

World Journal of *Gastroenterology*

World J Gastroenterol 2013 January 21; 19(3): 321-430





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2010-2013

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World Journal of Gastroenterology is now indexed in Current Contents[®]/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch[®]), Journal Citation Reports[®], Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports[®], Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor[®] Score: 0.06035 (5/74).

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NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

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PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China

Fax: +852-31158812
Telephone: +852-58042046
E-mail: bjpgoffice@wjgnet.com
<http://www.wjgnet.com>

PUBLICATION DATE
January 21, 2013

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Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

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Clinicopathological characteristics in the differential diagnosis of hepatoid adenocarcinoma: A literature review

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Received: May 7, 2012 Revised: July 16, 2012

Accepted: August 14, 2012

Published online: January 21, 2013

raise the suspicion of HAC, and characteristic pathological immunohistochemical stains can help with the differential diagnosis. Novel immunohistochemical markers may be useful to clearly differentiate HAC from HCC. Once metastatic HAC is diagnosed, the primary tumor origin should be identified for adequate treatment. The majority of HAC originates from the stomach, so pan-endoscopy should be arranged first.

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Key words: Hepatocellular carcinoma; Alpha-fetoprotein-producing tumor; Alpha fetoprotein; Gastric adenocarcinoma; Pathology

Su JS, Chen YT, Wang RC, Wu CY, Lee SW, Lee TY. Clinicopathological characteristics in the differential diagnosis of hepatoid adenocarcinoma: A literature review. *World J Gastroenterol* 2013; 19(3): 321-327 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/321.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.321>

Abstract

Hepatoid adenocarcinoma (HAC) is a rare but important special type of extrahepatic adenocarcinoma with clinicopathological presentation mimicking hepatocellular carcinoma (HCC), and prompt and correct diagnosis can be a challenge, especially in endemic areas with a high incidence of HCC. To date, HAC has only been reported in case series or single case reports, so we aimed to review the clinicopathological characteristics of HAC to obtain a more complete picture of this rare form of extrahepatic adenocarcinoma. All the articles about HAC published from 2001 to 2011 were reviewed, and clinicopathological findings were extracted for analysis. A late middle-aged male with high serum α -fetoprotein and atypical image finding of HCC should

INTRODUCTION

Hepatoid adenocarcinoma (HAC) is a rare but important special type of extrahepatic adenocarcinoma with clinicopathological presentation mimicking hepatocellular carcinoma (HCC). HAC was first reported as an α fetoprotein (AFP)-producing tumor in 1970^[1], and Ishikura *et al*^[2,3], in a report of seven AFP-producing gastric adenocarcinomas (GAC), proposed the term hepatoid adenocarcinoma due to the high AFP levels, ranging from 4730 to 700 000 ng/mL in serum. A serum AFP level higher than 200 ng/mL has been one of the screening criteria for HCC in international guidelines, and HCC may be diagnosed when liver tumors with high serum AFP are found among patients with liver cirrhosis^[4,5]. Because of high serum AFP levels among patients with HAC, identification of tumor origin may be difficult, especially when liver tumors arise

ing from HAC metastasis are initially found. Distinguishing between HAC and HCC is important, especially in areas with a high incidence of chronic hepatitis B, chronic hepatitis C and HCC.

HAC can originate from different organs such as the stomach^[6], gallbladder^[7], colon^[8], lung^[9] and urinary bladder^[10], and the stomach is the most common origin of tumors in the literature. Finding the primary tumor origin of HAC is crucial, and prompt and adequate treatment may improve outcomes of patients. However, HCC with various extrahepatic metastases such as to the stomach has been reported^[11], and determining the tumor origin may become a challenge for physicians, especially when clinicopathological findings of liver tumors are not typical for HCC. Pathological findings in immunohistochemical (IHC) stains may help in the differential diagnosis of HAC, and some specific IHC stains such as cytokeratin (CK)19, AFP, Hep-Par 1 and palate, lung, and nasal epithelium carcinoma-associated protein (PLUNC) have been used in the literature^[12]. A systemic literature review regarding differential diagnosis of HAC by IHC stains is lacking, and the IHC presentations of HAC, HCC and common GAC (except HAC) are compared in this review.

The majority of HAC originates from the stomach, and the incidence of gastric HAC among GAC cases was reported to be 0.3%-1% in some recent articles^[6,13,14]. The clinical features of gastric HAC are old age, aggressive clinical course, poor survival and frequent metastasis to the liver and lymph nodes, and data have suggested that HAC of the stomach has a poorer prognosis than common GAC^[14]. Detection of tumors in the early stage, followed by surgical resection is still considered to be the only way to cure HAC. Because clinical presentations of HAC differ from those of common GAC, studies focusing on gastric HAC may be important in the future, and clinical characteristics may provide useful clues for further research. Moreover, a review of HAC originating from organs other than stomach is still lacking. To date, HAC has only been reported in case series or single case reports, so we aimed to review the clinicopathological characteristics of HAC to obtain a more complete picture of this rare form of extrahepatic adenocarcinoma.

LITERATURE REVIEW

All articles cited in the Medline/PubMed database from January 2001 to December 2011 were searched to identify relevant medical literature, and the search terms included "hepatoid adenocarcinoma", "AFP-producing tumor", " α fetoprotein-producing tumor" and " α fetoprotein-producing gastric cancer". In addition, a manual search of all relevant articles was conducted. All studies with full text were included. The search limits were: (1) type of article: all types; (2) languages: English; (3) species: humans; (4) sexes: both male and female; (5) subsets: all types and fields; (6) ages: all ages; and (7) search field tags: titles. Studies without pathological confirmation of HAC or without clinical data were excluded.

A total of 98 articles were initially identified from the

literature search. Among the 98 articles, 32 were excluded because the diagnosis was not confirmed as HAC, and 13 were excluded because the full text was not available. In total, 217 patients with HAC were included in our review. (All 53 reviewed articles are listed in Supplementary References online).

CLINICAL CHARACTERISTICS

The clinical features of HAC cases are summarized in Table 1. Most HAC originated from the stomach (83.9%), and origins other than the stomach, including gallbladder (3.7%), uterus (3.2%), lung (2.3%) and urinary bladder (1.8%), were rare. Moreover, HAC originating from the esophagus and peritoneum was very rare (0.9%), and HACs of the rectum, transverse colon, testis, ovary, jejunum or ureter were only reported in single case report form ($< 0.5\%$). The mean age of HAC patients was 63.0 ± 12.8 years, with a range of 21 to 100 years. Patients with HAC were usually male and the male-to-female ratio was 2.3:1. Most patients had elevated serum AFP (84.8%), with a range from less than 1.0 to 475 000 ng/mL.

When HAC was diagnosed, metastases from original organs were usually found. The most common sites of metastasis were lymph nodes (57.5%), followed by liver (46.3%) and lung (3.4%) metastasis. Liver and gastric tumors were often found simultaneously, and liver tumors could be detected before gastric tumors. As in common gastric cancer, surgical resection was the primary curative treatment in HAC. Most patients received surgical resection (80.2%), even when lymph node or distant metastases were found. In addition, about half of patients (52.1%) received adjuvant chemotherapy following surgical resection. In our literature review, survival data were not reported for all patients, but we were able to analyze data from 125 cases with various tumor origins. The median survival of all HAC patients was 12 mo, and 64 (51.2%) patients died within the first 12 mo.

PATHOLOGICAL CHARACTERISTICS

Hematoxylin and eosin stain

HAC usually showed morphologic similarity to HCC in histology, and polygonal tumor cells were found in hematoxylin and eosin (HE) stains. Polygonal tumor cells proliferating in both trabecular and intestinal-like structures could be found (Figure 1), but definite diagnosis of HAC was difficult only based on findings in histology. Further IHC stains were usually done for differential diagnosis.

Immunohistochemical staining

The major IHC presentations of HAC tumor tissues are depicted in Table 2. Positive AFP stains (Figure 2A) were found in the majority of HAC (91.6%), but positive Hep Par 1 stains were only found in some HAC patients (38.1%) (Figure 2B). In addition, positive carcinoembryonic antigen (CEA) stains were found in most HAC patients (78.7%). Among epithelial markers, all HAC tumors were

Table 1 Clinical characteristics of patients with hepatoid adenocarcinoma in the literature review *n* (%)

	Number	Male (total), <i>n</i>	Female (total), <i>n</i>	Ratio M:F	Age (range), yr	Elevated serum AFP	Lymph node metastasis	Liver metastasis	Lung metastasis	Other metastasis	Operation	Chemotherapy	Median survival (range), mo
Total	217 (100)	135 (194)	59 (194)	2.3:1	63 (21-100)	78 (84.8)	122 (57.5)	94 (46.3)	7 (3.4)	16 (7.9)	138 (80.2)	49 (52.1)	12 (1.2-66)
Stomach	182 (83.9)	121 (159)	38 (159)	3.2:1	63.3 (28-100)	56 (87.5)	115 (63.9)	84 (48.8)	5 (2.9)	9 (5.2)	107 (77.5)	34 (50)	13 (1.2-66)
Gallbladder	8 (3.7)	2	6	1:03	66.1 (55-76)	3 (75)	2 (28.6)	3 (42.9)	0 (0)	1 (14.3)	7 (100)	2 (28.6)	12 (5-20)
Uterus	7 (3.2)	-	7	-	69.4 (61-86)	7 (100)	2 (28.6)	2 (28.6)	1 (14.3)	1 (14.3)	7 (100)	5 (71.4)	22 (12-36)
Lung	5 (2.3)	4	1	4:01	54.8 (49-68)	2 (60)	2 (40)	1 (20)	NA	2 (40)	5 (100)	2 (50)	15 (2-45)
Urinary bladder	4 (1.8)	4	-	-	70 (61-85)	4 (100)	NA	NA	NA	NA	4 (100)	NA	19 (12-26)
Esophagus	2 (0.9)	1	1	1:01	60 (44-76)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	2 (100)	1 (50)	N/A
Peritoneum	2 (0.9)	1	1	1:01	46.5 (21-72)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	2 (100)	2 (100)	6
Retropertitoneum	1 (< 0.5)	1	-	-	47	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	N/A
Testis	1 (< 0.5)	1	-	-	36	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	NA	28
Ovary	1 (< 0.5)	-	1	-	59	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	N/A
Jejunum	1 (< 0.5)	-	1	-	40	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	NA	N/A
Transverse colon	1 (< 0.5)	-	1	-	59	NA	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	N/A
Rectum	1 (< 0.5)	-	1	-	50	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	NA	N/A
Ureter	1 (< 0.5)	-	1	-	80	NA	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	N/A

AFP: α -fetoprotein; NA: Not available.

Table 2 Immunohistochemical staining characteristics of patients with hepatoid adenocarcinoma in the literature review *n* (%)

	Number	AFP	Hep Par1	CEA	CK18	CK19	CK20	CK7	AE1/AE3	α 1-AT	CD10	GPC3
Total	217 (100)	177 (91.6)	24 (38.1)	37 (78.7)	22 (100)	27 (100)	6 (28.6)	4 (15.4)	12 (92.3)	10 (91)	5 (62.5)	10 (100)
Stomach	182 (83.9)	156 (90.6)	12 (26.7)	27 (75.0)	17 (100)	16 (100)	5 (38.5)	0 (0)	10 (100)	7 (87.5)	0 (0)	10 (100)
Gallbladder	8 (3.7)	5 (83.3)	4 (66.7)	3 (75)	1 (100)	6 (100)	0 (0)	2 (33.3)	1 (100)	1 (100)	3 (100)	NA
Uterus	7 (3.2)	6 (85.7)	NA	1 (100)	NA	NA	NA	NA	NA	NA	1 (100)	NA
Lung	5 (2.3)	4 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (50)	2 (0)	NA	NA	1 (100)	NA
Urinary bladder	4 (1.8)	4 (100)	4 (100)	4 (100)	NA	NA	NA	NA	NA	NA	NA	NA
Esophagus	2 (0.9)	2 (100)	1 (50)	1 (100)	NA	NA	NA	NA	NA	2 (100)	NA	NA
Peritoneum	2 (0.9)	1 (50)	1 (100)	NA	1 (100)	NA	0 (0)	NA	1 (50)	NA	NA	NA
Retropertitoneum	1 (< 0.5)	NA	NA	NA	NA	NA	NA	NA	0 (0)	NA	NA	NA
Testis	1 (< 0.5)	1 (100)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ovary	1 (< 0.5)	1 (100)	NA	1 (100)	NA	NA	NA	NA	NA	NA	NA	NA
Jejunum	1 (< 0.5)	1 (100)	1 (100)	NA	1 (100)	1 (100)	0 (0)	0 (0)	NA	NA	NA	NA
Transverse colon	1 (< 0.5)	NA	NA	NA	NA	1 (100)	0 (0)	0 (0)	NA	NA	NA	NA
Rectum	1 (< 0.5)	1 (100)	1 (100)	NA	1 (100)	1 (100)	0 (0)	0 (0)	NA	NA	NA	NA
Ureter	1 (< 0.5)	1 (100)	NA	NA	NA	1 (100)	0 (0)	NA	NA	NA	0 (0)	NA

CEA: Carcinoembryonic antigen; CK: Cytokeratin; AE1/AE3: Anti-pancytokeratin AE1/AE3; CK18: Cluster of differentiation 18; α 1-AT: α 1-Antitrypsin; GPC3: Glypican 3; AFP: α -fetoprotein; NA: Not available.

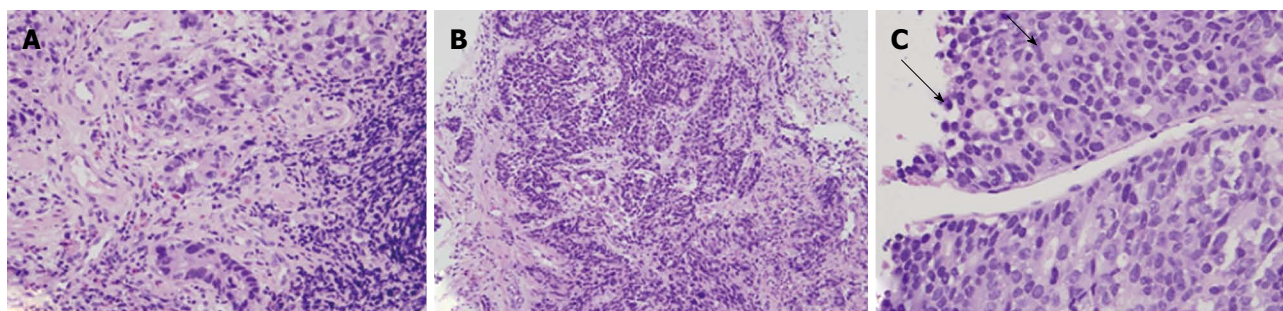


Figure 1 Presentations of hematoxylin and eosin stains: Gastric hepatoid adenocarcinoma. A: Polygonal cells with glandular pattern; B: Trabecular pattern; C: Metastatic liver tumor tissue from gastric hepatoid adenocarcinoma showed focal glandular pattern (arrows).

