AVH has been reduced to 20% from the first variceal bleed[4]. Bleeding from AVH carries a high risk of mortality during the first 5 d, with a gradual decline in risk over the next 4-6 wk[5]. The prediction and evaluation of adequate hemostasis by non-endoscopic methods are desired by treating physicians. Many criteria and definitions to evaluate failure to control and prevent variceal bleeding were developed in the Baveno Consensus Workshops Ⅰ-Ⅲ but failed in clinical application due to cumbersome procedures and calculations[6-10]. Further developments in this subject identified an independent factor the "Adjusted Blood Requirement Index (ABRI)" in the Baveno Workshop Ⅳ[11]. ABRI was developed to determine adequate control or failure to control variceal hemorrhage. An ABRI value of $\geqslant 0.75$ at any point time was defined as a failure to control variceal bleeding[11].

Failure of AVB control leads to increased mortality. Thus ABRI could be used to assess the risk of mortality. A correlation between ABRI and mortality has not been evaluated in a prospectively designed study. We have reported its correlation with outcome in a retrospective analysis previously[12]. As there are no reports of a prospective evaluation of ABRI and its relation to mortality, there is a need to assess this correlation in our settings.

This study was designed to evaluate the correlation between ABRI and outcome at the 7th day after hospital admission as improved or expired in acute variceal bleeding.

MATERIALS AND METHODS

All cirrhotic patients who presented with AVB were included. Informed consent was obtained from all patients. Patients with a history of previous band ligation or sclerotherapy, hepatocellular carcinoma and the presence of peptic ulcer or gastrointestinal (GI) malignancy on endoscopy were excluded. Patients were managed as per standard protocol of acute variceal bleeding[10]. Blood samples were taken for Complete Blood Counts, Prothrombin Time, Liver Function Tests and albumin before the start of therapy. Child's Pugh Class assessment was carried out. All patients were given terlipressin 2 mg *iv* initial dose and followed by 1 mg/6 h for 3 d. The number of blood units transfused was noted and endoscopic variceal band ligation (EVBL) was performed within 24 h of admission. Study endpoint was patient outcome (alive or expired at the 7th day after admission). The ABRI value was calculated using the following formula[11]: ABRI = blood units transfused/[(final hematocrit - initial hematocrit) + 0.01]. Child's Pugh score was calculated using the formula[13] shown in Table 1.

Sample size

Sample size was estimated using the following parameters: Level of Significance (α) = 5%; Power of test (1-β) = 80%; Test value of population proportion (P_0) = 20% (0.2); Anticipated value of population proportion (P_a) = 30% (0.3); Sample size (n) = 109.

Statistical analysis

mean ± SD was calculated for age. Median and inter-quartile range were calculated for the number of blood units transfused and ABRI. Frequencies of gender, Child's Pugh Class and outcome were calculated. ABRI values $\geqslant$ 0.75 were recoded into a new variable as uncontrolled while ABRI values < 0.75 were recoded as controlled and their frequency estimated. χ^2 test was performed for outcome with ABRI control status and Child's Pugh Class was carried out with continuity correction and likelihood ratio applied where indicated. A receiver operative characteristic (ROC) curve of ABRI was plotted for expiry. Log transformation of variable ABRI was carried out as it was not normally distributed and then used for Pearson's Bivariate correlation with outcome. The significance level was set at $P \leqslant 0.05$. SPSS version 17.0 was used for statistical analysis.

Table 1 Child's Pugh score was calculated (using formula)

Parameter	Numerical score		
	1	2	3
Ascites	None	Slight	Moderate to severe
Encephalopathy	None	Slight to moderate	Moderate to severe
Bilirubin (mg/dL)	< 2.0	2-3	> 3.0
Albumin (g/dL)	> 3.5	2.8-3.5	< 2.8
Prothrombin time (prolonged in seconds)	1-3 s	4-6 s	> 6.0

Child's Pugh Class A = 5-6 points; Child's Pugh Class B = 7-9 points; Child's Pugh Class C = 10-15 points.

Table 2 Cross tabulation of ABRI groups with outcome

		Outcome		Total
		Alive	Expired	
ABRI groups	Controlled	27	0	27
	Uncontrolled	67	19	86
Total		94	19	113

RESULTS

A total of 113 patients fulfilling the inclusion/exclusion criteria were inducted. These included 76 (67.3%) male (44.3 ± 11.8 years) and 37 (32.7%) female (44.1 ± 9.4 years). Terlipressin was given to 111 patients (98.2%) immediately on admission. EVBL was performed in 105 (92.9%) patients. The assessment on admission showed that 18 (15.9%) were in Child's Pugh Class A; 82 (72.6%) in Child's Pugh Class B and 13 (11.5%) were in Child's Pugh Class C. The median number of blood transfusions given was 3.0 pints and the inter-quartile range was 2.0. The median ABRI was 1.3 with an inter-quartile range of 1.1. The number of patients with ABRI $\geqslant$ 0.75 was 86 (76.1%) showing a failure to control variceal bleeding according to ABRI criteria. Outcome at the 7th day after admission showed that 94 (83.2%) patients were alive while 19 (16.8%) patients had expired during this period. Cross tabulation of outcome (alive and expired) with ABRI status [controlled (< 0.75) and uncontrolled ($\geqslant$ 0.75)] showed that no patients had expired in the ABRI controlled group (Table 2). χ^2 test with continuity correction gave a significance value of $P = 0.017$. A similar cross tabulation with Child's Pugh Class showed that the highest percentage of patients expired in Child's Pugh Class C while no patients with Child's Class A expired (Table 3). χ^2 test with the Likelihood Ratio gave significant differences in the frequencies of expiry with Child's Pugh Class ($P < 0.0001$). A ROC curve was plotted using expiry as a state variable (Figure 1). The area under the curve was significantly large at 0.848 ($P < 0.0001$; 95% CI: 0.75-0.95). The sensitivity and specificity of the ABRI cutoff value of 0.75 in our study was 100% and 73.4%, respectively. The correlation of

Table 3 Cross tabulation of Child's Pugh Class with outcome

			Outcome		Total
			Alive	Expired	
Child's Pugh Class	Class A	Count	18	0	18
		% within Child's Pugh Class	100.0%	0.0%	100.0%
	Class B	Count	71	11	82
		% within Child's Pugh Class	86.6%	13.4%	100.0%
	Class C	Count	5	8	13
		% within Child's Pugh Class	38.5%	61.5%	100.0%
Total		Count	94	19	113
		% within Child's Pugh Class	83.2%	16.8%	100.0%

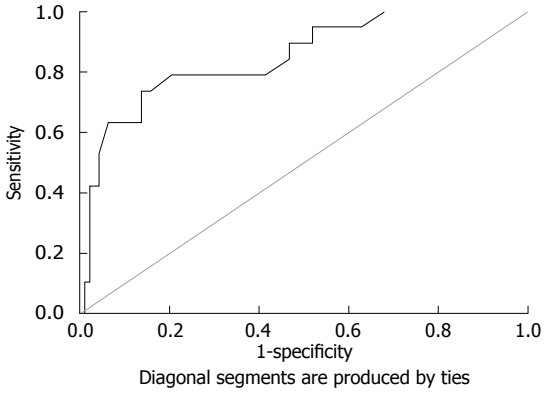

Figure 1 ROC curve of ABRI against expiry.

ABRI with outcome was analyzed by Pearson's Bivariate Correlation test. As the data of ABRI was skewed and not normally distributed its Log_{10} transformation was used. The results showed a significant correlation between ABRI and expiry with $P < 0.0001$.

DISCUSSION

This study showed a significant correlation between ABRI and the 7th day outcome in patients with AVH. This is a very significant finding as it is important to predict the outcome at an initial stage of management and ABRI suggests whether the variceal hemorrhage has been arrested at any point during management. Our study also established its correlation with mortality. Earlier validation studies of ABRI were retrospective[11,12]. The current study is prospective and designed more specifically to assess the correlation of ABRI with mortality which has not been previously studied. The correlation of higher ABRI scores with mortality was significant and this simple to use parameter should be used to assess failure to control bleeding and risk of mortality. The number of units of blood transfused and hematocrit levels, if used alone, are not good criteria to assess variceal bleeding control. We also used pharmacological and endoscopic interventions and the combined effect of these interventions was reflected in the outcome which was also observed in other reports from this region[14]. About 70% of patients rebleeding within 2 years, thus managing the index bleed properly and obliteration of varices can decrease rebleeding[15,16].

Many scoring systems have been derived to predict the outcome of upper GI hemorrhage. The Rockall score is one such scoring system for predicting rebleeding and mortality which also showed good correlation[17,18]. Limitations of the Rockall score are that it is rather difficult to use with the requirement of more parameters as compared to ABRI and it is not variceal bleeding specific, but designed for both variceal and non-variceal bleeding[18,19]. Another popular scoring system, the Child's Pugh score predicts all cause morbidity and mortality in cirrhotic patients but is not specific for variceal hemorrhage[20].

In conclusion, among the many predictive scoring systems in cirrhotic patients, ABRI is specific for variceal hemorrhage and correlates strongly with mortality and is a good indicator of the failure of variceal hemorrhage control.

COMMENTS

Background

In a developing country like Pakistan, hepatitis B and C are the most important causes of cirrhosis. The frequency of varices is very high in cirrhotic patients, nearly 40% of patients with compensated cirrhosis and 60% of patients with decompensated cirrhosis have varices. Mortality from variceal bleeding is still high at 20%. The prediction of mortality risk is difficult and available scores are difficult to calculate and thus do not enjoy wide acceptability and application.

Research frontiers

Adjusted Blood Requirement Index (ABRI) is a score which is used to determine the failure to control variceal bleeding. In this study it was correlated with the outcome of mortality.

Innovations and breakthroughs

Many scoring systems are in use to predict the outcome of upper gastrointestinal hemorrhage. The Rockall score is one such scoring system for predicting rebleeding and mortality but it is rather difficult to use. Another scoring system, the Child's Pugh score predicts all cause morbidity and mortality in cirrhotic patients but is not specific for variceal hemorrhage. The ABRI is a variceal hemorrhage-specific score and is easy to use.

Applications

ABRI: A validated tool to determine variceal bleeding control also correlates well with mortality in such patients.

Peer review

Many scoring systems have been described to predict the prognosis of upper gastrointestinal hemorrhage like Rockall and Child's Pugh but these have limitations. In practice, it is useful to predict the outcome at admission of patients with acute variceal hemorrhage. This study showed a significant correlation between ABRI and expiry. The methodology is correct. This work deserves to be published to stimulate other teams over the world to perform the same study with a large number of patients.

REFERENCES

1 **Burroughs AK**, Triantos CK, O'Beirne J, Patch D. Predictors of early rebleeding and mortality after acute variceal hemorrhage in patients with cirrhosis. *Nat Clin Pract Gastroenterol Hepatol* 2009; **6**: 72-73

2 **Mashud I**, Khan H, Khattak AM. Relative frequency of hepatitis B and C viruses in patients with hepatic cirrhosis at DHQ Teaching Hospital D. I. Khan. *J Ayub Med Coll Abbottabad* 2004; **16**: 32-34

3 **Schepis F**, Cammà C, Niceforo D, Magnano A, Pallio S, Cinquegrani M, D'amico G, Pasta L, Craxì A, Saitta A, Raimondo G. Which patients with cirrhosis should undergo endoscopic screening for esophageal varices detection? *Hepatology* 2001; **33**: 333-338

4 **Turnes J**, Garcia-Pagan JC, Abraldes JG, Hernandez-Guerra M, Dell'Era A, Bosch J. Pharmacological reduction of portal pressure and long-term risk of first variceal bleeding in patients with cirrhosis. *Am J Gastroenterol* 2006; **101**: 506-512

5 **Burroughs AK**, Mezzanotte G, Phillips A, McCormick PA, McIntyre N. Cirrhotics with variceal hemorrhage: the importance of the time interval between admission and the start of analysis for survival and rebleeding rates. *Hepatology* 1989; **9**: 801-807

6 **North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices**. Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A prospective multicenter study. *N Engl J Med* 1988; **319**: 983-989

7 **de Franchis R**. Developing consensus in portal hypertension. *J Hepatol* 1996; **25**: 390-394

8 **de Franchis R**. Updating consensus in portal hypertension: report of the Baveno III Consensus Workshop on definitions, methodology and therapeutic strategies in portal hypertension. *J Hepatol* 2000; **33**: 846-852

9 **de Franchis R**. Evaluation and follow-up of patients with cirrhosis and oesophageal varices. *J Hepatol* 2003; **38**: 361-363

10 **de Franchis R**. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2005; **43**: 167-176

11 **Duvnjak M**, Barsić N, Tomasić V, Jukić LV, Lerotić I, Pavić T. Adjusted blood requirement index as indicator of failure to control acute variceal bleeding. *Croat Med J* 2006; **47**: 398-403

12 **Zuberi BF**, Riaz MF, Sultan BA, Gobindram P, Farooq A, Qadeer R, Memon AR, Afsar S. Correlation of adjusted blood requirement index with treatment intervention and outcome in patients presenting with acute variceal bleeding. *J Dow Uni Health Sci* 2007; **1**: 65-68

13 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231

14 **Varghese J**, Cherian JV, Solomon R, Jayanthi V. Predictors of variceal bleed among patients with liver cirrhosis in the era of sclerotherapy. *Singapore Med J* 2008; **49**: 239-242

15 **Bureau C**, Vinel JP. Management of failures of first line treatments. *Dig Liver Dis* 2008; **40**: 343-347

16 **Bosch J**, Abraldes JG, Berzigotti A, Garcia-Pagan JC. Portal hypertension and gastrointestinal bleeding. *Semin Liver Dis* 2008; **28**: 3-25

17 **Sarwar S**, Dilshad A, Khan AA, Alam A, Butt AK, Tariq S, Ahmad I. Predictive value of Rockall score for rebleeding and mortality in patients with variceal bleeding. *J Coll Physicians Surg Pak* 2007; **17**: 253-256

18 **Sanders DS**, Carter MJ, Goodchap RJ, Cross SS, Gleeson DC, Lobo AJ. Prospective validation of the Rockall risk scoring system for upper GI hemorrhage in subgroups of patients with varices and peptic ulcers. *Am J Gastroenterol* 2002; **97**: 630-635

19 **Tham TC**, James C, Kelly M. Predicting outcome of acute non-variceal upper gastrointestinal haemorrhage without endoscopy using the clinical Rockall Score. *Postgrad Med J* 2006; **82**: 757-759

20 **Sarwar S**, Khan AA, Tarique S. Comparison of MELD, Child Pugh score and Rockall score for predicting rebleeding and in-hospital mortality in patients of variceal bleeding. *J Coll Physicians Surg Pak* 2008; **18**: 524-525

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BRIEF ARTICLES

Local anesthesia with ropivacaine for patients undergoing laparoscopic cholecystectomy

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Abstract

AIM: To investigate the effect of pain relief after infusion of ropivacaine at port sites at the end of surgery.

METHODS: From October 2006 to September 2007, 72 patients undergoing laparoscopic cholecystectomy (LC) were randomized into two groups of 36 patients. One group received ropivacaine infusion at the port sites at the end of LC and the other received normal saline. A visual analog scale was used to assess postoperative pain when the patient awakened in the operating room, 6 and 24 h after surgery, and before discharge. The amount of analgesics use was also recorded. The demographics, laboratory data, hospital stay, and perioperative complications were compared between the two groups.

RESULTS: There was no difference between the two groups preoperatively in terms of demographic and laboratory data. After surgery, similar operation time, blood loss, and no postoperative morbidity and mortality were observed in the two groups. However, a significantly lower pain score was observed in the patients undergoing LC with local anesthesia infusion at 1 h after LC and at discharge. Regarding analgesic use, the amount of meperidine used 1 h after LC and the total used during admission were lower in patients undergoing LC with local anesthesia infusion. This group also had a shorter hospital stay.

CONCLUSION: Local anesthesia with ropivacaine at the port site in LC patients significantly decreased postoperative pain immediately. This explains the lower meperidine use and earlier discharge for these patients.

Key words: Prospective randomized trial; Local anesthesia; Ropivacaine; Normal saline; Laparoscopic cholecystectomy

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INTRODUCTION

Since 1987, laparoscopic cholecystectomy (LC) has been the favored treatment for gallbladder lesions[1]. Historically, contraindications to LC have included obesity, pregnancy, acute cholecystitis, and cardiovascular disease. With experience gained from laparoscopic surgery, LC has been attempted successfully and has become the procedure of choice in each subgroup of patients[2,3]. A major benefit of laparoscopy in upper gastrointestinal surgery results from avoidance of an upper abdominal incision. LC has proven benefits of less pain and improved pulmonary function tests compared with small-incision cholecystectomy[4-6]. However, assessment of postoperative stay and return to normal activity has shown conflicting results[5-7]. Many trials have assessed methods of reducing pain after LC, and several aspects of pain

after laparoscopy have been addressed[8-11]. These studies have concentrated on the mechanism of pain or focused on gynecological procedures, with an emphasis on the role of non-steroidal anti-inflammatory drugs (NSAIDs)[8]. Some of the reviews have demonstrated the heterogeneity of randomized controlled trials and have concluded that pain after LC is multifactorial[11]. Although, many methods of analgesia produce short-term benefits, this does not equate with earlier discharge or improved postoperative function.

This prospective and randomized controlled trial aimed to clarify the impact of infusion of local analgesia at the port site after LC on pain relief and postoperative outcome.

MATERIALS AND METHODS

From October 2006 to September 2007, 72 patients undergoing LC by the authors (Yeh CN, Yeh TS, and Chao TC) at the Department of Surgery, Chang Gung Memorial Hospital, Taiwan were included in this prospective randomized controlled trial. The study subjects were adult patients who had been referred for elective LC for gallbladder lesions. The diagnostic work-up for patients with gallbladder lesions before LC included history taking, physical examination, abdominal ultrasonography, abdominal computed tomography, and magnetic resonance cholangiopancreatography. The study was approved by the local institutional review board of Chang Gung Memorial Hospital and all patients gave informed consent before taking part in the study.

Randomization and treatment

The randomization was centralized and used a random permuted block design. Eligible patients were aged 20-85 years, not pregnant, and had adequate hematological, hepatic and renal function. The exclusion criteria were as follows: immunosuppressive drug therapy within the previous 6 mo; an immunosuppressive condition, including AIDS; autoimmune disorders; organ transplantation; radiotherapy or chemotherapy within the previous 6 mo; and insulin-dependent diabetes mellitus (type 1). Discharge from the wards was the primary endpoint. Clinical features, laboratory data, operative outcomes, pain score, and analgesic requirement were analyzed and compared between the ropivacaine and saline groups. Hospital stay was defined as the number of days from operation to the actual date of hospital discharge. Surgical mortality was defined as death that occurred within 1 mo after surgery.

Seventy-two patients were included in the study and randomized into a control or local anesthesia with ropivacaine (LA) group. All 72 patients received general anesthesia with the same protocol by one of the authors (Lin CC, anesthesiologist). The LA group received 1.0% ropivacaine 20 mL at the port site after wound closure (6 mL for epigastric port, 6 mL for umbilical port, and 4 mL for each working port). The control group received 0.9% normal saline 20 mL at the port site after wound closure (6 mL for epigastric port, 6 mL for umbilical port, and 4 mL for each working port). Ropivacaine or normal saline was applied to the skin, subcutis, fascia, and parietal peritoneum through the port sites at the end of surgery.

Table 1 Demographic data of 72 patients undergoing LC with and without local anesthesia infusion at the port site

	Control (n = 36)	LA (n = 36)	P
Age (yr)	48.4 ± 13.0	50.6 ± 12.4	0.461
Gender (M:F)	13:23	6:30	0.061
Previous abdominal operation history (+)	10 (27.8)	11 (30.6)	0.795
Associated disease (+)	16 (44.4)	12 (33.3)	0.334
Diagnosis			0.991
Gall stone	25 (69.4)	26 (72.2)	
Gall stone and AC	3 (8.3)	3 (8.3)	
Gall stone and CC	6 (16.7)	5 (13.9)	
Gall bladder polyp	2 (5.6)	2 (5.6)	
ASA grade	1.7 ± 0.6	1.5 ± 0.6	0.106
Operation time (min)	84.7 ± 31.3	78.5 ± 33.1	0.417
Blood loss (cc)	35.9 ± 84.8	32.4 ± 58.2	0.688
Conversion rate	0	0	NA
Post-operative drain	3 (8.3)	4 (11.1)	0.691
Morbidity rate	0	0	NA
Mortality rate	0	0	NA
Hospital stay (d)	2.8 ± 2.7	1.1 ± 0.3	0.001

LC: Laparoscopic cholecystectomy; M: Male; F: Female; AC: Acute cholecystitis; CC: Chronic cholecystitis; NA: Not available.

Patient monitoring and testing

A visual analog scale (VAS) with a 10-cm vertical score ranged from "no pain" to "worst possible pain". The VAS was used to assess postoperative pain when the patient awakened in the operating room (about 1 h after surgery), then after 6 and 24 h, and before discharge. The pain score was recorded by the authors (Lee HL, Liu YY, Wang SY, Tsai CY, and Yeh CN). Pain intensity was estimated using a VAS and the amount of analgesics used. The biochemistry data, operative time, hospital stay, and perioperative complications were recorded.

Statistical analysis

All data are presented as the percentage of patients or mean ± SD. Numerical data were compared by independent two-sample t test or paired two-sample t test. Pearson χ^2 test and Fisher exact test were used for nominal variables. All statistical analyses were performed using the SPSS computer software (Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Clinical features, laboratory data, and operative outcomes

Table 1 summarizes the demographic data of patients with gallbladder lesions receiving LC without local anesthesia (control group) and with local anesthesia (LA group). Both groups shared a similar age distribution and sex ratio. The two groups displayed no significant difference in ratio of previous abdominal operation, etiology of disease, and operative indications. The LA group had similar American Society of Anesthesiologists

Table 2 Laboratory data of 72 patients undergoing LC with and without local anesthesia infusion at the port site (mean ± SD)

	Control (n = 36)	LA (n = 36)	P
Hemoglobin (g/dL)	13.4 ± 1.8	14.1 ± 1.7	0.634
WBC (/μL)	7317.1 ± 2898.6	6617.3 ± 2226.8	0.681
BUN (mg/dL)	14.7 ± 8.5	14.1 ± 3.4	0.634
Creatinine (mg/dL)	1.1 ± 0.7	0.9 ± 0.1	0.130
Bilirubin (direct) (mg/dL)	0.27 ± 0.14	0.26 ± 0.14	0.713
Bilirubin (total) (mg/dL)	0.87 ± 0.77	0.66 ± 0.32	0.158
AST (IU/L)	29.8 ± 31.7	21.9 ± 18.8	0.206
ALT (IU/L)	36.9 ± 48.8	26.8 ± 25.1	0.269
ALP (IU/L)	86.2 ± 88.4	74.1 ± 38.8	0.422

BUN: Blood urea nitrogen; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase.

Table 3 Difference in pain scale and analgesia use of 72 patients undergoing LC (control *versus* local anesthesia) (mean ± SD)

	Control (n = 36)	LA (n = 36)	P
Pain analogue scale			
1-h post LC	6.8 ± 2.2	5.6 ± 2.0	0.021
6-h post LC	4.5 ± 1.7	3.9 ± 1.5	0.112
24-h post LC	2.7 ± 1.5	2.1 ± 1.1	0.039
Discharge post LC	1.7 ± 0.8	1.3 ± 0.6	0.020
Meperidine requirement (mg)			
1-h post LC	25.9 ± 21.3	13.0 ± 16.8	0.006
6-h post LC	22.9 ± 21.3	16.2 ± 26.5	0.347
24-h post LC	11.4 ± 30.0	5.4 ± 19.7	0.314
Discharge post LC	5.7 ± 26.5	0	0.211
Total amount	65.9 ± 79.7	34.6 ± 37.8	0.040
Acetaminophen requirement (500 mg/tablet)			
1-h post LC	0.06 ± 0.24	0.05 ± 0.23	0.955
6-h post LC	0.60 ± 0.74	0.32 ± 0.58	0.081
24-h post LC	0.57 ± 0.92	0.54 ± 0.80	0.879
Discharge post LC	0.20 ± 0.53	0.03 ± 0.16	0.073
Total amount	1.43 ± 1.56	0.95 ± 1.13	0.139

grade, operative time, operative blood loss, postoperative drain insertion, and complication rates as the control group. No 30-d mortality occurred in this study. The LA group had a significantly shorter hospital stay than the control group (1.1 ± 0.3 d *vs* 2.8 ± 2.7 d, $P = 0.001$). Table 2 displays the laboratory data of the 72 patients. No significant difference was noted between the two groups.

Evaluation of pain relief

Table 3 and Figure 1 compare the pain intensity and analgesic requirement between the control and LA groups. Both groups achieved gradual pain relief after surgery in terms of VAS for pain and need for analgesics. However, the LA group experienced significantly less pain at 1 and 24 h after surgery and at discharge when compared with the control group. Furthermore, the LA group had less meperidine use at 1 h and total meperidine use after LC. However, there was no significant difference in acetaminophen use between the two groups (Figures 2-4).

DISCUSSION

Postoperative pain associated with LC is less intense and lasts a shorter time than that seen with open cholecystectomy. This explains why patients can be discharged and returned to their normal daily activities earlier[12]. However, as seen in this study, LC is not a pain-free procedure. Pain remains a prevalent complaint of the

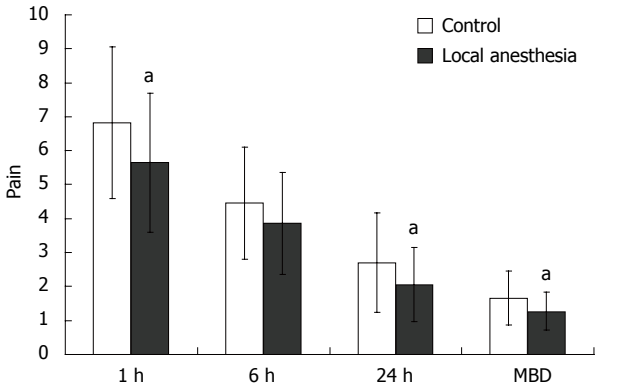

Figure 1 Pain evaluation of LC patients in the LA and control groups. $^aP < 0.05$ compared with the respective patient group at each time point after surgery. Significant difference in pain score was noted between the LA and control groups, except at 6 h after surgery. All data were presented as mean ± SD. MBD: May be discharged.

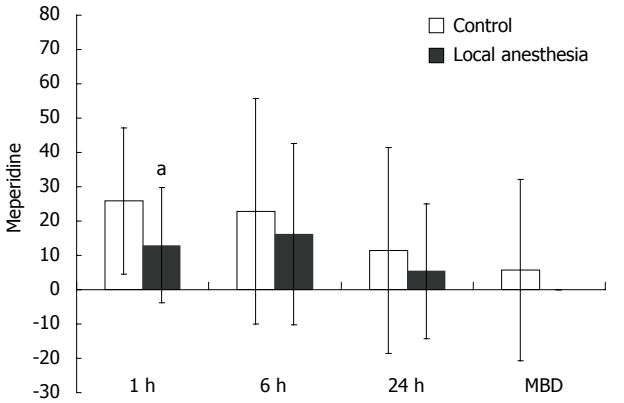

Figure 2 Meperidine required for LC patients in the LA and control groups. $^aP < 0.05$ when compared with the respective patient group at each time point after surgery, compared with the respective patient group before surgery. Significant difference was noted between the LA and control groups at 1 h after surgery. All data were presented as mean ± SD.

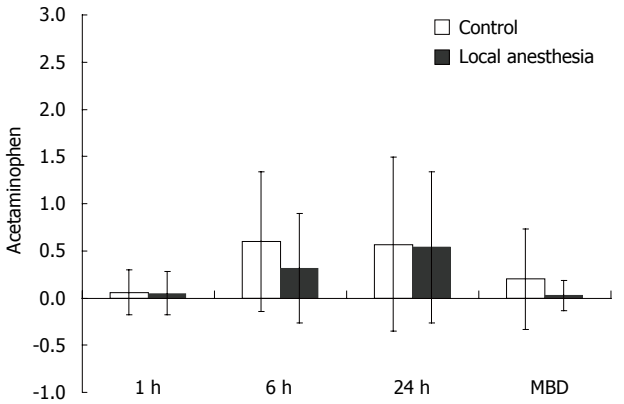

Figure 3 Acetaminophen required for LC patients in the LA and control groups. No significant difference was noted between the LA and control groups after surgery. All data were presented as mean ± SD.

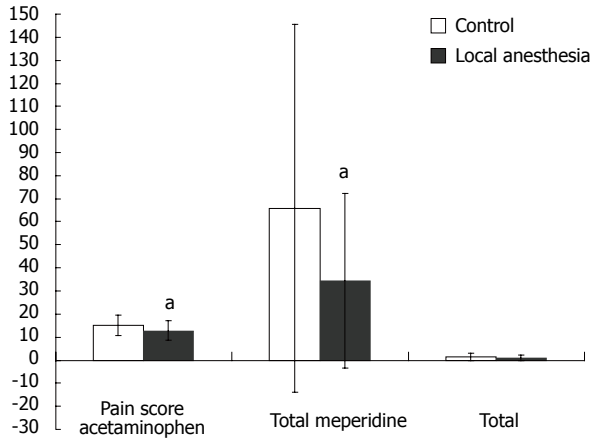

Figure 4 Pain evaluation and total analgesic requirement in LC patients in the LA and control groups. [a]*P* < 0.05 when compared with the respective patient group after surgery. Significant difference was noted for pain and meperidine requirement between the LA and control groups after surgery. All data were presented as mean ± SD.

early postoperative period after LC. This study clearly showed that pain reached a peak within the first few hours following the operation but diminished during the next 2-3 d, demonstrated by the distribution of the pain score and parenteral analgesic requirement. It has been reported that incisional pain is more intense than visceral pain and is dominant during the first 48 h after LC[13].

Several mechanisms have been proposed for generation of pain following laparoscopy: ruptured blood vessels caused by rapid distension of the peritoneum; traumatic traction on the nerves; release of inflammatory molecules; trauma to the abdominal wall, and when the gallbladder is removed from the abdomen; pneumoperitoneum created by use of CO_2; maintenance of high abdominal pressure; irritation of the phrenic nerve; and application of cold CO_2[14]. This explains why no consensus can be reached regarding effective postoperative pain relief in patients undergoing LC, because pain is multifactorial[11]. Although a number of studies have been conducted in an effort to reduce postoperative pain after surgery, the results have varied.

Postoperative pain control is directed at early mobilization, recovery, and discharge. However, pain also plays a major role in the metabolic and endocrine response, and is instrumental in the impairment of postoperative pulmonary function. Various methods have been investigated for reducing postoperative pain, such as local anesthesia[15], intraperitoneal infiltration of local anesthesia[16], preoperative administration of anti-inflammatory drugs[17], utilizing CO_2 at body temperature, applying intrapleural morphine[18], and combined use of NSAIDs and opioids[19].

Our findings indicated that infiltrating ropivacaine after surgery through the port site reduced pain intensity, the number of patients requiring postoperative analgesics, and hospital stay. Administering local anesthesia at the end of surgery offered a longer time delay to the need for analgesics, compared with patients who did not receive postoperative local anesthesia. Furthermore, patients who received local anesthesia at the end of surgery required significantly lower doses of analgesics than patients who did not receive local anesthesia. This is explained by the fact that pain intensity was less among patients who received local anesthesia at the end of the surgery than among those who did not.

Ropivacaine is a new long-acting local anesthetic that was developed after the emergence of bupivacaine-related severe toxicity. The agent is a pure left-isomer and, based on its three-dimensional structure, it has less toxic potential on the central nervous system and the heart[20]. Several clinical studies have evaluated its toxicology and clinical profiles: theoretically and experimentally, some differences can be seen, but reflection of these characteristics in clinical practice has not been evident. However, the reduced toxic potential of the pure left-isomer supports its use in clinical situations in which the risk of systemic toxicity related to overdosing or unwanted intravascular injection is high, such as during epidural or peripheral nerve blocks. Adverse effects associated with the use of local anesthesia, such as allergic reactions and local tissue, cardiovascular, central nervous system and systemic toxicity, were reported as rare in one previous study[20], and we did not observe any adverse effect related to the use of local anesthesia. Generally, the present study confirms earlier evidence that, in patients with gallbladder lesion undergoing LC, local anesthesia infusion is more effective when applied at the end of an operation than at the start.

Local anesthesia with ropivacaine infusion at the port site in LC patients at the end of surgery significantly decreased postoperative pain immediately. This short-term benefit explains the lower parenteral analgesic use and earlier discharge for LC patients with local anesthesia infusion. However, another clinical trial including multiple factors regarding pain after LC should be conducted.

COMMENTS

Background

Although laparoscopic cholecystectomy (LC) is a less painful procedure than open cholecystectomy, patients still felt wound pain after surgery. We tried to improve postoperative pain relief by the use of local anesthesia.

Research frontiers

Local anesthesia is used for postoperative analgesia and is effective. However, there have been few randomized studies performed. Good postoperative pain control will improve quality of life after LC.

Innovations and breakthroughs

Pain after LC may be caused by personal factors, duration of operation, intraperitoneal pressure, and the gallbladder lesion concerned. We used a prospective randomized trial to demonstrate that postoperative pain control improved by adding local anesthesia.