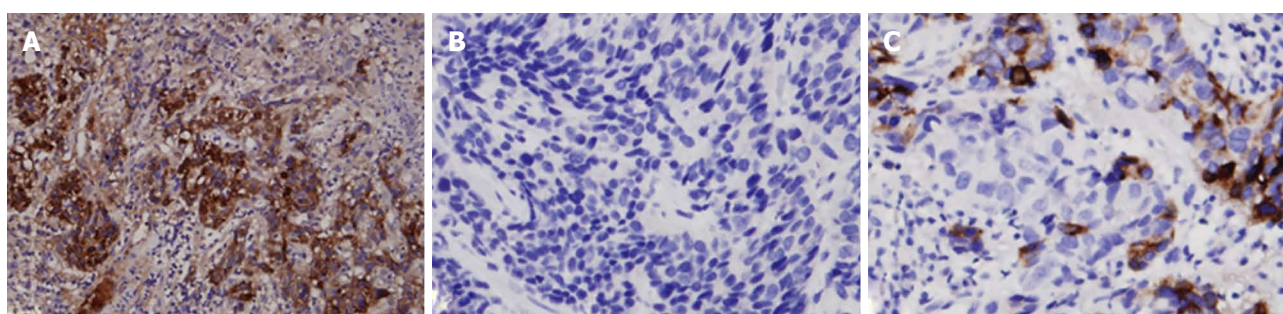


Figure 2 Presentations of immunohistochemical stains: Gastric hepatoid adenocarcinoma. A: Positive finding in α fetoprotein stain; B: Metastatic liver tumor tissue from gastric hepatoid adenocarcinoma showed negative for Hep Par 1 stain; C: Metastatic liver tumor tissue from gastric hepatoid adenocarcinoma showed positive for glypican 3 stain.

positive for both CK18 and CK19 stains (100%), and only a small proportion of HAC patients showed positive stains for CK20 (25%) and CK7 (15.4%). High proportions of HAC tumors were positive for pancytokeratin (AE1/AE3) stain (92.3%) and α 1-antitrypsin (α 1-AT) stain (91%), and 62.5% of HAC patients were positive for CD10 antigen. In addition, positive glypican 3 (GPC-3) staining (Figure 2C) was found in all HAC patients (100%).

DIFFERENTIAL DIAGNOSIS

Clinical presentation

Although the stomach is the most common origin of HAC, the clinical presentation of HAC may vary greatly depending on the anatomic location of the tumor. Among gastric HAC cases, clinical symptoms were usually non-specific, such as fatigue, weight loss, nausea, poor appetite, emesis, diarrhea, epigastric distress, epigastric mass, abdominal pain or abdominal distension^[15-17]. It is difficult to make a differential diagnosis from the clinical symptoms alone.

HAC is usually found in late middle-aged males, and elevated serum AFP is commonly noted among patients with HAC. Due to similar clinicopathological features, HAC may closely resemble and even be indistinguishable from HCC. Serum AFP has long been used for the screening and diagnosis of HCC, and high AFP in HAC may confuse physicians^[4,5]. HAC may be incorrectly diagnosed as HCC, especially in endemic areas with a high incidence of HCC. AFP may be elevated in various con-

ditions including HAC, so AFP is not suggested for diagnosis of HCC nowadays^[18]. Diagnosis of HCC should be based on dynamic image studies (such as computed tomography or magnetic resonance imaging) and/or liver tumor biopsy, and the typical dynamic image of HCC shows intense arterial uptake followed by “washout” of contrast in the venous and/or delayed phases^[18]. Even in liver tumors with elevated AFP, secondary malignancies still should be considered especially when the presentations of imaging studies are atypical, and liver tumor biopsy is recommended for differential diagnosis. In addition, risk factors such as liver cirrhosis, chronic hepatitis B or chronic hepatitis C infection can usually be found among patients with HCC, but those risk factors may not be observed among patients with HAC. Once AFP-producing metastatic liver tumors are found, an intensive search for primary tumor origin should be initiated.

HAC is a very aggressive neoplasm with metastasis in a high proportion of patients at the time of diagnosis. Lymph nodes and the liver are the most common sites of metastasis. Once metastatic HAC is suspected, panendoscopy should be arranged because the stomach is the most common origin of HAC. HCC with metastasis to the stomach has been reported^[11], and a clinical challenge of differentiating primary tumor origin arises when liver and gastric tumors are found simultaneously. Pathology markers, especially IHC stains, may provide clues to make a correct diagnosis. In addition, finding the tumor origin may be challenging when no tumor can be detected in the liver and/or stomach, so other organs

Table 3 Comparison of positive rates of commonly-used key immunohistochemical stains in hepatocellular carcinoma, hepatoid adenocarcinoma, and common gastric adenocarcinoma

IHC stains	HCC ¹	HAC ²	Common GAC ^{3,4}
AFP	8.2%-37%	92.1%	0%-0.8%
GPC-3	84%	100%	3.4%
Hep Par 1	73%-93%	38.1%	4%-47%
CK19	8.2%-15%	100%	83.6%-89.7%

IHC: Immunohistochemical; HCC: Hepatocellular carcinoma; HAC: Hepatoid adenocarcinoma; GAC: Gastric adenocarcinoma. ¹HCC: AFP stain in 218 cases^[16,29,30], GPC-3 stain in 56 cases^[30], Hep Par 1 stain in 308 cases^[16,29,32] and CK19 stain in 233 cases^[16,29,33]; ²HAC data source: Data based on our review in Table 2; ³Common GAC data source: AFP stain in 122 cases^[34,35], GPC-3 stain in 118 cases^[35], Hep Par 1 stain in 108 cases^[22,32], CK19 stain in 384 cases^[33,36]; ⁴Common GAC means GAC except HAC.

such as the gallbladder, urogenital tract, lung and intestine should be carefully checked.

As a result of the high rate of metastasis and a disappointing response to chemotherapy in HAC, poor patient survival can be predicted. Data have suggested that HAC has a poorer prognosis than more common types of tumors^[14], and more than half of patients died within the first 12 mo in our literature review. There has been no systemic therapy proven to be effective for HAC until now, but target therapy such as sorafenib is recommended as a standard treatment in HCC with extrahepatic metastasis to prolong patient survival^[18].

Pathological presentation

Because HAC bears a striking morphologic similarity to HCC in histology and IHC stains, HCC could be mistakenly diagnosed when liver tumors are the only initial finding, especially in regions with a high incidence of HCC. However, HAC and HCC differ in histologic presentation, and specific findings suggesting HAC may provide important clues. Kodama *et al.*^[19] described two histologic types of HAC: one was the medullary type, characterized by polygonal cells arranged in a solid nest or sheets, with scattered large pleomorphic or multinucleated giant cells, and the other was well-differentiated papillary or tubular type with clear cytoplasm. However, two types of HAC sometimes coexist in a single tumor. If polygonal tumor cells proliferating in both trabecular and intestinal-like structures are found in HE staining, HAC should be considered (Figure 1).

HAC usually secretes some special chemomediators, so it could be characterized by some IHC stains (Table 2). As an AFP-producing tumor, HAC is commonly > 90% stained for AFP regardless of organ origin (Figure 2A). Although AFP is a characteristic stain for identifying HAC, it can also cause confusion in differentiating HAC from HCC. AFP, a well-known tumor marker of HCC, is usually secreted by HCC and gonadal tumors, and AFP can be positive in tissues of these tumors^[20,21]. Hep Par 1, a highly specific marker of HCC, may help in the differ-

ential diagnosis between HAC and HCC (Figure 2B), but some HAC tumors can still present with positive staining. In addition, although a low percentage of gastric HAC was stained positive for Hep Par 1 (Table 2), high percentages of non-gastric HAC such as HAC of the gallbladder and urinary bladder were stained positive. However, due to the small case numbers of non-gastric HAC, large cohort studies are needed to confirm this finding. Moreover, other tumors such as adrenal cortical carcinoma, yolk sac tumor, colonic adenocarcinoma, lung carcinoma, ovarian carcinoma and endocervical adenocarcinoma may be occasionally positive for Hep Par 1 stain^[22].

The proportion of HAC with a positive CEA stain was high in both gastric (75%) and non-gastric HAC (75%-100%), but it can also be positive in HCC and other metastatic adenocarcinoma of the liver^[23] arising from the gastrointestinal tract such as the colon^[24,25], stomach^[26] and pancreas^[27]. Among epithelial markers, AE1/AE3, CK18 and CK19 are usually positive for HAC (92.3%, 100% and 100%, respectively), but the proportions of positive CK20 and CK7 stains are low (25% and 15.4%, respectively). CK7 and CK20 have diagnostic value in differentiating the tumors of unknown primary sites, and epithelial neoplasms with negative staining for CK7 and CK20 include adrenal cortical carcinoma, germ cell tumor, prostate carcinoma, renal cell carcinoma, and HCC^[28]. In the differential diagnosis between HAC and HCC for AFP-producing liver tumors, HAC is strongly suggested if AE1/AE3, CK18 and CK19 stains show strong positive findings.

Regarding other IHC stains for HAC such as α 1-AT, α 1-antichymotrypsin, CD10, CDX2, MUC1, PLUNC and GPC-3 have been reported to be promising in the differential diagnosis of HAC^[12]. For example, Sentani *et al.*^[29] reported that positive PLUNC staining was observed in all six gastric HAC, and PLUNC staining was also detected in both liver metastases. Furthermore, PLUNC staining was not observed in 52 cases of HCC, and it was a novel marker that might well distinguish HAC from HCC. However, case numbers in the literature were small (Table 2), and further studies to confirm sensitivity and specificity will be crucial.

In Table 3, we summarize and compare positive rates of commonly-used key IHC stains among HCC, HAC, and common GAC (except HAC) from our literature review^[16,29,36] of the most common clinical malignancies needing differential diagnosis. Although the AFP stain can be positive in both HCC and HAC, it is overwhelmingly positive in HAC (91.6%) in our review. In other words, tumors with a negative AFP stain are rare in HAC, and they usually do not indicate HAC. Moreover, AFP staining is almost always negative in common GAC (0%-0.8%) in the literature, and it may be a useful tool to confirm HAC-type GAC. Similar to the AFP stain, the GPC-3 stain can be used in the differential diagnosis because of its low positive rate in common GAC but high positive rate in HAC (Figure 2C). However, although GPC-3 and AFP stains can be positive in both HCC and

HAC, HCC with a negative AFP stain may be confirmed by GPC-3 staining. Hep Par 1 is another important stain with a high positive rate in HCC, but it can also be positive in some HAC (38.1%) in our review. The Hep Par 1 stain is usually negative in common GAC, and liver tumors with a negative Hep Par 1 stain suggest metastasis from other origins such as the stomach. Epithelial marker CK19 stain has an almost 100% positive rate in HAC, and it is also frequently positive in common GAC. However, the positive rate of CK19 in HCC is very low, and HCC is usually not suggested with a positive CK19 stain in liver tumors. In summary, HAC is a special type of extrahepatic adenocarcinoma with pathological presentation mimicking HCC, and HAC should be suspected once polygonal tumor cells proliferating in both trabecular and intestinal-like structures are found in HE staining. There is no single IHC stain that can completely differentiate HAC from HCC, and a panel of IHC stains, such as CK19, PLUNC, Hap-Par 1 and CEA, with detailed clinical history and endoscopic findings are essential for definitive diagnosis.

CONCLUSION

HAC is a rare but important special type of extrahepatic adenocarcinoma with clinicopathological presentation mimicking HCC, and prompt and correct diagnosis can be a challenge, especially in endemic areas with a high incidence of HCC. A late middle-aged male with high serum AFP and atypical image findings of HCC should raise the suspicion of HAC, and characteristic pathological IHC stains can help in differential diagnosis. Novel IHC markers may be useful to clearly differentiate HAC from HCC. Once metastatic HAC is diagnosed, a search for the primary tumor origin should be initiated for adequate treatment. The majority of HAC arise from the stomach, so panendoscopy should be arranged first.

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P-Reviewers Currò G, Zhang JZ, Kobayashi T **S-Editor** Shi ZF
L-Editor Cant MR **E-Editor** Zhang DN



Alpha-fetoprotein: A controversial prognostic biomarker for small hepatocellular carcinoma

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Supported by The Swiss National Science Foundation, No. 32003B-134963/1, to Dr. Montecucco F; EU FP7 AtheroRemo, No. 201668; and Swiss National Science Foundation, No. 310030B-133127, to Dr. Mach F

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Received: October 15, 2012 Revised: November 23, 2012

Accepted: December 20, 2012

Published online: January 21, 2013

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Key words: Hepatocellular carcinoma; Alpha-fetoprotein; Prognosis; Cirrhosis; Biomarker

Asrih M, Lenglet S, Mach F, Montecucco F. Alpha-fetoprotein: A controversial prognostic biomarker for small hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(3): 328-330 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/328.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.328>

INVITED COMMENTARY ON HOT ARTICLES

The incidence of hepatocellular carcinoma (HCC) has rapidly increased in the last decade to reach the sixth most common cancers worldwide^[1]. HCC most often develops in patients with chronic liver disease, usually cirrhosis. Several therapies for HCC have been developed and can be divided into four categories: surgical interventions (tumor resection and liver transplantation), percutaneous interventions (ethanol injection, radiofrequency thermal ablation), transarterial interventions (embolization, chemoperfusion, or chemoembolization) and drugs including gene and immune therapy. However, the diagnosis and treatment of a HCC at an early stage remain the most relevant strategies to improve prognosis^[2]. Several pathophysiological biomarkers have been investigated to predict HCC recurrence after liver transplantation^[3]. In particular, among different gene, RNA and protein targets, the circulating levels of alpha-fetoprotein (AFP) before the liver transplantation have been recently shown to be independent predictors of HCC recurrence^[3]. Firstly described by Abelev *et al*^[4], AFP is a large serum glycoprotein, belonging to the class of onco-development protein^[5]. It is synthesized by embryonic liver, the fetal intestinal tract and the vitelline sac cells. Similar to albumin, AFP binds and transports a large variety of ligands, such as fatty

Abstract

The assessment of the prognosis in patients with early hepatocellular carcinoma represents a hot-topic issue that requires further improvements and clarifications. The life expectancy of the patients has been shown to depend on several clinical and histological parameters (such as patient's general conditions, macroscopic tumor morphology and histopathology). Recently, the prognostic role of some biomarkers [i.e., alpha-fetoprotein (AFP)] has been also investigated with controversial findings mainly on the assessment of patient survival. The study by Giannini *et al* failed to show a prognostic value of AFP on survival of patients with well-compensated cirrhosis and small hepatocellular carcinoma. Since the study presents some limitations, a larger clinical trial is needed to clarify the potential prognostic role of serum AFP levels in these patients.

acids, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin, and various others drugs^[6,7]. Previous findings by Toso *et al.*^[8], showing that a combined score with total tumor volume (TTV) and AFP levels (> 400 ng/mL) was capable of predicting posttransplant survival, further supported the clinical prognostic use of this biomarker in transplanted patients. In this analysis of 6478 adult recipients of an isolated first liver transplant included in the Scientific Registry of Transplant Recipients database, the authors suggested that more traditional Milan criteria might be less efficient especially in patients with large TTV^[8]. Despite these promising results, the clinical use of AFP has been also shown to present some important limitations in sensitivity and specificity. In fact, low AFP levels have been described in HCC patients, while high levels might be detected in hepatic cirrhosis without HCC^[2]. For instance, Sangiovanni *et al.*^[9] have found that the level of AFP was increased in only 38% patients developing small HCC nodules. Indeed, AFP might be also useful in detecting other tumors (such as germ cell tumors of the ovary and testis and colorectal cancer)^[10,11]. Thus, although currently recommended as a fundamental parameter for HCC screening in patients with cirrhosis^[2,12], the prognostic capability of AFP in patients with HCC remains controversial and with important limitations.