Applications

This local anesthesia procedure can be used routinely in other kinds of laparoscopic surgery to reduce postoperative pain.

Peer review

The authors reported that local anesthesia with ropivacaine infusion was beneficial for LC. The present study was concerned mainly with anesthesia. However, this paper is interesting and instructive for surgeons. The presentation and readability of the manuscript are good.

REFERENCES

1 **Cuschieri A**, Dubois F, Mouiel J, Mouret P, Becker H, Buess G, Trede M, Troidl H. The European experience with laparoscopic cholecystectomy. *Am J Surg* 1991; **161**: 385-387
2 **Yeh CN**, Chen MF, Jan YY. Laparoscopic cholecystectomy in 226 cirrhotic patients. Experience of a single center in Taiwan. *Surg Endosc* 2002; **16**: 1583-1587
3 **Yeh CN**, Chen MF, Jan YY. Laparoscopic cholecystectomy for 58 end stage renal disease patients. *Surg Endosc* 2005; **19**: 915-918
4 **McMahon AJ**, Russell IT, Ramsay G, Sunderland G, Baxter JN, Anderson JR, Galloway D, O'Dwyer PJ. Laparoscopic and minilaparotomy cholecystectomy: a randomized trial comparing postoperative pain and pulmonary function. *Surgery* 1994; **115**: 533-539
5 **Barkun JS**, Barkun AN, Sampalis JS, Fried G, Taylor B, Wexler MJ, Goresky CA, Meakins JL. Randomised controlled trial of laparoscopic versus mini cholecystectomy. The McGill Gallstone Treatment Group. *Lancet* 1992; **340**: 1116-1119
6 **Squirrell DM**, Majeed AW, Troy G, Peacock JE, Nicholl JP, Johnson AG. A randomized, prospective, blinded comparison of postoperative pain, metabolic response, and perceived health after laparoscopic and small incision cholecystectomy. *Surgery* 1998; **123**: 485-495
7 **McMahon AJ**, Russell IT, Baxter JN, Ross S, Anderson JR, Morran CG, Sunderland G, Galloway D, Ramsay G, O'Dwyer PJ. Laparoscopic versus minilaparotomy cholecystectomy: a randomised trial. *Lancet* 1994; **343**: 135-138
8 **Alexander JI**. Pain after laparoscopy. *Br J Anaesth* 1997; **79**: 369-378
9 **Schoeffler P**, Diemunsch P, Fourgeaud L. [Ambulatory celioscopy] *Cah Anesthesiol* 1993; **41**: 385-391
10 **Mouton WG**, Bessell JR, Otten KT, Maddern GJ. Pain after laparoscopy. *Surg Endosc* 1999; **13**: 445-448
11 **Wills VL**, Hunt DR. Pain after laparoscopic cholecystectomy. *Br J Surg* 2000; **87**: 273-284
12 **Alexander JI**. Pain after laparoscopy. *Br J Anaesth* 1997; **79**: 369-378
13 **Lee IO**, Kim SH, Kong MH, Lee MK, Kim NS, Choi YS, Lim SH. Pain after laparoscopic cholecystectomy: the effect and timing of incisional and intraperitoneal bupivacaine. *Can J Anaesth* 2001; **48**: 545-550
14 **Inan A**, Sen M, Dener C. Local anesthesia use for laparoscopic cholecystectomy. *World J Surg* 2004; **28**: 741-744
15 **Bisgaard T**, Klarskov B, Kristiansen VB, Callesen T, Schulze S, Kehlet H, Rosenberg J. Multi-regional local anesthetic infiltration during laparoscopic cholecystectomy in patients receiving prophylactic multi-modal analgesia: a randomized, double-blinded, placebo-controlled study. *Anesth Analg* 1999; **89**: 1017-1024
16 **Szem JW**, Hydo L, Barie PS. A double-blinded evaluation of intraperitoneal bupivacaine vs saline for the reduction of postoperative pain and nausea after laparoscopic cholecystectomy. *Surg Endosc* 1996; **10**: 44-48
17 **Alexander DJ**, Ngoi SS, Lee L, So J, Mak K, Chan S, Goh PM. Randomized trial of periportal peritoneal bupivacaine for pain relief after laparoscopic cholecystectomy. *Br J Surg* 1996; **83**: 1223-1225
18 **Gharaibeh KI**, Al-Jaberi TM. Bupivacaine instillation into gallbladder bed after laparoscopic cholecystectomy: does it decrease shoulder pain? *J Laparoendosc Adv Surg Tech A* 2000; **10**: 137-141
19 **Motamed C**, Bouaziz H, Franco D, Benhamou D. Analgesic effect of low-dose intrathecal morphine and bupivacaine in laparoscopic cholecystectomy. *Anaesthesia* 2000; **55**: 118-124
20 **Casati A**, Putzu M. Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? *Best Pract Res Clin Anaesthesiol* 2005; **19**: 247-268

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Detection and evaluation of antibodies against neutrophil-activating protein of *Helicobacter pylori* in patients with gastric cancer

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Abstract

AIM: To detect and evaluate the antibodies against *Helicobacter pylori* (*H pylori*) neutrophil-activating protein (HP-NAP) in patients with gastric cancer and other gastroduodenal diseases.

METHODS: Recombinant HP-NAP was prepared from a prokaryotic expression system in *Escherichia coli*. Serum positivity and level of HP-NAP-specific antibodies in sera from 43 patients with gastric cancer, 28 with chronic gastritis, 28 with peptic ulcer, and 89 healthy controls were measured by rHP-NAP-based ELISA. rHP-NAP-stimulated production of interleukin-8 (IL-8) and growth-related oncogene (GRO_α) cytokines in the culture supernatant of SGC7901 gastric epithelial cells was also detected.

RESULTS: The serum positivity and mean absorbance value of HP-NAP-specific antibodies in the gastric cancer group (97.7% and 1.01 ± 0.24) were significantly higher than those in the chronic gastritis group (85.7% and 0.89 ± 0.14, $P < 0.005$) and healthy control group (27.7% and 0.65 ± 0.18, $P < 0.001$). The sensitivity and specificity of ELISA for the detection of HP-NAP-specific antibodies were 95.5% and 91.5%, respectively. HP-NAP could slightly up-regulate IL-8 production in gastric epithelial cell lines but had no effect on GRO_α production.

CONCLUSION: Infection with virulent *H pylori* strains secreting HP-NAP is associated with severe gastroduodenal diseases, and HP-NAP may play a role in the development of gastric carcinoma. rHP-NAP-based ELISA can be used as a new method to detect *H pylori* infection. The direct effect of HP-NAP on gastric epithelial cells may be limited, but HP-NAP may contribute to inflammatory response or carcinogenesis by activating neutrophils.

Key words: *Helicobacter pylori*; *Helicobacter pylori* neutrophil-activating protein; Gastric cancer; Peptic ulcer; Chronic gastritis

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INTRODUCTION

Helicobacter pylori (*H pylori*), a microaerophilic Gram-negative bacterium, infects the stomach of more than 50% of human population worldwide and is a major cause of chronic gastritis and peptic ulcer. Furthermore, it is associated with gastric adenocarcinoma and gastric B cell lymphoma. In 1994, the World Health Organization classified *H pylori* infection as a definite (class 1) carcinogen[1]. *H pylori* colonization is followed by infiltration of neutrophils, macrophages and lymphocytes in gastric mucosa. The degree of mucosal damage is closely associated with the extent of

neutrophil infiltration[2-4].

Multiple bacterial virulence factors, such as vacA, cagA and lipopolysaccharide (LPS), can modulate *H pylori*-induced inflammation. *H pylori* neutrophil-activating protein (HP-NAP), a 150-kDa iron-binding protein, is a ball-shaped dodecamer formed by four-helix bundled subunits with its sequence similar to that of bacterioferritins and DNA binding proteins[5,6]. It has been designated as a neutrophil-activating factor because it promotes the adherence of neutrophils to endothelial cells and stimulates production of reactive oxygen species (ROS) in neutrophils by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in plasma membrane[7-11]. Satin *et al*[12] demonstrated that the purified recombinant HP-NAP is chemotactic for human neutrophils and monocytes, induces surface expression of β_2-integrin, which mediates endothelial transmigration, adhesion and accumulation of leucocytes at the site of *H pylori* infection. Recombinant HP-NAP induces the production of ROS by neutrophils *via* a cascade of intracellular activation events, including increased cytosolic calcium and phosphorylation of proteins, leading to the assembly of functional NADPH oxidase on neutrophil plasma membrane. In addition, HP-NAP increases the synthesis of tissue factor and secretion of type 2 inhibitor of plasminogen activator in monocytes[13,14], contributing to the inflammation of gastric mucosa by fibrin deposition. These studies indicate that HP-NAP is a virulence factor relevant to the pathogenic effect of *H pylori*.

It was recently reported that HP-NAP promotes a Th1 immune response by inducing the expression of IL-12 and IL-23 in neutrophils and monocytes, and also elicits an antigen-specific Th1-polarized T cell response in gastric mucosa of *H pylori*-infected patients *in vivo*[15]. It has been shown that HP-NAP is able to shift antigen-activated human T cells from a Th2 to a Th1 cytotoxic phenotype characterized by production of IFN-γ and TNF-α[16]. Additionally, the majority of infected patients have antibodies against this antigen, and vaccination of mice with HP-NAP induces protection against a subsequent challenge with *H pylori*[12]. Therefore, HP-NAP is an immune modulator promoting Th1 immune responses and an important vaccine candidate antigen[17,18].

Since HP-NAP is a powerful stimulant for the production of ROS, mediating damage to DNA and enhancing cell turnover[19], it may be a risk factor for *H pylori*-associated gastric cancer. Currently, it is uncertain whether HP-NAP is related to the occurrence of gastric cancer. Interleukin-8 (IL-8) and growth-related oncogene (GRO_α) are members of the CXC chemokine family that induce neutrophil chemotaxis and activation. It has been shown that IL-8 and GRO_α levels are elevated in *H pylori*-infected gastric mucosa[20]. In addition, *H pylori* water soluble surface proteins up-regulate the expression of IL-8 and GRO_α mRNA and protein by neutrophils[21]. Whether HP-NAP contributes to the inflammatory response or carcinogenesis by up-regulating IL-8 and GRO_α production in *H pylori*-infected gastric mucosa remains unknown. An understanding of the relation between HP-NAP and gastric cancer and the molecular mechanism(s) underlying HP-NAP-induced diseases should lead to improved approaches to the effective control of *H pylori*-associated gastric cancer.

In the present study, recombinant HP-NAP was prepared from a prokaryotic expression system in *Escherichia coli*, and the *napA* gene in 20 *H pylori* clinical isolates from South China was detected by PCR. The seropositivity and level of HP-NAP-specific antibodies in sera from 43 patients with gastric cancer, 28 with chronic gastritis, 28 with peptic ulcer, and 89 healthy controls were measured by rHP-NAP-based ELISA. The production of IL-8 and GRO_α cytokines in culture supernatant from SGC7901 gastric epithelial cells stimulated by rHP-NAP was also detected.

MATERIALS AND METHODS

Preparation of bacterial and gastric epithelial cell lines

H pylori NCTC11639 strain was stored at -70℃ in our department. Bacteria were routinely cultured on Columbia agar plates supplemented with 10% defibrinated sheep blood, 0.004% triphenyltetrazolium chloride, and Dent selective supplement (Oxoid, Basingstoke, UK) at 37℃ for 3 d under a microaerophilic atmosphere containing 50 mL/L O_2, 150 mL/L CO_2 and 800 mL/L N_2. Several colonies were then picked up and inoculated into 20 mL of Brucella broth (Becton Dickinson, Cockeysville, MS) containing 0.1% β-cyclodextrin supplemented with 5% (v/v) fetal calf serum. After 24 h, 2 mL of culture was transferred to 40 mL of fresh medium, and the same process was repeated twice. Finally, 1 mL of the incubated medium containing the bacterial cells, most of which were spiral rather than coccoid, was plated on Brucella agar (Becton Dickinson) containing 10% (v/v) defibrinated sheep blood and cultured at 37℃ for an additional 3 d in a microaerophilic atmosphere containing 50 mL/L O_2, 150 mL/L CO_2 and 800 mL/L N_2. Bacterial cells were harvested, washed twice with cold phosphate-buffered saline (PBS, 25 mmol/L sodium phosphate, pH 7.2, 0.9% NaCl), and then sedimented by centrifugation at 5000 × *g* for 10 min at 4℃. The cell pellet was stored at -80℃.

Human gastric epithelial cells (SGC7901) were cultured at 37℃ in RPMI-1640 (Gibco, USA) containing 10% FBS (Gibco) in a humidified atmosphere containing 50 mL/L CO_2, and plated at 10^6 cells/well in 24-well plates. The medium was changed every 3 d and replaced with RPMI-1640 without serum before experiment.

Collection of serum samples from infected and healthy individuals

H pylori infection was diagnosed by histological examination of endoscopic biopsy specimens and CLO testing. Forty-three serum samples were collected from patients with gastric cancer at Southern Hospital, Guangzhou, China. The age of patients ranged 27-83 years. Twenty-eight serum samples were also collected from patients with peptic ulcer or chronic gastritis at Southern Hospital. The age of patients ranged 28-67 years. Finally, 89 serum samples were collected from healthy blood donors at the age of 18-70 years.

Cloning and purification of NAP

Genomic DNA of *H pylori* was prepared using a Takara kit (Takara, Japan) according to its manufacturer's instructions. The extracted genomic DNA was then used as a template for amplification of the NAP coding region using a Taq DNA polymerase PCR kit (Takara, Japan)[22,23]. Two primer sequences corresponding to the 5' and 3' ends of the coding gene, including *Eco*RI and *Xho*I restriction sites, were used: P1: 5'CCGGAATTCA TGAAAACATTTGAA-3', P2: 5'CCGCTCGAGTTAA GCCAAATGGGC-3'.

The PCR product was cloned into the expression vector pGEX-4T-1 (Amersham Biosciences). The plasmid was then transformed into *E. coli* strain TOP10 (Invitrogen BV, Leek, The Netherlands), and NAP expression was induced with 1 mmol/L isopropyl-β-d-1-thiogalactopyranoside when the cells were grown to the log phase at room temperature. After 4 h, the cells were harvested by centrifugation and washed with ice-cold PBS containing 5 mmol/L EDTA and 2 mmol/L PMSF. All subsequent procedures were performed at 4℃. The NAP-GST fusion protein was purified by glutathione-sepharose 4B column chromatography.

Screening for seropositive individuals with IgG antibodies against *H pylori* in healthy subjects

Anti-*H pylori* IgG antibodies in serum samples were assayed by indirect ELISA using a diagnostic kit (BIOCUP, Shenzhen, China). According to the instructions, serum (l00 μL, diluted 1/100 in PBS) was added to ELISA plates pre-coated with purified *H pylori* antigens in duplicate and incubated for 1 h. Controls consisted of wells with PBS alone, *H pylori* negative and positive serum, which were considered blank, negative and positive controls, respectively. Peroxidase-conjugated anti-human IgG (1/5000) was added and incubated for 30 min, after which the plates were washed with PBS, and color reaction was initiated by the addition of TMB (100 μL). After 10 min, the reaction was terminated by the addition of 1 mol/L H_2SO_4 (100 μL). The plate reader was calibrated to the blank well and the absorbance at 450 nm was read. Cut-off value (C.O.) = 2.1 × Nc (Nc = the mean absorbance value for three negative controls). Samples with absorbance > 0.21 were considered positive. The anti-*H pylori* IgG seropositive individuals were considered to be *H pylori*-infected healthy individuals.

Detection of antibodies against HP-NAP in serum by ELISA

Recombinant HP-NAP was prepared and purified, and ELISA was carried out as previously described[24-27]. Briefly, immunoplates (Nunc, Denmark) were coated with rNAP (5μg/well) and incubated overnight at 4℃. After washed three times with a washing buffer containing PBS (pH 7.2) and 0.1% Tween 20, the plates were blocked with 200 μL of 10% bovine serum in PBS and incubated in a moist chamber for 1 h at 37℃, then washed three times with a washing buffer containing PBS (pH 7.2) and 0.1% Tween 20. One hundred microlitre of serum samples (1:100) from patients or healthy individuals was then added to the microtiter wells, and the plates were incubated at 37℃ for 60 min. After washed with PBS, 100 μL of secondary antibody (goat anti-human IgG-HRP, 1:10 000) was added to each well and incubated for 60 min at 37℃. After washed with PBS, 100 μL of TMB/H_2O_2 substrate was added to the wells and incubated at room temperature for about 10 min. The reaction was terminated by adding 100 μL of 2 mol/L H_2SO_4. The absorbance of each well was read at 450 nm. Samples with absorbance > 0.78 were considered positive. Each sample was tested in duplicate.

Selection of cut-off values for antibodies against HP-NAP in human serum

A receiver operating curve (ROC) was plotted to calculate the cut-off values for antibodies against HP-NAP at a 95% accuracy level[26]. In addition, the cut-off values were determined by mean plus 2 × SD and mean plus 3 × SD, derived from *P* values in healthy individuals.

Detection of the *napA* gene in *H pylori* clinical isolates by PCR

Genomic DNA of 20 *H pylori* clinical isolates was prepared and used as a template for amplification of the NAP coding region with two primer sequences as preciously described[28]. PCR was carried out in a final volume of 60 μL. A preliminary denaturation step at 95℃ for 5 min was followed by 30 amplification cycles, each consisting of denaturation at 94℃ for 30 s, annealing at 50℃ for 30 s, extension at 72℃ for 45 s, and a final extension at 72℃ for 7 min. Then, 10 μL of amplicon was transferred onto a 1.5% (w/v) agarose gel in 1 × TBE buffer, and electrophoresis was performed at 80 V for 30 min in a DNA submarine plate. The gel was then stained with ethidium bromide.

IL-8 and GRO$_\alpha$ production in human gastric epithelial cells stimulated with rHP-NAP

Before use, human gastric epithelial cells (SGC7901) growing in 24-well plates were washed and replaced with RPMI-1640 without serum, and then stimulated with 10 μg rHP-NAP for 12 h and 36 h, respectively. The supernatant was centrifuged to remove particulate debris and stored in aliquots at -70℃. IL-8 and GRO$_\alpha$ concentrations in the culture supernatant were measured by ELISA using a Quantikine immunoassay kit (R&D, USA) according to its manufacturer's instructions. Before detection, the culture supernatant was filtered through a sterile membrane filter (0.22 μm. pore size).

Statistical analysis

Statistical analyses were conducted using the SPSS software package. One-way ANOVA was performed to assess differences among groups. *Post hoc* multiple comparisons between different infectious groups were done by LSD analysis. Comparisons between the seropositivity of anti-HP-NAP antibodies in patients with gastric cancer and other gastroduodenal diseases were done by chi-square test.

RESULTS

Expression and purification of recombinant protein

The gene encoding for HP-NAP was obtained by PCR amplification using genomic DNA extracted from *H pylori* as a template. Agarose gel electrophoresis analysis of the PCR product is shown in Figure 1.

A 435 bp DNA fragment was detected, which corresponded to the size of the HP-NAP gene. The DNA sequence of the hypothetical HP-NAP gene of *H pylori* was determined (data not shown) and submitted to GenBank (accession No. DQ341279). To facilitate purification, HP-NAP was expressed as a GST fusion protein in *E. coli* Top10 cells. Expression of the recombinant NAP-GST was examined by SDS-PAGE. A pronounced band with an approximate molecular weight of 44 kDa appeared in the supernatant of cell lysate after induction but not in control cells, suggesting that the fusion protein can be successfully expressed in bacterial cells (Figure 2).

The purified NAP-GST fusion protein was further confirmed by Western blotting. Serum from *H pylori*-infected patients specifically recognized the recombinant NAP-GST fusion protein, while negative serum did not (Figure 3).

Selection of cut-off values for HP-NAP-specific antibodies

A cut-off value of 0.78 was determined for ELISA of HP-NAP antibodies using ROC analysis (Figure 4). The area under the ROC curve was 0.97. Overall, ELISA yielded a sensitivity of 95.5% (95% confidence interval) and a specificity of 91.5% (95% confidence interval) for the detection of antibodies against HP-NAP in serum.

Comparison between seropositivity for anti-*H pylori* IgG and anti-HP-NAP antibodies in healthy subjects

Anti-*H pylori* IgG seropositivity was detected in 47 of the 89 healthy subjects who were considered *H pylori*-infected healthy individuals. As shown in Table 1, the anti-*H pylori* IgG and anti-HP-NAP seroposivity in healthy individuals was 52.8% (47/89) and 14.6% (13/89), respectively. The anti-*H pylori* IgG seropositivity was much higher than the anti-HP-NAP antibody seropositivity ($P < 0.005$). The anti-HP-NAP antibody seropositivity, however, was 27.7% (13/47) in *H pylori*-infected healthy individuals.

Detection of antibodies against HP-NAP in patients with gastric cancer and other gastroduodenal diseases by ELISA

The seropositivity for antibodies against HP-NAP in gastric cancer and peptic ulcer patients was 97.7% (42/43) and 92.8% (26/28), respectively, while the seropositivity for antibodies against HP-NAP in chronic gastritis patients was 85.7% (24/28). The seropositivity for antibodies against HP-NAP in infected healthy controls was 27.7%. The seropositivity for HP-NAP-specific antibodies in gastric cancer patients was higher than that in chronic gastritis patients ($P < 0.05$) and in infected healthy controls ($P < 0.01$). The difference in the mean absorbance value for antibodies against HP-NAP between groups was also confirmed by one-way

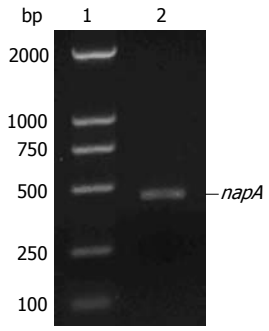

Figure 1 Agarose gel electrophoresis of *napA* gene amplified by PCR. Lane 1: DL2000 DNA marker; lane 2: *H pylori napA* gene (435 bp).

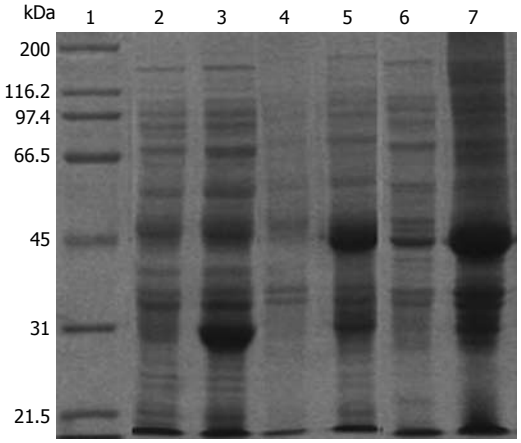

Figure 2 SDS-PAGE analysis of the expression of pGEX-4T-1/HP-NAP in *E. coli*. Lane 1: Protein marker; Lane 2: pGEX-4T-1 before induction; Lane 3: pGEX-4T-1 after induction; Lane 4: pGEX-4T-1/HP-NAP before induction; Lane 5: pGEX-4T-1/HP-NAP after induction; Lane 6: Supernatant of *E. coli* after induction; Lane 7: Lysate of *E. coli* after induction.

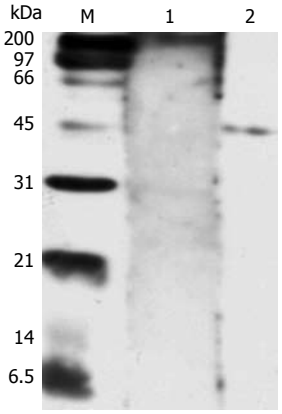

Figure 3 Western blotting analysis of HP-NAP reactivity with the positive serum from *H pylori*-infected patients. Lane 1: Serum from a control patient not infected with *H pylori*; Lane 2: Serum from a patient infected with *H pylori*; M: Protein marker.

Table 1 Positivity comparison between *H pylori* -specific IgG and HP-NAP-specific antibodies in healthy individuals *n* (%)

	Total number tested	Positivity
HP-specific IgG Abs	89	47 (52.8)
HP-NAP-specific Abs (total healthy persons)	89	13 (14.6)[1]
HP-NAP-specific Abs (infected healthy persons)	47	13 (27.7)

[1]$P < 0.005$ *vs* infected healthy persons; Abs: Antibodies.

Table 2 Demonstration of antibodies against HP-NAP in serum from *H pylori*-infected patients by ELISA *n* (%)

Patient group	Positivity for HP-NAP antibodies	Negativity for HP-NAP antibodies	Absorbance (mean ± 2SD) (cut-off value 0.78)	Range
Gastric cancer group (n = 43)	42 (97.7)	1 (2.3)	1.01 ± 0.24	0.748-1.269
Peptic ulcer group (n = 28)	26 (92.8)	2 (7.1)	0.98 ± 0.32	0.771-1.265
Chronic gastritis group (n = 28)	24 (85.7)	4 (14.3)	0.89 ± 0.14	0.711-1.122
Healthy persons (HP-IgG positive n = 47)	13 (27.7)	34 (72.3)	0.65 ± 0.18	0.451-0.948

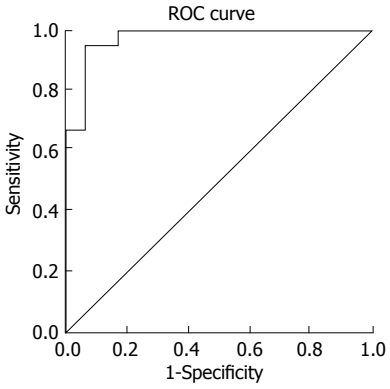

Figure 4 Receiver operating characteristic curve analysis showing the cut-off value for anti-HP-NAP antibodies in serum from patients with gastric cancer, peptic ulcer, chronic gastritis and healthy controls, respectively.

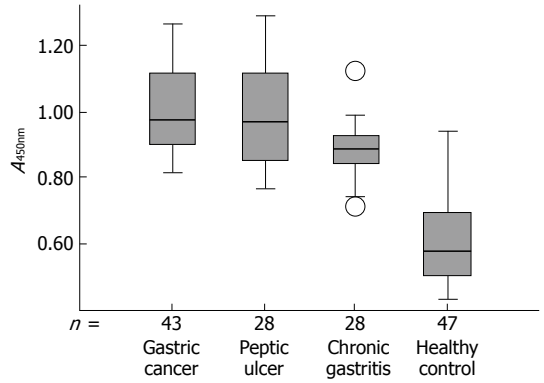

Figure 5 Box plot demonstrating the level of antibodies against HP-NAP in serum from patients with gastric cancer, peptic ulcer, chronic gastritis and healthy controls, respectively. The box plot showing the 5th and 95th percentiles (bars), the 75th and 25th percentiles (boxes), and the median (bars in boxes), respectively. *n*: Number of individuals in each group.

ANOVA analysis (F = 4.014, P = 0.023). The mean absorbance value for anti-HP-NAP antibodies in gastric cancer patients was 1.01 ± 0.24 (range 0.748-1.269), significantly higher than that in chronic gastritis patients (0.89 ± 0.14, range 0.711-1.122, $P < 0.005$) and in infected healthy controls (0.65 ± 0.18, range 0.451-0.948, $P < 0.001$). There was no significant difference, however, in the mean absorbance value for anti-HP-NAP antibodies between patients with gastric cancer (1.01 ± 0.24, range 0.748-1.269) and peptic ulcer (0.98 ± 0.32, range 0.771-1.265) (Table 2).

Box plots of the antibodies against HP-NAP in sera from patients with gastric cancer, peptic ulcer, chronic gastritis, and healthy controls are shown in Figure 5, with the 90th percentile range and 75th and 25th percentiles.

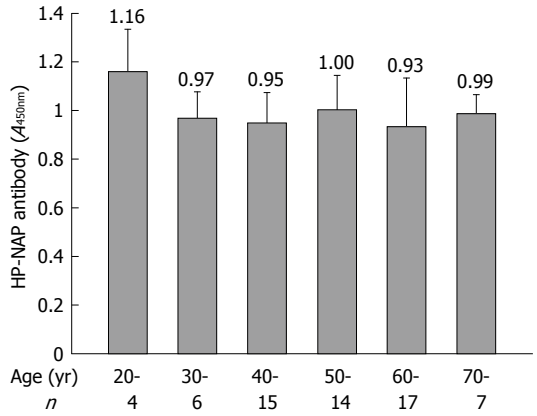

Figure 6 Comparison of HP-NAP antibody levels in different age groups.

Comparison of HP-NAP antibody levels in different age groups

Most *H pylori*-associated diseases occurred in subjects at the ages of 40-70 years (53/63). The mean absorbance value for anti-HP-NAP antibodies was not significantly different in patients at different ages (Figure 6).

Detection of napA gene in all H pylori clinical isolates

The *napA* gene was detected by PCR in 20 *H pylori* clinical isolates. All examined *H pylori* strains carried the *napA* gene. The electrophoresis results of PCR products of the *napA* gene from 8 representative *H pylori* strains are shown in Figure 7.

IL-8 and GROα production in human gastric epithelial cells stimulated with rHP-NAP

The IL-8 and GROα levels in culture supernatant of human gastric epithelial cell line SGC7901 were measured by ELISA, showing that HP-NAP slightly up-regulated the IL-8 production (Table 3) but had no effect on GROα production by gastric epithelial cells (data not shown).

DISCUSSION

HP-NAP, an important virulence factor for *H pylori*, induces adherence of neutrophils to gastric epithelial cells and causes an inflammatory reaction by activating neutrophils. In the present study, we cloned and expressed HP-NAP, which was then used as an antigen to detect HP-NAP-specific antibodies in serum samples. The seropositivity for anti-HP-NAP antibodies was detected in 89 healthy subjects and compared with that for anti-*H pylori* IgG antibodies in the same subjects. Meanwhile,

Table 3 IL-8 level in culture supernatant of human gastric epithelial cell line SCG7901 (pg/mL)

Time (h)	IL-8 level		
	10 μg/mL NAP	1 μg/mL LPS	Control
12	10.10[b]	8.07	3.55
36	30.14[b]	30.54	11.28

[b]$P < 0.01$ *vs* control.

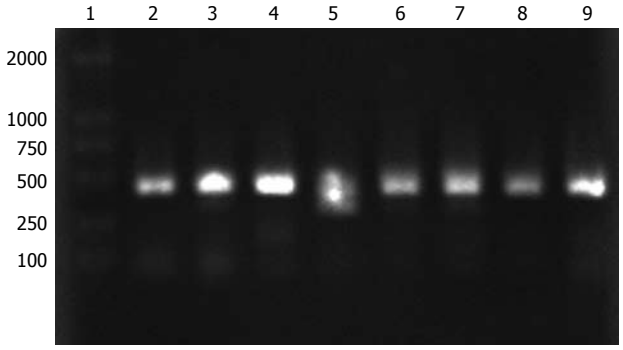

Figure 7 Agarose gel electrophoresis analysis of PCR product of *napA* gene from *H pylori* clinic strains. Lane 1: DL2000 DNA marker; Lanes 2-9: *napA* gene (435 bp) from eight *H pylori* clinic strains.

the seropositivity and levels of HP-NAP-specific antibodies in 43 patients with gastric cancer, 28 with chronic gastritis, 28 with peptic ulcer, were measured by rHP-NAP-based ELISA. Moreover, the *napA* gene in 20 *H pylori* clinical isolates from South China was detected by PCR. The production of IL-8 and GROα cytokines in the culture supernatant of SGC7901 gastric epithelial cells stimulated with rHP-NAP was also detected.

The seropositivity for anti-*H pylori* IgG was 52.8% (47/89) in healthy subjects, which is in agreement with the average prevalence (about 50%-60%) of *H pylori* in the adult Chinese population. The 47 subjects who were serum positive for anti-*H pylori* IgG were considered to be *H pylori*-infected healthy individuals. The seropositivity for anti-HP-NAP antibodies was 27.7% (13/47) in *H pylori*-infected healthy individuals, due to variations in HP-NAP expression in different *H pylori* strains. It has been shown that the neutrophil adhesion-promoting activity in different *H pylori* strains varies considerably[29,30], suggesting that HP-NAP is differently expressed in different *H pylori* strains[31]. Our results also show that the mean absorbance value for anti-HP-NAP antibodies was not significantly different in different age groups. Our work and other studies, however, have found that the *napA* gene is present in all *H pylori* clinical isolates.

H pylori is a clear (class 1) carcinogen. HP-NAP stimulates neutrophils to infiltrate gastric mucosa and subsequently causes ROS production by activating NADPH oxidase in plasma membrane. It was reported that ROS can cause a variety of DNA lesions and produce mutations in mammalian cells[32]. ROS production is significantly decreased in gastric mucosa of patients with *H pylori* successfully eradicated[33]. It has also been shown that HP-NAP can be involved in extravasation of leukocytes, and ROS can play a role in the carcinogenic process in gastric mucosa during chronic *H pylori* infection[34,35], indicating that HP-NAP may be a risk factor for *H pylori*-associated gastric cancer.