The article by Giannini *et al.*^[13] performed a retrospective analysis from the Italian Liver Cancer group and showed that serum AFP levels did not predict survival in a prospective cohort of 205 patients with well-compensated cirrhosis and small (< 3 cm) hepatocellular carcinoma (Child-Pugh class A and Eastern Cooperative Group Performance Status 0) treated with curative intent. Therefore, accordingly to the Child-Pugh score, which is used to assess the prognostic of the liver disease, and the status that indicates the severity of the disease, the patients for this study exhibit a very early stage of HCC. Interestingly, AFP failed to predict survival also when patients were sub-divided into three groups and analyzed accordingly to their alpha-fetoprotein serum levels (normal: 0-20 ng/mL, *n* = 116; altered: 21-200 ng/mL, *n* = 71; diagnostic: > 200 ng/mL, *n* = 18) at the time of HCC diagnosis. Authors also indicate some potential limitations underlying the study results. AFP levels were within the normal range in a large portion of the cohort, thus confirming the previously discussed limitations in sensitivity of this marker in patients with early HCC and cirrhosis^[14]. These low AFP levels also contributed to determine a very low statistical power (22%) to detect differences in survival rates between patient groups. In addition, the stratification from AFP levels revealed a statistically significant difference in male gender between groups. Since AFP levels have been shown to be potentially influenced by the patient gender (increased values in women)^[15], this point might represent a critical concern affecting the relevance of the negative results. Finally, potential comorbidities (such as human immunodeficiency virus co-infection previously shown to potentially influence AFP

levels) were also not reported^[16]. These limitations have been correctly considered by authors, who concluded that the evaluation of more accurate biomarkers (other than AFP) might improve the assessment of prognosis in patients with early HCC. We believe that the study by Giannini *et al.*^[13] critically confirmed important concerns on strategies targeting a single prognostic biomarker in early HCC. In fact, given several existing confounders and its potential association with HCC nodule size, AFP levels might be more perceptible and relevant in patients with advanced HCC^[17]. In particular, the changes from AFP basal levels might be also useful for monitoring the treatment efficacy^[18]. In agreement with Giannini *et al.*^[13], we support a multifactorial approach to improve the prognostic prediction in patients with early HCC. In addition, we would also suggest that larger prospective studies with appropriate statistical power are needed to really evaluate the role of AFP and other biomarkers in these patients.

In conclusion, we believe that the study by Giannini *et al.*^[13], focused on a hot-topic issue in the hepatological field, importantly contributed to the current debate on the clinical use of serum biomarkers in disease prognosis. Considering the substantial study limitations, larger clinical trials are needed to better evaluate the prognostic role of AFP in early HCC. The matter is opened for future studies and discussion.

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P- Reviewers Vivarelli M, Andrisani OM, Uversky V

S- Editor Gou SX **L- Editor** A **E- Editor** Li JY



Clinical significance of heterotopic gastric mucosal patch of the proximal esophagus

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Author contributions: Chong VH conceived the idea and collated data through literature search, drafted the article for intellectual content and approved the final version.

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Received: May 23, 2012 Revised: July 30, 2012

Accepted: August 16, 2012

Published online: January 21, 2013

Abstract

Heterotopic gastric mucosa of the proximal esophagus (HGMPE), also referred to as "inlet patch" or "cervical inlet patch", is a salmon colored patch that is usually located just distal to the upper esophageal sphincter. HGMPE is uncommon with endoscopic studies reporting a prevalence ranging from less than one percent to 18%. Most HGMPE are asymptomatic and are detected incidentally during endoscopy for evaluations of other gastrointestinal complaints. Most consider HGMPE as clinically irrelevant entity. The clinical significance of HGMPE is mainly acid related or neoplastic transformation. The reported prevalence of laryngopharyngeal reflux symptoms varies from less than 20% to as high as 73.1%. However, most of these symptoms are mild. Clinically significant acid related complications such as bleeding, ulcerations, stricture and fistulization have been reported. Although rare, dysplastic changes and malignancies in association with HGMPE have also been reported. Associations with Barrett's esophagus have also been reported but the findings so far have been conflicting. There are still many areas that are unknown or not well understood and these include the natural history of HGMPE, risk factors for complications, role of *Helicobacter pylori* infection and factors associated with

malignant transformations. Follow-up may need to be considered for patients with complications of HGMPE and surveillance if biopsies show intestinal metaplasia or dysplastic changes. Despite the overall low incidence of clinically relevant manifestations reported in the literature, HGMPE is a clinically significant entity but further researches are required to better understand its clinical significance.

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Key words: Cervical inlet patch; Laryngopharyngeal reflux; Globus pharyngeus; Neoplasms; Barrett's esophagus

Chong VH. Clinical significance of heterotopic gastric mucosal patch of the proximal esophagus. *World J Gastroenterol* 2013; 19(3): 331-338 Available from: URL: <http://www.wjg-net.com/1007-9327/full/v19/i3/331.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.331>

INTRODUCTION

Heterotopic gastric mucosa of the esophagus (HGMPE), also commonly referred to as "inlet patch" or "cervical inlet patch", is an island of ectopic gastric mucosa that is found in the proximal esophagus^[1,2]. Rarely, they can also been found in the other part of the esophagus^[2,3]. HGMPE is widely considered to be congenital in nature. However, it has also been proposed to be an acquired condition^[1,4,5].

The reported incidence varies from less than one percent^[6] to 13.8% in endoscopic studies^[7]. Autopsy study has reported higher incidence of up to 70%^[1,2]. Commonly reported symptoms include laryngopharyngeal reflux (LPR) symptoms and the prevalence has been reported to be as high as 73.1%^[8]. However, most of these symptoms are mild and the management depends on the severity of symptoms. A clinico-pathologic classification has been proposed which categorized HGMPE into five distinct groups based on their clinical, endoscopic and

histological characteristics (Table 1)^[1]. Serious and significant complications of HGMPE have been reported in both adults and the pediatric population^[1,2,9]. Interestingly, other common upper aero-digestive disorders have also been linked to HGMPE^[2,9]. The clinical significance of HGMPE remains debated due to the limited number of publications. This article reviews the literature and discusses the clinical manifestations and significance of HGMPE in clinical practice.

BACKGROUND

Pathogenesis

Currently, there are three proposed theories on the development of HGMPE^[1,2,4,5]. The most widely accepted theory is that HGMPE is congenital in origin. During the development of the esophagus and at 24 wk of gestation (equivalent to 90 mm crown rump length during fetal development), squamous lining replaces the columnar lining starting from the mid esophagus moving in both directions. The proximal esophagus is usually the last part to get stratification and this account for the common finding of heterotopic gastric mucosa in the proximal esophagus^[1,2]. Although less common, ectopic mucosa can also be found in the other parts including the distal esophagus^[2,3]. Another proposed theory involves metaplastic transformation of the squamous lining to columnar from chronic acid injury as seen in Barrett's esophagus^[4]. Proliferation of remnant pluripotent cells as result of acid injury has been proposed as the underlying pathogenesis. The third theory involves rupture of proximal esophageal retention cystic glands^[5].

The most common histological type is the cardia or oxyntic type mucosa followed by the antral mucosa. Depending on the mucosa type, some HGMPE can produce acid^[10-12], and given their close proximity to the laryngopharyngeal complex can easily reflux into this area to cause symptoms. The laryngeal mucosa is also particularly susceptible to acid injury, even with mildly acidic secretion. Non-acidic secretions such as mucus have also been shown to induce laryngopharyngeal symptoms^[13].

HGMPE can be colonized by *Helicobacter pylori* (*H. pylori*) and the prevalence have been reported to be as high as 82%^[14]. The infection rates probably correlate with the prevalence of *H. pylori* infection in the general population. Although the role of *H. pylori* in HGMPE remains uncertain, one can probably surmise that it can cause changes similar to those seen in the stomach. Inflammatory and histological changes such as atrophy, metaplasia, dysplasia and carcinoma of the HGMPE have also been reported^[2,15].

Endoscopic characteristics

HGMPE is often missed during endoscopy as the proximal esophagus is often neglected or briefly examined during routine endoscopic examination. On endoscopy, HGMPE appears as a salmon colored ovoid or round patch that is usually distinct from the surrounding esophageal squamous mucosa (Figure 1A and B). It can be flat,

Table 1 Clinico-pathological classification for heterotopic gastric mucosa of the proximal esophagus

Category	Description	Symptoms/findings
I	Asymptomatic	None
II	Symptomatic	Laryngopharyngeal reflux
III	Symptomatic with benign complications	Strictures/webs/fistula/bleeding
IV	Intra-epithelial dysplasia	None/non-specific
V	Malignant transformation	Asymptomatic/dysphagia

elevated or depressed and the surface can be smooth or nodular. Most HGMPE are located on the lateral walls typically a few centimetres distal to the upper esophageal sphincter (16 and 21 cm from the incisors)^[1,2]. The sizes can vary from microscopic to as large as three to five centimeters. Most patients have a single patch while those with multiple patches, the patches tend to be small and are usually found within close proximity of other patches. On specialized imaging such as narrow band imaging, the HGMPE is clearly more visible (Figure 1C).

CLINICAL MANIFESTATIONS

The clinical manifestations of HGMPE can be broadly divided into non-neoplastic and neoplastic. The majority of patients found to have HGMPE are asymptomatic and the HGMPE are detected incidentally during evaluation for other gastrointestinal complaints. These patients are categorized to have type I HGMPE. The non-neoplastic manifestations (type II and III) such as LPR symptoms and strictures and bleeding are probably related to the acid produced by the patch. The least common manifestations are the histological or neoplastic changes (type IV and V)^[1,2]. With the exception of malignancy in the pediatric population, all these have been reported both in the adult^[1,2] and pediatric population^[16-20]. Acid related symptoms are often seen in younger patients whereas neoplastic manifestations have been reported mainly in the elderly population.

Acid-related manifestations

Symptoms associated with HGMPE include LPR symptoms such as regurgitation, dysphagia, hoarseness, globus, throat discomfort and chronic cough^[1,2]. Often dysphagia is located in the proximal esophagus or throat level. Associations with chronic ear or sinus problem have also been reported.

Symptoms in adult population

To date, there have only been a few studies that have reported comparisons of symptoms between patients with and without HGMPE. Published studies have so far reported mixed findings, some showing significantly higher LPR symptoms^[8,21-24] whilst others reported no significant difference^[15,25] (Table 2). The symptoms enquired and reported were not consistent between studies. Some reported the overall prevalence or limited to only

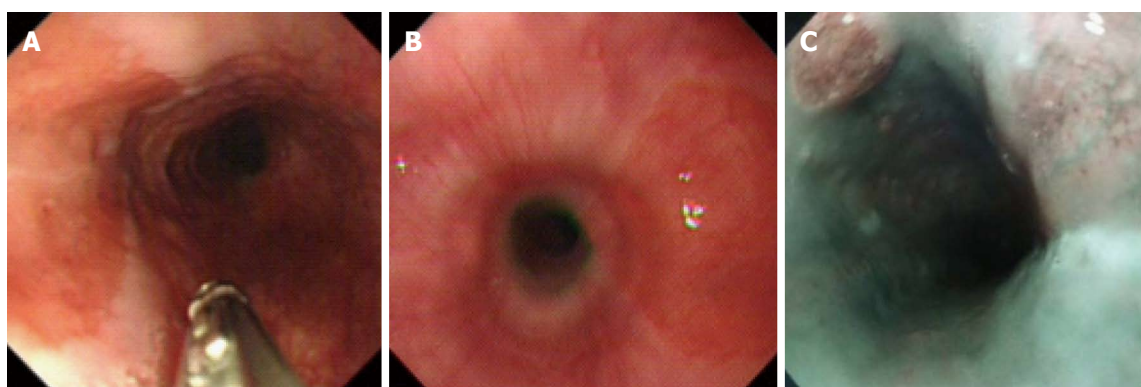


Figure 1 Endoscopic images of heterotopic gastric mucosa of the proximal esophagus. A: A large heterotopic gastric mucosa of the proximal esophagus (HGMPE) on the left lateral wall; B: A large HGMPE located on the right lateral wall; C: Small patch with a brownish hue located on the left lateral wall on neutral beam injection.

Table 2 Prevalence of symptoms reported to be associated with heterotopic gastric mucosa of the proximal esophagus in adult

Ref.	Symptoms reported	Prevalence of LPR symptoms (%)		P value
		HGMPE (+ve)	HGMPE (-ve)	
Chong <i>et al</i> ^[8]	Overall LPR symptoms	73.1	25.9	< 0.001
	Chronic cough	29.2	10.6	< 0.01
	Sore throat/hoarseness	54.2	11.7	< 0.01
	Globus	23.1	7.1	< 0.01
	Regurgitation	42.3	13.1	< 0.01
	Heartburn	50.0	22.5	< 0.01
Akbayir <i>et al</i> ^[15]	Upper esophageal and laryngopharyngeal symptoms	45	21.5	= 0.07
	Dysphagia	21	4.0	< 0.001
Baudet <i>et al</i> ^[21]	Dysphagia	39.4	0	< 0.05
Poyrazoglu <i>et al</i> ^[22]	Globus	78.6	0	< 0.05
Alagozlu <i>et al</i> ^[23]	Dyspepsia	88.2	97.8	NS
¹ Neumann <i>et al</i> ^[24]	Dysphagia/odynophagia	20.8	16.4	< 0.001
	Upper respiratory symptoms	2.5	0.9	< 0.001
	Globus	1.6	0.3	< 0.001
Weickert <i>et al</i> ^[25]	Recurrent hoarseness	9.1	5.6	= 0.5
	Dysphagia (any grade upper and lower)	15.2	9.4	NS
	Heartburn (any grade upper and lower)	15.2	9.4	NS

¹In the study by Neumann *et al*, the symptoms presented are symptom recorded as indications for endoscopy. HGMPE: Heterotopic gastric mucosa of the proximal esophagus; LPR: Laryngopharyngeal reflux; NS: Not significant.

one or few symptoms. However, most of the symptoms reported were mild. Prevalence rate of LPR symptoms as high as 73.1% has been reported^[8]. The largest study to date based on a database of 487 229 endoscopies carried out in non-referral centers in the United States showed that dysphagia or odynophagia, upper respiration symptoms suggestive of LPR and globus were significantly more common in patients with HGMPE^[24]. However, this study was limited by its retrospective nature and the assessment of only referral indications. The other studies were mainly from referral or specialized centers.

Symptoms in pediatric population

Currently, there are only a few studies on HGMPE in the pediatric population and are usually limited by small sample sizes^[9,16,18-20]. Most are in the form of case reports^[16-20]. To date, there is no endoscopic study that has assessed HGMPE in this population. Clinical manifestations are

different from the adult population. Common manifestations include laryngospasm^[16], respiratory symptoms^[17] and dysphagia from reflux or stricture^[9,20]. In one multi-centre study that had looked at 15 patients with a median age 9.5 years (range: 3.3-15 years) found that five patients had esophageal atresia, nine with gastroesophageal reflux disease (GERD) and asymptomatic in one. Dysphagia and food impaction were the most common manifestations reported in 14 patients. Six patients had respiratory or ear, nose, and throat symptoms^[9]. An autopsy study in the pediatric population showed that HGMPE was more common in younger age and was more significantly associated with unexplained death. Variend *et al*^[26] speculated that pulmonary aspiration of esophageal contents may have been the cause of death in some of the children.

Barrett's esophagus

Similarly to clinical symptoms, the association between

Table 3 Endoscopic studies reporting the associations between heterotopic gastric mucosa of the proximal esophagus and other endoscopic findings with special attention to Barrett's esophagus *n* (%)

Ref.	Prevalence of HGMPE	Findings
Positive association		
Avidan <i>et al</i> ^[4]	53 (1.1)	Significantly more reflux esophagitis (77 vs 50, $P = 0.023$), Barrett's esophagus (34 vs 9, $P < 0.001$), hiatus hernia (49 vs 30, $P < 0.05$) and gastric ulcer ($P < 0.05$)
Alagozlu <i>et al</i> ^[23]	68 (1)	On multivariate analysis, hiatus hernia, gastric ulcer and Barrett's esophagus remained significant Significantly more ($P < 0.05$) endoscopic Barrett's esophagus in patients with HGM (13.2 vs 2.4) but not with reflux esophagitis (10.3 vs 9.5) Hiatus hernia and duodenal ulcer were reported in 13.2% and 10.3% respectively but no comparisons were made
¹ Neumann <i>et al</i> ^[24]	870 (0.18)	Significantly more Barrett's mucosa on biopsy (9.7 vs 6.5, $P < 0.001$), adenocarcinoma arising from Barrett's mucosa (3.6 vs 0.7, $P < 0.01$) and reflux esophagitis (41.8 vs 49.7, $P < 0.001$)
Yuksel <i>et al</i> ^[27]	171 (1.8)	Significantly more reflux esophagitis (25.1 vs 5.6, $P < 0.001$) and histologically proven Barrett's esophagus (3.5 vs 0.5, $P < 0.000$) No difference in hiatus hernia
No association		
Borhan-Monesh <i>et al</i> ^[3]	64 (10)	No significant difference (all $P = \text{NS}$) between reflux esophagitis (34.3 vs 38.1) and Barrett's esophagus
Chong <i>et al</i> ^[8]	26 (5.6)	No significant difference (all $P = \text{NS}$) between esophageal, gastric and duodenal findings including Barrett's esophagus (3.8 vs 3.7), hiatus hernia (15.4 vs 12.2) and ulcers
Akbayir <i>et al</i> ^[15]	11 (1.67)	No significant difference (all $P = \text{NS}$): Barrett's esophagus (0 vs 0.9), hiatus hernia (0 vs 10), reflux esophagitis (27 vs 16) and duodenal ulcer (9 vs 7)
² Poyrazoglu <i>et al</i> ^[22]	33 (3.6)	No significant difference (all $P = \text{NS}$): Barrett's esophagus (0 vs 0.8), hiatus hernia (3 vs 9.1), reflux esophagitis (36.4 vs 34.8), gastric ulcer (3 vs 3) and duodenal ulcer (6.1 vs 6.8)
Weickert <i>et al</i> ^[25]	33 (11)	Overall prevalence: hiatus hernia ($n = 92$, 30.7%), reflux esophagitis ($n = 41$, 13.7%), Barrett's esophagus ($n = 3$, 1%), gastric ulcer ($n = 24$, 8%) and duodenal ulcer ($n = 22$, 7%), all $P = \text{NS}$
Jacobs <i>et al</i> ^[28]	33 (4.9)	Significant difference for reflux esophagitis (27.3 vs 11.4) but not for hiatus hernia (15.2 vs 12.5), Barrett's esophagus (6.1 vs 1.1) and any gastric or duodenal ulcer (15.2 vs 6.1)

Studies included are those with more than 10 patients with heterotopic gastric mucosa of the proximal esophagus (HGMPE). ¹The study by Neumann *et al* was based on analysis clinic-pathologic records of patients who had undergone upper gastrointestinal endoscopy with biopsies between January 2008 and December 2010 in private outpatient clinics and endoscopy centres in the United States; ²The study by Poyrazoglu *et al* compared the prevalence of other endoscopic findings between 33 patients with HGMPE with 132 matched controls without HGMPE. NS: Not significant.

HGMPE and Barrett's esophagus remains debated. Studies have reported conflicting results (Table 3). Avidan *et al*^[4] showed that patients with HGMPE had up to fivefold higher risk of Barrett's esophagus compared to patients without HGMPE. Three other studies^[23,24,27] have reported significant positive correlations while five studies that included more than 20 patients with HGMPE have not found any correlations^[3,8,22,25,28]. Two histological studies reported similar mucin staining patterns in Barrett's esophagus and HGMPE suggesting possible shared pathogenesis^[29,30]. However, Fuerle *et al*^[31] found some differences and postulated that the specialized columnar epithelium of Barrett's esophagus originates from a very immature multipotent gastrointestinal stem cell whereas HGMPE originates from remnant embryonic gastric mucosa located in the proximal esophagus

Non-laryngopharyngeal symptoms

Reports on symptoms associated with HGMPE are mainly on LPR symptoms^[2]. There is currently little information to suggest whether HGMPE is associated with non-LPR symptoms. Heartburn has been shown to be common in patients with HGMPE, even in those not found to have any erosive esophagitis^[4,8,32]. Explanations offered included symptoms induced by acid secretion flowing downward or increased sensitivity as seen in non-erosive esophagitis. Apart from proximal acid reflux independent of distal acid reflux, Korkut *et al*^[11]

also showed that some patients with HGMPE have signs of esophageal motor dysfunction based on manometry and 24 h dual probe pH study. They concluded that these abnormalities may be responsible for some of the symptoms experienced by patients with HGMPE. In another study, Rosztoczy *et al*^[32] reported several differences between patients with and without HGMPE. Patients with HGMPE had more and prolonged acid exposure in both proximal and distal esophagus, longer bile exposure time in the distal esophagus, reduced lower esophageal sphincter pressure with prolonged relaxation and decreased peristaltic wave amplitude. There was also increased number of simultaneous contractions in the esophageal body of patients with HGMPE. These suggest that patients with HGMPE also have motility disorders contributing to their symptoms.

Other non-neoplastic complications

Complications of HGMPE reported in the literature range from formations of strictures^[33-35], web^[36-38], ulceration with bleeding^[39], fistula formation with or without subcutaneous abscesses^[19,40-42], perforation^[43,44] and polyps^[45-47] (Table 4). HGMPE have been reported to be the possible cause of Plummer Vinson or Paterson Kelly Brown syndrome^[1,2,38]. The underlying pathogenesis is probably similar to those seen in acid-related gastric pathologies. In the pediatric population, HGMPE has also been associated with unexplained infant deaths^[26], dysphagia from strictures in the

Table 4 Complications of heterotopic gastric mucosa of the proximal esophagus reported in the literature

Clinico-pathological classification	Conditions	Status	Numbers based on PubMed literature search
Type III	Stricture	Reported	6
	Web	Reported	4
	Bleeding	Reported	1
	Fistula	Reported	4
	Perforation	Reported	2
	Polyp ¹	Reported	4
Type IV ²	Dysplasia		
	Low grade	Reported	None
	High grade	Reported	3 ^[32-34]
Type V ²	Adenocarcinoma		
	Early (pT1 tumor)	Reported	13 ^[35-48]
	Advanced	Reported	19 ^[33,49-54]

References for type III cases reported in the literature not included in the reference list. ¹Not included in the original classification proposed by von Rahden *et al*^[1]; ²Number of cases of neoplastic transformation identified through literature searches up till December 2011.

proximal esophagus^[9,18,20] or spasm^[16] and recurrent neck abscesses secondary to fistula^[19].

Histology progressions or neoplastic transformation

Overall, significant histological non-malignant changes or malignancies in HGMPE are extremely rare. Histological changes such as chronic inflammation^[3,14], atrophy, intestinal metaplasia^[14], and dysplasia^[48-50] as seen in the stomach have been reported mainly in the adult population. However intestinal metaplasia has also been reported in children^[17]. Macha *et al*^[17] reported that 8.3% ($n = 2/24$) of their pediatric patients with HGMPE had intestinal metaplasia detected on histology. Neoplastic transformations have only been reported in the adult population^[2,36-39]. A summary of the reported significant histological or malignancies in association with HGMPE are shown in Table 4.

Since the first case reported by Carrie *et al*^[51] in 1950, there have only been 43 cases of adenocarcinoma^[52-54] in association with HGMPE reported in the literature to date (Table 4). To date, Neumann *et al*^[24] in the largest series of endoscopies done in non-specialised centers did not encounter any cases of HGMPE with malignancy whereas Alagozlu *et al*^[23] encountered a case each of adenocarcinoma and low grade dysplasia among 64 patients with HGMPE. Based on these two studies, it can be estimated that the incidence of malignancies among patients with HGMPE ranges between 0 and 1.56%^[23,24]. However, the incidence is likely to be much lower than the estimate from the latter study.

Based on a review of 43 cases reported in the literatures by Akanamu *et al*^[54], majority of those affected were men (88.4%) with a median age of 60.4 years (range 35 to 85 years). Most of the lesions were advanced at diagnosis especially in the earlier reported. Early lesions (T1 lesion) accounted for 14 cases^[54]. The lesions were ulcerated in 31.2%, protruding in 30.2% and polypoidal in 18.6%.

Dysphagia was the most common complaint (74.4%) with smaller proportion detected through surveillance. Interestingly, acid-related symptoms were not common. Smoking appeared to be a risk factor.

Associations with extra-esophageal neoplasms

To date, association with extra-esophageal neoplasms have only been reported twice^[55,56]. Basseri *et al*^[55] reported the case of a 22-year-old lady with asthma and GERD diagnosed with laryngeal adenocarcinoma. She later developed a stricture proximal to a HGMPE (type III). In another report, Satoh *et al*^[56] reported the case of a 44-year-old lady with hypopharyngeal carcinoma with a HGMPE (2.0 cm × 1.5 cm) bordering the tumor. Chronic irritation from acid was proposed as the contributing cause to the development of the tumors.

OVERVIEW

HGMPE was first reported by Schmidt *et al*^[1] as an aberrant gastric fundus-type epithelium located in the proximal esophagus. Despite this, the number of publications on this entity has remained low compared to the other esophageal disorders. Therefore it is not surprising that controversies remain regarding the clinical significance of this entity. Despite the lack of data, HGMPE is clinically significant and the clinical significance is related to acid-related manifestations, mucosal changes and malignant transformations.

The clinical manifestations of HGMPE are best summarized by the classification proposed by von Rahden *et al*^[1]. Clinically significant manifestations which have been reported in only a small proportion of patients are categorized as type II, III, IV and V HGMPE. The most common is the type II with clinical symptoms of LPR^[1,8]. However, some do not consider type II HGMPE as significant as the symptoms experienced are mostly mild^[8,13]. On the other hand, these symptoms are chronic and very troublesome for a small minority of patients.

It is currently uncertain if the symptoms are acid-related given that studies have shown that only a small proportion of symptomatic patient have documented acid secretion from the HGMPE^[10,31]. However, one can probably surmise that there is a causal association. Similar to the stomach or heterotopic gastric mucosa found in Meckel's diverticulum, excess acid production can lead to acid-related symptoms and complications. Patients with HGMPE have also been shown to have motility disorders and this may contribute to the symptomology of HGMPE^[11,32].

Symptomatic patients with HGMPE typically reports LPR symptoms; globus pharyngeus, sore throat, hoarseness, chronic cough, throat clearing and dysphagia^[57]. This is not surprising given the close proximity of HGMPE with the laryngopharyngeal complex. The laryngopharyngeal complex is particularly sensitive and even a small amount of weakly acidic secretion can cause symptoms^[57,58]. Symptoms can also occur with non-acidic secretions^[4]. Presence of gastric enzymes such as pepsin

also contributes to irritations^[58].

The prevalence of the various LPR symptoms in association with HGMPE varies from less than 20% to as high as 73.1%^[2]. Comparisons of published studies are difficult due to differences in the study methodologies. Symptoms enquired were not always consistent with some studies enquiring on limited number of symptoms or not specifying the symptoms enquired. Furthermore, most studies had small sample sizes.