In the present study, the seropositivity and mean absorbance value for HP-NAP-specific antibodies in gastric cancer patients (97.7% and 1.01 ± 0.24) were significantly higher than those in chronic gastritis patients (85.7% and 0.89 ± 0.14, $P < 0.005$) and in infected healthy controls (0.65 ± 0.18, range 0.451-0.948, $P < 0.001$). There was no difference, however, in the seropositivity or mean absorbance value for HP-NAP antibodies between the patients with gastric cancer and peptic ulcer, indicating that HP-NAP specific antibodies are correlated with severe gastroduodenal diseases and HP-NAP may contribute to the pathogenesis of *H pylori*-associated gastric cancer. Our results also show that HP-NAP had a strong antigenicity, and the majority of infected patients produced antibodies against HP-NAP.

ELISA for the detection of antibodies against HP-NAP in this study had a sensitivity of 95.5% and a specificity of 91.5%. Since the level of HP-NAP-specific antibodies may be correlated with the severity of *H pylori* infection, ELISA can be used in immunodiagnostic assay for *H pylori*-infection and in screening for a high-risk population with *H pylori*-associated gastric cancer.

In this study, HP-NAP slightly up-regulated IL-8 production by gastric epithelial cell lines, but had no effect on GROα production, suggesting that the direct effect of HP-NAP on gastric epithelial cells may be limited, but HP-NAP may contribute to the inflammatory response or carcinogenesis by activating neutrophils.

In conclusion, infection with virulent *H pylori* strains secreting HP-NAP is associated with severe gastroduodenal diseases and HP-NAP may play a role in the development of gastric carcinoma. rHP-NAP-based ELISA can be used as a new method to detect *H pylori* infection. We have recently developed a monoclonal antibody against HP-NAP, which might be used to detect HP-NAP expression in gastric mucosa. It would be desirable to carry out a comparative analysis to define the characteristic differences in HP-NAP expression between patients with gastric cancer and other gastroduodenal diseases. Further characterization of the interactions between HP-NAP and gastric cancer should aid in the development of novel strategies against *H pylori*-associated gastric cancer.

COMMENTS

Background

Helicobacter pylori (H pylori) infection increases the risk of developing peptic ulcer, gastric adenocarcinoma, and gastric B cell lymphoma. *H pylori* neutrophil-activating protein (HP-NAP), a virulence factor, promotes the adherence of neutrophils to gastric mucosa endothelial cells and stimulates high production of reactive oxygen species (ROS) in neutrophils. Since ROS can mediate DNA damage and enhance cell turnover, HP-NAP may be a risk factor for *H pylori*-associated gastric cancer. To prevent *H pylori*-related cancer, mass screening for and treatment of *H pylori* infection are cost-effective. Whether HP-NAP is related to the occurrence of gastric cancer is still uncertain.

Research frontiers

HP-NAP is a virulence factor for *H pylori* infection and a vaccine candidate antigen. The majority of infected patients have antibodies against this antigen,

and vaccination of HP-NAP in mice can protect against a subsequent challenge with *H pylori*. *H pylori* colonization is followed by infiltration of neutrophils, macrophages and lymphocytes in gastric mucosa. The degree of mucosal damage is closely correlated with the extent of neutrophil infiltration. It has been shown that HP-NAP can be involved in the extravasation of leukocytes, and ROS can play a role in the carcinogenic process of gastric mucosa during chronic *H pylori* infection.

Innovations and breakthroughs

An understanding of the relation between HP-NAP and gastric cancer should lead to improved approaches to the effective control of *H pylori*-associated gastric cancer. Seropositivity and mean absorbance value for HP-NAP-specific antibodies in gastric cancer patients were significantly higher than those in chronic gastritis patients and in infected healthy controls. There was no difference, however, in serum positivity or mean absorbance value for HP-NAP antibodies between patients with gastric cancer and peptic ulcer. These findings indicate that HP-NAP specific antibodies are correlated with severe gastroduodenal diseases and HP-NAP may contribute to the pathogenesis of *H pylori*-associated stomach cancer.

Applications

ELISA used for the detection of antibodies against HP-NAP in this study had a sensitivity of 95.5% and a specificity of 91.5%. Since the level of HP-NAP-specific antibodies may be correlated with the severity of *H pylori* infection, ELISA can be used to screen for a high-risk population with *H pylori*-associated gastric cancer.

Terminology

H pylori, a gram-negative microaerophilic bacterium, causes a long-term mild inflammation of stomach lining and is strongly linked to the development of duodenal and gastric ulcers and stomach cancer. Over 50% of the world's population harbor *H pylori* in their upper gastrointestinal tract, and infection is more prevalent in developing countries.

Peer review

In this study, the authors detected and evaluated the level of antibodies against HP-NAP in patients with gastric cancer and other gastroduodenal diseases. The results suggest that HP-NAP-specific antibodies are correlated with severe gastroduodenal diseases and HP-NAP may contribute to the pathogenesis of *H pylori*-associated stomach cancer. ELISA can be used to screen for a high-risk population with *H pylori*-associated gastric cancer.

REFERENCES

1 **Pounder RE**, Ng D. The prevalence of Helicobacter pylori infection in different countries. *Aliment Pharmacol Ther* 1995; **9** Suppl 2: 33-39

2 **D'Elios MM**, Montecucco C, de Bernard M. VacA and HP-NAP, Ying and Yang of Helicobacter pylori-associated gastric inflammation. *Clin Chim Acta* 2007; **381**: 32-38

3 **Polenghi A**, Bossi F, Fischetti F, Durigutto P, Cabrelle A, Tamassia N, Cassatella MA, Montecucco C, Tedesco F, de Bernard M. The neutrophil-activating protein of Helicobacter pylori crosses endothelia to promote neutrophil adhesion in vivo. *J Immunol* 2007; **178**: 1312-1320

4 **Brisslert M**, Enarsson K, Lundin S, Karlsson A, Kusters JG, Svennerholm AM, Backert S, Quiding-Järbrink M. Helicobacter pylori induce neutrophil transendothelial migration: role of the bacterial HP-NAP. *FEMS Microbiol Lett* 2005; **249**: 95-103

5 **Zanotti G**, Papinutto E, Dundon W, Battistutta R, Seveso M, Giudice G, Rappuoli R, Montecucco C. Structure of the neutrophil-activating protein from Helicobacter pylori. *J Mol Biol* 2002; **323**: 125-130

6 **Cooksley C**, Jenks PJ, Green A, Cockayne A, Logan RP, Hardie KR. NapA protects Helicobacter pylori from oxidative stress damage, and its production is influenced by the ferric uptake regulator. *J Med Microbiol* 2003; **52**: 461-469

7 **Allen LA**. The role of the neutrophil and phagocytosis in infection caused by Helicobacter pylori. *Curr Opin Infect Dis* 2001; **14**: 273-277

8 **Dundon WG**, Nishioka H, Polenghi A, Papinutto E, Zanotti G, Montemurro P, Del GG, Rappuoli R, Montecucco C. The neutrophil-activating protein of Helicobacter pylori. *Int J Med Microbiol* 2002; **291**: 545-550

9 **Teneberg S**, Miller-Podraza H, Lampert HC, Evans DJ Jr, Evans DG, Danielsson D, Karlsson KA. Carbohydrate binding specificity of the neutrophil-activating protein of Helicobacter pylori. *J Biol Chem* 1997; **272**: 19067-19071

10 **Evans DJ Jr**, Evans DG, Takemura T, Nakano H, Lampert HC, Graham DY, Granger DN, Kvietys PR. Characterization of a Helicobacter pylori neutrophil-activating protein. *Infect Immun* 1995; **63**: 2213-2220

11 **Montecucco C**, de Bernard M. Molecular and cellular mechanisms of action of the vacuolating cytotoxin (VacA) and neutrophil-activating protein (HP-NAP) virulence factors of Helicobacter pylori. *Microbes Infect* 2003; **5**: 715-721

12 **Satin B**, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, Tonello F, Kelleher D, Rappuoli R, Montecucco C, Rossi F. The neutrophil-activating protein (HP-NAP) of Helicobacter pylori is a protective antigen and a major virulence factor. *J Exp Med* 2000; **191**: 1467-1476

13 **Montemurro P**, Barbuti G, Dundon WG, Del Giudice G, Rappuoli R, Colucci M, De Rinaldis P, Montecucco C, Semeraro N, Papini E. Helicobacter pylori neutrophil-activating protein stimulates tissue factor and plasminogen activator inhibitor-2 production by human blood mononuclear cells. *J Infect Dis* 2001; **183**: 1055-1062

14 **Ljungh A**. Helicobacter pylori interactions with plasminogen. *Methods* 2000; **21**: 151-157

15 **Amedei A**, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, Tasca E, Azzurri A, D'Elios MM, Del Prete G, de Bernard M. The neutrophil-activating protein of Helicobacter pylori promotes Th1 immune responses. *J Clin Invest* 2006; **116**: 1092-1101

16 **D'Elios MM**, Amedei A, Cappon A, Del Prete G, de Bernard M. The neutrophil-activating protein of Helicobacter pylori (HP-NAP) as an immune modulating agent. *FEMS Immunol Med Microbiol* 2007; **50**: 157-164

17 **Nishioka H**, Baesso I, Semenzato G, Trentin L, Rappuoli R, Del Giudice G, Montecucco C. The neutrophil-activating protein of Helicobacter pylori (HP-NAP) activates the MAPK pathway in human neutrophils. *Eur J Immunol* 2003; **33**: 840-849

18 **Montemurro P**, Nishioka H, Dundon WG, de Bernard M, Del Giudice G, Rappuoli R, Montecucco C. The neutrophil-activating protein (HP-NAP) of Helicobacter pylori is a potent stimulant of mast cells. *Eur J Immunol* 2002; **32**: 671-676

19 **Shimoyama T**, Fukuda S, Liu Q, Nakaji S, Fukuda Y, Sugawara K. Helicobacter pylori water soluble surface proteins prime human neutrophils for enhanced production of reactive oxygen species and stimulate chemokine production. *J Clin Pathol* 2003; **56**: 348-351

20 **Sieveking D**, Mitchell HM, Day AS. Gastric epithelial cell CXC chemokine secretion following Helicobacter pylori infection in vitro. *J Gastroenterol Hepatol* 2004; **19**: 982-987

21 **Kim JS**, Jung HC, Kim JM, Song IS, Kim CY. Helicobacter pylori water-soluble surface proteins activate human neutrophils and up-regulate expression of CXC chemokines. *Dig Dis Sci* 2000; **45**: 83-92

22 **Niccolai A**, Fontani S, Kapat A, Olivieri R. Maximization of recombinant Helicobacter pylori neutrophil activating protein production in Escherichia coli: improvement of a chemically defined medium using response surface methodology. *FEMS Microbiol Lett* 2003; **221**: 257-262

23 **Kang QZ**, Duan GC, Fan QT, Xi YL. Fusion expression of Helicobacter pylori neutrophil-activating protein in E.coli. *World J Gastroenterol* 2005; **11**: 454-456

24 **Xiang Z**, Bugnoli M, Ponzetto A, Morgando A, Figura N, Covacci A, Petracca R, Pennatini C, Censini S, Armellini D. Detection in an enzyme immunoassay of an immune response to a recombinant fragment of the 128 kilodalton protein (CagA) of Helicobacter pylori. *Eur J Clin Microbiol Infect Dis* 1993; **12**: 739-745

25 **Kashyap RS**, Rajan AN, Ramteke SS, Agrawal VS, Kelkar SS, Purohit HJ, Taori GM, Daginawala HF. Diagnosis of

tuberculosis in an Indian population by an indirect ELISA protocol based on detection of Antigen 85 complex: a prospective cohort study. *BMC Infect Dis* 2007; **7**: 74

26 **Tang JW**, Rohwäder E, Chu IM, Tsang RK, Steinhagen K, Yeung AC, To KF, Chan PK. Evaluation of Epstein-Barr virus antigen-based immunoassays for serological diagnosis of nasopharyngeal carcinoma. *J Clin Virol* 2007; **40**: 284-288

27 **Ohkusu K**. Cost-effective and rapid presumptive identification of gram-negative bacilli in routine urine, pus, and stool cultures: evaluation of the use of CHROMagar orientation medium in conjunction with simple biochemical tests. *J Clin Microbiol* 2000; **38**: 4586-4592

28 **Greco G**, Corrente M, Martella V, Pratelli A, Buonavoglia D. A multiplex-PCR for the diagnosis of contagious agalactia of sheep and goats. *Mol Cell Probes* 2001; **15**: 21-25

29 **Backert S**, Kwok T, Schmid M, Selbach M, Moese S, Peek RM Jr, König W, Meyer TF, Jungblut PR. Subproteomes of soluble and structure-bound Helicobacter pylori proteins analyzed by two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* 2005; **5**: 1331-1345

30 **Sabarth N**, Hurwitz R, Meyer TF, Bumann D. Multiparameter selection of Helicobacter pylori antigens identifies two novel antigens with high protective efficacy. *Infect Immun* 2002; **70**: 6499-6503

31 **Thoreson AC**, Hamlet A, Celik J, Byström M, Nyström S, Olbe L, Svennerholm AM. Differences in surface-exposed antigen expression between Helicobacter pylori strains isolated from duodenal ulcer patients and from asymptomatic subjects. *J Clin Microbiol* 2000; **38**: 3436-3441

32 **Drake IM**, Mapstone NP, Schorah CJ, White KL, Chalmers DM, Dixon MF, Axon AT. Reactive oxygen species activity and lipid peroxidation in Helicobacter pylori associated gastritis: relation to gastric mucosal ascorbic acid concentrations and effect of H pylori eradication. *Gut* 1998; **42**: 768-771

33 **Tari A**, Kitadai Y, Sumii M, Sasaki A, Tani H, Tanaka S, Chayama K. Basis of decreased risk of gastric cancer in severe atrophic gastritis with eradication of Helicobacter pylori. *Dig Dis Sci* 2007; **52**: 232-239

34 **Momynaliev KT**, Rogov SI, Selezneva OV, Chelysheva VV, Akopian TA, Govorun VM. Comparative analysis of transcription profiles of Helicobacter pylori clinical isolates. *Biochemistry* (Mosc) 2005; **70**: 383-390

35 **Wang G**, Hong Y, Olczak A, Maier SE, Maier RJ. Dual Roles of Helicobacter pylori NapA in inducing and combating oxidative stress. *Infect Immun* 2006; **74**: 6839-6846

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Association between Bmi1 and clinicopathological status of esophageal squamous cell carcinoma

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Abstract

AIM: To investigate the clinicopathological roles of Bmi1 in esophageal squamous cell carcinoma (ESCC).

METHODS: Quantitative real-time polymerase chain reaction and immunohistochemical staining for Bmi1 were performed in cancerous and adjacent non-cancerous paraffin-embedded esophageal specimens.

RESULTS: The Bmi1 expression level was unaffected by gender and age. The level of Bmi1 mRNA in ESCC was significantly higher than that in the adjacent non-cancerous tissues (2.181 ± 2.158 *vs* 0.931 ± 0.894, $P = 0.0152$), and its over-expression was aggressively associated with lymph node metastasis (3.580 ± 2.487 *vs* 1.703 ± 0.758, $P = 0.0003$), poorer cell differentiation ($P = 0.0000$) and advanced pathological stage (3.827 ± 2.673 *vs* 1.590 ± 0.735, $P = 0.0001$). The patients were divided into high-expression and low-expression groups based on the median expression level of Bmi1 mRNA, and a shorter overall survival time in the former group was observed. Immunohistochemistry for Bmi1 oncoprotein showed diffusely positive, focally positive and negative expression in 44, 16 and 10 of 70 ESCC cases, respectively, compared with three, two and five of 10 adjacent non-cancerous cases ($P = 0.027$). The positive rate of the oncoprotein in samples of histological grade Ⅲ was higher than that of grade Ⅱ ($P = 0.031$), but its expression had no relation to the lymph node metastasis and pathological staging. In 70 ESCC samples, Bmi1 showed high intense expression in the cytoplasm and less or even no expression in the nucleus.

CONCLUSION: Bmi1 was over-expressed in ESCC. Increased Bmi1 mRNA expression was significantly associated with ESCC progression, and the oncoprotein was largely distributed in the cytoplasm of tumor cells.

Key words: Esophageal squamous cell carcinoma; Bmi1; Quantitative real-time polymerase chain reaction; Immunohistochemistry; Clinicopathology

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INTRODUCTION

Esophageal cancer is one of the most frequently occurring malignancies and the seventh leading cause of cancer-related deaths in the world. It exhibits considerable geographic variation, and 95% of tumors are esophageal squamous cell carcinoma (ESCC)[1]. Besides the impact of the environment, the process of esophageal tumorigenesis at the molecular level is related to disorders of cell amplification, differentiation, senescence and apoptosis. The genetic bases underlying esophageal tumorigenesis have been partly understood in the past few years, including a loss of the anti-oncogene p53 and over-expression of epidermal growth

factor receptor or c-Myc[2]. However, other molecular mechanisms involved in esophageal tumorigenesis progress are still largely unknown.

Bmi1, located in 10p11.23, is a member of the polycomb group (PcG) and a component of the polycomb repressive complex 1. It was initially identified as an oncogene cooperating with c-Myc in the generation of lymphomas in double transgenic mice[3-5]. Several lines of evidence imply that Bmi1 plays an important role in the regulation of cell proliferation and senescence and is required for maintenance of adult hematopoietic and neural stem cells[6-9]. *Bmi1* gene amplification is observed mainly in mantle cell lymphomas[10], and recent serial studies have shown that Bmi1 is overexpressed in many somatic solid tumors such as colon carcinoma, non-small cell lung cancer, breast cancer, head and neck squamous cell carcinoma and gastric carcinoma[11-15], and it may be of diagnostic and prognostic relevance. However, to date, no report about the role of Bmi1 in ESCC has been made. The up-regulation of c-Myc and the down-regulation of p53 and p16 in ESCC[2] tissues make it plausible that Bmi1 may play an important role in the initiation and development of ESCC. This study was designed to investigate Bmi1 expression in ESCC tissues and its impact on patients with ESCC.

MATERIALS AND METHODS

Ethics

The use of study specimens for analyses was approved by the Research Ethics Committee of Nanjing Medical University. Informed written consent was obtained from all the patients.

Case selection

From June 1997 to February 2000, 80 ESCC and 15 adjacent non-cancerous paraffin-embedded samples were obtained from the tumor center of Nanjing First Hospital affiliated to Nanjing Medical University. There were 52 male and 28 female patients with a mean age of 60 years (range: 41-82). The patients were given preoperative examination including biopsy for diagnosis, barium X-ray, CT and ultrasonic endoscopy for clinical staging, and no treatment was given before operation. Radical resection was performed in each patient, and all the samples underwent postoperative pathological examination. There were 54 cases of stage Ⅰ-Ⅱ and 26 cases of stage Ⅲ-Ⅳ cancer according to the American Joint Committee on Cancer staging manual (AJCC, 2002)[16]. With regard to postoperative histological results, 16 were well-differentiated, 40 moderately differentiated and 24 poorly differentiated. Another 70 ESCC and 10 non-cancerous paraffin-embedded samples were enlisted from January 2002 to December 2003 in the same institution. There were 48 male and 22 female patients with a mean age of 61 years (range: 38-89). All the patients were assessed for physiological ability and endoscopy and CT scan were performed for clinical staging prior to routine surgery for ESCC. The postoperative pathological examination found 56 cases of stage Ⅰ-Ⅱ and 14 cases of Ⅲ-Ⅳ cancer according to AJCC (2002) pTNM standards[16]. Clinical follow-up after surgery and diagnosis was based on periodic visits (every 3 mo during the first year, every 6 mo the second year, and then yearly until relapse).

RNA extraction and quantitative real-time polymerase chain reaction (PCR)

Real-time quantitative PCR was performed on paraffin-embedded sections from 80 ESCC patients and 15 adjacent non-cancerous samples. Briefly, total RNA was extracted by Recover All Total Nucleic Acid Isolation kit (Ambion), and 10 mg of DNase-treated total RNA was used for reverse transcription with Superscript Ⅲ (Invitrogen, Carlsbad, CA, USA). An aliquot representing 100 ng input RNA was amplified by quantitative real-time PCR using the TaqMan PCR reagent kit and assay-on-demand gene expression products (FAM/Sybr, Foster City, CA, USA). RNA extracted from a non-cancerous lesion in one patient was used as a standard. After reverse transcription, standard cDNA was serially diluted to obtain five standard solutions for use in PCR to generate the reference curve. Sequences of the Bmi1 bidirectional primers were designed using Primer 5.0 rotor-gene 6.0 (Corbett Research) as follows: Bmi1 sense 5'-GTATTCCC TCCACCTCTTCTTG-3', Bmi1 antisense 5'-TGCTGAT GACCCATTTACTGAT-3'. House-keeping gene: β-actin sense 5'-CCTGTACGCCAACACAGTGC-3', antisense 5'-ATACTCCTGCTTGCTGATCC-3'. Quantitative real-time PCR was carried out in a Rotor-Gene 3000 PCR kit (Corbett Research) with 10000 × Syber Green (Molecular Probes). After reverse transcription, standard cDNA was serially diluted to obtain five standard solutions for use in PCR to generate the reference curve. The relative amount of cDNA in each sample was measured by interpolation using the standard curve (Figure 1), and then the relative ratio of Bmi1 to β-actin (housekeeping gene) expression was calculated for each ESCC sample.

Immunohistochemistry

Histopathological evaluation was performed on 4-μm slides stained with hematoxylin and eosin (HE) (Figure 2). Commercially available rabbit monoclonal antibodies against Bmi-1 (1:100, Santa Cruz Biotechnology) were used as primary antibodies. A paraffin section of the ESCC sample was deparaffinized and rehydrated in graded alcohol to water. Antigenic enhancement was performed by submerging in citrate buffer (pH 6.0) and microwaving. Endogenous peroxide activity was quenched by applying 0.3% hydrogen peroxide for 10 min, followed by incubation with 1% BSA to block the non-specific binding. The primary monoclonal anti-Bmi1 antibody was incubated for 60 min at 37℃. After washing, the tissue section was reacted with the biotinylated anti-rabbit IgG, and visualized using a Dako Envision System horseradish peroxidase for monoclonal antibodies. The slides were immersed in the prepared diaminobenzidine solution, which produces a brown precipitate at the level of the antigen-primary antibody. Slides were then counterstained with hematoxylin, dehydrated through alcohols of increasing concentration, placed in xylene, coverslipped using Permount, and

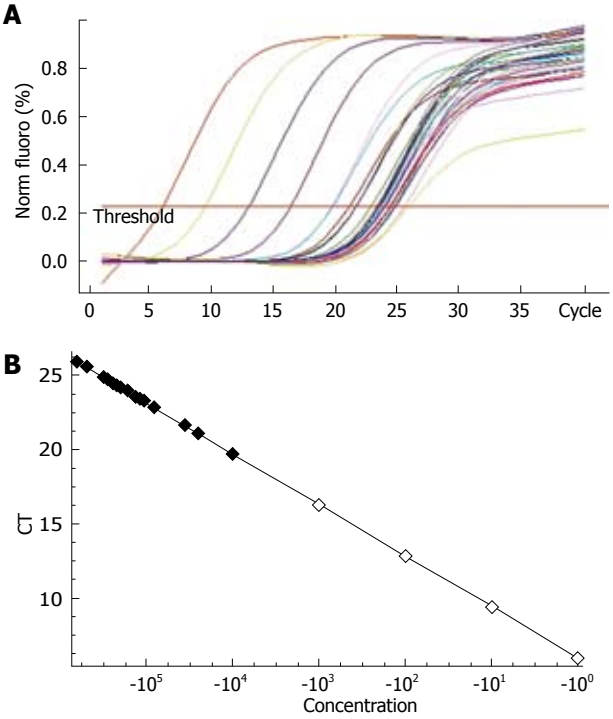

Figure 1 Amplification curve (A) and standard curve (B) of quantitative real-time PCR for Bmi1 mRNA. Total RNA was extracted for subsequent reverse transcription, and standard cDNA was serially diluted to obtain five standard solutions (1×10^{-1}, 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5}) for use in PCR to generate the reference curve, slope rate of the straight line (a) = -3.42089, intercept (b) = 5.97517, correlation coefficient (*r*) = 0.9996. The strength of Bmi1 and β-actin was directly generated by the machine.

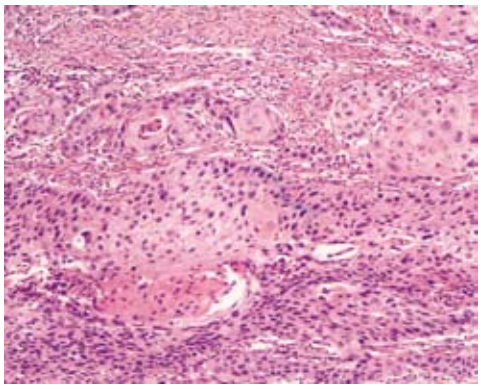

Figure 2 Staining of ESCC tissues. The tumor cells of cancerous tissues were stained as violet in the nucleus and pink in the cytoplasm (× 100).

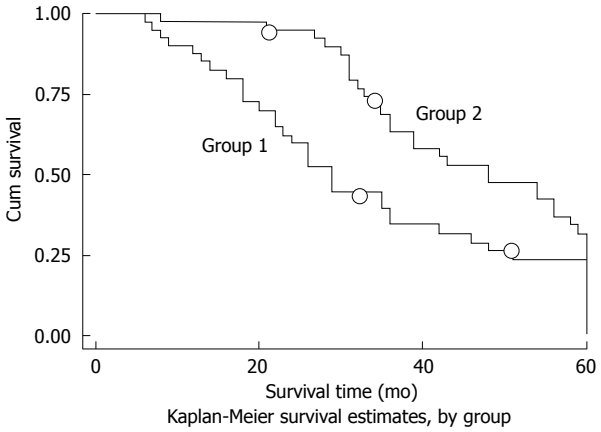

Figure 3 Effects of Bmi1 mRNA expression on prognosis of ESCC patients. Two patients did not complete the 5-year follow-up in each group (small circles). The survival rate was higher in the down-expression group (group 2) than in the up-expression group (group 1), $\chi^2 = 4.41$, $P = 0.0356$.

analyzed under light microscopy. Each section was evaluated by at least two independent professional pathologists, the distribution of Bmi-1 was scored on a semi-quantitative scale, the percentage of positive tumor cells was recorded and divided as follows: negative (< 10% of tumor cells positive), locally positive (10%-50% of tumor cells positive), and diffusely positive (> 50% of tumor cells positive).

Statistical analysis

Data were expressed as mean ± SD and analyzed using the Stata v9.0-CYGiSO bin (Computer Resource Center, USA). The significance of differences among groups was determined by Student's *t* test and χ^2 test or Fisher's exact test. The difference in free survival between groups was analyzed by the Kaplan-Meier method and log-rank test. The starting point for calculating free survival was the date of surgery, and the endpoint was the date of death. Statistical significance was assessed at the two sided 5% level, and *P* values less than 0.05 were considered statistically significant.

RESULTS

Quantitative real-time PCR analysis

Clinical follow-up was made in 76 patients. The comparative expression levels were determined as a ratio between the Bmi1 and the housekeeping gene (β-actin) to correct for variation in the amounts of mRNA. The 5-year survival rate was 37.46%. The Bmi1 mRNA level was higher in the cancerous tissues than that in the non-cancerous tissues (2.181 ± 2.158 *vs* 0.931 ± 0.894, $P = 0.0152$). The Bmi1 expression level was unaffected by gender and age. The expression level of Bmi1 mRNA was much lower at the Ⅰ-Ⅱ stage than that at the Ⅲ-Ⅳ stage, which varied inversely with the differentiation grade, and was lower in cases without metastatic lymph nodes than in those with metastatic lymph nodes (Table 1). Based on the detection of Bmi-1 median expression level (1.085), patients were divided into the down-expression group (Bmi1 mRNA level < 1.085) and the up-expression group (Bmi1 mRNA level > 1.085), and the accumulated survival rate was higher in the former than that in the latter (Figure 3).

Protein analysis

Bmi1 oncoprotein expression was diffusely positive, focally positive and negative in 44, 16 and 10 of 70 ESCC cases, respectively. Compared with three, two and five of 10 adjacent non-cancerous cases, Bmi1 protein was significantly increased in ESCC samples ($P = 0.027$). Analysis of protein localization in ESCC cells was made, and the tumor cells with Bmi1 staining were divided

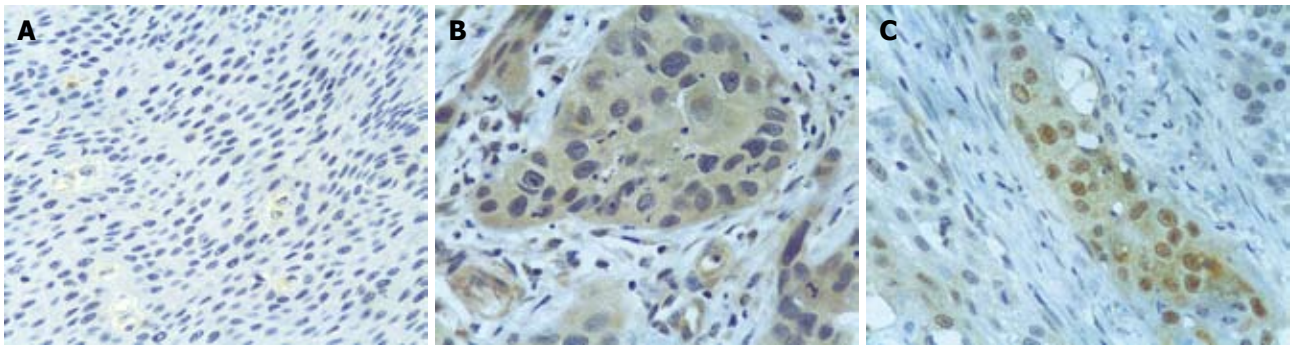

Figure 4 Immunohistochemical staining of ESCC using antibody to Bmi-1. A: Bmi1 protein expression in adjacent non-cancerous tissues (× 400); B: Cytoplasm staining of Bmi1 in ESCC cells (× 400); C: Intense Bmi-1 staining in the nucleus in ESCC cells (× 400). The brown staining under light microscopy indicates positivity.

Table 1 Associations of Bmi-1 mRNA expression in ESCC tissues with clinicopathological characteristics (mean ± SD)

Parameter	Bmi-1 mRNA	*P*
Age (yr)		
≥ 60	2.312 ± 2.171	0.3816
< 60	2.167 ± 2.045	
Gender		
Male	2.402 ± 2.359	0.2133
Female	1.770 ± 1.690	
Lymph node metastases		
Yes	3.580 ± 2.487	0.0003
No	1.703 ± 0.758	
Stage		
Ⅲ/Ⅳ	3.827 ± 2.673	0.0001
Ⅰ/Ⅱ	1.590 ± 0.735	
Histological grade		
Ⅰ	0.881 ± 0.418	0.000
Ⅱ	1.858 ± 0.979	
Ⅲ	3.580 ± 2.487	

Quantitative real-time PCR was employed to identify Bmi1 mRNA expression. The comparative expression levels were determined as a ratio between Bmi1 and housekeeping gene (β-actin) to correct variation in the amount of mRNA.

Table 2 Relationship between Bmi-1 protein expression in ESCC tissues and clinicopathological status

Parameter	> 50%	10%-50%	< 10%	*P*
Age (yr)				
≥ 60	23	9	4	0.784
< 60	21	7	6	
Histological grade				
Ⅰ	7	3	3	0.079
Ⅱ	28	5	6	
Ⅲ	9	8	1	
Lymph node metastases				
+	11	9	3	0.073
-	33	7	7	
Stage				
Ⅲ/Ⅳ	9	4	1	0.691
Ⅰ/Ⅱ	35	12	9	
Location				
Nucleus	0	7	8	0.000
Cytoplasm	30	18	16	
Both nucleus and cytoplasm	5	15	20	

ESCC was subjected to immunohistochemistry using antibodies to Bmi1. χ^2 test was used to detect the difference between Bmi1 oncoprotein and clinicopathological status.

into three categories, referring to both the nucleus and cytoplasm. In all 70 tested ESCC tissues, Bmi1 presented highly intense expression in both nucleus and cytoplasm with varied degrees, accompanied by less or even no expression in the nucleus, with significant differences (Figure 4). The positivity of the oncoprotein in samples of histological grade Ⅲ was more frequent than that of grade Ⅱ, but no significant differences were observed between other differentiated grades ($P = 0.031$). No relationship was found between the Bmi1 protein expression and lymph node metastases, pathological staging and cell differentiation (Table 2). Clinical follow-up was made in 67 patients. The 5-year survival rate was 40.01%. There was no statistical difference in survival rates between groups according to the Bmi1 expression ($P = 0.1704$).

DISCUSSION

ESCC is a major cause of morbidity and mortality worldwide[1], and it is significant to identify a biological genetic molecular marker related to its pathophysiological processes.

Epigenetic aberrations, the heritable changes in gene expression that occur in chromatin structure including DNA methylation, histone post-translational modifications and nucleosomal remodeling, rather than the DNA sequence, are involved in cancer development[17-20]. Bmi1, the first PcG protein found, is a chromatin modifier implicated in the tumorigenesis through negatively regulating the gene expression such as the INK4A locus, which is thought to regulate p53 and the Rb signaling pathway in cooperation with c-myc[5,6,19,21]. In this retrospective study, we examined the Bmi1 expression and investigated its impact on ESCC patients.

Bmi1 mRNA expression was significantly higher in the ESCC samples than in the adjacent non-cancerous tissues, and so was Bmi1 protein expression, which indicated that Bmi1 plays an important role in the development of ESCC, and has diagnostic value.

Dirks[22] has reported that Bmi1-deficient tumors may be less aggressive because they have fewer stem cells. Bmi1 expression is also found inversely correlated with the differentiation grade of clear cell carcinoma

and is involved in tumor progression[23]. Our data are in agreement with the findings by previous publications that the acquisition of metastatic ability of tumor cells is considered a late event in the evolution of malignant tumors. We found that the Bmi1 mRNA expression was higher in the stage Ⅰ/Ⅱ tissues than in stage Ⅲ/Ⅳ, significantly lower in patients without metastatic lymph nodes, and inversely related to cell differentiation. The oncoprotein was more frequently observed in tissues with poorer differentiation. These results suggest that Bmi1 expression may not be required for initiation of ESCC but is required for its progression. It may be a guide for postoperative therapy and a differentiation marker in ESCC with high malignancy. Furthermore, we discovered that the accumulated survival of patients in the up-expression group was much shorter than that of patients in the down-expression group, which may predict survival in ESCC patients.

It was interesting to note that Bmi1 protein expression was negatively correlated with malignant grade, including lymph node metastasis and advanced pathological stage. This may have been because the samples for protein analysis were obtained at different periods than those for mRNA analysis, which resulted in a different selection bias. Also, the number of lymph nodes resected by different operators varied, and the lymph nodes removed during surgery for pathological diagnosis may have been misdiagnosed as metastatic lymph nodes.

The PcG protein Bmi1 showed abundant nuclear expression in prostate cancer, colorectal cancer and gastric carcinoma[11,15,24]. However, in our study, cytoplasmic staining appeared in most of the tumor cells with less or even no expression in the nucleus alone, which suggests that Bmi1 produces a marked effect on the development of ESCC, mainly in the cytoplasm. This is inconsistent with the PcG pathway activation hypothesis that states that increased Bmi1 expression in cancer cells is associated with elevated levels of H2AubiK119 and H3metK27 histones, which suppress the expression of the INK4a/ARF locus in the nucleus[21].

The mechanisms of Bmi1 up-expression that induce adverse pathological and clinical features in ESCC patients are poorly understood. Some previous studies have shown that Bmi1 expression is a potential escape mechanism and associated with markedly increased likelihood of treatment failure and disease relapse after surgery[25,26]. Qin *et al*[27] have found that down-regulation of Bmi-1 enhances 5-fluorouracil-induced apoptosis in nasopharyngeal carcinoma cells and have suggested that the combination of 5-FU treatment and Bmi-1 depletion might be a potential clinical strategy for cancer chemotherapy.

However, we believe that further investigations on larger series of ESCC patients, including clinical follow-up and novel molecular techniques, are needed to confirm our conclusions. Whether Bmi1 can be used for accurate prediction of ESCC and its potential chemosensitivity to current pharmaceutical treatment needs further study.

COMMENTS

Background

Bmi1, a member of the polycomb group and a component of the polycomb repressive complex1, has been considered as a oncogene involved in many solid and hematological malignant tumors, and it may be of diagnostic and prognostic relevance. However, to date, no report about the role of Bmi1 in esophageal squamous cell carcinoma (ESCC) has been made. However, the up-regulation of c-Myc and the down-regulation of p53 and p16 in ESCC tissues make it plausible that Bmi1 may play an important role in the initiation and progression of ESCC.

Research frontiers

This research, for the first time, investigated the expression of Bmi1 and its clinicopathological role in patients with ESCC.

Innovations and breakthroughs

A significant upregulation of Bmi1 was observed in cancerous tissues in contrast to adjacent non-cancerous paraffin-embedded esophageal specimens at the mRNA and the protein level by quantitative real-time polymerase chain reaction (PCR) and immunohistochemistry. Furthermore, overexpression of Bmi1 positively correlated with lymph node metastases, pathological stage and differentiation grade at the mRNA level.

Applications

Bmi1 may act as a guide for the postoperative therapy and a differentiation marker of ESCC with high malignancy, and for prediction of the survival of ESCC patients.

Peer review

The authors investigated the expression of Bmi1 at the mRNA and protein level in patients with ESCC in comparison with healthy adjacent tissue, by real-time PCR and immunohistochemistry. They observed a significant up-regulation of Bmi1 in tumor tissues in contrast to healthy control tissues. Up-regulation correlated positively with lymph node metastases, stage and histological grading at the mRNA level. However, the histological analysis showed no such correlation. The authors conclude that Bmi1 expression is common in ESCC and may serve as a marker to predict lymph node metastasis and survival in ESCC patients.

REFERENCES

1 **Fisichella PM**, Patti MG. Esophageal cancer: eMedicine: oncology, 2009-03-04. Available from: URL: http://emedicine.medscape.com/article/277930-overview

2 **Kuwano H**, Kato H, Miyazaki T, Fukuchi M, Masuda N, Nakajima M, Fukai Y, Sohda M, Kimura H, Faried A. Genetic alterations in esophageal cancer. *Surg Today* 2005; **35**: 7-18

3 **Haupt Y**, Alexander WS, Barri G, Klinken SP, Adams JM. Novel zinc finger gene implicated as myc collaborator by retrovirally accelerated lymphomagenesis in E mu-myc transgenic mice. *Cell* 1991; **65**: 753-763

4 **Haupt Y**, Bath ML, Harris AW, Adams JM. bmi-1 transgene induces lymphomas and collaborates with myc in tumorigenesis. *Oncogene* 1993; **8**: 3161-3164

5 **Jacobs JJ**, Scheijen B, Voncken JW, Kieboom K, Berns A, van Lohuizen M. Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes Dev* 1999; **13**: 2678-2690

6 **Jacobs JJ**, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 1999; **397**: 164-168

7 **Park IK**, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ, Clarke MF. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 2003; **423**: 302-305

8 **Molofsky AV**, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SJ. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* 2003; **425**: 962-967

9 **Molofsky AV**, He S, Bydon M, Morrison SJ, Pardal R.

Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways. *Genes Dev* 2005; **19**: 1432-1437

10 **Beà S**, Tort F, Pinyol M, Puig X, Hernández L, Hernández S, Fernandez PL, van Lohuizen M, Colomer D, Campo E. BMI-1 gene amplification and overexpression in hematological malignancies occur mainly in mantle cell lymphomas. *Cancer Res* 2001; **61**: 2409-2412

11 **Kim JH**, Yoon SY, Kim CN, Joo JH, Moon SK, Choe IS, Choe YK, Kim JW. The Bmi-1 oncoprotein is overexpressed in human colorectal cancer and correlates with the reduced p16INK4a/p14ARF proteins. *Cancer Lett* 2004; **203**: 217-224

12 **Vonlanthen S**, Heighway J, Altermatt HJ, Gugger M, Kappeler A, Borner MM, van Lohuizen M, Betticher DC. The bmi-1 oncoprotein is differentially expressed in non-small cell lung cancer and correlates with INK4A-ARF locus expression. *Br J Cancer* 2001; **84**: 1372-1376

13 **Guo WJ**, Zeng MS, Yadav A, Song LB, Guo BH, Band V, Dimri GP. Mel-18 acts as a tumor suppressor by repressing Bmi-1 expression and down-regulating Akt activity in breast cancer cells. *Cancer Res* 2007; **67**: 5083-5089

14 **Prince ME**, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF, Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 2007; **104**: 973-978

15 **Liu JH**, Song LB, Zhang X, Guo BH, Feng Y, Li XX, Liao WT, Zeng MS, Huang KH. Bmi-1 expression predicts prognosis for patients with gastric carcinoma. *J Surg Oncol* 2008; **97**: 267-272

16 **Greene FL**, American Joint Committee on Cancer, American Cancer Society. AJCC cancer staging manual. 6th ed. New York: Springer, 2002: 91-98

17 **Fraga MF**, Esteller M. DNA methylation: a profile of methods and applications. *Biotechniques* 2002; **33**: 632, 634, 636-649

18 **Quina AS**, Buschbeck M, Di Croce L. Chromatin structure and epigenetics. *Biochem Pharmacol* 2006; **72**: 1563-1569

19 **Sparmann A**, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer* 2006; **6**: 846-856

20 **Cao R**, Tsukada Y, Zhang Y. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. *Mol Cell* 2005; **20**: 845-854

21 **Shilatifard A**. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu Rev Biochem* 2006; **75**: 243-269

22 **Dirks P**. Bmi1 and cell of origin determinants of brain tumor phenotype. *Cancer Cell* 2007; **12**: 295-297

23 **Kozakowski N**, Soleiman A, Pammer J. BMI-1 expression is inversely correlated with the grading of renal clear cell carcinoma. *Pathol Oncol Res* 2008; **14**: 9-13

24 **van Leenders GJ**, Dukers D, Hessels D, van den Kieboom SW, Hulsbergen CA, Witjes JA, Otte AP, Meijer CJ, Raaphorst FM. Polycomb-group oncogenes EZH2, BMI1, and RING1 are overexpressed in prostate cancer with adverse pathologic and clinical features. *Eur Urol* 2007; **52**: 455-463

25 **Liu S**, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res* 2005; **7**: 86-95

26 **Berezovska OP**, Glinskii AB, Yang Z, Li XM, Hoffman RM, Glinsky GV. Essential role for activation of the Polycomb group (PcG) protein chromatin silencing pathway in metastatic prostate cancer. *Cell Cycle* 2006; **5**: 1886-1901

27 **Qin L**, Zhang X, Zhang L, Feng Y, Weng GX, Li MZ, Kong QL, Qian CN, Zeng YX, Zeng MS, Liao DF, Song LB. Downregulation of BMI-1 enhances 5-fluorouracil-induced apoptosis in nasopharyngeal carcinoma cells. *Biochem Biophys Res Commun* 2008; **371**: 531-535

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Polymorphisms of alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 and esophageal cancer risk in Southeast Chinese males

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Abstract

AIM: To evaluate the impact of alcohol dehydrogenase-2 (ADH2) and aldehyde dehydrogenase-2 (ALDH2) polymorphisms on esophageal cancer susceptibility in Southeast Chinese males.

METHODS: Two hundred and twenty-one esophageal cancer patients and 191 healthy controls from Taixing city in Jiangsu Province were enrolled in this study. ADH2 and ALDH2 genotypes were examined by polymerase chain reaction and denaturing high-performance liquid chromatography. Unconditional logistic regression was used to calculate the odds ratios (OR) and 95% confidence interval (CI).

RESULTS: The ADH G allele carriers were more susceptible to esophageal cancer, but no association was found between ADH2 genotypes and risk of esophageal cancer when disregarding alcohol drinking status. Regardless of ADH2 genotype, ALDH2G/A or A/A carriers had significantly increased risk of developing esophageal cancer, with homozygous individuals showing higher esophageal cancer risk than those who were heterozygous. A significant interaction between ALDH2 and drinking was detected regarding esophageal cancer risk; the OR was 3.05 (95% CI: 1.49-6.25). Compared with non-drinkers carrying both ALDH2 G/G and ADH2 A/A, drinkers carrying both ALDH2 A allele and ADH2 G allele showed a significantly higher risk of developing esophageal cancer (OR = 8.36, 95% CI: 2.98-23.46).

CONCLUSION: Both ADH2 G allele and ALDH2 A allele significantly increase the risk of esophageal cancer development in Southeast Chinese males. ALDH2 A allele significantly increases the risk of esophageal cancer development especially in alcohol drinkers. Alcohol drinkers carrying both ADH2 G allele and ALDH2 A allele have a higher risk of developing esophageal cancer.

Key words: Alcohol dehydrogenase-2; Aldehyde dehydrogenase-2; Gene polymorphisms; Alcohol drinking; Esophageal cancer

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INTRODUCTION

There is epidemiological evidence showing that alcohol intake is associated with increased esophageal cancer risk[1]. Acetaldehyde, the oxidative metabolite of ethanol, is recognized to be carcinogenic in animals and suspected to have similar effects in humans[2]. Ethanol is oxidized to acetaldehyde and then to acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), both of which have genetic

polymorphisms. People homozygous for the ALDH2*2 allele (Glu487Lys, Lys or A allele) do not have any ALDH activity. Heterozygous individuals carrying the reference (G) and variant (A) alleles (ALDH2*1/2, or A/G) show only 1/16 of the activity seen in ALDH2*1 homozygotes (G/G)[3-5]. ADH2*2 allele (Arg47His, His or A allele) encodes a superactive subunit of ADH2 and that superactive ADH2*2 homodimer has about a 40 times higher Vmax than the less-active ADH2*1/2*1 form of ADH2[3,4]. Therefore, shortly after alcohol drinking, individuals carrying both variant ADH2 and ALDH2 would accumulate a large amount of aldehyde that cannot be efficiently oxidized to the non-toxic acetic acid. Different combinations of ADH2 and ALDH2 genotypes may influence the individual susceptibility to cancer.

Taixing city, located in the middle part of Jiangsu Province, China, has relatively high incidence and mortality rates for esophageal cancer (in 2005, the age-adjusted mortality rate was 53.66 per 100 000 for esophageal cancer). Our previous study has shown that more than 40% of adult residents in Taixing drink wine and that drinking is a risk factor for esophageal cancer in this area[6]. We have also shown relationships between ALDH2 and the risk of esophageal cancer, but no statistically significant association was found[7]. In this study, we increased the sample size to define the individual and combined roles of ADH2, ALDH2 polymorphisms and drinking habits in the risk analysis for esophageal cancer development in Southeast Chinese males.

MATERIALS AND METHODS

Study subjects

We recruited male patients who were histopathologically diagnosed as having esophageal carcinoma from January 2005 to December 2006. Population-based male controls were recruited from healthy residents in the villages or towns where cases resided. All study subjects have completed a questionnaire administrated by a trained interviewer, covering residential, occupational, social, living style, psychological and economical factors. The interviewer then collected blood samples of subjects from a peripheral vein after obtaining their oral informed consents. The collected blood samples were shipped to the public health center within a day. Buffy coat was then separated and stored at -30℃. We defined a drinker as a person who drinks at least once per week (alcohol intake more than 40 g) and continuously drinks for at least half the year. A few patients and residents refused to participate in our study, but the overall response rate was 97% for patients and 95% for controls, respectively. The Ethics Committee of Jiangsu Provincial Institute of Cancer Research approved this study. Associations could not be assessed in women because of sparse drinking habits.

DNA extraction and genotyping of ADH2 and ALDH2

Whole blood was collected into EDTA-coated tubes and centrifuged for 15 min. The buffy coat layer was isolated. Genomic DNA was extracted from 200 μL of buffy coat using a Qiagen QIAamp DNA blood mini kit (QIAGEN Inc., Valencia, CA). Genotyping of ADH2 and ALDH2 was determined by polymerase chain reaction (PCR) and denaturing high-performance liquid chromatography (DHPLC).

The sequences of primers used in this study are F: 5'-GGGCTTTAGACTGAATAACCTTGG-3' and R: 5'-AGGGAAAGAGGAAACTCCTGAA-3' for ADH2 Arg47His, and F: 5'-TGCTATGATGTGTTTGGAGCC-3' and R: 5'-GGCTCCGAGCCACCA-3' for ALDH2 Glu487Lys. Reactions were carried out in a total volume of 25 μL containing 20 pmol of each primer, 0.25 mmol/L each dNTPs, 2.0 mmol/L $MgCl_2$, 2.5 μL 10 × buffer, 1 IU hotTag polymerase and 0.5 μL genomic DNA. PCR conditions were as follows: denaturation at 95℃ for 7 min, followed by 35 cycles at 95℃ for 30 s, at 62℃ for 30 s, at 72℃ for 30 s, and a final extension at 72℃ for 5 min. The products were denatured at 94℃ for 4 min, and their temperature was declined to 25℃ step by step according to 0.1℃/s.

Transgenomic WAVE DNA fragment analysis system (WAVE-300, Transgenomic, USA) and associated WAVEMAKER software were used for genotyping. An aliquot (5 μL) of the PCR products was directly injected into a DNASep column. The column mobile phase for sample elution consisted of a mixture of buffer A [0.1 mol/L triethylammonium acetate (TEAA)] and buffer B (0.1 mol/L TEAA with 25% acetonitrile). Samples were eluted at a linear gradient of buffer B over a 4.5-min period at a constant flow rate of 0.9 mL/min. For each DNA region, DHPLC conditions were established by a titration analysis at 1-3℃ above and below the mean melting temperature predicted by software simulation. There were three genotypes: namely G/G, G/A, and A/A, for ADH2 Arg47His and ALDH2 Glu487Lys, respectively.

Statistical analysis

All analyses were done with the SAS (version 6.02) and Epi-info (version 6.04) statistical package. Odds ratios (OR) and 95% confidence intervals (CI) were adjusted by unconditional logistic regression analysis. Gene-environment interactions were evaluated by additive model and expressed in terms of synergy index (S) and attributable proportions of interaction (API)[8]. The Mantel-Haenszel χ^2 method was used to test for significant associations between the ADH2 or ALDH2 genotype and cancer risk.

RESULTS

Four hundred and twelve Jiangsu males were enrolled in this study. Numbers of subjects were 221 cases with esophageal cancer and 191 controls (Table 1). The proportional distributions of age, occupation, education, smoking and drinking did not significantly differ between cases and controls, but the proportional distributions

Table 1 Background characteristics of cases and their controls

	Controls *n* (%)	Cases *n* (%)	χ^2 MH	*P*
Age (yr)				
<50	10 (4.53)	8 (4.19)		
50-59	57(25.79)	60 (31.41)		
60-69	98 (44.34)	80 (41.88)		
> 70	56 (25.34)	43 (22.52)	0.91	0.341
Total	221	191		
Income (yuan/year per person)				
Ten years before	2097	3040		< 0.01
Recent years	3629	4746		< 0.01
Drinking status				
Non-drinker	96 (43.44)	94 (49.21)		
Drinker	125 (56.56)	97 (50.79)	1.38	0.24
Smoking status				
Non-smoker	70 (31.67)	58 (30.37)		
Smoker	151 (68.33)	133 (69.63)	0.78	0.08

Table 2 ORs and their 95% CIs for esophageal cancer with reference to ALDH2 and ADH2 polymorphisms

	Controls *n* (%)	Cases *n* (%)	OR[1] (CI)	OR[2] (CI)
ALDH2 genotype				
G/G	90 (40.73)	114 (59.69)	1.00	1.00
G/A	89 (40.27)	66 (34.55)	1.71 (1.10-2.66)	1.70 (1.08-2.68)
A/A	42 (19.00)	11 (5.96)	4.84 (2.25-10.61)	5.69 (2.51-12.18)
G/A+A/A	131 (59.27)	77 (40.51)	2.15 (1.43-3.26)	2.19 (1.43-3.34)
ADH2 genotype				
A/A	106 (47.96)	108 (56.54)	1.00	1.00
A/G	96 (43.44)	75 (39.27)	1.30 (0.85-1.99)	1.21 (0.79-1.86)
G/G	19 (8.60)	8 (4.19)	2.42 (1.02-5.77)	2.78 (1.06-7.29)
A/G+G/G	115 (52.04)	83 (43.46)	1.41 (0.96-2.08)	1.34 (0.89-2.04)
Allele frequencies				
ALDH2				
G	269 (60.86)	294 (76.96)	1.00	
A	173 (39.14)	88 (23.04)	2.15 (1.57-2.95)	
ADH2				
A	308 (69.68)	291 (76.18)	1.00	
G	134 (30.32)	91 (23.82)	1.39 (1.01-1.92)	

[1]Crude OR; [2]Adjusted odds ratios (ORs) were adjusted for income.

of income (ten years before and recent years) were significant lower in cases than in controls (4.52 and 3.64 for *T* value, $P < 0.01$).

As shown in Table 2, the frequency of ALDH2 G/G, G/A and A/A genotypes were 40.73%, 40.27% and 19.00% in cases and 59.69%, 34.55% and 5.96% in controls respectively. The distribution of the ALDH2 genotypes was significant different between controls and cases ($\chi^2 = 22.30$, $P < 0.01$). The frequency of ADH2 A/A, A/G and G/G genotypes demonstrated no significant differences between cases and controls ($\chi^2 = 4.92$, $P = 0.085$). The allelic distribution of ADH2 and ALDH2 polymorphisms was in Hardy-Weinberg equilibrium ($P > 0.05$).

As for income-adjusted odds ratio, compared with ALDH2 G/G carriers, the OR was 1.70 (95% CI: 1.08-2.68) for G/A carriers, 5.69 (95% CI: 2.51-12.18) for the A/A carriers and 2.19 (95% CI: 1.43-3.34) for the two genotypes combined. Compared with the subjects with ADH2 A/A genotype, subjects with G/G genotypes had an increased OR of 2.78 (95% CI: 1.06-7.29). As for allelic comparison, the OR was 2.15 (95% CI: 1.57-2.95) for ALDH2 A allele and 1.39 (95% CI: 1.01-1.92) for ADH2 G allele carriers.

Table 3 Interaction between ALDH2 and ADH2 genotype and the ORs for esophageal cancer

ADH2	ALDH2	Cases	Controls	OR[1] (95% CI)	OR[2] (95% CI)
A/A	G/G	44	68	1.00	1.00
G/A+G/G	G/G	46	46	1.55 (0.89-2.70)	1.46 (0.79-2.70)
A/A	G/A	40	33	1.87 (1.03-3.40)	1.93 (0.99-3.75)
G/A+G/G	G/A	49	33	2.29 (1.28-4.11)	2.10 (1.13-3.91)
A/A	A/A	22	7	4.98 (1.91-12.33)	5.28 (1.88-14.83)
G/A+G/G	A/A	20	4	7.73 (2.48-24.13)	12.22 (2.62-56.91)

[1]Crude OR; [2]ORs were adjusted for income.

Regardless of ADH2 genotype, ALDH2G/A or A/A carriers were found to have significantly increased risk of developing esophageal cancer. ALDH2 A/A homozygotes have higher esophageal cancer risk than ALDH2G/A heterozygotes. As compared to the subjects with ADH2A/A and ALDH2 G/G genotypes (double wild type), those with variant alleles for both ADH2 (G allele) and ALDH2 (A allele) had a significantly increased OR. ALDH2 A/A homozygotes who were also carrying ADH2 G allele had the highest OR of 12.22 (95% CI: 2.62-56.91) (Table 3).

The ALDH2 A/A genotype alone showed a moderate increase of esophageal cancer risk in both drinkers and non-drinkers (Table 4). No significant relationship was found in analysis of ADH2 genotypes. Compared with non-drinkers with both ALDH2 G/G and ADH2 A/A genotypes, drinkers with ALDH2 A and ADH2 G alleles showed a significantly elevated risk of esophageal cancer (OR = 8.36, 95% CI: 2.98-23.46)

The OR for esophageal cancer among alcohol drinkers with ALDH2 A allele was markedly increased to 3.05 (95% CI: 1.49-6.25) compared to non-drinkers with ALDH2 G/G genotypes (Table 5). A significant gene-environment interaction between alcohol drinking and ALDH2 was observed for esophageal cancer risk (S = 2.93). The population attributable risk due to alcohol drinking by ALDH2 A allele carriers was estimated to be 41% for esophageal cancer (API = 0.41).

DISCUSSION

Our previous studies showed that drinking was associated with increased esophageal, stomach and liver cancer in Taixing[6]. We also found that it was not ADH2 but ALDH2 polymorphisms that had a significant interaction with heavy alcohol consumption in the development of hepatocellular carcinoma (HCC)[9]. In the present study, both ADH2 G allele and ALDH2 A allele significantly increased the risk of esophageal cancer development. ALDH2 A allele significantly increases the risk of esophageal cancer development

Table 4 Analysis of ALDH2 and ADH2 genotypes and risk of esophageal cancer with reference to drinking habits

Genotypes		Non-drinker			Drinker		
		Cases	Controls	OR[1] (95% CI)	Cases	Controls	OR[1] (95% CI)
ALDH2							
G/G		26	42	1.00	64	72	1.00
G/A		43	44	1.29 (0.65-2.55)	46	22	2.47 (1.27-4.82)
A/A		27	8	4.67 (1.63-13.38)	15	3	8.63 (2.07-35.95)
G/A+A/A		70	52	1.78 (0.94-3.37)	61	25	3.08 (1.65-5.78)
ADH2							
A/A		50	53	1.00	56	55	1.00
G/A		42	38	1.31 (0.70-2.46)	54	37	1.18 (0.64-2.16)
G/G		4	3	2.10 (0.35-12.54)	15	5	2.90 (0.85-9.90)
G/A+G/G		46	41	1.37 (0.74-2.54)	69	42	1.36 (0.76-2.43)
ALDH2	ADH2						
G/G	A/A	10	28	1.00	34	40	2.02 (0.79-5.17)
G/A+A/A	G/A+G/G	30	27	2.84 (1.10-7.31)	39	10	8.36 (2.98-23.46)

[1]ORs were adjusted for income.

Table 5 Interaction between alcohol drinking and ALDH2 genotype and the ORs for esophageal cancer

Genotype[1]	Drinker[2]	Cases	Controls	OR[1] (95% CI)
-	-	26	42	1.00
-	+	64	72	0.92 (0.48-1.78)
+	-	70	52	1.78 (0.94-3.37)
+	+	61	25	3.05 (1.49-6.25)

[1]-: ALDH2G/G; +: ALDH2G/A and A/A; ORs were adjusted for income; [2]-: Non-drinker; +: Drinker.

especially in alcohol drinkers. Alcohol drinkers carrying both ADH2 G allele and ALDH2 A allele have a higher risk of developing esophageal cancer.

There is no doubt that the differences in environment exposures/lifestyle influence the genetic susceptibility to cancer. There have been a lot of papers regarding the relationship between ADH2 and ALDH2 polymorphisms and esophageal cancer susceptibility. Chao *et al*[10] found that Chinese alcoholic patients with the ADH G and ALDH2 A allele were more susceptible to esophageal cancer. Many studies found that the inactive ALDH2 genotypes had a significantly increased risk for developing esophageal cancer and that a gene-environment interaction exists between alcohol drinking and the inactive ALDH2 genotypes[2,11-16]. Boonyaphiphat *et al*[17] did not find ALDH2 increased the risk significantly (OR of ALDH G/A 1.57, 95% CI: 0.89-2.76). However, the combined at risk genotypes, ADH A/A and ALDH G/A increased risk by four-fold and heavy drinkers > 60 g/d harboring ADH A/A or ALDH G/A had about an 11-fold increased risk. Our previous study showed no statistically significant association between ALDH2 and esophageal cancer susceptibility[7]. However, in this study with a larger sample size, we found that the ALDH2 A allele showed a moderately increased risk for esophageal cancer as compared with ALDH2 G/G carriers, and significant gene-environment interactions between alcohol drinking and ALDH2 were observed regarding esophageal cancer risk (S = 2.93). The population attributable risk due to alcohol drinking by ALDH2 A allele carriers was estimated to be 41% for esophageal cancer. Yokoyama *et al*[18] also found that an extraordinarily high proportion of excessive risk for esophageal cancer in Japanese males can be attributed to drinking by persons with inactive heterozygous ALDH2 (68.5%). Aldehyde dehydrogenase-2 generates acetic acid from acetaldehyde metabolism and its activity correlates with *in vivo* acetaldehyde concentration. Thus, diminished ALDH2 enzyme activity and consequent higher concentrations of acetaldehyde can be risk factors for esophageal cancer. In this study, we, for the first time, report that ALDH2 A/A homozygotes have higher esophageal cancer risk than ALDH2 G/A homozygotes, which is consistent with the different ALDH2 enzyme activity resulting from A/A and G/G genotypes. Literature has shown that after drinking, the blood acetaldehyde concentrations in those with ALDH2 A/A and G/A were 19- and 6-fold higher than in those with G/G genotype, respectively[19].

In this study, we found the ADH G allele carriers were more susceptible to esophageal cancer, but no association was found between ADH2 genotypes and risk of esophageal cancer when disregarding drinking status. Compared with non-drinkers carrying both ALDH2 G/G and ADH2 A/A, drinkers carrying both ALDH2 A allele and ADH2 G allele showed a significantly higher risk of developing esophageal cancer (OR = 8.36, 95% CI: 2.98-23.46). The inactive ADH2 genotype has also been demonstrated to enhance the risk of esophageal cancer among alcoholics and the general population. The inactive ALDH2 genotype and ADH2 genotype carriers have higher risk of developing esophageal cancer, especially among alcohol drinkers[11,13-18]. These findings conflicts with those demonstrating that the enzyme activity in ADH G allele was much higher than that of A allele. Yoshihara *et al*[20] showed that there were no significant differences in blood ethanol and acetaldehyde concentrations between volunteers with ADH2*1 and without ADH2*1. Thus, the mechanism of the ADH2 polymorphism involved

in esophageal cancer risk may be associated with, not acetaldehyde, but a direct involvement of ethanol.

In summary, this study found that polymorphisms of the ADH2 and ALDH2 genes were significantly associated with the risk of esophageal cancer in Southeast Chinese males. Significant gene-environment interactions between alcohol drinking and ALDH2 were observed in esophageal cancer risk. Significant interactions between ADH2 and ALDH2 polymorphisms were also observed. These findings can provide additional information about the role of alcohol in esophageal cancer risk in Chinese populations. For individuals with ALDH2 A/A or G/A genotypes, reducing alcohol consumption may help lower their risk for esophageal cancer.

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COMMENTS

Background

Esophageal cancer is the most common cancer in China. There is epidemiological evidence that alcohol intake is associated with an increased esophageal cancer risk. Alcohol dehydrogenase-2 (ADH2) and aldehyde dehydrogenase-2 (ALDH2) have a strong impact on alcohol metabolism. The authors' previous study has shown that more than 40% of adult residents in Taixing drink wine and that drinking is a risk factor for esophageal cancer in this area. However, no statistically significant association between ALDH2 and the risk of esophageal cancer was found. In this study, the authors increased the sample size to define the individual and combined roles of ADH2, ALDH2 polymorphisms and drinking habits in the risk for esophageal cancer development in Chinese males.

Innovations and breakthroughs

The present study showed that polymorphisms of the ALDH2 genes were significantly associated with the risk of esophageal cancer in Southeast Chinese males. Significant gene-environment interactions between alcohol drinking and ALDH2 were observed in esophageal cancer risk. Significant interactions between ADH2 and ALDH2 polymorphisms were also observed.

Applications

This research showed the genetic risk factors and the role of gene-environment interactions in identifying individuals at risk of esophageal cancer, which have certain theoretical and application values for studying the etiology of esophageal cancer and its prevention.

Terminology

ADH2: A zinc-containing enzyme which oxidizes primary and secondary alcohols or hemiacetals in the presence of NAD. In alcoholic fermentation, it catalyzes the final step of reducing aldehyde to alcohol in the presence of NADH and hydrogen. ALDH2: An enzyme that oxidizes aldehyde in the presence of NAD+ and water to acid and NADH. Genetic polymorphisms: The regular and simultaneous occurrence of two or more discontinuous genotypes in a single interbreeding population. The concept includes differences in genotypes ranging in size from a single nucleotide site to large nucleotide sequences visible at a chromosomal level.

Peer review

This study provides more information on the ADH2 and ALDH2 polymorphisms of esophageal cancer in Southeast Chinese males and the findings support the previous results that the risk of esophageal cancer increases in subjects carrying ADH2*1 allele (G allele) and ALDH2*2 allele (A allele) in an overall population, and especially ALDH2*2 allele (A allele) in alcohol drinkers. These epidemiological findings might help construct a prevention strategy against esophageal cancer.