In clinical practice, we also often encounter patients with LPR symptoms without any heartburn. Such patients are categorized as having extra-esophageal manifestations of GERD. Some of these patients clearly do not have acid exposure in the distal esophagus and as such, LPR symptoms in association with HGMPE should be considered a separate entity separate from GERD.

Associations with neoplastic changes and importantly malignant transformation are reported and these represent the most significant associations of HGMPE. However, the numbers reported is still very low and are considered extremely rare. Interestingly, a large number of the reported cases especially in the non-English literatures have been from Japan^[54]. Whether, HGMPE with malignant transformations is more common in the Japanese population is unknown. It may be related to better screening program and increased detection rate. Most of the reported cases were advanced at diagnosis especially in the earlier studies. However, there were more early cancers (T1 lesions) reported in the latter part. Men seem to be more at risk and smoking appears to be a risk factor^[54]. Dysphagia was the main complaint leading to detection of tumors.

Association with Barrett's esophagus is another area that remains debated. A widely accepted theory is based on a shared pathogenesis with both Barrett's esophagus and HGMPE being congenital in origin but with differences in pathogenesis in the later part of the development. However, this had been based on western population where the prevalence of Barrett's esophagus is much higher compared to the East. Further large population studies will be required and help shade some lights into this controversy. Associations with other acid related complications such as erosions, ulcerations, bleeding, perforation, fistula and stricture formations have been documented^[1,8]. Possible link between HGMPE and unexplained death in children have also been reported^[26].

Currently, there are still many issues regarding HGMPE that remain unresolved or unknown. These ranges from the origin of HGMPE, exact correlation with Barrett's esophagus, role of *H. pylori* infection in symptoms manifestations, lack of natural history data and significance of mucosal changes in the HGMPE and its relation to neoplastic transformations.

Given that HGMPE mainly manifests with upper autodigestive symptoms, it is not unexpected that only clinicians from certain specialties such as the gastroenterologists, otorhinolaryngologists and rarely upper gastrointestinal surgeons will encounter such patients. How-

ever, it is important that other clinicians from the other specialties to be aware of this entity especially when they come across patients with troublesome LPR symptoms. Establishing a diagnosis is important as it can provide reassurance. This may also lead to symptoms improvement and avoid any further unnecessary investigations or consultations. As the awareness of HGMPE increase, more asymptomatic or symptomatic cases will be diagnosed and with the ageing population, it is very likely the number of cases of neoplastic transformations will also increase, albeit a small increase.

CONCLUSION

The clinical significance of HGMPE lies mainly with its capacity to produce acid and mucosal progressions to dysplasia and frank neoplastic transformation. Currently, there are still many areas of HGMPE that are not well understood and further research are required. Symptomatic patients should to be treated and those found to have metaplasia or dysplasia may need to be considered for surveillance.

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P-Reviewers Quesada BM, Morelli L **S-Editor** Gou SX
L-Editor A **E-Editor** Zhang DN



Diagnostic value of AFP-L3 and PIVKA- II in hepatocellular carcinoma according to total-AFP

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Supported by The Industrial Core Technology Development Program funded by the Ministry of Knowledge Economy, No. 10033183

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Received: July 16, 2012 Revised: October 25, 2012

Accepted: November 6, 2012

Published online: January 21, 2013

Abstract

AIM: To evaluate diagnostic value of α -fetoprotein (AFP)-L3 and prothrombin induced by vitamin K absence- II (PIVKA- II) in hepatocellular carcinoma (HCC).

METHODS: One hundred and sixty-eight patients during routine HCC surveillance were included in this study. Of the 168 patients, 90 (53.6%) had HCC including newly developed HCC ($n = 82$) or recurrent HCC after treatment ($n = 8$). Sera were obtained during their first evaluation for HCC development and at the time of HCC diagnosis before commencing HCC

treatment. HCC was diagnosed by histological examination, appropriate imaging characteristics-computed tomography or magnetic resonance imaging. Control sera were collected from 78 patients with benign liver disease (BLD), which were obtained during routine surveillance with a suspicion of HCC. AFP, AFP-L3 and PIVKA- II were measured in the same serum by microchip capillary electrophoresis and liquid-phase binding assay on a micro-total analysis system Wako i30 auto analyzer. The performance characteristics of three tests and combined tests for the diagnosis of HCC were obtained using receiver operating characteristic curves in all populations and subgroups with AFP < 20 ng/mL.

RESULTS: Of 90 HCC patients, 38 (42.2%) patients had AFP < 20 ng/mL, 20 (22.2%) patients had AFP 20-200 ng/mL and 32 (35.6%) patients had AFP > 200 ng/mL. Of the 78 BLD patients, 74 (94.9%) patients had AFP < 20 ng/mL. After adjustment for age and HBV infection status, AFP-L3 levels were higher in HCC than in BLD among patients with low AFP levels (< 20 ng/mL) ($P < 0.001$). In a total of 168 patients, areas under the curve (AUC) for HCC were 0.879, 0.887, 0.801 and 0.939 for AFP, AFP-L3, PIVKA- II and the combined markers, respectively. The combined AUC for three markers showed higher value than the AUCs of individual marker ($P < 0.05$). AFP-L3 had higher AUC value than PIVKA- II for HCC detection in entire patients ($P = 0.043$). With combination of AFP-L3 (cut-off > 5%) and PIVKA- II (cut-off > 40 AU/L), the sensitivity were 94.4% and specificity were 75.6% in all patients. In 112 patients with low AFP levels (< 20 ng/mL), AUCs of AFP-L3, PIVKA- II and combine AFP-L3 and PIVKA- II tests were 0.824, 0.774 and 0.939, respectively. AFP-L3 with a cut-off value of 5% showed sensitivity of 71.1% and specificity of 83.8%, and PIVKA- II with a cut-off value of 40 AU/L had sensitivity of 57.9% and specificity of 95.9% in patients with low AFP levels. The combination of AFP-L3 and PIVKA- II increased the sensitivity and specificity up to 92.1% and 79.7%, respectively, in low AFP group. Combined markers detected 81.8% of early stage HCC (Union for Inter-

national Cancer Control stage I), 86.7% of small sized tumor (< 2 cm) and 91.7% of single tumor of HCC in the low AFP group. In multivariate analysis, AFP-L3 was correlated with AFP and tumor size, and PIVKA-II was correlated with laboratory tests including serum aspartate aminotransferase, total bilirubin, platelets and albumin levels. PIVKA-II had no correlation with AFP, AFP-L3 or tumor characteristics.

CONCLUSION: Combined determination of AFP-L3 and PIVKA-II could improve the diagnostic value for HCC detection in patients with or without increased AFP levels.

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Key words: α -fetoprotein; Prothrombin induced by vitamin K absence-II; Hepatocellular carcinoma; Diagnosis; Tumor marker

Choi JY, Jung SW, Kim HY, Kim M, Kim Y, Kim DG, Oh EJ. Diagnostic value of AFP-L3 and PIVKA-II in hepatocellular carcinoma according to total-AFP. *World J Gastroenterol* 2013; 19(3): 339-346 Available from: URL: <http://www.wjg-net.com/1007-9327/full/v19/i3/339.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.339>

INTRODUCTION

Early detection of hepatocellular carcinoma (HCC) is important as the treatment of HCC with surgical resection, liver transplantation or percutaneous ablation can be curative at early stage. Currently, the recommended screening strategy includes measurement of serum α -fetoprotein (AFP) levels and an abdominal ultrasound every 3-6 mo for the detection of HCC at an earlier stage^[1-4]. Usually, a serum AFP level of 20 ng/mL is considered as a cut-off value to differentiate HCC from non-HCC^[5]. However, serum AFP level has a high false-negative rate for the detection of small or early stage tumors^[2,6-9], and it is often markedly elevated in patients with either cirrhosis or those with exacerbated chronic hepatitis without HCC^[10,11].

Therefore, additional tumor markers including fucosylated fraction of AFP (AFP-L3) fraction^[12,13] and prothrombin induced by vitamin K absence-II (PIVKA-II) or Des- γ -carboxyprothrombin^[14-16] have been suggested, and the surveillance program with these markers have been mostly organized by Japanese study groups^[1,17]. The lens culinaris agglutinin-reactive, AFP-L3 has been reported to be highly specific for HCC^[5,18-20] and in predicting prognosis^[21-24]. Recently, a highly sensitive analytical system using the technically advanced microfluidics-based separation system has been introduced^[25]. With this micro-total analysis system (μ TAS), AFP-L3 can be measured accurately at very low AFP concentrations under 10 ng/mL with high sensitivity^[17,19,25,26]. PIVKA-II is an abnormal protein and some reports have indicated

the improved specificity of PIVKA-II over AFP in the diagnosis of HCC^[2,5,27-29]. The combination of AFP-L3 and PIVKA-II seems to improve the diagnostic value for HCC patients, and these additive assays are especially recommended for patients with low AFP levels according to previous Japanese studies^[19,26,27]. However, diagnostic values of these markers are controversial and different cut-off values have been suggested depending on the study design^[5,20,26]. In addition, the clinical values of these markers in combination are not conclusive yet, and the clinical and laboratory factors which possibly effect the measured values of AFP-L3 and PIVKA-II have not been sufficiently investigated.

In this study, we compared the levels of AFP, AFP-L3 and PIVKA-II in benign liver diseases (BLD) patients and HCC patients, and evaluated the individual and combined diagnostic values of AFP-L3 and PIVKA-II for HCC detection according to the total AFP levels and tumor characteristics. The clinical and laboratory factors affecting AFP-L3 and PIVKA-II were also analyzed.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 168 patients during routine HCC surveillance at the Seoul St. Mary's Hospital, Seoul, South Korea, during the period between April and November 2011. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital. Of the 168 patients, 90 (53.6%) had HCC including newly developed HCC ($n = 82$) or recurrent HCC after treatment ($n = 8$). Sera were obtained during their first evaluation for HCC development and at the time of HCC diagnosis before commencing HCC treatment. HCC was diagnosed by histological examination, appropriate imaging characteristics-computed tomography or magnetic resonance imaging. Tumor stage was assessed by the Union for International Cancer Control (UICC) staging system, which is based on tumor number, size, vascular invasion and metastasis^[4,30]. Control sera were collected from 78 patients with BLD, which were obtained during routine surveillance with a suspicion of HCC. All patients were diagnosed by imaging study and were followed up for 7-16 mo after sampling to confirm non-HCC.

Measurement of AFP, AFP-L3 and PIVKA-II

AFP, AFP-L3 and PIVKA-II were measured in the same serum by microchip capillary electrophoresis and liquid-phase binding assay on a μ TAS Wako i30 auto analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The measuring range was 0.3-1000 ng/mL for AFP and 5-50 000 AU/L for PIVKA-II. AFP-L3 levels were calculated in sera with AFP levels over 0.3 ng/mL. The total imprecision for two levels of the three markers were less than 5% CVs in 20 d of evaluation. Serum samples were stored at -80 °C until tested. All processes were performed automatically and followed the manufacturer's instructions.

Table 1 Demographics and laboratory test values of patients with benign liver disease and with hepatocellular carcinoma *n* (%)

	BLD (<i>n</i> = 78)	HCC (<i>n</i> = 90)	<i>P</i> value
Age (mean ± SD), yr	55.6 ± 12.5	59.7 ± 10.1	0.020
Male	51 (65.4)	64 (71.1)	0.506
Cause of disease			
HBV	34 (43.6)	61 (67.8)	0.002
HCV	9 (11.5)	9 (10.0)	0.749
Alcohol	5 (6.4)	12 (13.3)	0.139
NANBNC	1 (1.3)	8 (8.9)	0.038
Benign mass	10 (12.8)		
Others	19 (24.4)		
AFP < 20 ng/mL	74 (94.9)	38 (42.2)	
AFP 20-200 ng/mL	4 (5.1)	20 (22.2)	
AFP > 200 ng/mL	0 (0)	32 (35.6)	
Tumor stage			
I / II / III/IVa/IVb		16 (17.8)/31 (34.4)/ 19 (21.1)/18 (20.0)/6 (6.7)	
Tumor size			
< 2/2-5/> 5 cm		25 (27.8)/33 (36.7)/32 (35.6)	
Tumor number			
Single/multiple		40 (44.4)/50 (55.6)	
AFP level	6.9 ± 13.5	473.9 ± 746.0	< 0.001
(mean ± SD, ng/mL)			
AFP-L3 level	2.3 ± 4.9	40.0 ± 35.7	< 0.001
(mean ± SD, %)			
PIVKA- II level	20.0 ± 31.2	4469 ± 11 553.8	< 0.001
(mean ± SD, AU/L)			

HBV: Hepatitis B virus; HCV: Hepatitis C virus; NANBNC: Non-hepatitis A, non-hepatitis B, non-hepatitis C virus; AFP: α -fetoprotein; PIVKA- II: Prothrombin induced by vitamin K absence- II; BLD: Benign liver disease; HCC: Hepatocellular carcinoma.

Statistical analysis

Statistical analysis were performed with SPSS version 12.0 (SPSS, Chicago, IL) and Med-Calc version 12. 2. 1. 0 (MedCalc Software, Mariakerke, Belgium). Comparisons were made using *t* test. The performance characteristics of three tests and combined tests for the diagnosis of HCC were obtained using receiver operating characteristic (ROC) curves in all populations and subgroups with AFP < 20 ng/mL. The sensitivity and specificity of these three markers for the diagnosis of HCC were calculated in all the groups and subgroups according to the total AFP levels. A logistic regression model was performed in SPSS on the three markers and ROC curves analysis was performed for area under the curve (AUC) values for the combined markers. All *P* values were 2-tailed with *P* < 0.05 considered statistically significant.

RESULTS

Patient characteristics

Table 1 shows the demographics of the study population. The most common cause of liver disease was hepatitis B virus (HBV) infection in both BLD and HCC patients. HCC patients had a higher frequency of males and HBV infection than that of BLD patients (*P* < 0.05). Of the 90 HCC patients, 38 (42.2%) patients had AFP < 20 ng/mL, 20 (22.2%) patients had AFP 20-200 ng/mL and

32 (35.6%) patients had AFP > 200 ng/mL. Of the 78 BLD patients, 74 (94.9%) patients had AFP < 20 ng/mL. In four patients with non-HCC and increased AFP levels (45.2-72.8 ng/mL), PIVKA- II levels were less than 40 AU/L, but AFP-L3 levels were above 10%. The levels of AFP, AFP-L3 and PIVKA- II in BLD and HCC patients are plotted with respect to the AFP levels (Figure 1). In patients with AFP < 20 ng/mL, AFP-L3 levels were higher in HCC than that in BLD (*P* < 0.001). Regarding the patients with total AFP levels of 20-200 ng/mL, not AFP-L3 but PIVKA- II was able to discriminate HCC from BLD (*P* = 0.010). After adjustment for age (> 50 and < 50 years) and HBV infection status, AFP-L3 levels were still higher in HCC than in BLD among patients with low AFP levels (< 20 ng/mL) (*P* < 0.001).