REFERENCES

1 **Gemma S**, Vichi S, Testai E. Individual susceptibility and alcohol effects:biochemical and genetic aspects. *Ann Ist Super Sanita* 2006; **42**: 8-16

2 **Matsuo K**, Hamajima N, Shinoda M, Hatooka S, Inoue M, Takezaki T, Tajima K. Gene-environment interaction between an aldehyde dehydrogenase-2 (ALDH2) polymorphism and alcohol consumption for the risk of esophageal cancer. *Carcinogenesis* 2001; **22**: 913-916

3 **Bosron WF**, Li TK. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology* 1986; **6**: 502-510

4 **Bosron WF**, Lumeng L, Li TK. Genetic polymorphism of enzymes of alcohol metabolism and susceptibility to alcoholic liver disease. *Mol Aspects Med* 1988; **10**: 147-158

5 **Enomoto N**, Takase S, Yasuhara M, Takada A. Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. *Alcohol Clin Exp Res* 1991; **15**: 141-144

6 **Ding JH**, Li SP, Gao CM, Zhou J N, Su P, Wu JZ, Zhang Y, Liu YT, Zhou XF, Ding BG, Wang RH. On population based case-control study of upper digestive tract cancer. *Zhongguo Gonggong Weisheng* 2001; **17**: 319-320

7 **Ding JH**, Wu JZ, Li SP, Gao CM, Zhou JN, Su P, Liu YT, Zhou XF, Ding BG, Wang RH. Polymorphisms of aldehyde Dehydrogenase-2 genotypes and alcohol consumption for the susceptibility of the liver cancer, stomach cancer and esophageal cancer. *Zhongguo Zhongliu* 2002; **11**: 450-452

8 **Tan SK**, Qiu XQ, Yu HP, Zeng XY, Zhao YN, Hu L. [Evaluation of the risk of clonorchiasis inducing primary hepatocellular carcinoma] *Zhonghua Gan Zang Bing Za Zhi* 2008; **16**: 114-116

9 **Ding J**, Li S, Wu J, Gao C, Zhou J, Cao H, Su PS, Liu Y, Zhou X, Chang J. Alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 genotypes, alcohol drinking and the risk of primary hepatocellular carcinoma in a Chinese population. *Asian Pac J Cancer Prev* 2008; **9**: 31-35

10 **Chao YC**, Wang LS, Hsieh TY, Chu CW, Chang FY, Chu HC. Chinese alcoholic patients with esophageal cancer are genetically different from alcoholics with acute pancreatitis and liver cirrhosis. *Am J Gastroenterol* 2000; **95**: 2958-2964

11 **Wu CF**, Wu DC, Hsu HK, Kao EL, Lee JM, Lin CC, Wu MT. Relationship between genetic polymorphisms of alcohol and aldehyde dehydrogenases and esophageal squamous cell carcinoma risk in males. *World J Gastroenterol* 2005; **11**: 5103-5108

12 **Yang CX**, Matsuo K, Ito H, Hirose K, Wakai K, Saito T, Shinoda M, Hatooka S, Mizutani K, Tajima K. Esophageal cancer risk by ALDH2 and ADH2 polymorphisms and alcohol consumption: exploration of gene-environment and gene-gene interactions. *Asian Pac J Cancer Prev* 2005; **6**: 256-262

13 **Chen YJ**, Chen C, Wu DC, Lee CH, Wu CI, Lee JM, Goan YG, Huang SP, Lin CC, Li TC, Chou YP, Wu MT. Interactive effects of lifetime alcohol consumption and alcohol and aldehyde dehydrogenase polymorphisms on esophageal cancer risks. *Int J Cancer* 2006; **119**: 2827-2831

14 **Yokoyama A**, Muramatsu T, Omori T, Yokoyama T, Matsushita S, Higuchi S, Maruyama K, Ishii H. Alcohol and aldehyde dehydrogenase gene polymorphisms and oropharyngolaryngeal, esophageal and stomach cancers in Japanese alcoholics. *Carcinogenesis* 2001; **22**: 433-439

15 **Guo YM**, Wang Q, Liu YZ, Chen HM, Qi Z, Guo QH. Genetic polymorphisms in cytochrome P4502E1, alcohol and aldehyde dehydrogenases and the risk of esophageal squamous cell carcinoma in Gansu Chinese males. *World J Gastroenterol* 2008; **14**: 1444-1449

16 **Yang SJ**, Wang HY, Li XQ, Du HZ, Zheng CJ, Chen HG, Mu XY, Yang CX. Genetic polymorphisms of ADH2 and ALDH2 association with esophageal cancer risk in southwest China. *World J Gastroenterol* 2007; **13**: 5760-5764

17 **Boonyaphiphat P**, Thongsuksai P, Sriplung H, Puttawibul P. Lifestyle habits and genetic susceptibility and the risk of esophageal cancer in the Thai population. *Cancer Lett* 2002;

186: 193-199

18 **Yokoyama A**, Kato H, Yokoyama T, Tsujinaka T, Muto M, Omori T, Haneda T, Kumagai Y, Igaki H, Yokoyama M, Watanabe H, Fukuda H, Yoshimizu H. Genetic polymorphisms of alcohol and aldehyde dehydrogenases and glutathione S-transferase M1 and drinking, smoking, and diet in Japanese men with esophageal squamous cell carcinoma. *Carcinogenesis* 2002; **23**: 1851-1859

19 **Mizoi Y**, Yamamoto K, Ueno Y, Fukunaga T, Harada S. Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenases in individual variation of alcohol metabolism. *Alcohol Alcohol* 1994; **29**: 707-710

20 **Yoshihara E**, Ameno K, Nakamura K, Ameno M, Itoh S, Ijiri I, Iwahashi K. The effects of the ALDH2*1/2, CYP2E1 C1/C2 and C/D genotypes on blood ethanol elimination. *Drug Chem Toxicol* 2000; **23**: 371-379

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Diagnostic effect of capsule endoscopy in 31 cases of subacute small bowel obstruction

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Abstract

AIM: To evaluate the effectiveness and safety of capsule endoscopy (CE) in patients with recurrent subacute small bowel obstruction.

METHODS: The study was a retrospective analysis of 31 patients referred to hospital from January 2003 to August 2008 for the investigation of subacute small bowel obstruction, who underwent CE. The patients were aged 9-81 years, and all of them had undergone gastroscopy and colonoscopy previously. Some of them received abdominal computed tomography or small bowel follow-through.

RESULTS: CE made a definitive diagnosis in 12 (38.7%) of 31 cases: four Crohn's disease (CD), two carcinomas, one intestinal tuberculosis, one ischemic enteritis, one abdominal cocoon, one duplication of the intestine, one diverticulum and one ileal polypoid tumor. Capsule retention occurred in three (9.7%) of 31 patients, and was caused by CD (2) or tumor (1). Two with retained capsules were retrieved at surgery, and the other one of the capsules was spontaneously passed the stricture by medical treatment in 6 mo. No case had an acute small bowel obstruction caused by performance of CE.

CONCLUSION: CE provided safe and effective visualization to identify the etiology of a subacute small bowel obstruction, especially in patients with suspected intestinal tumors or CD, which are not identified by routine examinations.

Key words: Capsule endoscopy; Small bowel obstruction; Capsule retention

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INTRODUCTION

Small bowel obstruction is a frequent cause of acute abdomen. The definitive diagnostic rate is not high using traditional radiographic evaluation, such as plain film radiography, abdominal computed tomography (CT), or small bowel follow-through. Some reports have demonstrated that capsule endoscopy (CE) is superior to radiographic examination and push enteroscopy in the investigation of intestinal diseases, especially for obscure gastrointestinal bleeding or suspected Crohn's disease (CD)[1-4]. Although capsule retention is a relatively infrequent complication, small bowel obstruction and strictures have been considered contraindications to CE. It is interesting to note that there is a controversy about this contraindication in the literature. The goal of the present study was to evaluate the safety and effectiveness of CE in patients with small bowel obstruction.

MATERIALS AND METHODS

Subjects

Between January 2003 and August 2008, 31 patients underwent CE for the investigation of small bowel obstruction, who had previously received gastroscopy and colonoscopy, abdominal CT or small bowel follow-

Table 1 Clinical findings and outcomes of CE or surgery

Patient	Gender/age (yr)	Surgical history/ NSAID use	Prior examinations	GI transit time (min)	CE or surgical findings	Follow-up (mo)
1	M/43	None	EGD, colonoscopy (-), AXR	319	Abdominal cocoon	17
2	F/18	Appendectomy	EGD, colonoscopy (+), AXR	387	CD	53
3	M/74	None	EGD, colonoscopy (-), AXR	329	Normal	54
4	F/69	None	EGD, colonoscopy, SBFT (-), AXR	295	Normal	53
5	M/54	None	Colonoscopy, SBFT (±), AXR	CE retention	CD	29
6	M/9	Intussusception	EGD, colonoscopy (-), AXR	205	Normal	16
7	F/67	None	EGD, colonoscopy (-), AXR	Not pass	Ischemic enteritis	Lost in 1[1]
8	F/36	None	EGD, colonoscopy, CTE (-), AXR	308	Normal	27
9	M/46	None	CTE (±), SBFT (±), AXR	461	Tumor	15
10	F/37	Abdominal delivery	EGD, colonoscopy, SBFT (-), AXR	247	Normal	17
11	F/52	None	EGD, colonoscopy, CTE, SBFT (-)	CE retention	Tumor	2
12	F/52	None	EGD, colonoscopy, CTE, SBFT (-)	Not pass	Normal	1
13	F/62	None	EGD, colonoscopy, CTE, SBFT (-)	Not pass	Normal	2
14	M/57	None	EGD, CTE, SBFT (-), AXR	324	Normal	33
15	M/31	None	EGD, colonoscopy (-), AXR	250	Normal	32
16	F/32	None	EGD, colonoscopy (±), CTE (-)	446	Normal	30
17	M/53	None	CTE (+), EGD/colonoscopy (-),	346	Normal	33
18	F/31	Abdominal delivery	EGD/colonoscopy (-), US/CTE (+)	425	TB	30
19	M/22	None	EGD/colonoscopy, SBFT (-), AXR	340	Normal	3
20	M/46	Small bowel resection	EGD/colonoscopy, CTE (-), AXR	296	Normal	3
21	M/81	None	Colonoscopy (-), AXR	378	Normal	Lost[2]
22	F/54	Tubal ligation	EGD/colonoscopy, CTE (-)	458	Normal	41
23	M/75	None	EGD/colonoscopy (-), AXR	327	Normal	Death in 24
24	M/53	None	EGD/colonoscopy (-), AXR	421	CD	51
25	M/60	None	EGD/colonoscopy, SBFT (-)	465	Intestinal diverticulum	5
26	F/52	None	EGD/colonoscopy, CTE (±)	465	Normal	16
27	M/32	None	EGD/colonoscopy (-), AXR	293	Normal	39
28	F/54	Tubal ligation	EGD/colonoscopy, SBFT (-)	354	Normal	36
29	F/59	None	EGD/colonoscopy, MRI (-), AXR	349	Ileal polypoid tumor	Lost[2]
30	M/9	None	EGD/colonoscopy, CTE (-), AXR	Not pass	Duplication of intestine	12
31	F/65	None	EGD/colonoscopy, SBFT (-), AXR	CE retention	CD	14

EGD: Esophagogastroduodenoscopy; AXR: Abdominal X-ray; MRI: Magnetic resonance imaging; CE: Capsule endoscopy; CTE: CT enterography; SBFT: Small bowel follow-through; US: Ultrasound; CD: Crohn's disease. (±): Suspected positive; (+): Positive; (-): Negative; Not pass: The capsule did not pass the ileocecal valve within the duration of the examination, but was not retained. GI: Gastrointestinal. [1]The patient was lost to follow-up 1 mo after surgery. [2]The follow-up was missed after CE examination.

through more than once. All previous radiological and endoscopic examinations could not identify clear etiology.

Materials

CE (Given M2A, Giving Imaging Ltd, Yoqneam, Israel) measuring 11 mm × 26 mm, which magnify images eight times, has a battery life of 6-8 h. It is used in conjunction with an imaging system including a data recorder and interpretative workstation. Continuous video-images are transmitted at a rate of two frames per second.

Methods

A total of 1121 patients underwent CE between January 2003 and August 2008. Most of them underwent CE for the evaluation of obscure bleeding or suspected CD. We identified 31 patients presenting with symptoms consistent with small bowel obstruction, and abdominal X-ray showed incomplete intestinal obstruction. All the 31 patients who were aware of an increased risk for capsule retention and the possibility for surgery received CE examination, when the symptoms of intestinal obstruction were relieved by conservative management. All the patients gave written informed consent. The medical data were retrospectively analyzed, including age, sex, medical and surgical history, follow-up, and radiographic, routine endoscopic and CE examinations.

RESULTS

The mean age of these 31 patients was 47.12 ± 18.38 years (range 9-81 years); 18 of the subjects were male and 13 were female. Seventeen of them were out-patients, 14 were in-patients, and nine had surgical histories before capsule examinations were performed. All of them had undergone gastroscopy and colonoscopy previously, but the results were negative. Twenty-three of them had undergone CT enterography or small bowel follow-through, and positive or suspected results were found in six cases. Four of the six patients achieved definitive diagnoses by CE examination, surgical or pathological biopsy, and the remaining two were false-positive.

The average gastric emptying time was 43.8 ± 36.1 min (range 4-131 min). In 15 of the 31 patients, the capsule passed the ileocecal valve within the duration of the examination. The mean small bowel transit time (based on 24 patients) was 332.2 ± 86.7 min (range 167-484 min, Table 1). In 28 of the 31 patients, the capsule was evacuated in 3 d. Capsule retention occurred in three

Table 2 Abnormalities detected on CE in patients with small bowel obstruction

Detected abnormalities (12)	Gender/age (yr)	CE retention (time)	Therapy	Post-CE obstructive symptom
CD (4)				
	F/18	No	Medical therapy	None
	M/54	Yes (1 wk)	Surgical resection	None
	M/53	No	Medical therapy	None
	F/65	Yes (6 mo)	Medical therapy	None
Tumor (2)				
Ileal neuroendocrine carcinoma	M/46	No	Surgical resection	None
Jejunal adenocarcinoma	F/52	Yes (2 wk)	Surgical resection	None
Intestinal tuberculosis (1)	F/31	No	Medical therapy	None
Ischemic enteritis (1)	F/67	No	Surgical resection	None
Abdominal cocoon (1)	M/43	No	Surgical resection	None
Intestinal diverticulum (1)	M/60	No	Medical therapy	None
Ileal polypoid tumor (1)	F/59	No	Lost to follow-up	None
Duplication of intestine (1)	M/9	No	Surgical resection	None

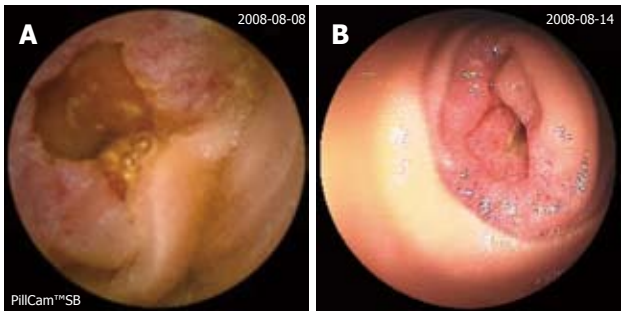

Figure 1 CE and double air-balloon endoscopic images of stenosis. A: CE shows an annuliform mass in the intestine; B: Double air-balloon endoscopy also shows an annuliform mass, but we failed to retrieve the retained capsule.

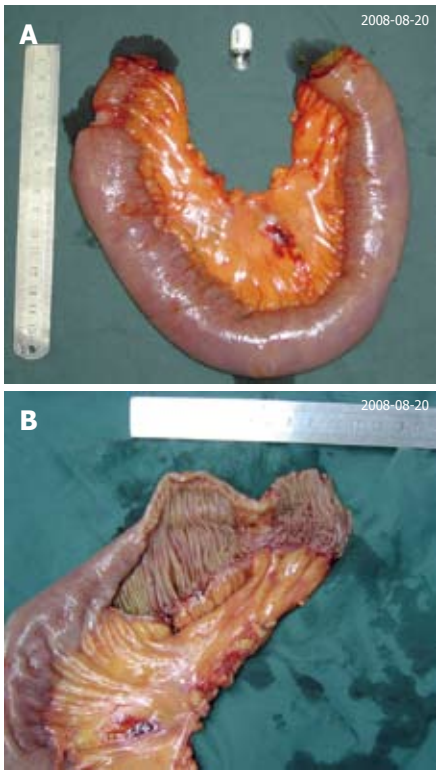

Figure 2 Surgical images of stenosis. A: A retained capsule was removed at surgery; B: An obvious stenosis was caused by jejunal adenocarcinoma.

(9.7%) cases, caused by CD or tumor, of which, were retrieved at surgery, and the other one of the capsules was spontaneously passed the stricture by medical treatment in 6 mo. None of the cases showed any symptoms of acute or subacute obstruction during CE examination.

CE disclosed definitive intestinal disease in 12 (38.7%) of the 31 patients, including four CD, two carcinoma, one intestinal tuberculosis, one ischemic enteritis, one abdominal cocoon, one intestinal duplication, one small-intestinal diverticulum and one ileal polypoid tumor (Table 2). Single or multiple ulcers were found in six patients. In three of the six, CD was diagnosed by CE images and clinical manifestations, and obvious symptom relief was achieved through treatment with mesalazine. In one of the six patients, capsule was retrieved at surgery which had not passed the stricture for 7 d, and the replacement showed CD. In another of the six patients, multiple ulcers were found with CE and double-balloon enteroscopy. CD was firstly considered according to the endoscopic findings and clinical data, but medical treatment with mesalazine did not relieve the patient's symptoms. The later BUS and CT scans showed multiple retroperitoneal lymph node enlargement, meanwhile, the purified protein derivative test was found to be positive. Pathological analysis of biopsy specimens obtained from these lymph nodes indicated tuberculosis. The patient's symptoms were relieved significantly by anti-tuberculosis treatment, therefore, intestinal tuberculosis was diagnosed. The remainder of the six was demonstrated abdominal cocoon at surgery. In one elderly patient, intestinal mucosal erosion and bleeding were found at CE examination. Later, exploratory laparotomy was performed for advanced identification of etiology and therapy, which indicated superior mesenteric artery embolus. In another case, the capsule images presented abnormal intestinal motility and CT scan showed mural thickening of the distal ileum, and finally, ileal neuroendocrine carcinoma was diagnosed by surgery. In a pediatric case, CE also showed abnormal intestinal motility. The child was treated surgically because of failure of medical treatment, which indicated duplication of the intestine. The CE findings in the remaining two cases disclosed diverticulum of the small intestine and ileal polypoid tumor. In a female patient

whose CA199 increased clearly CT, BUS scan or air-barium double contract examination were negative, but CE and double air-balloon endoscopy showed an annuliform mass, which was demonstrated to be jejunal adenocarcinoma at later surgery (Figures 1 and 2).

None of the patients had other risk factors for stricture formation, such as long-term administration of non-steroidal anti-inflammatory drug (NSAIDs) and abdominal radiotherapy. The capsule images were normal in 19 of the 31 cases. Follow-up was missed in three of the 19 cases. An elderly patient in the remaining 16 died of pulmonary infection. Small bowel obstruction did not reappear in the other 15 cases during medical treatment in the follow-up period. However, adhesive ileus could not be excluded in four of the 14 patients who had a history of abdominal surgery. The capsule findings allowed a definitive diagnosis in 12 of the 31 cases: six patients accepted surgical treatment (one CD, two tumors, one ischemic enteritis, one abdominal cocoon, one duplication). Five patients (three CD, one intestinal tuberculosis, one intestinal diverticulum) were treated medically without surgery, and no recurrence of small bowel obstruction was found in these patients during follow-up.

DISCUSSION

CE is a novel diagnostic technique that has been used increasingly for analysis of many disorders of the small intestine, such as occult gastrointestinal bleeding, suspected CD, chronic diarrhea, and protein-losing enteropathy. Although small bowel obstruction has been considered a contraindication to CE, in our series, CE was documented to be very valuable and safe in identifying the etiology of small bowel obstruction, and it was also found to be easy to swallow, painless and well tolerated by these selected patients. CE findings allowed a definitive diagnosis in 12 (38.7%) out of the 31 cases, in which CD (4/31) was the major disease inducing stricture of the intestine. This cause was consistent with that in the study of Chiefetz and Lewis[5]. In contrast, some authors have reported that NSAID-induced stricture was the major cause of capsule retention[6,7]. Recently, Mason *et al*[8] have reported that intestinal mass or radiation enteritis are the main causes of subacute small bowel obstruction. The different causes of stricture may be associated with the indications of the patients.

The incidence of retention is closely related to the selected population. The incidence of retention varies from 0%-21% in the literature as a result of the different populations and indications for examination. The highest published rate (21%) was reported in the study of Chiefetz and Lewis[5], in which CD (2/19 cases) was also noted to be the major cause of retention. In another study with a total of 102 cases[9], the rate of retention was 13% (5/38) in patients with known CD, but only 1/64 cases with suspected CD had a retained capsule. The rate of capsule retention was very low in most studies, especially when the patients were selected without suspected small bowel obstruction or intestinal stricture. In the report of Barkin and Friedman[10], the incidence was 0.75% in a large study of 900 patients who had previously normal small intestines. Most recently, Li *et al*[7] have reported 14 cases of CE retention (1.4%) in 1000 capsule examinations. It was shown that tumors or NSAID strictures were the major etiology of retention in both of these studies. In our highly selected population, capsule retention occurred in 3 of 31 cases (two CD, one tumor).

Recently, dissolving patency capsules have been used in some studies to evaluate intestinal patency in patients with small bowel strictures, before video-CE (VCE). The patency capsule[11] is composed of lactose, remains intact in the gastrointestinal tract for 40-100 h post-ingestion, and disintegrates thereafter. Spada *et al*[12] have reported that 94% (30/34) of cases with small bowel stricture passed the intact or disintegrated capsule in the stools. Expulsion was confirmed in three cases by fluoroscopy, and the remaining patient withdrew consent to the study. In addition, VCE passed uneventfully through the small bowel stricture of all 10 patients who underwent VCE following patency capsule examination. The study of Spada *et al* has suggested that the patency capsule is a safe and effective tool for evaluation of functional patency of the small bowel, even when stricture has been indicated by traditional radiology. In another multicenter study[13], in all the 106 patients with strictures, no acute ileus was induced by Agile patency capsule. However, in the study of Bovin *et al*[14], in one of the 22 cases with suspected obstructive intestinal disease and/or radiological evidence of small-bowel strictures, impaction of an intact capsule led to ileus and emergency surgery. Similarly, in the study of Delvaux *et al*[15], of all 22 patients with known or suspected stenosis, the patency capsule induced a symptomatic intestinal occlusion in three patients, which was resolved spontaneously in one and required emergency surgery in two. It was shown that the start of dissolution at 40 h after ingestion was too late to prevent intestinal occlusion. Furthermore, the patency capsule can not detect stenosis and the etiology of small bowel obstruction.

Capsule retention has been defined as the presence of a capsule in the body for a minimum of 2 wk after ingestion, or when the capsule is retained in the bowel lumen indefinitely, unless targeted medical endoscopy or surgical intervention is initiated[16]. In our series, capsule retention occurred only in three cases, in which one of the capsules was spontaneously passed the stricture by medical treatment in 6 mo, and the other two retained capsules were retrieved at surgery. No acute small bowel obstruction occurred after administration of CE. The reported rate of acute abdomen induced by capsule is low. However, there is a controversy in the literature about the utility of capsule retention. In many studies, patients with a high risk of intestinal stricture were excluded for fear of capsule retention, which may have led to acute intestinal obstruction or surgical emergency. However, in most cases, capsule retention is symptomatic, although some patients accepted surgical therapy, which is safe and identifies or treats the underlying disease. Thus, some authors consider that capsule impaction is a valuable means of detecting significant stenosis that would benefit from

surgical management[5]. In addition, the retained capsule can be retrieved using double-balloon endoscopy[17,18]. Importantly, it is necessary to make the patients aware of the potential need for surgery before CE, although the risk for retention was low.

Based on our results, the most common etiology of small bowel obstruction was CD, followed by tumor. In our selected population, capsule retention was asymptomatic, which did not lead to surgical emergency. It is concluded that CE is a safe and effective tool for detecting etiology and stenosis of patients who have a history of small bowel obstruction, especially for the patients with suspected intestinal tumors or CD, which are not identified by routine examinations. Such results need future confirmation from prospective randomized studies.

COMMENTS

Background

Capsule endoscopy (CE) has been demonstrated to be superior to routine radiological examinations in the investigation of obscure gastrointestinal bleeding or suspected Crohn's disease (CD). Small bowel obstruction or strictures are considered to be a contraindication for CE in many centers. However, the accuracy of radiography in this situation has often been questioned.

Research frontiers

CE is now commonly performed for gastrointestinal bleeding of obscure origin or suspected CD. It is noted that the visualization of patients with suspected small bowel stenosis using traditional radiological methods is associated with high false-negative results and radiation doses. Recently, CE or patency capsule has been performed for suspected intestinal strictures in some studies, mainly for patients with known or suspected CD. However, reports about patients with subacute intestinal obstruction receiving CE are rare in the literature up till now.

Innovations and breakthroughs

At many centers, CE is considered to be contraindicated in suspected obstructive small bowel disease, for fear of capsule retention. In the present study, capsule retention occurred only in three cases (one of the capsules was spontaneously passed the stricture by medical treatment in 6 mo, the other two were retrieved at surgery), and no acute small bowel obstruction occurred after administration of CE. CE can be helpful in diagnosing subacute intestinal obstruction in patients with otherwise negative imaging studies, especially for patients with suspected intestinal tumors or CD.

Applications

CE is helpful in diagnosing subacute intestinal obstruction in patients with negative or uncertain imaging studies, which may become an appropriate indication for performing CE.

Terminology

Subacute small bowel obstruction is diagnosed in patients who present with symptoms consistent with small bowel obstruction, in whom abdominal X-ray shows incomplete intestinal obstruction. CE is a novel diagnostic technique that has been used increasingly for analysis of many disorders of the small intestine, such as occult gastrointestinal bleeding, suspected CD, chronic diarrhea, and protein-losing enteropathy.

Peer review

This paper describes CE in patients with small bowl obstruction. Although today the results of MRI of the intestine mostly offers the best chance of finding stenosis, CE is an interesting technique and should lead to a higher percentage of diagnosis.

REFERENCES

1 **Saperas E**, Dot J, Videla S, Alvarez-Castells A, Perez-Lafuente M, Armengol JR, Malagelada JR. Capsule endoscopy versus computed tomographic or standard angiography for the diagnosis of obscure gastrointestinal bleeding. *Am J Gastroenterol* 2007; **102**: 731-737

2 **Mylonaki M**, Fritscher-Ravens A, Swain P. Wireless capsule endoscopy: a comparison with push enteroscopy in patients with gastroscopy and colonoscopy negative gastrointestinal bleeding. *Gut* 2003; **52**: 1122-1126

3 **Ge ZZ**, Hu YB, Xiao SD. Capsule endoscopy and push enteroscopy in the diagnosis of obscure gastrointestinal bleeding. *Chin Med J* (Engl) 2004; **117**: 1045-1049

4 **Hara AK**, Leighton JA, Sharma VK, Fleischer DE. Small bowel: preliminary comparison of capsule endoscopy with barium study and CT. *Radiology* 2004; **230**: 260-265

5 **Cheifetz AS**, Lewis BS. Capsule endoscopy retention: is it a complication? *J Clin Gastroenterol* 2006; **40**: 688-691

6 **Sears DM**, Avots-Avotins A, Culp K, Gavin MW. Frequency and clinical outcome of capsule retention during capsule endoscopy for GI bleeding of obscure origin. *Gastrointest Endosc* 2004; **60**: 822-827

7 **Li F**, Gurudu SR, De Petris G, Sharma VK, Shiff AD, Heigh RI, Fleischer DE, Post J, Erickson P, Leighton JA. Retention of the capsule endoscope: a single-center experience of 1000 capsule endoscopy procedures. *Gastrointest Endosc* 2008; **68**: 174-180

8 **Mason M**, Swain J, Matthews BD, Harold KL. Use of video capsule endoscopy in the setting of recurrent subacute small-bowel obstruction. *J Laparoendosc Adv Surg Tech A* 2008; **18**: 713-716

9 **Cheifetz AS**, Kornbluth AA, Legnani P, Schmelkin I, Brown A, Lichtiger S, Lewis BS. The risk of retention of the capsule endoscope in patients with known or suspected Crohn's disease. *Am J Gastroenterol* 2006; **101**: 2218-2222

10 **Barkin JS**, Friedman S. Wireless capsule endoscopy requiring surgical intervention: the world's experience. *Am J Gastroenterol* 2002; **97**: S298

11 **Cheifetz SA**, Sachar D, Lewis B. Small bowel obstruction: indication or contraindication for capsule endoscopy. *Gastrointest Endosc* 2004; **59**: AB461

12 **Spada C**, Spera G, Riccioni M, Biancone L, Petruzziello L, Tringali A, Familiari P, Marchese M, Onder G, Mutignani M, Perri V, Petruzziello C, Pallone F, Costamagna G. A novel diagnostic tool for detecting functional patency of the small bowel: the Given patency capsule. *Endoscopy* 2005; **37**: 793-800

13 **Herrerias JM**, Leighton JA, Costamagna G, Infantolino A, Eliakim R, Fischer D, Rubin DT, Manten HD, Scapa E, Morgan DR, Bergwerk AJ, Koslowsky B, Adler SN. Agile patency system eliminates risk of capsule retention in patients with known intestinal strictures who undergo capsule endoscopy. *Gastrointest Endosc* 2008; **67**: 902-909

14 **Boivin ML**, Lochs H, Voderholzer WA. Does passage of a patency capsule indicate small-bowel patency? A prospective clinical trial? *Endoscopy* 2005; **37**: 808-815

15 **Delvaux M**, Ben Soussan E, Laurent V, Lerebours E, Gay G. Clinical evaluation of the use of the M2A patency capsule system before a capsule endoscopy procedure, in patients with known or suspected intestinal stenosis. *Endoscopy* 2005; **37**: 801-807

16 **Cave D**, Legnani P, de Franchis R, Lewis BS. ICCE consensus for capsule retention. *Endoscopy* 2005; **37**: 1065-1067

17 **Tanaka S**, Mitsui K, Shirakawa K, Tatsuguchi A, Nakamura T, Hayashi Y, Sakamoto C, Terano A. Successful retrieval of video capsule endoscopy retained at ileal stenosis of Crohn's disease using double-balloon endoscopy. *J Gastroenterol Hepatol* 2006; **21**: 922-923

18 **May A**, Nachbar L, Ell C. Extraction of entrapped capsules from the small bowel by means of push-and-pull enteroscopy with the double-balloon technique. *Endoscopy* 2005; **37**: 591-593

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BRIEF ARTICLES

Effect of two-channel gastric electrical stimulation with trains of pulses on gastric motility

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Abstract

AIM: To investigate the effect of two-channel gastric electrical stimulation (GES) with trains of pulses on gastric emptying and slow waves.

METHODS: Seven dogs implanted with four pairs of electrodes and equipped with a duodenal cannula were involved in this study. Two experiments were performed. The first experiment included a series of sessions in the fasting state with trains of short or long pulses, each lasted 10 min. A 5-min recording without pacing was made between two sessions. The second experiment was performed in three sessions (control, single-channel GES, and two-channel GES). The stimulus was applied *via* the 1st pair of electrodes for single-channel GES (GES *via* one pair of electrodes located at 14 cm above the pylorus), and simultaneously *via* the 1st and 3rd channels for two-channel GES (GES *via* two pairs of electrodes located at 6 and 14 cm above the pylorus). Gastric liquid emptying was collected every 15 min *via* the cannula for 90 min.

RESULTS: GES with trains of pulses at a pulse width of 4 ms or higher was able to entrain gastric slow waves. Two-channel GES was about 50% more efficient than single-channel GES in entraining gastric slow waves. Two-channel but not single-channel GES with trains of pulses was capable of accelerating gastric emptying in healthy dogs. Compared with the control session, two-channel GES significantly increased gastric emptying of liquids at 15 min (79.0% ± 6.4% *vs* 61.3% ± 6.1%, $P < 0.01$), 30 min (83.2% ± 6.3 % *vs* 68.2% ± 6.9%, $P < 0.01$), 60 min (86.9% ± 5.5 % *vs* 74.1% ± 5.9%, $P < 0.01$), and 90 min (91.0% ± 3.4% *vs* 76.5% ± 5.9%, $P < 0.01$).

CONCLUSION: Two-channel GES with trains of pulses accelerates gastric emptying in healthy dogs and may have a therapeutic potential for the treatment of gastric motility disorders.

Key words: Gastric electrical stimulation; Gastric slow waves; Gastric emptying; Gastrointestinal motility; Gastric pacing

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INTRODUCTION

Gastrointestinal functional or motor disorders are common, affecting 25% of the United State population[1,2] and 5%-10% of Asian population[3]. The patients often complain of a series of dyspeptic symptoms such as nausea and vomiting[4]. Gastric dysrhythmia has been observed in a variety of gastrointestinal motility disorders, including unexplained nausea and vomiting[4], gastroparesis[4,5], type II diabetes[2,5], early pregnancy[6], gastroesophageal reflux disease[7], after vagotomy and surgery[8] or after bone marrow or stem cell transplant[9]. In these circumstances, the frequency of gastric slow wave becomes either abnormally high (tachygastria) or low (bradygastria). Gastric emptying is delayed in

patients with gastroparesis and in about 30%-65% of patients with functional dyspepsia.

The commonly used medical therapy for gastroparesis is prokinetic agents, such as metoclopramide, cisapride, domperidom and erythromycin[10]. However, there are a considerable number of patients who are refractory to these medical therapeutic agents and side effects also limit their usage. While a number of studies have shown that some prokinetics have anti-dysrhythmic effects, but none of them has been developed for the normalization of gastric dysrhythmia[5,11].