ROC curve analysis of three markers for the detection of HCC

The ROC curves of AFP, AFP-L3 and PIVKA- II for the diagnosis of HCC in all of 168 patients are shown in Figure 2. The maximum AUCs for distinguishing between HCC and BLD were 0.879, 0.887 and 0.801 for AFP, AFP-L3 and PIVKA- II, respectively. AFP-L3 had higher AUC than PIVKA- II for HCC (*P* = 0.043). Using logistic regression model, the combined AUC for three markers was 0.939 and showed higher AUC values than the individual markers (*P* < 0.05).

In 112 patients with AFP < 20 ng/mL (HCC, *n* = 38; BLD, *n* = 74), the AUC for HCC detection was 0.824 for AFP-L3, 0.774 for PIVKA- II and 0.939 for the two combined markers. Combined AUC was significantly higher than each AUC (*P* < 0.05), and the AUC value was not different between AFP-L3 and PIVKA- II (*P* = 0.516) (Figure 3).

Sensitivity and specificity of three markers for HCC diagnosis

The sensitivities and specificities of the three markers for all patients and subgroups are shown in Table 2. In all patients, the total AFP showed a sensitivity of 78.9% and a specificity of 84.6% with a cut-off of 10 ng/mL. AFP-L3 with a cut-off value of 5% increased the sensitivity up to 82.2%. When the cut-off value for AFP-L3 and PIVKA- II were set at 10% and 40 AU/L, the specificities were improved up to 93.6% and 94.9%, respectively.

In the subgroups according to the total AFP levels (AFP < 20 ng/mL, *n* = 112; AFP < 200 ng/mL, *n* = 136), the sensitivity and specificity of AFP-L3 were close to 70% and 80% respectively with a cut-off of 5%, and there was no significant difference compared to that of the entire group. The sensitivity and specificity of PIVKA- II was approximately 60% and 95% respectively with a cut-off of 40 AU/L in all patients and subgroups.

To increase the diagnostic value for HCC detection, we combined the AFP-L3 and PIVKA- II results. If either AFP-L3 or PIVKA- II level was above the cut-off value, the combined result was defined as positive. With combination of AFP-L3 (cut-off > 5%) and PIVKA- II

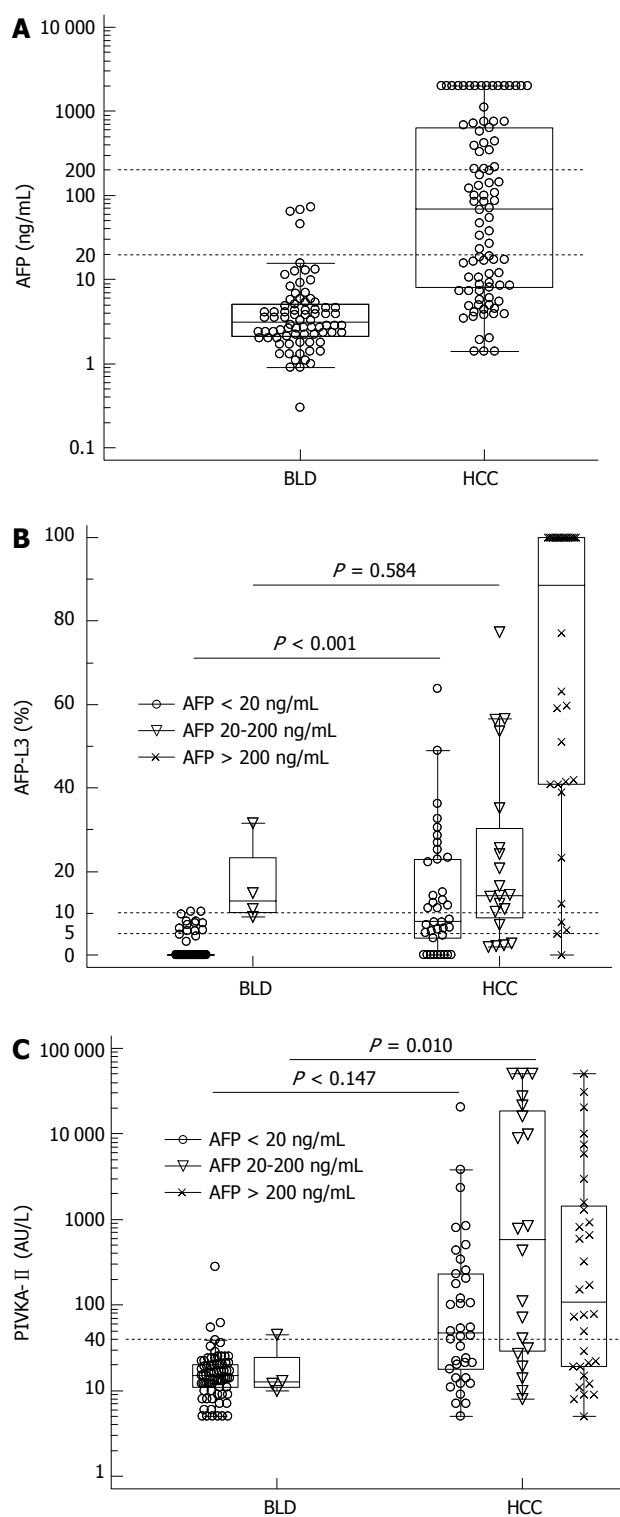


Figure 1 α -fetoprotein (A), α -fetoprotein-L3 (B) and prothrombin induced by vitamin K absence-II (C) values in benign liver disease and hepatocellular carcinoma patients. α -fetoprotein (AFP)-L3 and prothrombin induced by vitamin K absence (PIVKA)-II levels are plotted by AFP concentrations (AFP < 20 ng/mL, AFP 20-200 ng/mL and AFP > 200 ng/mL). In patients with AFP < 20 ng/mL, AFP-L3 levels were higher in hepatocellular carcinoma (HCC) ($P < 0.001$) (B). In patients with AFP 20-200 ng/mL, PIVKA-II showed higher levels in HCC ($P = 0.010$) (C). The horizontal dotted lines are cut-off values used for performance analysis in this study. BLD: Benign liver disease.

(cut-off > 40 AU/L), the sensitivities were 92.1%-94.4%

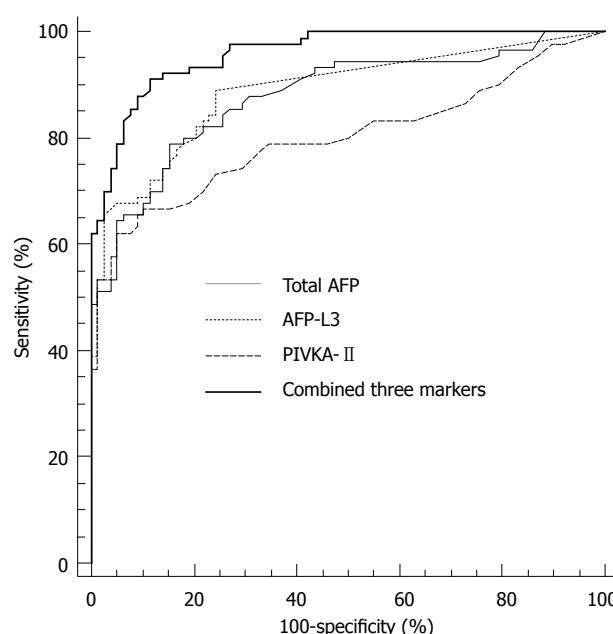


Figure 2 Receiver operating characteristic curves of total α -fetoprotein, α -fetoprotein-L3, prothrombin induced by vitamin K absence-II and three combined markers for the diagnosis of hepatocellular carcinoma in all patients. The area under the curve values were 0.879 for total α -fetoprotein (AFP), 0.887 for AFP-L3, 0.801 for prothrombin induced by vitamin K absence (PIVKA)-II and 0.959 for the three combined markers.

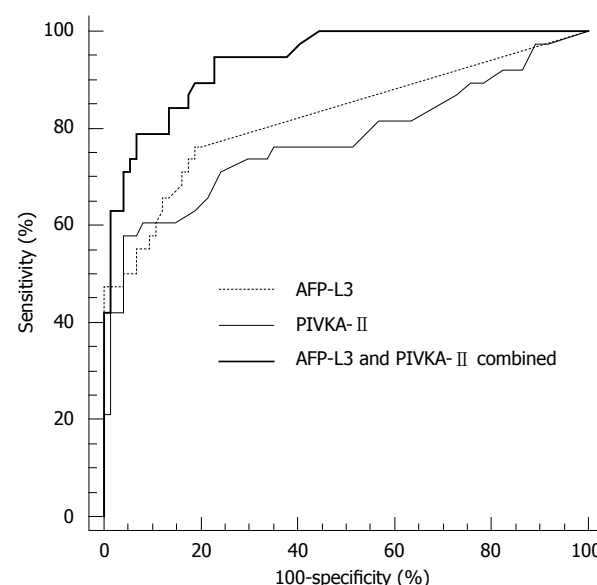


Figure 3 Receiver operating characteristic curves of α -fetoprotein-L3 and prothrombin induced by vitamin K absence-II for the diagnosis of hepatocellular carcinoma in patients with total α -fetoprotein < 20 ng/mL. The area under the curve values were 0.824 for α -fetoprotein (AFP)-L3, 0.774 for prothrombin induced by vitamin K absence (PIVKA)-II and 0.939 for the two combined markers.

and specificities were 75.6%-79.7% in all patients and subgroups. When a cut-off value was set at 10% for AFP-L3 and combined with PIVKA-II, the sensitivities were approximately 80% (78.9%-84.4%) and the specificities were maintained close to 90%.

Table 2 Performance of α -fetoprotein, α -fetoprotein-L3 and prothrombin induced by vitamin K absence-II in detecting hepatocellular carcinoma in all patients and in subgroups according to total α -fetoprotein levels: Total α -fetoprotein

		Cut-off value	Sensitivity	Specificity
Entire group (<i>n</i> = 168, 90 HCC)	AFP	10 ng/mL	78.9%	84.6%
	AFP-L3	5%	82.2%	79.5%
		10%	67.8%	93.6%
	PIVKA- II	40 AU/L	62.2%	94.9%
AFP < 20 ng/mL (<i>n</i> = 112, 38 HCC)	AFP-L3 or PIVKA- II	5% or 40 AU/L	94.4%	75.6%
		10% or 40 AU/L	84.4%	89.7%
	AFP-L3	5%	71.1%	83.8%
		10%	47.4%	97.3%
AFP < 200 ng/mL (<i>n</i> = 136, 58 HCC)	PIVKA- II	40 AU/L	57.9%	95.9%
	AFP-L3 or PIVKA- II	5% or 40 AU/L	92.1%	79.7%
		10% or 40 AU/L	78.9%	93.2%
	AFP-L3	5%	74.1%	79.5%
		10%	56.9%	93.6%
	PIVKA- II	40 AU/L	62.1%	94.9%
	AFP-L3 or PIVKA- II	5% or 40 AU/L	93.1%	75.6%
		10% or 40 AU/L	82.8%	89.7%

AFP: α -fetoprotein; PIVKA- II: Prothrombin induced by vitamin K absence- II; HCC: Hepatocellular carcinoma.

Sensitivity of AFP-L3 and PIVKA- II according to the tumor characteristics in patients with AFP < 20 ng/mL

To establish the sensitivity of AFP-L3 (cut-off 5%) and PIVKA- II (cut-off 40 AU/L) for the detection of small tumors and early stage HCC especially in 38 HCC patients with AFP < 20 ng/mL, we subgrouped the HCC patients according to the UICC stage (I, II, III, IVa, IVb), tumor size (< 2, 2-5, > 5 cm) and tumor number (single and multiple tumor) (Table 3). The sensitivity of AFP-L3 and PIVKA- II in UICC stage I - II (*n* = 27) was 63.6%-75.0% and 45.5%-56.3%. The combination of AFP-L3 and PIVKA- II increased the sensitivity up to 81.8%-100% (Table 3). In HCC patients with tumor size < 2 (*n* = 15) and 2-5 cm (*n* = 17), the sensitivity of AFP-L3 was 46.7% and 47.1%, respectively. The sensitivity of PIVKA- II for the detection of HCC with tumor size < 2 and 2-5 cm was 40.0% and 64.7%, and combination of AFP-L3 and PIVKA- II resulted in an enhancement of sensitivity up to 86.7% and 94.1%, respectively. With a combined test (AFP-L3 and PIVKA- II), 22 (91.7%) of 24 HCCs with a single tumor were detected.

Correlation analysis

To investigate the clinical and laboratory factors which may affect the measured values of AFP-L3 and PIVKA- II, we performed univariate and multivariate analyses. In univariate correlation analysis, there was a correlation between AFP-L3 and PIVKA- II. AFP-L3 and PIVKA- II levels were correlated with tumor stage (UICC), tumor size, aspartate aminotransferase (AST) and albumin. Tumor number and AFP levels were correlated with only AFP-L3 levels.

In multivariate analysis, only AFP and tumor size were significant correlates of AFP-L3 (Table 4). In terms of PIVKA- II, laboratory tests including serum AST, total bilirubin, platelets and albumin levels were correlated

Table 3 Sensitivity of α -fetoprotein-L3 and prothrombin induced by vitamin K absence-II according to the tumor characteristics in 38 hepatocellular carcinoma patients with α -fetoprotein < 20 ng/mL *n* (%)

		AFP-L3 <i>n</i> (> 5%)	PIVKA- II (> 40 AU/L)	AFP-L3 and PIVKA- II combined (> 5%, > 40 AU/L)
Tumor stage (UICC)				
I	11	7 (63.6)	5 (45.5)	9 (81.8)
II	16	12 (75.0)	9 (56.3)	16 (100)
III	9	6 (66.7)	6 (66.7)	8 (88.9)
IVa	2	2 (100)	2 (100)	2 (100)
Tumor size				
< 2 cm	15	7 (46.7)	6 (40.0)	13 (86.7)
2-5 cm	17	8 (47.1)	11 (64.7)	16 (94.1)
> 5 cm	6	3 (50.0)	5 (83.3)	6 (100)
Tumor number				
Single	24	16 (66.7)	12 (50.0)	22 (91.7)
Multiple	14	11 (78.6)	10 (71.4)	13 (92.7)

AFP: α -fetoprotein; PIVKA- II: Prothrombin induced by vitamin K absence- II; UICC: International Union Against Cancer; HCC: Hepatocellular carcinoma.

Table 4 Stepwise multiple linear regression analysis using dependent variables α -fetoprotein-L3 and prothrombin induced by vitamin K absence- II

Variable	AFP-L3		PIVKA- II	
	β coefficient	<i>P</i> value	β coefficient	<i>P</i> value
AFP	0.839	< 0.001		NS
Tumor size	0.190	0.002		NS
AST		NS	0.550	< 0.001
Total bilirubin		NS	0.336	< 0.001
Platelet		NS	0.566	0.001
Albumin		NS	-0.674	< 0.001

AFP: α -fetoprotein; PIVKA- II: Prothrombin induced by vitamin K absence- II; AST: Aspartate aminotransferase; NS: Not significant.

with the PIVKA- II results. However, PIVKA- II had no correlation with AFP, AFP-L3 or tumor characteristics.

DISCUSSION

In patients at risk for developing HCC, systematic screening is required for detection of small tumors at an early stage. Although the diagnostic strategy for early detection of HCC has been changed with the technical development in imaging diagnosis^[3], measurement of tumor markers for HCC at regular intervals is still a common practice. AFP is the most widely used tumor marker for HCC. However, recent studies reported that AFP lacks adequate sensitivity and specificity for effective surveillance^[6,9-11]. Our data showed that 38 (42.2%) of 90 HCC patients had AFP < 20 ng/mL, and 4 (5.1%) of 78 non-HCC patients had AFP > 20 ng/mL, which support the inadequacy of AFP as a tumor marker shown by previous reports. Therefore, we examined the individual and combined diagnostic values of AFP-L3 and PIVKA- II for HCC according to the AFP levels (< 20 ng/mL and

< 200 ng/mL), and investigated the clinical and laboratory factors that influence the measured values of AFP-L3 and PIVKA- II.

The AFP-L3 fraction has been reported to be more sensitive than AFP for small sized or early stage HCC^[20,31]. AFP-L3 is also known to be highly specific for HCC and reflect tumor characteristics including poor differentiation or malignant invasion^[32-35]. The present data of all patients showed that the AUC value of AFP-L3 for HCC detection was 0.887, which was not different with the AUC value of AFP (0.879), but was higher than the AUC of PIVKA- II. In focusing on the patients with AFP < 20 ng/mL, AFP-L3 levels were significantly higher in HCC than in non-HCC, and the sensitivity of AFP-L3 for HCC was 71.1% and 47.4% with cut-offs of 5% and 10%, respectively. These values are higher than those reported in previous studies showing sensitivity of 25%-50% with cut-offs between 5% and 10%^[19,20]. The high specificity for HCC is an important advantage of AFP-L3 measurement and higher sensitivity without a concomitant reduction in specificity is useful in clinical settings. In the present study, the specificities of AFP-L3 for HCC were 79.5%-97.3% in all patients or in patients with low AFP levels. The values are similar to those in previous reports showing the specificities of AFP-L3 for HCC as over 85% among patients with low total AFP-levels^[19,20]. Overall, in patients with low AFP levels, AFP-L3 (cut-off 5%) had reasonable AUC value, sensitivity and specificity (0.824, 71.1% and 83.8%, respectively) which are similar or improved results compare to previously reported data^[19,36].

In the present study, PIVKA- II alone had sensitivities and specificities close to 60% and 95% in all patients and patients with low AFP levels. PIVKA- II was shown to have a higher specificity in previous studies^[2,5,27], and all four patients with non-HCC and increased AFP/AFP-L3 levels showed lower values of PIVKA- II in the present study. This suggests that the PIVKA- II test may be useful in detecting false-positive AFP results.

The levels of AFP-L3 and PIVKA- II had no correlation between them and these two may be compensative markers reflecting a different developmental form of HCC^[37]. In multivariate analysis, AFP-L3 correlated with total AFP and tumor size. In contrast, PIVKA- II had no correlation with AFP or tumor characteristics, but was correlated with some liver function tests including serum AST, tuberculosis, platelet and albumin.

In our study, the combination of PIVKA- II and AFP-L3 improved the sensitivity close to 90% and showed significantly increased AUC (0.939) even in patients with low AFP. In addition, the combined tests showed approximately 90% sensitivity for the detection of early stage, small sized or single HCC tumors in patients with low AFP levels. These results suggest that these two biomarkers should be measured simultaneously and in combination with imaging tests to improve the diagnostic accuracy.

Potential limitations of our study include the small number of newly diagnosed HCC patients, lack of ran-

domization and relative short-term follow-up. Our results should be validated with a larger number of patients with age- and sex-matched control subjects. Since AFP-L3 has been suggested as a prognostic marker in patients with HCC^[22,25,26], future studies using longitudinal AFP-L3 data analysis or studies regarding the effect of AFP-L3 effect on the survival rate of HCC patients after treatment are needed. Despite these limitations, the present study indicates that AFP-L3 and PIVKA- II are useful additive tumor markers for the diagnosis of HCC.

In conclusion, combined determination of AFP-L3 and PIVKA- II improved the diagnostic value for HCC detection in patients with or without increased AFP levels. The utility of improved surveillance protocol based on these tumor markers needs to be investigated.

COMMENTS

Background

In patients at risk for developing hepatocellular carcinoma (HCC), systematic screening is required for detection of small tumors at an early stage. Although, α -fetoprotein (AFP) is the most widely used tumor marker for HCC, AFP lacks adequate sensitivity and specificity for effective surveillance. Therefore, additional tumor markers including fucosylated fraction of AFP (AFP-L3) fraction and prothrombin induced by vitamin K absence- II (PIVKA- II) have been suggested.

Research frontiers

The clinical values of AFP, AFP-L3 and PIVKA- II in combination are not conclusive yet, and the clinical and laboratory factors which possibly affect the measured values of AFP-L3 and PIVKA- II have not been sufficiently investigated. In this study, the authors evaluated the individual and combined diagnostic values of AFP-L3 and PIVKA- II for HCC detection according to the total AFP levels and tumor characteristics. The clinical and laboratory factors affecting AFP-L3 and PIVKA- II were also analyzed.

Innovations and breakthroughs

In the study, the combination of PIVKA- II and AFP-L3 improved the sensitivity close to 90% and showed significantly increased areas under the curve (0.939) even in patients with low AFP. In addition, the combined tests showed approximately 90% sensitivity for the detection of early stage, small sized or single HCC tumors in patients with low AFP levels. In multivariate analysis, AFP-L3 correlated with total AFP and tumor size. In contrast, PIVKA- II had no correlation with AFP or tumor characteristics, but was correlated with some liver function tests including serum aspartate aminotransferase, tuberculosis, platelet and albumin. The present study indicates that AFP-L3 and PIVKA- II are useful additive tumor markers for the diagnosis of HCC.

Applications

Combined determination of AFP-L3 and PIVKA- II could improve the diagnostic value for HCC detection in patients with or without increased AFP levels. The utility of improved surveillance protocol based on these tumor markers needs to be investigated.

Peer review

This study confirmed that the combined determination of AFP-L3 and PIVKA- II improved the diagnostic value for HCC detection in patients with or without increased AFP levels. The authors performed the multivariate analysis and revealed that AFP-L3 and PIVKA- II had no correlation between them and might be useful additive and compensate tumor markers for the diagnosis of HCC. The results are interesting and can be used to elucidate the different development form of HCC.

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P- Reviewers Aspinall RJ, Yang SF, Kataoka H
S- Editor Wen LL **L- Editor** A **E- Editor** Li JY



Epidemiology of perforated peptic ulcer: Age- and gender-adjusted analysis of incidence and mortality

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Received: October 24, 2012 Revised: December 12, 2012

Accepted: December 20, 2012

Published online: January 21, 2013

Abstract

AIM: To investigate the epidemiological trends in incidence and mortality of perforated peptic ulcer (PPU) in a well-defined Norwegian population.

METHODS: A retrospective, population-based, single-center, consecutive cohort study of all patients diagnosed with benign perforated peptic ulcer. Included were both gastric and duodenal ulcer patients admitted to Stavanger University Hospital between January 2001 and December 2010. Ulcers with a malignant neoplasia diagnosis, verified by histology after biopsy or resection, were excluded. Patients were identified from the

hospitals administrative electronic database using pertinent ICD-9 and ICD-10 codes (K25.1, K25.2, K25.5, K25.6, K26.1, K26.2, K26.5, K26.6). Additional searches using appropriate codes for relevant laparoscopic and open surgical procedures (e.g., JDA 60, JDA 61, JDH 70 and JDH 71) were performed to enable a complete identification of all patients. Patient demographics, presentation patterns and clinical data were retrieved from hospital records and surgical notes. Crude and adjusted incidence and mortality rates were estimated by using national population demographics data.

RESULTS: In the study period, a total of 172 patients with PPU were identified. The adjusted incidence rate for the overall 10-year period was 6.5 per 100 000 per year (95%CI: 5.6-7.6) and the adjusted mortality rate for the overall 10-year period was 1.1 per 100 000 per year (95%CI: 0.7-1.6). A non-significant decline in adjusted incidence rate from 9.7 to 5.6 occurred during the decade. The standardized mortality ratio for the whole study period was 5.7 (95%CI: 3.9-8.2), while the total 30-d mortality was 16.3%. No difference in incidence or mortality was found between genders. However, for patients ≥ 60 years, the incidence increased over 10-fold, and mortality more than 50-fold, compared to younger ages. The admission rates outside office hours were high with almost two out of three (63%) admissions seen at evening/night time shifts and/or during weekends. The observed seasonal variations in admissions were not statistically significant.

CONCLUSION: The adjusted incidence rate, seasonal distribution and mortality rate was stable. PPU frequently presents outside regular work-hours. Increase in incidence and mortality occurs with older age.

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Key words: Perforated peptic ulcer; Epidemiology; Incidence; Mortality; Seasonal variation

Thorsen K, Søreide JA, Kvaløy JT, Glomsaker T, Søreide K. Epidemiology of perforated peptic ulcer: Age- and gender-adjusted analysis of incidence and mortality. *World J Gastroenterol* 2013; 19(3): 347-354 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/347.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.347>

INTRODUCTION

Each year peptic ulcer disease (PUD) affects 4 million people around the world^[1]. Complications are encountered in 10%-20% of these patients and 2%-14% of the ulcers will perforate^[2,3]. Perforated peptic ulcer (PPU) is a quite rare, but life threatening disease and the mortality varies from 10%-40%^[2,4-6]. Females account for more than half the cases, they are older and have more comorbidity than their male counterparts^[6]. Main etiologic factors include use of non-steroidal anti-inflammatory drugs (NSAIDs), steroids, smoking, *Helicobacter pylori* (*H. pylori*) and a diet high in salt^[3,7]. All these factors have in common that they affect acid secretion in the gastric mucosa. Defining the exact etiological factor in any given patient may often be difficult, as more than one risk factor may be present and they tend to interact^[8]. While previous reports have shown a seasonal variation in the incidence of PPU, others have failed to find such a pattern^[9-11].

The incidence rate of PPU has, with some fluctuations, been fairly stable in Northern Europe for decades with reported annual incidence rates of about 4-11 per 100 000 per year^[12,13]. Several studies have investigated this up to the beginning of the 21st century, however there is hardly any data on incidence reports from this area the last decade. Due to the high mortality and morbidity rates from PPU, it is of importance to understand the epidemiology to, if possible, enable preventive measures.

The aim of this study was to investigate the recent epidemiological incidence trends and presentation of benign perforated gastroduodenal peptic ulcer in a well-defined Norwegian population.

MATERIALS AND METHODS

Study population

In this retrospective study all patients diagnosed with benign perforated peptic ulcer (either gastric ulcer or duodenal ulcer) admitted to Stavanger University Hospital (SUH) between January 2001 and December 2010 were included. Ulcers with a malign neoplasia diagnosis, verified by histology after biopsy or resection, were excluded.

SUH serves as the only hospital in the greater Stavanger area and has a primary mixed urban and rural catchment area of about 330 000 (per January, 2012). The "twin cities" of Stavanger and Sandnes are together the third most populated area in Norway, and Stavanger is the densest populated city in Norway.

Patients were identified from the hospitals admin-

istrative electronic database using pertinent ICD-9 and ICD-10 codes (K25.1, K25.2, K25.5, K25.6, K26.1, K26.2, K26.5, K26.6). Additional searches using appropriate codes for various surgical procedures (JDA 60 gastrotomy, JDA 61 laparoscopic gastrotomy, JDH 70 duodenotomy, JDH 71 laparoscopic duodenotomy) were done to enable a complete identification of all patients. Patient demographics and clinical data were retrieved from hospital records and surgical notes. Crude and adjusted mortality rates were estimated.

Definitions

Incidence calculations: The crude incidence is the number of new cases of perforated peptic ulcer in our region per 100 000 persons per year. The adjusted incidences are the crude rates adjusted for age and gender *vs* the total Norwegian population using Statistics Norway. The crude death rate is the total number of deaths in the study population per 100 000 persons per year. The adjusted mortality rate is the crude death rate adjusted for age and gender *vs* the total Norwegian population using Statistics Norway.

The standardized mortality ratio is the number of observed deaths within 30 d of hospital admission in the study group divided by the number of expected deaths in a general population with the same age and sex distribution.

Ulcer localisation was regarded as gastric when present anywhere in the stomach, including prepyloric and pyloric ulcers. Postpyloric ulcers were classified as duodenal. One ulcer located in an anastomosis after a gastrectomy was regarded as a gastric ulcer. The diagnosis and exact localisation was made at operation in most cases, while some were verified at postoperative endoscopy.

Comorbidity was defined as any concomitant disease at the time of admittance for PPU, including cardiovascular disease, pulmonary disease, autoimmune disorders and known or previous cancer disease in patient history.

NSAIDs included acetylic acid as part of the NSAID group in this study.

Seasons were classified as spring (March, April and May); late spring/summer (May, June, July and August); summer (June, July and August); autumn (September, October and November); and winter (December, January and February).

Evening/night time was defined as the hours between 16:30 and 07:30 all days of the week, including Saturday and Sunday.

Study ethics

The study was approved as a quality control assurance according to general guidelines provided by the Regional Ethics Committee Vest (REK Vest).