Gastric electrical stimulation (GES) or pacing has been under investigation as a potential therapy for gastrointestinal motility disorders[12,13]. A number of studies have been performed to investigate the effect of gastric pacing, but the majority of them seem to indicate that gastric pacing is able to entrain gastric slow waves[14-20], accelerate gastric emptying in patients with gastroparesis[21,22] or in animal model of gastroparesis[18-20]. Three distinct methods have been used in GES, including long pulse stimulation, short pulse stimulation, and stimulation with trains of short or long pulses. In long-pulse stimulation, the pulse width is in the order of milli-seconds and the stimulation frequency is usually in the vicinity of the physiological frequency of gastric slow wave[23-25]. In short pulse stimulation, the pulse width is substantially shorter and is in the order of a few hundred micro-seconds. The stimulation frequency is usually a few times higher than the physiological frequency of gastric slow wave[12,26]. It has been reported that long-pulse stimulation can normalize gastric dysrhythmia, entrain the slow wave[13,17,25,27,28], accelerate gastric emptying in human beings and dogs, and short-pulse stimulation is effective against nausea and vomiting with no or little effect on gastric dysrhythmia, slow waves, or gastric emptying[18,26]. Trains of pulses are composed of a repetitious train of pulses and are derived from the combination of two signals: a continuous signal with a high frequency (in the order of 5-100 Hz) and a control signal to turn the pulses on and off, such as x seconds "on" and y seconds "off". This kind of stimulation has been frequently used in electroacupuncture[29]. Most previous studies were performed using long- or short-pulse GES in patients and in animal model of gastroparesis. Commercially available implantable stimulators are capable of generating short pulses or trains of pulses but not long pulses that are technically difficult to produce. That is, long pulse GES is practically not feasible or much less feasible than GES of pulse trains and has to be replaced by GES with trains of pulses. Accordingly, it is important to study whether the GES with trains of pulses is able to mimic the functions of long pulse GES. However, to the best of our knowledge, few studies have investigated the effect of GES with trains of pulses on gastric motility, such as gastric slow waves and gastric emptying.

This study was to investigate the effect of GES with trains of pulses on gastric slow waves and gastric emptying in health dogs.

MATERIALS AND METHODS

Animal preparation

Seven healthy female beagle dogs, weighing 14-21 kg, were used in this study. After an overnight fasting, the dogs were anesthetized with 2% sodium thiopental (0.6 mL/kg, intravenous) and underwent abdominal surgery. Their tongue color, pulse rate and breath rate were monitored. Four pairs of stainless steel cardiac pacing wires were implanted on the serosal surface of stomach in an arching line along the greater curvature. The most distal pair was placed 2 cm above the pylorus, and the distance between adjacent pairs of electrodes was 4 cm. The bipolar electrodes in each pair were 0.5 cm apart. The electrodes were affixed to the gastric serosa with an unabsorbable suture in the seromuscular layer of stomach. The wires were brought out through the anterior abdominal wall, channeled subcutaneously along the left side of the trunk, and placed outside the skin for pacing or recording gastric myoelectric activity. Each dog was equipped with a duodenal cannula 20 cm beyond the pylorus for the assessment of gastric liquid emptying. The study was initiated after the dogs were completely recovered from surgery, usually 2 wk after surgery. The Animal Care and Use Committee of the Union Hospital of Tongji Medical College approved the surgical and experimental protocols.

Experimental protocol

The study was composed of two experiments using the following protocols. Experiment 1 was designed to assess the optimal stimulation parameters (lowest stimulation energy) to entrain gastric slow waves in the dogs. The dogs were fasted overnight and received no medication before the study. A 30-min baseline recording was made *via* all electrodes in the stomach. Then, an adjustable multi-channel electrical stimulator (model A300, World Precisio Instruments, Sarasota, Florida) was used for stimulation in a constant current mode, and the stimulus consisted of periodic trains of bipolar pulses with adjustable pulse widths. In order to get effective pacing parameters for the entrainment, a series of sessions with various pacing parameters were performed in the fasting state, 10 min each session. A 5-min recording without pacing was made between two consecutive pacing sessions. The pulse width was gradually increased (0.3 ms, 0.5 ms, 0.7 ms, 0.9 ms, 1 ms, 2 ms, 3 ms, 4 ms …) until entrainment of gastric slow waves was achieved. Other parameters for GES were fixed. The stimulus was delivered *via* the 1st channel for single-channel GES (a train on-time of 3 s and off time of 8 s, a pulse frequency of 30 Hz, an amplitude of 5 mA) or *via* both the 1st and 3rd channels for two-channel (channel one: 3 s-on and 8 s-off, 30 Hz, 2 mA; channel three: the same as channel one except for pulse amplitude of 1.6 mA). With this setting, the frequency of pulse train was about 5.5 trains/min, which is similar to the physiological frequency of gastric slow wave. Time delays among different channels were determined

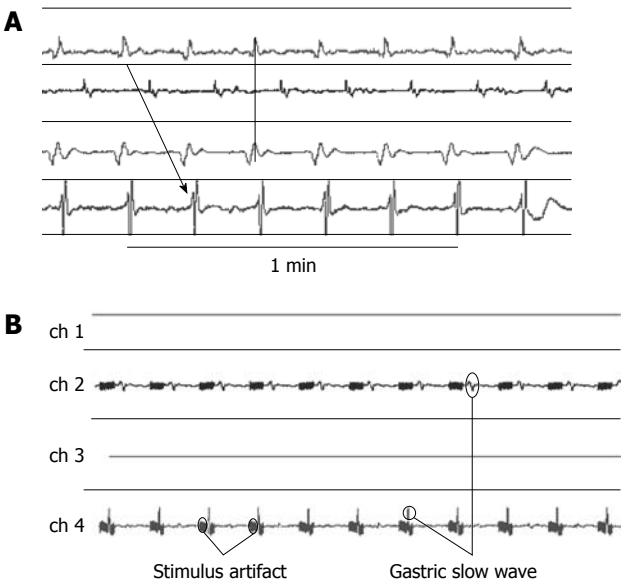

Figure 1 Gastric slow waves at the baseline and with two-channel GES (A), and recordings of gastric slow waves at baseline during 2-channel GES *via* the first and third channels (B).

based on the propagation speed of intrinsic gastric slow waves during the baseline recording. The peak of slow waves occurred simultaneously at the 1st and 3rd channels (a phase shift of 360 degree). Accordingly, stimulation applied in these two channels was synchronic or simultaneous (Figure 1).

Experiment 2 was to investigate the effect of two-channel GES with trains of pulses on gastric emptying. The stimulation parameters were determined as in experiment 1. The study was performed in three sessions (control, single-channel GES, two-channel GES) on three separate days (at least 3 d apart) in a randomized order. Each session consisted of four consecutive 30-min periods of gastric slow wave recordings. During each study session, the dogs were fed with a liquid meal composed of 43 g Nutrison (Nutricia, Holland) and 100 mg phenol red mixed with 100 mL water, immediately after a 30-min baseline recording in the fasting state (the dogs were fasted for 12 h or more). The total volume was 237 mL with a total energy of 250 kcal (6 g fat, 40 g carbohydrate, and 9 g protein). The emptied chyme containing gastric secretion and the ingested liquid meal were collected every 15 min *via* the intestinal cannula for 90 min. The collected volume and the amount of phenol red in each collection were used for the assessment of gastric emptying. Session two was the same as session one, except that GES was performed *via* the 1st channel during the entire postprandial period (Figure 2) with a train on-time of 3 s and off time of 8 s, a pulse frequency of 30 Hz, an amplitude of 5 mA, and width of 4 ms (the optimal pulse width obtained from experiment 1). Session three was the same as session two, except that GES was performed *via* channels one and three (pulse amplitude of 2 mA for channel one and 1.6 mA for channel three). The reduced pulse amplitude in the distal (channel three) stimulation channel was designed to avoid retrograde propagation of stimulation.

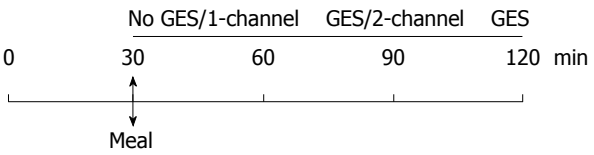

Figure 2 Experiment protocol.

Recording and assessment of gastric slow waves

A multi-channel recorder (AcqknowledgeIII, EOG 100A, Biopac System, Inc. Santa Barbara, CA) was used to record gastric slow waves *via* the serosal electrodes during the entire study. All signals were displayed on a computer monitor and saved on the hard disk with an IBM-compatible 486PC. The low and high cutoff frequencies of the amplifier were 0.05 and 35 Hz, respectively. The most distal recording was used to identify whether gastric slow waves are entrained with GES. Complete entrainment was defined as the frequency of gastric slow waves that was the same as the pacing frequency and phase-locked with the pacing stimulus. The percentage of entrainment of gastric slow waves was defined as the ratio of difference between the recorded slow wave frequency during pacing (f) and the intrinsic frequency before pacing (fi), and the difference between the pacing frequency (fp) and the intrinsic frequency before pacing. It was represented as % of entrainment = (f - fi)/(fp - fi)[28].

Gastric emptying

The test liquid meal contained 100 mg of phenol red as a marker, and gastric emptying was determined by assessment of the amount of phenol red in each collection of gastric effluent as previously described[19]. During the study, the volume of each collection was recorded and a sample of 5 mL was taken and stored in a freezer. At the end of study, these samples were analyzed using a spectrophotometer to detect the amount of phenol red in each sample. Gastric emptying was assessed by calculating the amount of phenol red recovered from each collection of gastric effluent.

Statistical analysis

Results were reported as mean ± SE. The analysis of variance (ANOVA) was used to assess the difference in three sessions of gastric emptying. Paired Student *t* test was used to investigate the differences in gastric emptying between the stimulation and control sessions. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of GES with trains of pulses on gastric slow waves

Gastric slow waves were entrained in each dog with single-channel or two-channel GES using trains of pulses with a greater pulse width. The relation between the entrainment of slow waves and stimulation pulse width is presented in Figure 3. The percentage of entrainment of gastric slow waves with single- or two-channel GES

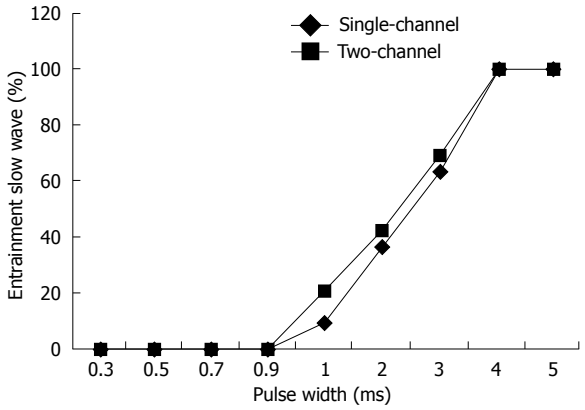

Figure 3 Percentage of slow wave entrainment with GES as a function of stimulation pulse width.

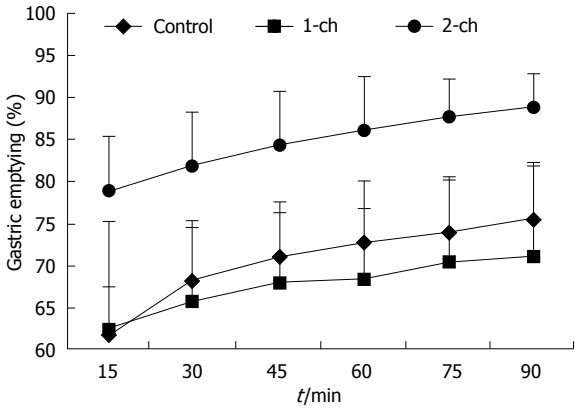

Figure 4 Gastric emptying in the control sessions and sessions with single-channel or two-channel GES. $P < 0.01$ (2-ch *vs* control).

was 7.75% ± 1.62% or 19.25% ± 9.04% at 1 ms, 36.62% ± 5.75% or 42.82% ± 5.45% at 2 ms, 62.34% ± 7.38% or 67.75% ± 9.80% at 3 ms and 100% ± 0% or 100% ± 0% at 4 ms, respectively. A complete entrainment was achieved with GES with a pulse width of 4 ms or greater. Some typical recordings at the baseline and during GES are shown in Figure 1. The entrainment of gastric slow waves usually occurred a few minutes after gastric pacing as demonstrated by the fact that the slow waves were phase-locked with the pacing stimulus a few minutes after the initiation of pacing.

To compare the stimulation energy required to completely entrain the gastric slow waves, the following formula was used for the calculation of stimulation energy (E): E = (cycles/min) × (frequency) × (pulse width) × (amplitude)^2. Accordingly, the minimum energy required by single-channel GES was 16 500 ms × mA^2, whereas that for the two-channel GES was 8421.6 ms × mA^2, which represents about 51.04% of the energy required by single-channel GES or a saving of 48.96% of energy.

Effect of GES with trains of pulses on gastric emptying

Two-channel GES with trains of long pulses (pulse width: 4 ms) could accelerate gastric emptying in the healthy dogs ($P < 0.01$, ANOVA) (Figure 4). Compared with the control session, two-channel GES significantly increased gastric emptying of liquids at 15 min (79.0% ± 6.4% *vs* 61.3% ± 6.1%, $P = 0.001$), 30 min (83.2% ± 6.3 % *vs* 68.2% ± 6.9%, $P = 0.005$), 60 min (86.9% ± 5.5% *vs* 74.1% ± 5.9%, $P = 0.010$), and 90 min (91.0% ± 3.4% *vs* 76.5% ± 5.9%, $P < 0.0037$), respectively, after feeding. However, no significant difference was noted in gastric emptying between single-channel GES and control sessions.

DISCUSSION

In the present study, GES with trains of wider pulses (width ⩾ 4 ms) but not short pulse could entrain gastric slow waves. Two-channel GES but not single-channel GES, significantly accelerated gastric emptying of liquids in healthy dogs.

Most previous studies showed that long pulse GES can entrain gastric slow waves in human beings and animals[19,20,22-25,27]. None of these studies has investigated the effect of GES with trains of pulses on gastric slow waves. It has been shown that GES with trains of short pulses can improve symptoms, such as nausea and vomiting of patients with gastroparesis[24,26], but cannot entrain gastric slow waves or normalize gastric dysrhythmia. In this study, GES with trains of pulses entrained gastric slow waves as long as the width of pulses in the train was 4 ms or greater. The energy required to completely entrain gastric slow waves with two-channel GES was less than that with single-channel GES, which might be due to the fact that each stimulation was responsible for entraining slow waves in a smaller region (about 50%) of the stomach with two-channel GES, compared with single-channel GES.

Conventionally, long-pulse GES is performed using a single pair of electrodes or single-channel GES. It has been reported that single-channel GES with long pulses accelerates gastric emptying in patients with gastroparesis[22] and in animal models of gastroparesis[30], and has no effect on gastric emptying in healthy dogs[18,19,30]. Recent studies on the effect of multi-channel GES on gastric emptying and entrainment of slow waves indicate that multi-channel stimulation with long pulses is more efficient than single-channel stimulation for the entrainment of slow waves, and can accelerate gastric emptying[18-20,31]. It has been shown that four-channel long pulse GES can accelerate gastric emptying in healthy dogs[18], whereas two-channel long pulse GES can normalize vasopressin-induced delayed gastric emptying in dogs[19]. To date, no study is available on the effect of multi-channel GES with trains of pulses on gastric emptying. In the present study, we used single-channel (14 cm above the pylorus) and two-channel (6 and 14 cm above the pylorus) GES to investigate their effect on gastric emptying. Compared with the control session, two-channel but not single-channel GES with trains of pulses significantly accelerated gastric emptying, which is consistent with the previous findings.

It is well known that gastric emptying of liquid and solid occurs separately, involving different areas

of stomach. It is believed that the antrum undergoes orderly peristaltic contractions and acts as a pump, while the proximal segment functions as a reservoir[32]. Gastric emptying demands accommodated motion of the proximal and distal stomach. The motility of stomach follows an orderly pattern in which gastric peristaltic contractions are phase-locked with gastric pacesetter potentials, which sweep distally from the corpus toward the pylorus. It is also known that gastric contractions are controlled by gastric slow waves. Multi-channel GES more accurately mimics the natural propagation and characteristics of gastric slow waves[18,30], thus controlling gastric contractions more effectively.

In this study, two-channel GES with trains of pulses entrained gastric slow waves and accelerated gastric emptying in healthy dogs, suggesting that two-channel GES with trains of pulses might be applicable in treatment of gastroparesis and normalization of gastric dysrhythmia. Technically, it is more feasible to make an implantable stimulator using trains of pulses than using repetitive long pulses due to the current charge balance. Accordingly, GES with trains of pulses is technically more attractive than long pulse GES. Currently, most commercially available implantable stimulators use trains of pulses. However, none of them is able to generate pulses with a width of 4 ms or greater. Therefore, new hardware design and development are needed before two-channel GES with trains of pulses can be used in clinical practice.

In conclusion, entrainment of gastric slow waves is feasible using GES with trains of pulses at a pulse width of 4 ms or greater. Two-channel GES with trains of pulses can accelerate gastric emptying in healthy dogs and may have a therapeutic potential for the treatment of gastric motility disorders.

COMMENTS

Background

Gastric dysrhythmia and delayed gastric emptying have been observed in a variety of gastric motility disorders. Treatment options for such disorders include medical therapy, surgical therapy, and nutritional support.

Research frontiers

Gastric electrical stimulation (GES) or pacing has been under investigation as a potential therapy for gastrointestinal motility disorders. However, few studies are available on the effect of two-channel GES with trains of pulses on gastric slow waves and gastric emptying.

Innovations and breakthroughs

In this study, the authors used single-channel (14 cm above the pylorus) and two-channel (6 and 14 cm above the pylorus) GES to investigate their effect on gastric emptying. Compared with the control session, two-channel but not single-channel GES with trains of pulses significantly accelerated gastric emptying, which is consistent with the previous findings.

Applications

Two-channel GES could entrain gastric slow waves and accelerate gastric emptying in healthy dogs, suggesting that two-channel GES with trains of pulses can be used in treatment of gastroparesis and normalization of gastric dysrhythmia.

Terminology

GES with trains of pulses, composed of a repetitious train of pulses, is derived from the combination of a continuous pulse singal with a high frequency (in the order of 5-100 Hz) and a control signal to turn the pulses on and off, such as x seconds "on" and y seconds "off". This kind of stimulation has been used in electroacupuncture.

Peer review

The authors have demonstrated that entrainment of gastric slow waves is feasible with GES of trains of pulses at a pulse width of 4 ms or greater and two-channel GES with trains of pulses can accelerate gastric emptying in healthy dogs, thus having a therapeutic potential for the treatment of gastric motility disorders.

REFERENCES

1 **Feldman M**, Schiller LR. Disorders of gastrointestinal motility associated with diabetes mellitus. *Ann Intern Med* 1983; **98**: 378-384

2 **Horowitz M**, Maddox AF, Wishart JM, Harding PE, Chatterton BE, Shearman DJ. Relationships between oesophageal transit and solid and liquid gastric emptying in diabetes mellitus. *Eur J Nucl Med* 1991; **18**: 229-234

3 **Chang FY**, Lu CL. Irritable bowel syndrome in the 21st century: perspectives from Asia or South-east Asia. *J Gastroenterol Hepatol* 2007; **22**: 4-12

4 **Chen J**, McCallum RW. Gastric slow wave abnormalities in patients with gastroparesis. *Am J Gastroenterol* 1992; **87**: 477-482

5 **Koch KL**, Stern RM, Stewart WR, Vasey MW. Gastric emptying and gastric myoelectrical activity in patients with diabetic gastroparesis: effect of long-term domperidone treatment. *Am J Gastroenterol* 1989; **84**: 1069-1075

6 **Riezzo G**, Pezzolla F, Darconza G, Giorgio I. Gastric myoelectrical activity in the first trimester of pregnancy: a cutaneous electrogastrographic study. *Am J Gastroenterol* 1992; **87**: 702-707

7 **Cucchiara S**, Salvia G, Borrelli O, Ciccimarra E, Az-Zeqeh N, Rapagiolo S, Minella R, Campanozzi A, Riezzo G. Gastric electrical dysrhythmias and delayed gastric emptying in gastroesophageal reflux disease. *Am J Gastroenterol* 1997; **92**: 1103-1108

8 **Dauchel J**, Schang JC, Kachelhoffer J, Eloy R, Grenier JF. Gastrointestinal myoelectrical activity during the postoperative period in man. *Digestion* 1976; **14**: 293-303

9 **Xu X**, Mandanas RA, Lin X, Chen JD. Impaired gastric slow wave rhythmicity in patients after bone marrow or stem cell transplant. *Dig Dis Sci* 2002; **47**: 1746-1751

10 **McCallum RW**. Cisapride: a new class of prokinetic agent. The ACG Committee on FDA-related matters. American College of Gastroenterology. *Am J Gastroenterol* 1991; **86**: 135-149

11 **Chen JD**, Ke MY, Lin XM, Wang Z, Zhang M. Cisapride provides symptomatic relief in functional dyspepsia associated with gastric myoelectrical abnormality. *Aliment Pharmacol Ther* 2000; **14**: 1041-1047

12 **Abell T**, McCallum R, Hocking M, Koch K, Abrahamsson H, Leblanc I, Lindberg G, Konturek J, Nowak T, Quigley EM, Tougas G, Starkebaum W. Gastric electrical stimulation for medically refractory gastroparesis. *Gastroenterology* 2003; **125**: 421-428

13 **Hocking MP**, Vogel SB, Sninsky CA. Human gastric myoelectric activity and gastric emptying following gastric surgery and with pacing. *Gastroenterology* 1992; **103**: 1811-1816

14 **Sarna SK**, Daniel EE. Electrical stimulation of small intestinal electrical control activity. *Gastroenterology* 1975; **69**: 660-667

15 **Qian L**, Lin X, Chen JD. Normalization of atropine-induced postprandial dysrhythmias with gastric pacing. *Am J Physiol* 1999; **276**: G387-G392

16 **Lin ZY**, McCallum RW, Schirmer BD, Chen JD. Effects of pacing parameters on entrainment of gastric slow waves in patients with gastroparesis. *Am J Physiol* 1998; **274**: G186-G191

17 **Kelly KA**. Pacing the gut. *Gastroenterology* 1992; **103**: 1967-1969

18 **Chen JD**, Xu X, Zhang J, Abo M, Lin X, McCallum RW, Ross

B. Efficiency and efficacy of multi-channel gastric electrical stimulation. *Neurogastroenterol Motil* 2005; **17**: 878-882
19 **Song G**, Hou X, Yang B, Liu J, Qian W, Chen JD. Two-channel gastric electrical stimulation accelerates delayed gastric emptying induced by vasopressin. *Dig Dis Sci* 2005; **50**: 662-668
20 **Song GQ**, Hou X, Yang B, Sun Y, Qian W, Chen JD. A novel method of 2-channel dual-pulse gastric electrical stimulation improves solid gastric emptying in dogs. *Surgery* 2008; **143**: 72-78
21 **Bellahsène BE**, Lind CD, Schirmer BD, Updike OL, McCallum RW. Acceleration of gastric emptying with electrical stimulation in a canine model of gastroparesis. *Am J Physiol* 1992; **262**: G826-G834
22 **McCallum RW**, Chen JD, Lin Z, Schirmer BD, Williams RD, Ross RA. Gastric pacing improves emptying and symptoms in patients with gastroparesis. *Gastroenterology* 1998; **114**: 456-461
23 **Familoni BO**, Abell TL, Voeller G, Salem A, Gaber O. Electrical stimulation at a frequency higher than basal rate in human stomach. *Dig Dis Sci* 1997; **42**: 885-891
24 **Chen JD**, Qian L, Ouyang H, Yin J. Gastric electrical stimulation with short pulses reduces vomiting but not dysrhythmias in dogs. *Gastroenterology* 2003; **124**: 401-409
25 **Hou X**, Song GQ, Yang B, Sun Y, Qian W, Chen JD. Effects of gastric electrical stimulation with short pulses and long pulses on gastric dysrhythmia and signs induced by vasopressin in dogs. *Dig Dis Sci* 2008; **53**: 630-635
26 **Song G**, Hou X, Yang B, Sun Y, Liu J, Qian W, Chen JD. Efficacy and efficiency of gastric electrical stimulation with short pulses in the treatment of vasopressin-induced emetic responses in dogs. *Neurogastroenterol Motil* 2006; **18**: 385-391
27 **Forster J**, Sarosiek I, Delcore R, Lin Z, Raju GS, McCallum RW. Gastric pacing is a new surgical treatment for gastroparesis. *Am J Surg* 2001; **182**: 676-681
28 **Lin X**, Peters LJ, Hayes J, Chen JD. Entrainment of segmental small intestinal slow waves with electrical stimulation in dogs. *Dig Dis Sci* 2000; **45**: 652-656
29 **Qian L**, Peters LJ, Chen JD. Effects of electroacupuncture on gastric migrating myoelectrical complex in dogs. *Dig Dis Sci* 1999; **44**: 56-62
30 **Lin XM**, Ouyang H, Zhu HB, Chen JD. Multi-channel electrical stimulation is more effective and efficient than single channel stimulation for the entrainment of gastric myoelectrical activity. *Gastroenterology* 2000; **118**: A393
31 **Xu J**, Ross RA, McCallum RW, Chen JD. Two-channel gastric pacing with a novel implantable gastric pacemaker accelerates glucagon-induced delayed gastric emptying in dogs. *Am J Surg* 2008; **195**: 122-129
32 **Horowitz M**, Dent J. The study of gastric mechanics and flow: a Mad Hatter's tea party starting to make sense? *Gastroenterology* 1994; **107**: 302-306

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CASE REPORT

Adult hereditary fructose intolerance

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Abstract

Hereditary fructose intolerance (HFI) is an under-recognized, preventable life-threatening condition. It is an autosomal recessive disorder with subnormal activity of aldolase B in the liver, kidney and small bowel. Symptoms are present only after the ingestion of fructose, which leads to brisk hypoglycemia, and an individual with continued ingestion will exhibit vomiting, abdominal pain, failure to thrive, and renal and liver failure. A diagnosis of HFI was made in a 50-year-old woman on the basis of medical history, response to IV fructose intolerance test, demonstration of aldolase B activity reduction in duodenal biopsy, and molecular analysis of leukocyte DNA by PCR showed homozygosity for two doses of mutant gene. HFI may remain undiagnosed until adult life and may lead to disastrous complications following inadvertent fructose or sorbitol infusion. Several lethal episodes of HFI following sorbitol and fructose infusion have been reported. The diagnosis can only be suspected by taking a careful dietary history, and this can present serious complications.

Key words: Adults; Fructose intolerance; Diet; Fructose; Sorbitol

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INTRODUCTION

Hereditary fructose intolerance (HFI) is an autosomal recessive inborn error of metabolism that results from a deficiency of fructose 1-phosphate aldolase in the liver, intestine and kidney.

The estimated incidence is 1 in 20 000 live births[1] and the carrier frequency is 1 in 70, but the prevalence of HFI in adults is unknown. The clinical symptoms were first described by Chambers and Pratt in 1956[2].

Affected individuals fail to metabolize fructose completely in the liver, intestine and kidneys because of deficiency of fructose 1-phosphate aldolase and ingestion of fructose, sorbitol or sucrose causes abdominal pain, vomiting and symptomatic hypoglycemia. The syndrome typically appears in the newborn at the time of weaning from the breast when food containing sucrose or fructose is given. Continued ingestion results in poor feeding, growth retardation, gradual liver and kidney failure acidosis, and eventually death[3]. Affected children soon develop an aversion to all foods and protect themselves by self-imposed fructose and sucrose restriction.

The strict dietary exclusion leads to normal growth and longevity. Nevertheless, complete elimination of this sugar from the diet is difficult to achieve, especially for undiagnosed adults, without professional advice. These people may suffer symptoms throughout life and represent a diagnostic challenge for attending physicians. Furthermore, potentially lethal complications may result from inadvertent infusion of fructose- or sorbitol-containing solutions in a hospital setting[4,5].

CASE REPORT

A 50-year-old German woman presented with a long life history of aversion to sugary foods. She reported being breast fed until the age of 2 years, and her mother said that she refused the usual sucrose-containing formulas. She described nausea, vomiting, diffuse abdominal pain and hypoglycemic symptoms even after the smallest amount of sugar or fruit. Her 2-year-old brother died after receiving an intravenous infusion in hospital, while her parents and three siblings are asymptomatic. She takes no regular medications. On examination, she

Table 1 FTT using 250 mg/kg body weight showing glucose, phosphate, uric acid and magnesium levels in the blood following injection of fructose

Time (min)	Glucose (mg/dL)	Phosphate (mmol/L)	Uric acid (mg/dL)	Magnesium (mmol/L)
0	87	1.000	5.1	0.72
15	67	0.77	6.74	0.84
30	61	0.64	7.74	0.8
45	60	0.80	7.58	0.88
60	68	0.83	7.58	0.91

had mild thoracic scoliosis with no neurological defect. Otherwise, physical examination was unremarkable.

Results of laboratory investigations including full blood count, urea, creatinine, electrolytes, full biochemical profile, amylase lipase and lipid studies, liver function tests and insulin level were within normal ranges. A fructose tolerance test (FTT) using 250 mg fructose per kilogram body weight was performed. At 0, 15, 30, 45 and 60 min after fructose injection, blood samples were taken for analysis of glucose, phosphate, uric acid and magnesium. A typical abnormal FTT was observed after the infusion, i.e. a drop in serum glucose and serum phosphate and rise in serum uric acid and magnesium concentration occurred (Table 1).

Thirty minutes after fructose injection, she developed significant dizziness, sweating, tremor and abdominal pain that were closely observed, and by 60 min her symptoms improved. The diagnosis was further confirmed by histochemical analysis of an endoscopic biopsy specimen from the small intestine, which showed 70% reduction in aldolase B activity in the mucosa, and molecular analysis of leukocyte DNA extracted from a blood sample using PCR amplification revealed that she had inherited two doses of the mutant gene, one from each parent, as the cause of the disease.

DISCUSSION

Fructose is a natural component of many plants and is distributed widely among most fruits and vegetables. Fructose is metabolized primarily in the liver and to some extent in the kidney, small intestine and adipose tissue[6]. Deficiency in aldolase B in the liver, kidney and small intestine causes fructose intolerance[7]. After ingestion, fructose rapidly enters the hepatocytes where fructokinase phosphorylates it to fructose 1-phosphate. Fructose 1-phosphate accumulates in HFI because of deficiency of the enzyme fructose 1-phosphate aldolase, which splits fructose 1-phosphate into glyceraldehydes and dihydroxyacetone phosphate.

The accumulation of fructose 1-phosphate results in inhibition of other enzymes, namely phosphorylase, liver fructose 1-6 bisphosphate aldolase and fructokinase. This results in impaired glycogenolysis and glyconeogenesis, and may induce hypoglycemia[8]. Early exclusion of fructose and sucrose from the diet is accompanied by dramatic improvement; otherwise, growth is retarded and progressive liver and renal disease are likely, and may lead to death[9-11]. Diagnosis can be achieved by FTT and tissue diagnosis by direct assay of aldolase B activity in the liver, intestine or renal tissue. Recently, the use of PCR-based procedures has made the diagnosis simpler[12,13].

The infusion of fructose- or sorbitol-containing solutions in patients with unsuspected disease leads to potentially fatal hepatorenal failure. More than 20 cases have been reported in Germany where the use of fructose or sorbitol solutions is long established[14,15]. Our patient is alive at the age of 50 years with previously undiagnosed HFI, and did not have complications of the disease. This patient illustrates the importance of a careful dietary history and awareness of disease symptoms. In contrast, incorrect diagnosis and unawareness of possible pediatric problems in adult life may lead to catastrophic complications, while early recognition leads to effective management.

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REFERENCES

1 **Gitzelmann R**, Baerlocher K. Vorteile und Nachteile der Fruktose in der Nahrung. *Pädiat Fortbildk Praxis* 1973; **37**: 40-55
2 **Chambers RA**, Pratt RT. Idiosyncrasy to fructose. *Lancet* 1956; **271**: 340
3 **Gitzelmann R**, Steinmann B, Van den Berghe G. Disorders of fructose metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D. eds. The metabolic basis of inherited disease. 6th ed. New York: McGraw-Hill, 1989: 399-424
4 **Ali M**, Rosien U, Cox TM. DNA diagnosis of fatal fructose intolerance from archival tissue. *Q J Med* 1993; **86**: 25-30
5 **Cox TM**. Iatrogenic deaths in hereditary fructose intolerance. *Arch Dis Child* 1993; **69**: 413-415
6 **Froesch ER**. Disorders of fructose metabolism. *Clin Endocrinol Metab* 1976; **5**: 599-611
7 **Hers HG**, Joassin G. [Anomaly of hepatic aldolase in intolerance to fructose.] *Enzymol Biol Clin* (Basel) 1961; **1**: 4-14
8 **Burmeister LA**, Valdivia T, Nuttall FQ. Adult hereditary fructose intolerance. *Arch Intern Med* 1991; **151**: 773-776
9 **Collins J**. Metabolic disease. Time for fructose solutions to go. *Lancet* 1993; **341**: 600
10 **Krebs HA**, Woods HF, Alberti KGMM. Hyperlactataemias and lactic acidosis. *Essays Med Biochem* 1975; **1**: 81-104
11 **Lameire N**, Mussche M, Baele G, Kint J, Ringoir S. Hereditary fructose intolerance: a difficult diagnosis in the adult. *Am J Med* 1978; **65**: 416-423
12 **Cox TM**. An independent diagnosis. *BMJ* 1990; **300**: 1512-1514
13 **Steinmann B**, Gitzelmann R, Van den Berghe G. Disorders of fructose metabolism. In: Scriver C, Beaudet A, Sly W, Valle D, eds. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill, 2001: 1489-1520
14 **Steegmanns I**, Rittmann M, Bayerl JR, Gitzelmann R. [Adults with hereditary fructose intolerance: risks of fructose infusion] *Dtsch Med Wochenschr* 1990; **115**: 539-541
15 **Jamar S**, Evenepoel P, Kuypers D, Maes B, Vanrenterghem Y. A young patient with unexplained acute hepatorenal dysfunction. *Nephrol Dial Transplant* 2003; **18**: 1220-1222

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CASE REPORT

Drug-induced liver injury due to "natural products" used for weight loss: A case report

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Abstract

Taking herbal-extracts to lose weight is an underestimated health hazard. Often, these products contain active agents that can cause acute liver damage. In this case report, a 22-year-old female patient, who presented with a feature of cholestatic syndrome, was so sure that the "natural products" were not dangerous that she did not inform her physicians that she had taken them, making their task that much more challenging. Clinical presentation mimicked acute cholecystitis and the patient underwent a cholecystectomy. Surgery was without any consequences and complications, although it did not completely cure the illness. She later admitted to having taken herbal remedies and this led to the correct diagnosis of phytotherapy-related hepatotoxicity and a successful therapeutic approach. The true incidence of phytotherapy-related hepatotoxicity and its pathogenic mechanisms are largely unknown. It is important to increase the awareness of both clinicians and patients about the potential dangers of herbal remedies.

Key words: Drug-induced liver injury; Obesity; Herbal remedies; Cholecystitis

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INTRODUCTION

The desire to lose weight using "natural products" and the availability of these items can induce pathologies, the causes of which are often overlooked. The main problem with "natural products" is that the exact quantity and purity of a given ingredient contained in extracts of vegetable origin (mainly herbs) are largely unknown. It can happen that patients deny taking these products, thinking that they are "safe" because they are "natural" and thus physicians do not have the key facts to interpret dangerous pathologies. Here we report a case of a patient who experienced jaundice and pruritus, not assuming the "natural products" was at fault, with a clinical presentation highly suggestive of cholecystitis whose final diagnosis turned out to be drug-induced liver injury (DILI).

CASE REPORT

A 22-year-old obese (BMI 32) woman presented to hospital in May 2007 with a cholestatic syndrome of unknown origin. Her only declared pre-existing medication was paracetamol (500 mg daily), used as an analgesic for menstrual pain. Pre-admission blood tests were normal.

Symptoms included jaundice, pruritus, right upper quadrant pain and epigastric tenderness, accompanied

by fever of low grade, nausea, vomiting, dark urine and pale stools. At the time of admission routine liver enzyme tests revealed bilirubin 128 μmol/L (< 20 μmol/L), alkaline phosphatase (ALP) 1229 U/L (40-110 U/L), γ-glutamyltransferase (GGT) 293 U/L (< 50 U/L), Aspartic-aminotransferase (AST) 1378 U/L (< 45 U/L) and Alanine-aminotransferase (ALT) 1686 U/L (< 40 U/L). She had never consumed alcohol and there was no recent travel history. Viral serology for hepatitis and HIV were negative. An infection screen was carried out because our country has an increasing incidence of exotic illnesses due to recent immigration that can cause transient liver enzyme derangement. This panel included cytomegalovirus, Epstein-Barr virus, Flavivirus, Dengue virus, Ross River virus, Barmah Forest virus, Spotted fever virus, Scrub Typhus, and Leptospirosis serology; all of them were unremarkable. Serum copper and caeruloplasmin, α-fetoprotein, α-1 antitrypsin, iron deposits were in the normal range. Anti-nuclear antibodies, perinuclear antineutrophil cytoplasmic antibodies, antibodies to liver kidney microsomal antigen type-1, anti-mitochondrial and anti-smooth muscle antibodies were negative. Her complete blood count was slightly abnormal. In fact, a modest increase in WBCs without left shift was present on admission, concurrent with an increase in eosinophils (7%) and a decrease in lymphocyte counts.

Abdominal ultrasound revealed the presence of microcalculi in the gallbladder. No clear dilatation of the common bile duct was seen, but the exam was performed in the presence of marked abdominal meteorism.

A magnetic resonance cholangio-pancreatography (MRC) indicated a dilatation of the choledocus with a likely interruption of its terminal tract, with some evidence of microstones in the gallbladder (Figure 1). Consequently, physicians empirically treated this illness by imipenem/cilastatin (500/500 mg every 6 h) for seven days.

A negative history of drug use, the physical findings (i.e. right upper quadrant and epigastric tenderness in the absence of peritoneal findings), laboratory data (i.e. elevated levels of bilirubin, alkaline phosphatase, ALT, and γ-glutamyltransferase) were consistent with extrahepatic obstruction, suggesting stones complicated by acute cholecystitis. Therefore, to gain access to and/or remove impacted common bile duct (CBD) stones at the ampulla of Vater, the patient was submitted to a preoperative endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic sphincterotomy and extraction of sand-like stones.

Using this standard therapy, her laboratory data improved, with AST and ALT values of 1110 U/L and 1225 U/L, respectively. At this point, a laparoscopic operative CBD exploration with mini-invasive technique was planned.

The abdominal inspection showed the presence of extensive visceral adherences, as well as a general aspect of diffuse bowel inflammation. The gallbladder was reddish-colored with a thick wall and bled easily. This was suggestive of a complex abdominal pathology, therefore a conversion to an open procedure was chosen.

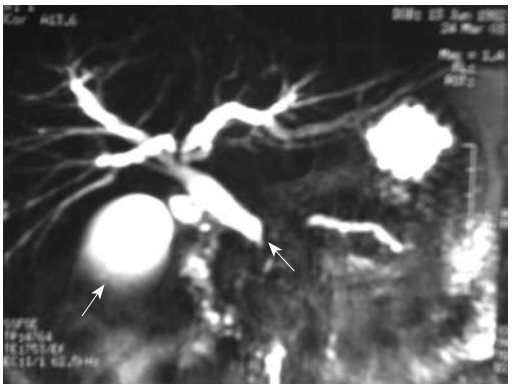

Figure 1 Magnetic resonance cholangio-pancreatography showing dilatation of the choledocus and mimicking an interruption of its terminal tract, with some evidence of microstones in the gallbladder (arrows).

Abdominal exploration and biliary manometry caused the surgeons to utilize an ante-grade trans-ampullary intra-operative endobiliary stenting. The macroscopic examination showed acute cholecystitis with moderate wall-thickening containing very dense bile and some small stones.

The postoperative course was pain-free and the patient was prematurely discharged with drastically reduced (though elevated compared to normal) enzymatic values of AST 108 U/L and ALT 156 U/L.

Four days later the patient underwent liver laboratory tests that underlined an elevation of the transaminases, with an AST level of 644 U/L, ALT of 810 U/L, AP of 806 U/L and bilirubin of 64 μmol/L, mainly unconjugated with a persistently draining T-tube.

An interrogation of the woman's relatives highlighted that the patient had taken several doses of a phyto-preparation in solution, bought from a store run by an herbalist, labeled as "herbal therapy for losing weight". Fortunately, the relatives found this preparation, containing "Lycopodium serratum and Chelidonium maius" at home. The Roussel Uclaf Causality Assessment Method, also known as Danan's international consensus criteria[1], was developed to quantify the strength of association between liver injury and herbal remedies and implicated the phyto-preparation as causing the injury. The case was adjudicated by three reviewers (DC, GT, MND) working independently and the patient was diagnosed as likely to be suffering from DILI. One month after given up taking the herbal remedy, all the liver parameters returned to normal values, without any apparent consequences. The case was re-adjudicated after a four-week follow-up by the same reviewers and the diagnosis was confirmed.

DISCUSSION

The "chelidonium majus" belongs to the family of papaveraceae; its roots contain the biologically active components chelerythrine and sanguinarine. The active "principia" are similar to those of opium, and have well-known hepatotoxic effects[2-4], although in animals an average daily oral dose of alkaloids up to 5 mg/kg has

been proven to be safe[5].

The herb "*Lycopodium serratum*" has several[6] active agents that can cause hepatotoxicity[7]. The hepatic damage caused by these agents, generally of a cholestatic type, is possibly mediated by an idiosyncratic or hypersensitivity reaction. Recently, a different hypothesis was proposed involving an impairment of mitochondrial respiration[8].

Although this is not the first case reported in the literature, its importance lies in the atypical presentation. Indeed, was this a case of misdiagnosis, the co-existence of two diseases or an uncommon manifestation of DILI with a clinical presentation mimicking an other disease? The results of liver laboratory tests and imaging studies were attributed to an earlier combination of symptomatic gallstones and cholangitis and the patient was treated accordingly. Unfortunately, a liver biopsy, which would have indicated the presence of canalicular cholestasis with bile plugs in dilated canaliculi, occasional portal tracts containing a prominent lymphocytic infiltrate with mild piecemeal necrosis, was not performed and consequently the opportunity for a definitive diagnosis was lost.

Gallstone disease remains one of the most common medical problems. The risk factors predisposing to gallstone formation include obesity, diabetes mellitus, estrogen and pregnancy, hemolytic diseases, and cirrhosis. Acute cholecystitis can carry the risk of complications, including empyema, perforation, abscess, peritonitis and sepsis. Acute cholecystitis also causes acute pain in the right upper quadrant (RUQ). However, cross-sectional imaging is essential, because more than one-third of patients with acute RUQ pain do not have acute cholecystitis. Today, laparoscopic cholecystectomy, laparoscopic common bile duct exploration, and endoscopic retrograde management of CBD stones play important roles in the treatment of gallstones, even though the treatment of choice remains cholecystectomy. However, when asymptomatic gallstones are detected during the evaluation of a patient, a prophylactic cholecystectomy is normally not indicated because of several factors. Only about 30% of patients with asymptomatic cholelithiasis will warrant surgery during their lifetime, suggesting that cholelithiasis is a relatively benign condition in some people.

The main question we should ask ourselves is: was surgery the right choice? Although the patient's symptoms and signs were extremely atypical for establishing the diagnosis of acute cholecystitis in this young immune-competent patient, her declaration of not having taken any other medications, including over-the-counter medications, herbal or traditional medicines, definitely misled physicians. The late admission by her parents allowed a correct diagnosis of DILI and not acute cholecystitis. Given the diagnosis of DILI, the patient took a further risk with anesthesia.

Should physicians have performed further studies before surgery? A CT cholangiogram would have shown contrast material being excreted by the renal tract, suggesting that the pathology concerned hepatocellular damage rather than a biliary obstruction.

Was the patient incautiously discharged from the surgery unit? The answer is probably yes, because the reduction of liver enzymatic activity caused surgeons to underestimate the pathology, with overconfidence in the previous diagnosis of cholecystitis.

There are many examples of hepatotoxicity induced by herbal remedies, which have been widely used in recent decades as weight loss agents. Germander (Teucrium chamaedrys) extracts cause DILI, probably mediated by furano neoclerodane diterpenoids[9]. Chaparral is a desert shrub traditionally used by Native Americans for treatment of several ailments. Recently, preparations of chaparral leaves have been marketed as weight loss agents. The mechanism of chaparral toxicity involves its active ingredient, nordihydroguaiaretic acid[10]. Kava (kava kava, awa, or kew), derived from the dried root and rhizome of Piper methysticum, has recently been marketed as an anxiolytic and mood enhancer. Recent studies from Europe have described cases of kava-associated hepatic injury. The mechanism of hepatic injury appears to be immune-mediated, with CYP2D6 deficiency perhaps being a risk factor[11]. *Herba Ephedrae* (from Ephedra sinica and other Ephedra species) is a traditional Chinese extract also used for treatment of asthma, nasal congestion, and fever. Although most adverse effects of *Herba Ephedrae* are cardiovascular or neurological, 4% of reports mentioned acute hepatitis. *Herba Ephedrae* contains phytochemicals, which are thought to strengthen its toxic activity[12].

In addition to the above supplements, liver injury has been attributed to other botanical agents. The pyrrolizidine alkaloids found in comfrey leaves and Heliotropium, Senecio, and Crotalaria species are known to cause veno-occlusive disease of the liver *via* a toxic effect[13]. Mixtures of valerian and skullcap (Valeriana officinalis and Scutellaria lateriflora) have induced hepatitis *via* alkylating agents. LipoKinetix was marketed as a dietary supplement for weight loss. Hepatic injury appears to be due to an idiosyncratic reaction, perhaps related to phenylpropanolamine[14]. Among other weight loss agents, Usnic acid should be suspected in case of severe hepatotoxicity[15].

In our case, the patient was sure the product was harmless and denied the use of a potentially dangerous product in her history, thus not allowing physicians to discover the etiology of the serious pathology from which she suffered. Only an accurate interrogation of relatives was able to discover the relationship between the herbal remedy and DILI.

The diagnostic approach was, in spite of the lack of a certain etiology, the most cautious possible; in fact, MRC and ERCP, perfectly framed into the clinical picture of this patient, are generally considered investigations of first level.

The prevalence of adverse drug reactions (ADR) in health care systems has generated immense interest in recent years. Some of these adverse events are completely unpredictable, but some result from medical errors, patient's negligence or ignorance, and may occur anywhere and at anytime in the health care processes. However, a majority of them may be preventable. The consequences of these ADRs might vary, from little or

no harm to ultimately being fatal to the patients.

Patient safety has received increased attention in recent years, but mostly with a focus on the epidemiology, rather than on practices that reduce (1) ADRs, (2) adverse events related to exposure to herbal remedies and dietary supplements and (3) invasive procedures in medical care involving a wide spectrum of diagnoses or conditions. Potential safety practices should be identified, based on preliminary surveys of the literature and expert consultation.

The misdiagnosis of DILI has many ramifications. These include medical and psychological implications for patients and their families, and financial and public health implications for health-care institutions.

The patient could have sued the health care practitioners (specifically the surgeons) if she felt she had been injured. However, successful medical malpractice lawsuits require proof of the following items: the care provided was below the ordinary standard of care that would be provided by similar health care practitioners under similar circumstances and the patient was harmed because of the deviation from the standard of care. In our case, concerns about lawsuits did not arise, because the physicians' actions were in the best interests of the patient. In fact, a good defence against malpractice lawsuits is to provide excellent medical care and to build close, trusting, and collaborative relationships with patients.

As a final consideration, patients should be especially cautious about using drugs, and should inform their doctor about any drugs or other substances they are taking, including prescription and over-the-counter medications, recreational drugs, herbal remedies, and nutritional supplements. Health care professionals are encouraged to report all ADRs, especially hepatotoxicity and to pay much more attention in prescribing and administering drugs.

For the vegetal abstracts, it should be mandatory to correctly describe their contents, taking in account the active ingredients, the real quantity per unit of product contained with the preparation, and to make clear any possible side effects.

In conclusion, the authors believe that a detailed, painstaking and meticulous history could have unveiled the underlying condition and the patient would not have been subjected to invasive and potentially harmful interventions. This is probably the most important learning point that emerged from this case report.

REFERENCES

1 **Danan G**, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol* 1993; **46**: 1323-1330

2 **Conti E**, De Checchi G, Mencarelli R, Pinato S, Rovere P. Lycopodium similiaplex-induced acute hepatitis: a case report. *Eur J Gastroenterol Hepatol* 2008; **20**: 469-471

3 **Stickel F**, Pöschl G, Seitz HK, Waldherr R, Hahn EG, Schuppan D. Acute hepatitis induced by Greater Celandine (Chelidonium majus). *Scand J Gastroenterol* 2003; **38**: 565-568

4 **Crijns AP**, de Smet PA, van den Heuvel M, Schot BW, Haagsma EB. [Acute hepatitis after use of a herbal preparation with greater celandine (Chelidonium majus)] *Ned Tijdschr Geneeskd* 2002; **146**: 124-128

5 **Kosina P**, Walterová D, Ulrichová J, Lichnovský V, Stiborová M, Rýdlová H, Vicar J, Krecman V, Brabec MJ, Simánek V. Sanguinarine and chelerythrine: assessment of safety on pigs in ninety days feeding experiment. *Food Chem Toxicol* 2004; **42**: 85-91

6 **Takayama H**, Katakawa K, Kitajima M, Yamaguchi K, Aimi N. Ten new Lycopodium alkaloids having the lycopodane skeleton isolated from Lycopodium serratum Thunb. *Chem Pharm Bull* (Tokyo) 2003; **51**: 1163-1169

7 **Woolf GM**, Petrovic LM, Rojter SE, Wainwright S, Villamil FG, Katkov WN, Michieletti P, Wanless IR, Stermitz FR, Beck JJ, Vierling JM. Acute hepatitis associated with the Chinese herbal product jin bu huan. *Ann Intern Med* 1994; **121**: 729-735

8 **Choy CS**, Cheah KP, Chiou HY, Li JS, Liu YH, Yong SF, Chiu WT, Liao JW, Hu CM. Induction of hepatotoxicity by sanguinarine is associated with oxidation of protein thiols and disturbance of mitochondrial respiration. *J Appl Toxicol* 2008; **28**: 945-956

9 **Stickel F**, Egerer G, Seitz HK. Hepatotoxicity of botanicals. *Public Health Nutr* 2000; **3**: 113-124

10 **Gordon DW**, Rosenthal G, Hart J, Sirota R, Baker AL. Chaparral ingestion. The broadening spectrum of liver injury caused by herbal medications. *JAMA* 1995; **273**: 489-490

11 **Russmann S**, Lauterburg BH, Helbling A. Kava hepatotoxicity. *Ann Intern Med* 2001; **135**: 68-69

12 **Lee MK**, Cheng BW, Che CT, Hsieh DP. Cytotoxicity assessment of Ma-huang (Ephedra) under different conditions of preparation. *Toxicol Sci* 2000; **56**: 424-430

13 **Whiting PW**, Clouston A, Kerlin P. Black cohosh and other herbal remedies associated with acute hepatitis. *Med J Aust* 2002; **177**: 440-443

14 **Lake CR**, Gallant S, Masson E, Miller P. Adverse drug effects attributed to phenylpropanolamine: a review of 142 case reports. *Am J Med* 1990; **89**: 195-208

15 **Sanchez W**, Maple JT, Burgart LJ, Kamath PS. Severe hepatotoxicity associated with use of a dietary supplement containing usnic acid. *Mayo Clin Proc* 2006; **81**: 541-544

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CASE REPORT

Primary hepatic carcinoid: A case report and literature review

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Abstract

Carcinoids are tumors derived from neuroendocrine cells and often produce functional peptide hormones. Approximately 54.5% arise in the gastrointestinal tract and frequently metastasize to the liver. Primary hepatic carcinoid tumors (PHCT) are extremely rare; only 95 cases have been reported. A 65-year-old man came to our attention due to occasional ultrasound findings in absence of clinical manifestations. His previous medical history, since 2003, included an echotomography of the dishomogeneous parenchymal area but no focal lesions. A computed tomography scan performed in 2005 showed an enhanced pseudonodular-like lesion of about 2 cm. Cholangio-magnetic resonance imaging identified the lesion as a possible cholangiocarcinoma. No positive findings were obtained with positron emission tomography. Histology suggested a secondary localization in the liver caused by a low-grade malignant neuroendocrine tumor. Immunohistochemistry was positive for anti chromogranin antibodies, Ki67 antibodies and synaptophysin. Octreoscan scintigraphy indicated intense activity in the lesion. Endoscopic investigations were performed to exclude the presence of extrahepatic neoplasms. Diagnosis of PHCT was established. The patient underwent left hepatectomy, followed by hormone therapy with sandostatine LAR. Two months after surgery he had a lymph nodal relapse along the celiac trunk and caudate lobe, which was histologically confirmed. The postoperative clinical course was uneventful, with a negative follow-up for hematochemical, clinical and radiological investigations at 18 mo post-surgery. Diagnosis of PHCT is based principally on the histopathological confirmation of a carcinoid tumor and the exclusion of a non-hepatic primary tumor. Surgical resection is the recommended primary treatment for PHCT. Recurrence rate and survival rate in patients treated with resection were 18% and 74%, respectively.

Key words: Carcinoid; Primary hepatic carcinoid; Neuroendocrine neoplasm; Therapy; Surgical treatment; Prognosis

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INTRODUCTION

Carcinoids are tumors of neuroendocrine origin, capable of producing functional peptide hormones. The literature has reported different classifications, mainly based on either anatomo-pathological and/or clinical criteria of neuroendocrine tumors of ubiquitous distribution. Several organs may be involved, such as the adrenal gland (pheochromocytoma), the thyroid (midollar carcinoma) and the lung, where microcytoma accounts

for 20%[1] while carcinoids represent 1%-2% of all pulmonary neoplasms in the typical variant (80%-90%) and atypical (10%-20%)[2,3].

About 54.5% of carcinoid tumors arise within the gastrointestinal system and frequently metastasize to the liver[4]. Primary hepatic carcinoid tumor (PHCT) is an extremely rare neoplasm affecting relatively young subjects with an average age of 45 years[5] and no gender predominance. Diagnosis of PHCT is mainly achieved through histological confirmation and exclusion of other sites of the disease[6].

Here, we report the case of an occasional finding of a hepatic lesion, which led to the diagnosis of PHCT after a complicated diagnostic process.

CASE REPORT

A 65-year-old man presented with hypertension, peripheral vascular disease, and statin treated dyslipidemia. His previous medical history, dating back to spring 2003, included an echotomography of the dishomogeneous parenchymal area with no focal lesions. A computed tomography (CT) scan showed no steatosis in the image area. In July 2005, a CT scan of the asymptomatic patient showed an enhanced a pseudonodular-like lesion of about 2 cm localized in hepatic segments Ⅱ-Ⅲ, with intra-hepatic biliary dilatation (Figure 1).

The patient was admitted to hospital for further clinical investigations. Blood chemical analyses showed no abnormalities, not even the presence of markers (CEA, CA 19-9, α FP). The laboratory results are shown in Table 1.

Due to the doubtful interpretations of the radiological findings, a magnetic resonance imaging (MRI) was carried out (Figure 2). The investigation revealed a pseudonodular mass of about 5 cm × 3 cm characterized by low signal intensity both on T1 and T1FS weighted images, as well as weak irregular high signal intensity on T2 and T2FS weighted images. Moreover, an intra-hepatic biliary dilatation was described at the source of the lesion, thus leading us to suspect a heteroplastic lesion similar to a cholangiocarcinoma.

The patient was therefore referred for an 18-fluorodeoxyglucose positron emission tomography (PET), which proved negative. This confirmed by histological examination of a biopsy sample from the lesion. The cytohistological and immunohistochemical picture proved to be consistent with a hepatic localization of a low-grade malignant neuroendocrine carcinoma (presence of anti-chromogranin and Ki 67 antibodies; positive for synaptophysin and S 100 protein) while serum markers were negative (CgA, NSE).

Octreotide scintigraphy using 111 In-pentetreotide (octreotide scan) confirmed the diagnosis as well as enhancing a marked hyperactivity near the lesion in the left hepatic lobe associated with adenopathy in the interaortocaval site (Figure 3A). The patient thus underwent further investigations to exclude metastases with extra hepatic lymph node involvement by endoscopic examinations of the gastroenteric tract (esophagus-gastro-duodenoscopy, colonoscopy, and capsular endoscopy) and analysis of the respiratory systema (bronchoscopy) which all proved negative.

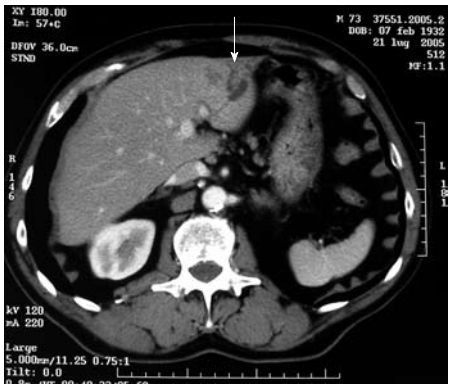

Figure 1 Abdominal CT scan (July 2005) showing a low-density pseudonodular area (arrow) of 2 cm with biliary dilatation.

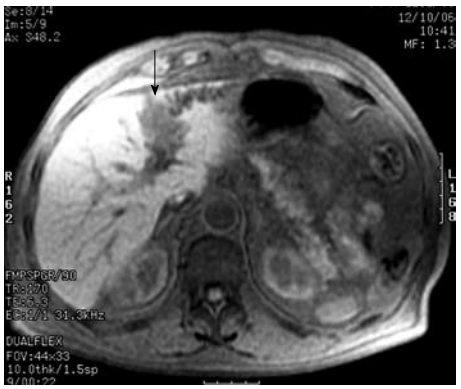

Figure 2 Abdominal MRI (August 2005) showing a pseudonodular mass (arrow) measuring about 5 cm × 3 cm of the hepatic segments Ⅱ-Ⅲ. At the bottom of the lesion, the biliary tree appears dilated.

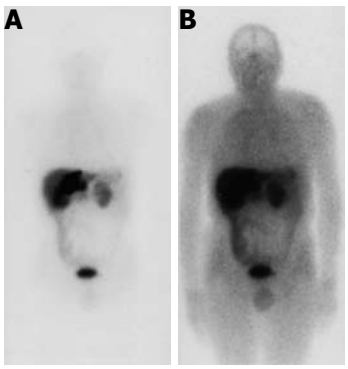

Figure 3 111 In-pentetreotide (octreotide) scintigraphy. A: Before hepatectomy: marked hyperactivity of the lesion is observed in the left hepatic lobe and interaortal adenopathy is observed; B: After hepatectomy; abnormal fluid accumulation of ligand in the epigastric region and dishomogeneous hepatic distribution.

The patient suffered no flushing, no abdominal pain, and no alvus alteration. Moreover, the patient only referred to some successive episodes of angioneurotic edema of the face in the previous 10 years, regardless of the disease in question. As a result, the patient underwent an uncomplicated left hepatic resection and appendectomy with an uneventful postoperative clinical course. Subsequently, octreotide therapy was

Table 1 Hematological values of the patient and normal range

Variable	Patient value	Normal value	Variable	Patient value	Normal value
Erythrocytes	4.93×10^6 U/L	4.2-5.4	Amylase	60 U/L	30-110
Leucocytes	8.16×10^3 U/L	4-10	Total proteins	8.3 g/dL	6.3-8.2
Hemoglobin	14.9 g/dL	12-16	Urea	79 mg/dL	10-50
Platelets	2.22×10^5 U/L	150-400	LDH	250 U/L	313-618
Creatinine	1.1 mg/dL	0.7-1.2	ESR	15 mm/s	1-30
PT	95%	70%-100%	CRP	3 mg/L	up to 3
Total bilirubin	1 mg/dL	0.2-1.3	CEA	2.6 ng/mL	up to 5
GOT	23 U/L	up to 40	CA 19-9	7 ng/mL	up to 37
GPT	22 U/L	9-56	α FP	3 ng/mL	up to 15
GGT	84 U/L	12-58	NSE	8.7 ng/mL	up to 14
ALP	73 U/L	38-126	CgA	72 ng/mL	20-98
Albumin	4.8 g/L	35-52	Anti HCV	Negative	
Cholinesterase	7926 U/L	4650-12220	HbsAg	Negative	
Total cholesterol	258 mg/dL	145-200	Triglycerides	125 mg/dL	50-170

PT: Prothrombin time; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase; GGT: Gamma glutamyl transpeptidase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; ESR: Erythrocyte sedimentation rate; CRP: C reactive protein; CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigenic determinant; α FP: α-fetoprotein; NSE: Neuron-specific enolase; Anti HCV: Antibodies for hepatitis C virus; HbsAg: Hepatitis B surface antigen; CgA: Chromogranin A.

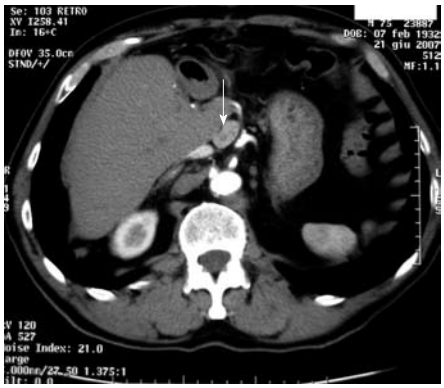

Figure 4 Abdominal CT scan. Results of left hepatectomy show an extended nodular mass measuring about 2.5 cm × 1.5 cm arranged on the back part of the caudate lobe, indicating a lymph node localization of disease.

administered subcutaneously *via* octreotide scan at 2 mo from resection which showed a dishomogeneous distribution of the radioactive drug in the liver site, without any focal images, which warranted further investigation (Figure 3B).

CT scan confirmed the presence of an extended nodular mass measuring about 2.5 cm × 1.5 cm on the back part of the caudate lobe, in close contact with the celiac tripod. The mass was attributable to lymph node recurrence of the disease (Figure 4). The patient showed no signs of evolutive chest disease or any other particular clinical signs.

After a strict clinical and instrumental follow-up period, jointly conducted by an oncologist and a surgeon, the patient's clinical and radiological picture remained stable, as demonstrated by the octreotide therapy. The patient then underwent a surgical lymph node exeresis. The histological finding was compatible with the lymph node metastasis of the neuroendocrine tumor (Figure 5). The postoperative clinical course was uneventful, with a negative follow-up for hematochemical, clinical and radiological investigations at 18 mo post surgery.

DISCUSSION

Neuroendocrine tumors cover a wide range of neoplasms that originate in the neuroendocrine cells that spread throughout the body. Recent studies have suggested an increase in the incidence of these tumors over time[7]. In particular, Maggard *et al*[4] reported a 6.3% increase in 1997 compared with 1973. This could be attributed to an enhanced classification of these tumors and better use of endoscopic techniques for screening purposes. In 1998, 90% of neuroendocrine tumors were reported to occur within the gastrointestinal tract, particularly at the level of the terminal ileum and appendix[8]. However, more recent studies have reported a less frequent gastroenteric involvement (54.%) followed, in decreasing order, by the lung (30.1%), pancreas (2.3%), reproductive system, (1.2%), biliary tract (1.1%), and head and neck (0.4%). As far as the gastrointestinal tract is concerned, a slighter greater involvement of the appendix has been reported compared other sites, such as the small bowel (44.7%) followed by the rectum (19.6%), appendix (16.7%) colon (10.6%) and stomach (7.2%)[4].

PHCTs are rare neuroendocrine tumors, representing 0.3% of all carcinoids, and were first described by Edmonson in 1958[9,10]. Recent studies reported a survey on 95 cases of PHCT[5,11]. The liver is the most frequently involved organ due metastatic disease from extrahepatic neuroendocrine tumors, thus justifying the physician's efforts in ruling out the presence of other diseases before confirming this organ as the primary nature of the tumor[5].

Indeed both the clinical exclusion criteria and the histological confirmation represent a diagnostic means to approach this rare disease.

The clinical onset of neoplasms is often aspecific and related to mass effect on the liver and adjacent organs. Likely symptoms include pain, weight loss, palpable mass, while less common is the classic carcinoid syndrome (skin flushing, abdominal pain and episodes

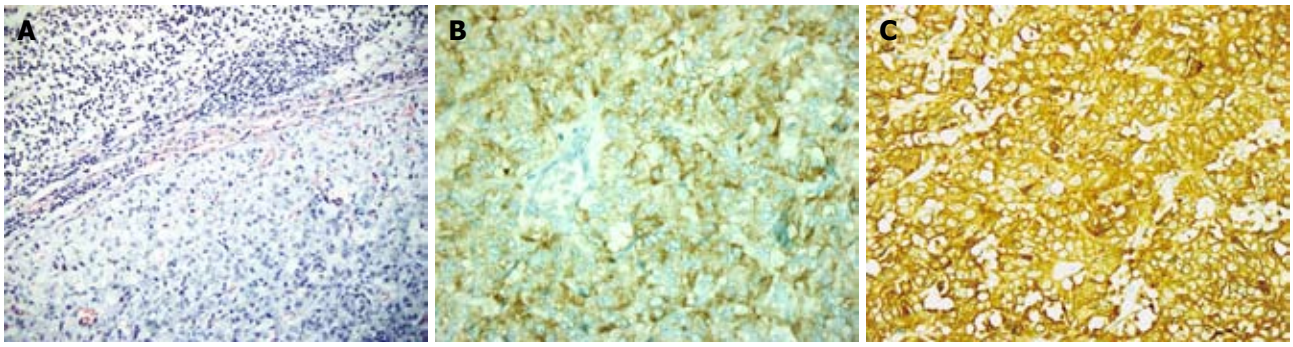

Figure 5 Histological and immunohistochemistry. Proliferation of average sized monomorphic epithelial cells collected in strings and glandular structures (A). Positive immunohistochemistry staining for CgA (B) and NSE (C).

of diarrhea) which are present in 5% of cases[5] and more frequently found in tumors which metastasize to the liver.

The clinical course of PHCT is generally painless compared to other neuroendocrine neoplasms with a more malignant progression. The latter may be characterized by varying degrees of pleomorphism, greater mitotic activity, vascular invasion and necrosis[12]. From the morphological point of view, in accordance with the literature, our patient presented well-differentiated PHCT with low-grade malignancy, minimum pleomorphism, low mitotic index and poor necrotic foci.

The first level diagnosis consisted of non-invasive imaging. A traditional ultrasound scan revealed a hyperecogenic mass containing multiple cystic lesions, while a CT scan confirmed a cystic pattern. Moreover, angiography might demonstrate multiple hypervascular and centrally located radiolucent areas[5]. Classical PET with fluorodeoxyglucose did not prove to be advantageous in neuroendocrine tumor imaging[13]. Thus, a serotonin precursor 11C-5 hydroxy tryptophan was developed as a tracer for PET-scanning, which can be concentrated within carcinoid tumors[13]. Findings using this application are encouraging, allowing the identification of the primary tumor in 84% of cases[14,15]. Primary carcinoid tumors and distant metastasis in patients affected by neuroendocrine tumors are better detected by otreoscan scintigraphy compared to the CT scan and the MRI[16].

The presence of somatostatin receptors within the tumoral cell is best suited for scintigraphy. There are five receptor subtypes (SSTR 1-5) each having different functional properties and binding specificity for the target tissue[16]. Octreotide binds with high affinity to the somatostatin subtype 2 receptor (SSTR 2), which is widely expressed on the cell surface with neuroendocrine characteristics. Certain diagnosis is, however, achieved by fine needle aspiration or biopsy. Immunohistochemistry confirms neuroendocrine origin of PHCT by detecting the markers CgA, NSE, chromostatin, CEA and synaptophysin[17]. Measurement of plasma CgA and repetition of the octreoscan scanning provide the basis for follow-up[5]. Although PHCT appears to be a low malignancy tumor with slow progression, treatment effectiveness and prognosis are difficult to establish owing to its rarity and subsequent lack of prospective data[18]. Surgical resection is the most commonly used therapy and it is considered the treatment of choice[19] in about 85% of primary hepatic carcinoids[18]. This procedure cannot be performed in the 10% of patients affected by metastatic hepatic carcinoid[20,21]. For these cases, as well as for non operable tumors, therapy with radionuclides and the somatostatin analog 177Lu-DOTA-Tyr3-octreotate, are the most modern and promising, not only in terms of stabilization but also with regard to disease regression with minimal toxicity[5,22]. Other therapeutic interventions have been tried for curative and palliative purposes, such as systemic chemotherapy, hepatic artery chemoembolization (only in cases of non resectable or recurrent disease), somatostatin hormone therapy or its analogs performed as a stand-alone therapy or as an adjunct to surgery[5,18]. Hormone therapy is indicated in carcinoids causing functional symptoms, however, no evidence is available as to the control of disease progression. Moreover, this therapy might only exert cytostatic effects[23-25]. Indeed, evidence does exist demonstrating that somatostatin analogs can inhibit tumour growth, at least for a certain period of time[26,27], but further studies are necessary to evaluate this effect.

Recent reports have demonstrated a favourable prognosis at 5 years in 74% of surgically treated cases with an 18% recurrence rate[19]. Post-resection perihepatic lymph node involvement has been infrequently reported in the literature without hepatic involvement, similarly to bone and lung metastasis[18,28].

In 2001, Iwao *et al*[18] analyzed 53 cases of PHCT reported in the English language literature. Accordingly, lymph node involvement occurs in 60% of cases. A case report of 2002[29] confirmed a case of metachrone lymph node metastasis after a 5-year follow-up in one case of surgically treated PHCT.

The case reported here is unique due to its discovery by chance during an abdominal scan which the patient was undergoing for other reasons.

The extremely long evolution, and the absolute lack of pathognomonic symptoms of the disease, resulted in successful diagnosis following a complex process lasting two years. Moreover, the diagnostic course was characterized by the physician's efforts to rule out

extrahepatic neoplasms with possible hepatic metastatic disease.

A diagnostic algorithm proposed by a study published in 2003[23] underlined the need for thorough research into neuroendocrine neoplasms of the small bowel (mid gut), large bowel (hind gut), bronchi (foregut) and pancreas (islet cell). In fact, a small sized lesion can metastasize extensively to liver tissues and might not be detected during a classic diagnostic approach.

In conclusion, a regular clinical and instrumental post surgical review is essential for identifying possible tumor recurrence as well as detecting previously unrecognised primary extrahepatic lesions.

REFERENCES

1 **Brambilla E**, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. *Eur Respir J* 2001; **18**: 1059-1068
2 **Giovanella L**. Tumori neuroendocrini: diagnosi e fisiopatologia clinica. Genova: Medical Systems, 1999: 7-102
3 **Harpole DH Jr**, Feldman JM, Buchanan S, Young WG, Wolfe WG. Bronchial carcinoid tumors: a retrospective analysis of 126 patients. *Ann Thorac Surg* 1992; **54**: 50-54; discussion 54-55
4 **Maggard MA**, O'Connell JB, Ko CY. Updated population-based review of carcinoid tumors. *Ann Surg* 2004; **240**: 117-122
5 **Modlin IM**, Kidd M, Latich I, Zikusoka MN, Shapiro MD. Current status of gastrointestinal carcinoids. *Gastroenterology* 2005; **128**: 1717-1751
6 **Fenwick SW**, Wyatt JI, Toogood GJ, Lodge JP. Hepatic resection and transplantation for primary carcinoid tumors of the liver. *Ann Surg* 2004; **239**: 210-219
7 **Yao JC**, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008; **26**: 3063-3072
8 **Caplin ME**, Buscombe JR, Hilson AJ, Jones AL, Watkinson AF, Burroughs AK. Carcinoid tumour. *Lancet* 1998; **352**: 799-805
9 **Edmondson HA**. Carcinoid tumor. In: Edmondson HA. Tumors of the liver and intrahepatic bile ducts (Atlas of Tumor Pathology). Washington: Armed Forces Institute of Pathology, 1958: 105-111
10 **Modlin IM**, Lye KD, Kidd M. A 5-decade analysis of 13,715 carcinoid tumors. *Cancer* 2003; **97**: 934-959
11 **Modlin IM**, Shapiro MD, Kidd M. An analysis of rare carcinoid tumors: clarifying these clinical conundrums. *World J Surg* 2005; **29**: 92-101
12 **Staren ED**, Gould VE, Warren WH, Wool NL, Bines S, Baker J, Bonomi P, Roseman DL, Economou SG. Neuroendocrine carcinomas of the colon and rectum: a clinicopathologic evaluation. *Surgery* 1988; **104**: 1080-1089
13 **Oberg K**, Eriksson B. Nuclear medicine in the detection, staging and treatment of gastrointestinal carcinoid tumours. *Best Pract Res Clin Endocrinol Metab* 2005; **19**: 265-276
14 **Hoegerle S**, Altehoefer C, Ghanem N, Koehler G, Waller CF, Scheruebl H, Moser E, Nitzsche E. Whole-body 18F dopa PET for detection of gastrointestinal carcinoid tumors. *Radiology* 2001; **220**: 373-380
15 **Orlefors H**, Sundin A, Garske U, Juhlin C, Oberg K, Skogseid B, Langstrom B, Bergstrom M, Eriksson B. Whole-body (11)C-5-hydroxytryptophan positron emission tomography as a universal imaging technique for neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and computed tomography. *J Clin Endocrinol Metab* 2005; **90**: 3392-3400
16 **Shi W**, Johnston CF, Buchanan KD, Ferguson WR, Laird JD, Crothers JG, McIlrath EM. Localization of neuroendocrine tumours with [111In] DTPA-octreotide scintigraphy (Octreoscan): a comparative study with CT and MR imaging. *QJM* 1998; **91**: 295-301
17 **Sundin A**, Eriksson B, Bergström M, Långström B, Oberg K, Orlefors H. PET in the diagnosis of neuroendocrine tumors. *Ann N Y Acad Sci* 2004; **1014**: 246-257
18 **Iwao M**, Nakamuta M, Enjoji M, Kubo H, Fukutomi T, Tanabe Y, Nishi H, Taguchi KI, Kotoh K, Nawata H. Primary hepatic carcinoid tumor: case report and review of 53 cases. *Med Sci Monit* 2001; **7**: 746-750
19 **Knox CD**, Anderson CD, Lamps LW, Adkins RB, Pinson CW. Long-term survival after resection for primary hepatic carcinoid tumor. *Ann Surg Oncol* 2003; **10**: 1171-1175
20 **Moertel CG**. Karnofsky memorial lecture. An odyssey in the land of small tumors. *J Clin Oncol* 1987; **5**: 1502-1522
21 **McEntee GP**, Nagorney DM, Kvols LK, Moertel CG, Grant CS. Cytoreductive hepatic surgery for neuroendocrine tumors. *Surgery* 1990; **108**: 1091-1096
22 **Kwekkeboom DJ**, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, Feelders RA, van Aken MO, Krenning EP. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *J Clin Oncol* 2008; **26**: 2124-2130
23 **Yao JC**, Vauthey JN. Primary and metastatic hepatic carcinoid: is there an algorithm? *Ann Surg Oncol* 2003; **10**: 1133-1135
24 **Rohaizak M**, Farndon JR. Use of octreotide and lanreotide in the treatment of symptomatic non-resectable carcinoid tumours. *ANZ J Surg* 2002; **72**: 635-638
25 **Bax ND**, Woods HF, Batchelor A, Jennings M. Octreotide therapy in carcinoid disease. *Anticancer Drugs* 1996; **7** Suppl 1: 17-22
26 **Saltz L**, Trochanowski B, Buckley M, Heffernan B, Niedzwiecki D, Tao Y, Kelsen D. Octreotide as an antineoplastic agent in the treatment of functional and nonfunctional neuroendocrine tumors. *Cancer* 1993; **72**: 244-248
27 **Faiss S**, Räth U, Mansmann U, Caird D, Clemens N, Riecken EO, Wiedenmann B. Ultra-high-dose lanreotide treatment in patients with metastatic neuroendocrine gastroenteropancreatic tumors. *Digestion* 1999; **60**: 469-476
28 **Nikfarjam M**, Muralidharan V, Christophi C. Primary hepatic carcinoid tumours. *HPB* (Oxford) 2004; **6**: 13-17
29 **Iimuro Y**, Deguchi Y, Ueda Y, Tanaka A, Iwasa Y, Ishihara M, Mizuta K, Yamamoto Y, Ikai I, Shimahara Y, Yamaoka Y. Primary hepatic carcinoid tumor with metachronous lymph node metastasis after long-term follow up. *J Gastroenterol Hepatol* 2002; **17**: 1119-1124

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CASE REPORT

Biliary drainage of the common bile duct with an enteral metal stent

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Abstract

In this case report we present an elderly patient who was referred to our hospital with recurrent episodes of cholangitis that persisted after placement of five metal stents for a distal common bile duct (CBD) stenosis. All metal stents were endoscopically removed from the CBD by forceps after balloon dilatation of the papilla. A profoundly dilated CBD with sludge and concrements was seen. To ensure adequate bile drainage an enteral metal stent was inserted in the CBD. This case shows that proximally migrated uncovered metal stents in the CBD can be safely removed endoscopically under certain circumstances. We suggest that in the case of a CBD drainage problem due to an extremely dilated CBD, placement of an enteral metal stent in the CBD could be considered, especially in patients who are unfit for surgery.

Key words: Cholangitis; Dilated common bile duct; Endoscopic retrograde cholangiopancreatography; Enteral metal stent; Metal stent removal

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INTRODUCTION

Biliary drainage of inoperable malignant bile duct obstruction by a metal stent is preferred over plastic stents due to longer patency[1]. Unfortunately, even metal stents can become dysfunctional[2]. Usually a second stent (metal or plastic) is placed[3] or an attempt to remove the stent endoscopically can be made[4]. We report a case of relapsing cholangitis after placement of 5 metal stents. Removal of the metal stents and insertion of an enteral stent in the common bile duct (CBD) regained adequate drainage.

CASE REPORT

An 84-year old woman was referred to our hospital with relapsing cholangitis since August 2007. Under the suspicion of a malignant distal CBD stricture with stones, a metal stent was placed. As drainage remained inadequate, the patient underwent several endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC) procedures with the subsequent placement of 5 uncovered metal 30 French 60 mm Wall stents (Boston Scientific Corporation, Natick, USA) and 2 externally draining PTC drains in the CBD, without clinical improvement.

The patient was referred to our centre in February 2008. Work-up by endoscopy and radiography showed five Wall stents in the CBD (four proximally and one was dislocated distally) and two externally draining 8 French percutaneous drains (Figure 1). To improve drainage the percutaneous drains were replaced by two internal/external 10 French drains. After endoscopic removal of the distally dislocated metal stent with the help of a snare, a large amount of biliary sludge with concrements was drained.

The patient recovered well, but two weeks later, she had to be re-admitted with bile leakage alongside the drains and a poor overall condition due to inadequate drainage (Figure 2).

During ERCP all metal stents were removed. We

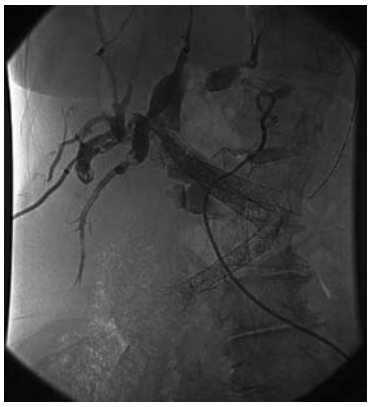

Figure 1 Radiography image. At time of presentation, sludge and stones in the proximal bile ducts with five metal stents in the CBD (four proximally and one distally) and two externally draining 8 French percutaneous drains.

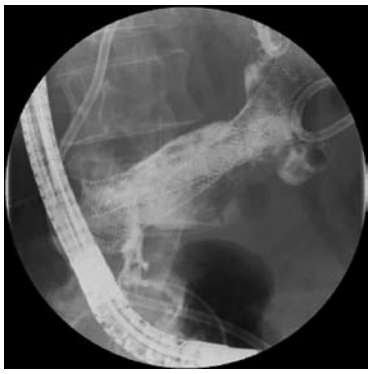

Figure 2 After endoscopic removal of the distal metal stent. The distal CBD was visualized with 4 metal stents proximally in an abnormal position in relation to the CBD.

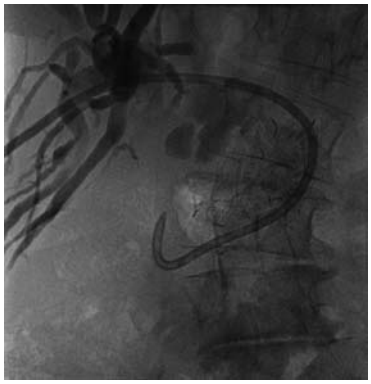

Figure 3 After placement of an enteral metal stent in the dilated CBD.

performed balloon dilatation (18 mm CRE balloon, Boston Scientific International S.A., Nanterre Cedex, France) of the sphincter and the relatively narrow distal CBD to 18 mm. We encountered four metal stents that had been overlapping. The stents were subsequently removed with foreign body forceps. With a balloon and crusher the majority of the concrements and debris was removed. Given the extreme dilatation of the CBD, up to 5 cm in diameter, with a profound angulation of the distal CBD and in retrospect no malignant stricture, an enteral metal stent was placed (WallFlex enteral duodenal stent 22 mm × 60 mm, Boston Scientific Corporation, Natic, USA) to prevent further obstruction by kinking[5] (Figure 3). Two internal/external drains were temporarily placed through the "enteral" stent to flush the bile ducts to remove the remaining debris.

Six months later our patient is still without signs of cholangitis.

DISCUSSION

In this case the following aspects were identified: After several interventions, the CBD drainage was still inadequate and the general condition of the patient excluded surgery as an option. To improve drainage, we removed all metal stents. The endoscopic removal of distal dislocated metal stents by forceps or a loop was previously described by Matsushita *et al*[4]. However, in our case we removed, in addition to the distally dislocated stent, a total of 4 metal stents that had migrated proximally in the CBD. To our knowledge, this has never been described. Usually these proximally migrated stents are fixed into their surroundings due to the radial expending force and ingrowth of bile duct epithelium. Secondly, these stents were located beyond the papilla which had to be dilated in order to reach the stents. Normally in the case of stent dysfunction, plastic stents or a second metal stent is introduced through the obstructing metal stent. However, the metal stents seemed to attribute to the increased angulation of the distal CBD and therefore had to be removed. In this particular case, safe removal was possible because the metal stents were free floating within an extremely dilated CBD. This left the patient with a widened CBD with a secondary distal angulation contributing to recurrent obstruction. Normally a hepaticojejunostomy would solve these problems, however, her general condition combined with her age forced us to look for other options. We chose to place a large diameter enteral metal stent in the distal CBD to avoid dislocation and kinking of the widened CBD. This procedure was successfully used by Diehl *et al*[5] to stent a wide CBD due to a choledochal cyst. In our patient we achieved adequate drainage, the stent remained in position and no recurrence of cholangitis has occurred for more than 6 mo.

Proximally migrated uncovered metal stents in the CBD can be safely removed endoscopically under certain circumstances. We suggest that in the case of a CBD drainage problem due to an extremely dilated CBD, placement of an enteral metal stent in the CBD could be considered, especially in patients who are unfit for surgery.

REFERENCES

1 **Davids PH**, Groen AK, Rauws EA, Tytgat GN, Huibregtse K. Randomised trial of self-expanding metal stents versus polyethylene stents for distal malignant biliary obstruction. *Lancet* 1992; **340**: 1488-1492

2 **Familiari P**, Bulajic M, Mutignani M, Lee LS, Spera G, Spada C, Tringali A, Costamagna G. Endoscopic removal of malfunctioning biliary self-expandable metallic stents. *Gastrointest Endosc* 2005; **62**: 903-910

3 **Bueno JT**, Gerdes H, Kurtz RC. Endoscopic management of occluded biliary Wallstents: a cancer center experience. *Gastrointest Endosc* 2003; **58**: 879-884

4 **Matsushita M**, Takakuwa H, Nishio A, Kido M, Shimeno N. Open-biopsy-forceps technique for endoscopic removal of distally migrated and impacted biliary metallic stents. *Gastrointest Endosc* 2003; **58**: 924-927

5 **Diehl DL**. Use of a 22-mm enteral Wallstent for biliary obstruction. *Gastrointest Endosc* 2006; **64**: 1003-1004; discussion 1004

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CASE REPORT

Solitary extramedullary plasmacytoma in retroperitoneum: A case report and review of the literature

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Abstract

Extramedullary plasmacytoma (EPM) is a plasma cell tumor arising outside of the bone marrow. Solitary EMP is an uncommon neoplasm and rarely occurs in the retroperitoneum and lacks distinctive clinical manifestations. We report a 26-year-old man with a solitary EMP in the retroperitoneum and discuss its clinical features, diagnosis and treatment.

Key words: Extramedullary plasmacytoma; Retroperitoneal neoplasm; Computed tomography; Histopathology

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INTRODUCTION

Extramedullary plasmacytoma (EMP), accounting for approximately 3% of all plasma cell neoplasms, results from uncontrolled plasma cell proliferation and consists of monoclonal plasmacytic infiltration without bone marrow involvement[1]. Approximately 80%-90% of EMPs involve mucosa-associated lymphoid tissue of the upper airway and 75% of them involve the nasal and paranasal regions, while retroperitoneal infiltration is very rare[2]. We report a 26-year-old man with a solitary EMP in the retroperitoneum.

CASE REPORT

A 26-year-old man was referred to our hospital with a history of abdominal distention and effort intolerance persisting for the previous 2 mo. He had no history of fever, weight loss, bladder or bowel dysfunction, and back pain. Physical examination revealed an irregular, firm, non-tender mass occupying almost the whole abdomen. He had no icterus or lymphadenopathy with normal tests. An abdominal computed tomography (CT) scanning showed a large heterogeneous mass in the right retroperitoneal region, surrounding the posterior portion of the right kidney and compressing the right kidney (Figure 1A). The tumor tissue was slightly enhanced after injection of a contrast medium (Figure 1B).

Laboratory test revealed 2.8 × 10^9/L white blood cells (WBC) (normal range 4-10 × 10^9/L), 119 g/L hemoglobin (Hb) (normal range 120-160 g/L), 122 × 10^9/L platelets (PLT) (normal range 100-300 × 10^9/L), and normal serum levels of creatinine, blood urea nitrogen, amylase, hepatic enzymes, electrolytes including calcium and phosphorus. No erythrocyte or protein was observed in his urine.

Fine needle aspiration cytology of the mass was not done because of refusal of his parents who were afraid of needle track implantation. Thereafter, an extensive resection of the tumor including extirpation of the right kidney, and part of the liver was performed. Postoperative recovery was uneventful.

Histopathology revealed a 30 cm × 16 cm × 10 cm tumor surrounding the posterior portion of the right kidney with adherent liver. Microscopy showed diffusive infiltration of polygonal cells in the retroperitoneum.

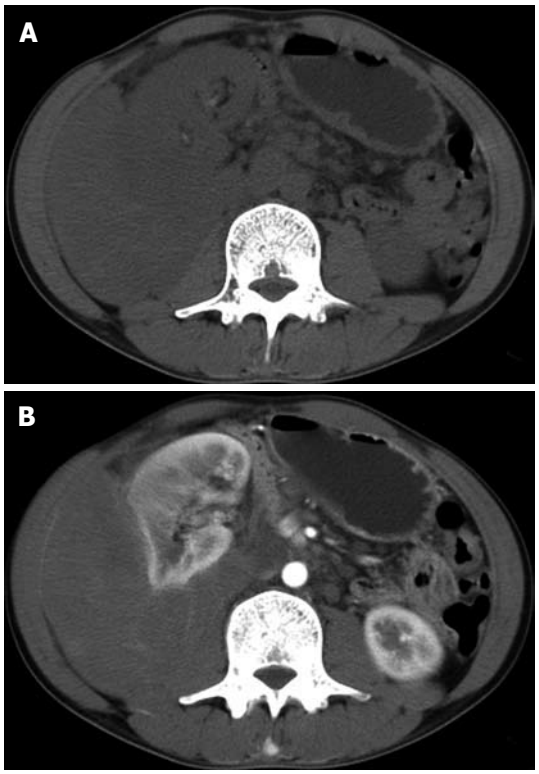

Figure 1 Abdominal computed tomography showing a large heterogeneous mass in the right retroperitoneal region, surrounding the posterior portion of the right kidney and compressing the right kidney (A), and slightly enhanced tumor tissue after injection of a contrast medium (B).

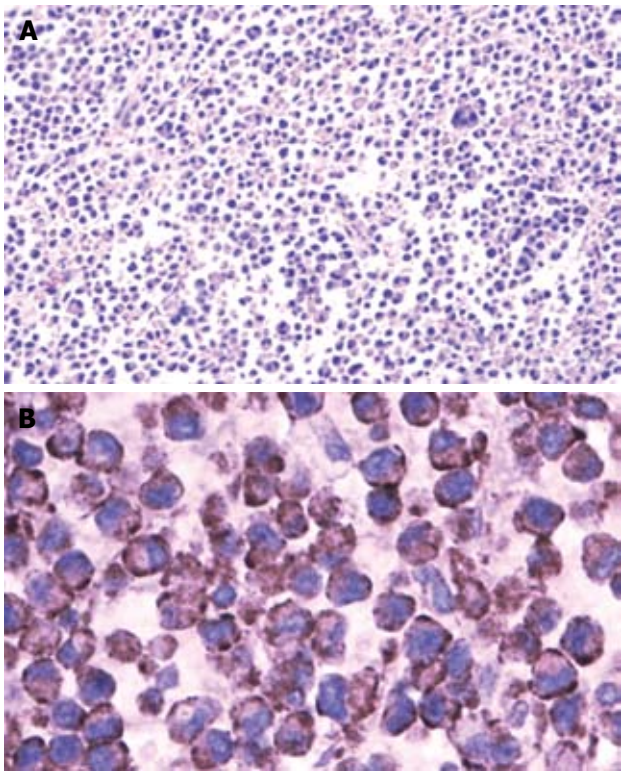

Figure 2 Tumor cells with homogenous amphophilic cytoplasm, whell-type and asymmetric nuclei, coarsely stippled chromatin, some acidophilic nucleoli, and occasional binucleate (A) (HE, × 100) and positive tumor cells for CD138 (B) (× 400) under microscope.

Homogeneous amphophilic cytoplasm, wheel-type and asymmetric nuclei, coarsely stippled chromatin, and some acidophilic nucleoli were observed in the tumor cells. Binucleate cells were also occasionally observed (Figure 2A). Immunohistochemistry demonstrated tumor cells were positive for CD138 (Figure 2B), Bcl-2 and VS38C, but negative for CD20, CD3, CD79a, CK, CD38, S-100, CD5, CK, myosin and CD10.

To confirm the diagnosis of EMP, further investigations were done after surgery. Serum IgG was 25.4 g/L (normal range 6.94-16.20 g/L). Serum IgA and IgM levels were within normal range. No Bence-Jones protein was detected in his urine. Iliac crest bone marrow aspiration and biopsy did not find any plasmacytic infiltration. A skeletal survey revealed no osteolytic lesions.

DISCUSSION

Solitary bone and extramedullary plasmacytomas are rare plasma cell proliferative disorders. Their diagnosis is based on the monoclonal plasma cell infiltration at a single disease site and the exclusion of systemic myeloma[3]. WBC and hemoglobin were slightly abnormal, and iliac crest bone marrow aspiration and biopsy showed no plasmacytic infiltration in our case. We ascribed these abnormalities to the fact that he worked as a painter for 8 years prior to surgery. Hematopathy can be found in workers exposed to benzene[4].

EMP occurs most commonly in the head and neck region, followed by gastrointestinal (GI) tract, central nervous system (CNS), thyroid, breast, parotid gland, testis, and lymph nodes[5].

Solitary EMP rarely occurs in the retroperitoneum. Cases of retroperitoneal EMP have different clinical manifestations, such as renal failure due to bilateral renal vein occlusion[6], flank pain, hematuria due to thrombosis of the renal vein[7], obstructive jaundice[8], abdominal distention and pain[9,10], and hyperamylasemia[11]. However, our patient presented with only abdominal distention and effort intolerance.

Retroperitoneal EMP should be differentially diagnosed from lymphoplasmacytic lymphoma and immunoblastic lymphoma[12]. Immunohistochemistry is used for its final diagnosis. In our case, microscopy showed that the tumor cells might be origined from plasmacytic cells confirmed by immunohistochemistry.

Preoperative CT scanning does not contribute to its differential diagnosis from other tumors, while preoperative angiography can indicate the vessels feeding the mass and the correlation to other vessels. Serum electrophoresis can help its diagnosis by finding the M band. However, we considered the mass as a common type of tumors, such as schwannoma, sarcoma before operation and serum electrophoresis was not done.

No clear guidelines for treatment of EMP are available due to its rarity and variable presentations. EMP is highly radiosensitive with excellent results (< 10% of local recurrences and about 50%-65% of patients remain free of disease for > 10 years)[13]. However, it is associated with a high morbidity particularly when

used for large retroperitoneal tumors. It was reported that there is no evidence that retroperitoneal EMP progresses one year after chemotherapy in combination with radiotherapy[14]. Chen *et al*[8] have reported a case of retroperitoneal EMP accompanying obstructive jaundice, who showed a complete response to sequential radiotherapy and chemotherapy.

Sharma *et al*[10] performed a complete surgical resection of a large bulky retroperitoneal EMP when the patient did not respond to chemotherapy, and found that the patient was symptom free 16 mo post surgery. Our patient did not receive chemotherapy or radiotherapy prior to operation. He was under observation 2 mo after surgery and remained asymptomatic when we wrote this paper.

In summary, EMP should be considered whenever a retroperitoneal soft tissue mass is identified.

REFERENCES

1 **Dimopoulos MA**, Kiamouris C, Moulopoulos LA. Solitary plasmacytoma of bone and extramedullary plasmacytoma. *Hematol Oncol Clin North Am* 1999; **13**: 1249-1257

2 **Ooi GC**, Chim JC, Au WY, Khong PL. Radiologic manifestations of primary solitary extramedullary and multiple solitary plasmacytomas. *AJR Am J Roentgenol* 2006; **186**: 821-827

3 **Dimopoulos MA**, Hamilos G. Solitary bone plasmacytoma and extramedullary plasmacytoma. *Curr Treat Options Oncol* 2002; **3**: 255-259

4 **Wocka-Marek T**, Zajac-Nedza M, Braszczyńska Z, Zygan U, Wójcik-Chrobok B, Lukas A, Klementys A. [Hematologic disorders in workers exposed to benzene and ethylbenzene] *Przegl Lek* 1988; **45**: 836-839

5 **Weber DM**. Solitary bone and extramedullary plasmacytoma. *Hematology Am Soc Hematol Educ Program* 2005; 373-376

6 **Marks ES**, Lee KM. Acute renal failure secondary to vascular occlusion by a retroperitoneal plasmacytoma. *Cancer* 1984; **53**: 1228-1229

7 **Kobayashi H**, Itoh T, Murata R, Tanabe M. Primary retroperitoneal plasmacytoma with tumor thrombus within the renal vein. *J Urol* 1992; **147**: 452-454

8 **Chen TC**, Wu JH, Ng KF, Lien JM, Hung CF. Solitary extramedullary plasmacytoma in the retroperitoneum. *Am J Hematol* 1998; **58**: 235-238

9 **Sered S**, Nikolaidis P. CT findings of perirenal plasmacytoma. *AJR Am J Roentgenol* 2003; **181**: 888

10 **Sharma LM**, Biswas G, Rai SS, Nair R, Gupta S, Parikh PM. Retro-peritoneal plasmacytoma: a case report and review of literature. *Indian J Cancer* 2004; **41**: 133-134

11 **Tsai YS**, Cheng HL, Lin JS, Tong YC, Chang KC. Retroperitoneal plasmacytoma associated with hyperamylasemia. *J Urol* 1999; **162**: 1681-1682

12 **El-Sharkawy MS**, Siddiqui N, Aleem A, Diab AA. Renal involvement in lymphoma: prevalence and various patterns of involvement on abdominal CT. *Int Urol Nephrol* 2007; **39**: 929-933

13 **Chao MW**, Gibbs P, Wirth A, Quong G, Guiney MJ, Liew KH. Radiotherapy in the management of solitary extramedullary plasmacytoma. *Intern Med J* 2005; **35**: 211-215

14 **Saito M**, Tsuchiya N, Iinuma M, Mitsumori K, Matsuura S, Shimoda N, Ohyama C, Satoh S, Sato K. [A case of retroperitoneal extramedullary plasmacytoma] *Hinyokika Kiyo* 2003; **49**: 735-739

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World J Gastroenterol 2009 May 21; 15(19): 2429
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Meetings

Events Calendar 2009

January 12-15, 2009
Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
Hong Kong Convention and Exhibition Centre, Hong Kong, China
19th Conference of the APASL
http://www.apasl2009hongkong.org/en/home.aspx

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel
Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)
http://www.easl.ch/

May 17-20, 2009
Denver, Colorado, USA
Digestive Disease Week 2009

May 29-June 2, 2009
Orange County Convention Center
Orlando, Florida
45th ASCO Annual Meeting
www.asco.org/annualmeeting

May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
McCormick Place, Chicago, IL
DDW 2009
http://www.ddw.org

June 17-19, 2009
North Bethesda, MD
Accelerating Anticancer Agent Development

June 20-26, 2009
Flims, Switzerland
Methods in Clinical Cancer Research (Europe)

June 24-27 2009
Barcelona, Spain
ESMO Conference: 11th World Congress on Gastrointestinal Cancer
www.worldgicancer.com

June 25-28, 2009
Beijing International Convention Center (BICC), Beijing, China
World Conference on Interventional Oncology
http://www.chinamed.com.cn/wcio2009/

July 5-12, 2009
Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

August 1-7, 2009
Vail Marriott Mountain Resort, Vail, CO, United States
Methods in Clinical Cancer Research

August 14-16, 2009
Bell Harbor Conference Center, Seattle, Washington, United States
Practical Solutions for Successful Management
http://www.asge.org/index.aspx?id=5040

September 23-26, 2009
Beijing International Convention Center (BICC), Beijing, China
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists(IASGO)
http://iasgo2009.org/en/index.shtml

September 27-30, 2009
Taipei, China
Asian Pacific Digestive Week
http://www.apdwcongress.org/2009/index.shtml

October 7-11, 2009
Boston Park Plaza Hotel and Towers, Boston, MA, United States
Frontiers in Basic Cancer Research

October 13-16, 2009
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009
Versailles, France
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009
Boston, MA, United States
The Liver Meeting

November 15-19, 2009
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009
London, UK
Gastro 2009 UEGW/World Congress of Gastroenterology
www.gastro2009.org

Global Collaboration for Gastroenterology

For the first time in the history of gastroenterology, an international conference will take place which joins together the forces of four pre-eminent organisations: Gastro 2009, UEGW/WCOG London. The United European Gastroenterology Federation (UEGF) and the World Gastroenterology Organisation (WGO), together with the World Organisation of Digestive Endoscopy (OMED) and the British Society of Gastroenterology (BSG), are jointly organising a landmark meeting in London from November 21-25, 2009. This collaboration will ensure the perfect balance of basic science and clinical practice, will cover all disciplines in gastroenterology (endoscopy, digestive oncology, nutrition, digestive surgery, hepatology, gastroenterology) and ensure a truly global context; all presented in the exciting setting of the city of London. Attendance is expected to reach record heights as participants are provided with a compact "all-in-one" programme merging the best of several GI meetings. Faculty and participants from all corners of the earth will merge to provide a truly global environment conducive to the exchange of ideas and the forming of friendships and collaborations.

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Instructions to authors

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Acknowledgments

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension,

insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
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