Statistical analysis

PASW Statistics 19.0 for Mac (SPSS Inc., Chicago, IL) was used for statistical analysis. Descriptive analyses were performed using χ^2 or Fisher's exact test where appropri-

Table 1 Patient characteristics according to ulcer localisation *n* (%)

	Duodenal ulcer <i>n</i> = 60	Gastric ulcer <i>n</i> = 112	Total <i>n</i> = 172	<i>P</i> value
Age				
< 60 yr	18 (30)	37 (33)	55 (32)	0.68
≥ 60 yr	42 (70)	75 (67)	117 (68)	
Gender				
Female	27 (45)	62 (55)	89 (52)	0.20
Male	33 (55)	50 (45)	83 (48)	
ASA status				
1	0	0	0	0.99
2	2 (3)	3 (3)	5 (3)	
3	34 (57)	63 (56)	97 (56)	
4	20 (33)	39 (35)	59 (34)	
5	4 (7)	7 (6)	11 (6)	
Social status				
Married	37 (62)	58 (52)	95 (55)	0.32
Divorced	3 (5)	8 (7)	11 (6)	
Widowed	9 (15)	28 (25)	37 (22)	
Single	6 (10)	7 (6)	13 (8)	
Unknown	5 (8)	11 (10)	16 (9)	
Smokers ¹	26 (54)	60 (69)	86 (64)	0.09
NSAID-use	26 (43)	50 (45)	76 (44)	0.87
Steroid-use	5 (8)	11 (10)	16 (9)	0.73
Comorbidity	45 (75)	97 (87)	142 (82)	0.06
Cardiovascular disease	27 (45)	50 (45)	77 (45)	0.89
Pulmonary disease	8 (13)	19 (17)	27 (16)	0.53
Autoimmune disease	13 (22)	18 (16)	31 (18)	0.36
Malignancy (past or current)	6 (10)	23 (21)	29 (17)	0.08
Past ulcer history	8 (13)	18 (16)	26 (15)	0.36
30-d mortality	14 (23)	14 (13)	28 (16)	0.07

P values are for trends across groups. Percentages may not add up due to rounding. ¹Data on smoking was missing in 37 (22%) of patients. Percentages are of patients with reported data (*n* = 135). ASA: American Society of Anesthesiology; NSAIDs: Non-steroidal anti-inflammatory drugs.

ate for dichotomous data, and Mann-Whitney *U* test for continuous data, where applicable. Poisson regression analysis was used to test for differences in crude rates between different periods and between different population groups. Time-trends were also tested by a Poisson time series model. Logistic regression was used to test for association with gender and comorbidity. All tests are two sided and *P* values < 0.050 were regarded as statistically significant.

RESULTS

Patient characteristics

A total of 172 patients with a perforated peptic ulcer were identified between 2001 and 2010. Median age was 68 years ranging from 18 to 100 years. There was an equal gender distribution (52% were women), but women were significantly older than men (median age of 73 years *vs* 62 years, respectively, *P* < 0.001). The number of women affected increased significantly with age across age groups, with only one in four patients aged < 50 years of age being female, compared to two-thirds of those > 70 years of age

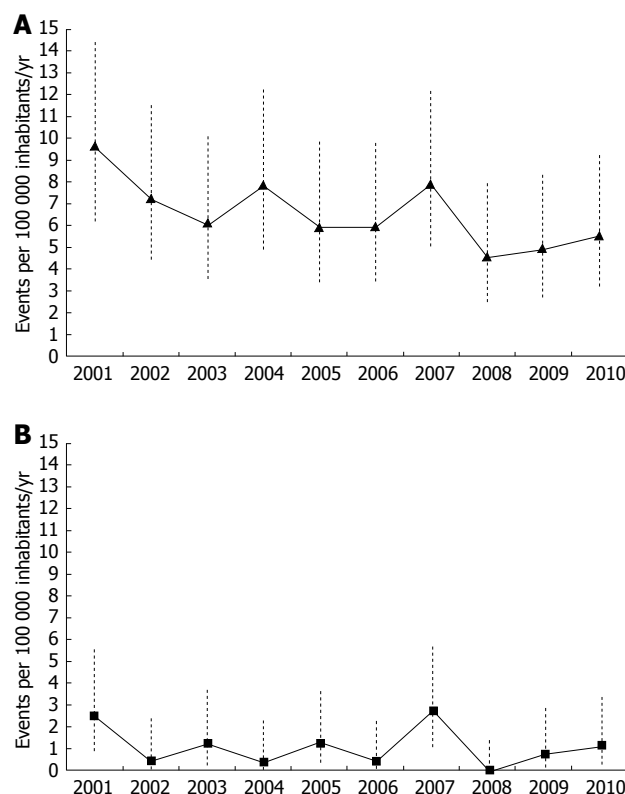


Figure 1 Incidence and mortality of perforated peptic ulcer from 2001-2010.

A: The incidence rates of perforated peptic ulcer (PPU) per 100 000 inhabitants per year, adjusted for age and gender; B: The mortality rate from PPU per 100 000 inhabitants per year, adjusted for age and gender.

being female (26% *vs* 65% women, respectively, *P* = 0.002). Further patient characteristics are given in Table 1.

Crude and adjusted incidence trends

The crude incidence rate of PPU during the study period was 5.6 per 100 000 per year and declined from 8.5 in 2001 to 4.6 in 2010. The adjusted incidence rate for the overall 10-year period was 6.5 per 100 000 per year (95%CI: 5.6-7.6) and declined from 9.7 to 5.6 over the decade, but neither crude nor adjusted incidence rate decline were statistically significant (Figure 1A). Patients ≥ 60 years of age accounted for 117 of 172 (68%), and remained the dominant age group throughout the decade.

When considering the incidence in specific age- and gender-groups, the adjusted incidence rate for men demonstrated considerable differences, with men < 60 years at 2.7 per 100 000 per year (95%CI: 1.9-3.8) compared to 22.1 (95%CI: 16.3-29.4) for men ≥ 60 years of age. A similar pattern was seen in women, with women < 60 years at 1.5 per 100 000 per year (95%CI: 0.9-2.4), while it was 26.1 for those aged ≥ 60 (95%CI: 20.3-32.9) for the whole period. This corresponds to an almost 10-fold increase for men and a corresponding 17-fold increase for women in incidence for those aged ≥ 60 years.

An indicated decline in PPU numbers seen in Figure 1A is evident (but not statistically significant) in those aged > 70 years when the decade was divided in two peri-

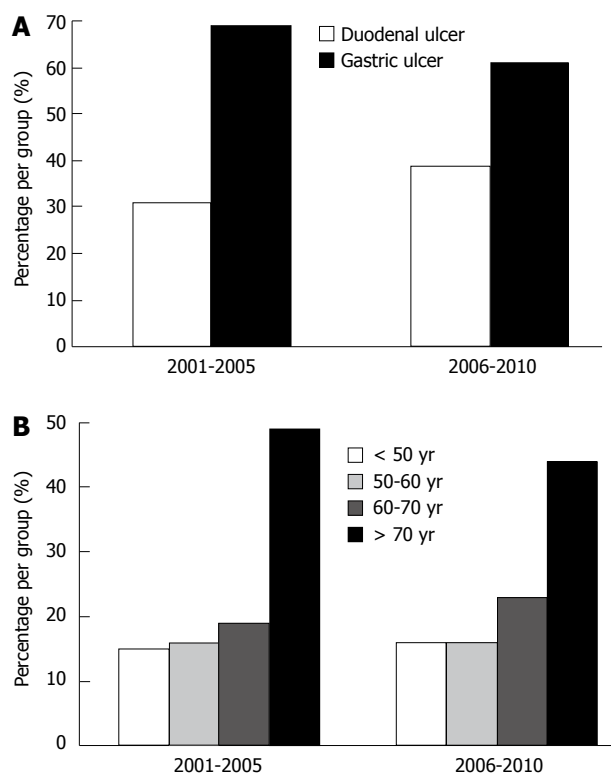


Figure 2 Localisation of ulcers and distribution of age-groups. A: Perforated peptic ulcer (PPU) localisation with admission rates over time. The decade is divided in two periods from 2001-2005 and 2006-2010; B: PPU presentation according to age groups (age in years; < 50, 50-59, 60-69, > 70) divided into the two time periods.

ods, as illustrated in Figure 2.

Crude and adjusted mortality trends

The crude mortality rate for the study period was 0.9 and the adjusted mortality rate for the overall 10-year period was 1.1 per 100 000 per year (95%CI: 0.7-1.6) and was stable during the whole period (Figure 1B). The standardized mortality ratio for the whole study period was 5.7 (95%CI: 3.9-8.2), while the total 30-d mortality was 16.3%.

The adjusted mortality rate for men < 60 years was 0.1 per 100 000 per year (95%CI: 0.0-0.43) and 4.6 (95%CI: 2.21-8.48) for men aged \geq 60 years. For women the adjusted mortality rate for the whole decade for those aged < 60 years was 0.1 (95%CI: 0.0-0.45) while it was 5.6 (95%CI: 3.13-9.22) for those aged \geq 60 years. This demonstrated negligible differences between genders, but an almost 50 to 60 times increase in mortality among the elderly age group.

Ulcer localisation

Gastric ulcers predominated (Figure 2A) and accounted for 112 of 172 (65%) patients in this study, but declined during the latter years of the period, while the frequency of duodenal ulcers remained stable, but increased somewhat the latter period (Figure 2). Prepyloric ulcers represented 61 of 112 (54%) of gastric ulcers and 21 of 112

(19%) were located in the pylorus. In the corpus/fundus area 12 of 112 (11%) ulcers were observed, while 8 of 112 (7%) were located in the antrum. One ulcer was located in an anastomosis and 9 of 112 (8%) ulcers were missing exact localisation in the stomach, but being classified as gastric ulcers at operation.

Age and comorbidity

Of those aged > 60 years of age 105 of 117 (90%) had comorbidity compared to 37 of 55 (67%) of those aged \leq 60 years ($P < 0.001$). Women also had significantly higher rate of comorbidity compared to men (91% *vs* 74%, respectively, $P = 0.002$), also when adjusted for age ($P = 0.036$).

Associated etiologic factors

Data on smoking was obtained in 135 of 172 patients. Of the 135 with confirmed information on smoking habits, 86 (64%) were smokers. Consequently, at least 50% of the total 172 patients were smokers, but likely this is an underestimate. Of those aged < 60 years of age 37 of 44 (84%) were smokers, while 49 of 91 (54%) of those aged \geq 60 were smokers ($P = 0.001$). The number of smokers registered was stable during the decade.

The number of NSAID users was stable during the decade studied and were used by 47 of 89 women and 29 of 83 men (53% *vs* 35%, respectively, $P = 0.018$). Also, NSAID-use was more common in those aged \geq 60 years (55 of 117; 47%), compared to the younger patients aged < 60 years (20 of 55; 36%), but this was not statistically significant.

Patterns of seasonal and circadian presentation

Patients were admitted at all hours and days during the week. While 70 of 172 patients (41%) were admitted during weekends (Friday, Saturday and Sunday), more than half (91 of 172; 53%) of the patients were admitted during evening/night time shifts and 109 of 172 (63%) patients were admitted either during evening/night time and/or on Saturday/Sunday. Notably, during this time a lower staff: patient ratio than during regular office hours is present.

PPU was more frequent in patients \geq 60 years of age (117 of 172; 68%) than < 60 years of age (55 of 172; 32%). This was particularly evident during the four months of late spring/summer. Poisson regression and time series analyses revealed no statistical significant differences regarding monthly variation or seasonal variation for PPU admittance patterns (Figure 3).

DISCUSSION

In this single-institution, population based study we found an adjusted incidence rate of 6.5 (95%CI: 5.5-7.5) per 100 000/year during this period. This is in concordance to several Northern European studies, most of them reporting an incidence between 4-11 per 100 000/year^[13-17].

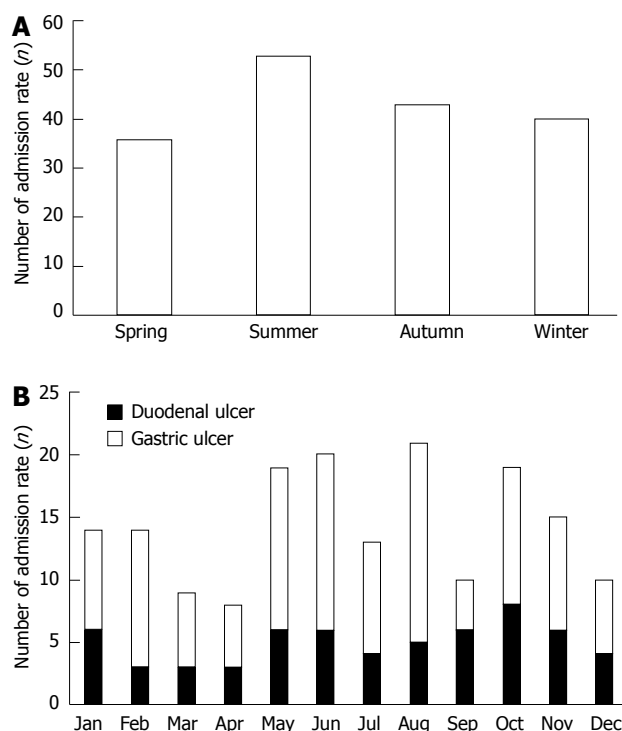


Figure 3 Seasonal and monthly variation in admissions. A: Seasonal variation in admissions overall; B: The monthly variation in admissions with numbers for gastric and duodenal ulcers, respectively.

Several factors may influence the incidence rates of PPU. For one, epidemiological studies have demonstrated that birth-cohorts born up to the 1930s were at higher risk of acquiring PPU than later birth cohorts^[15,18]. The reason for this is not known, but speculation of an overriding influence of *H. pylori* infection on the population, has been postulated as the main cause^[18]. However, *H. pylori* infection has been deemed of less importance in perforated peptic ulcer disease compared to that of uncomplicated peptic ulcer disease^[1,7]. However, the association between specific birth-cohorts and mortality from PUD has been quite convincing, and a decline in the incidence of PPU may thus be expected when the cohorts at risk disappear with time^[4,18]. This may have affected the incidence rates also in this study, which are rates comparable to other epidemiological studies from the turn of the century^[19], and which may reflect a stable scenario with a relatively low population at risk for PPU in the current century.

Another etiologic factor for perforated peptic ulcer is smoking^[20,21]. Prospective data from SUH between 1987 and 1993 observed that 58 of 104 (56%) of all PPU patients were smokers^[22], reflecting the current data. The percentage of smokers in the PPU population may reflect this as a known risk for ulcer disease and perforation. Notably, in 2011 the number of daily smokers in Norway (Figure 4) is reduced to half the number compared to 2001 data^[23], and consequently this risk factor has decreased dramatically over just a decade. The decrease in smoking habits is seen in all the age groups from 16-64 years, but most evident in the age group from 16-44

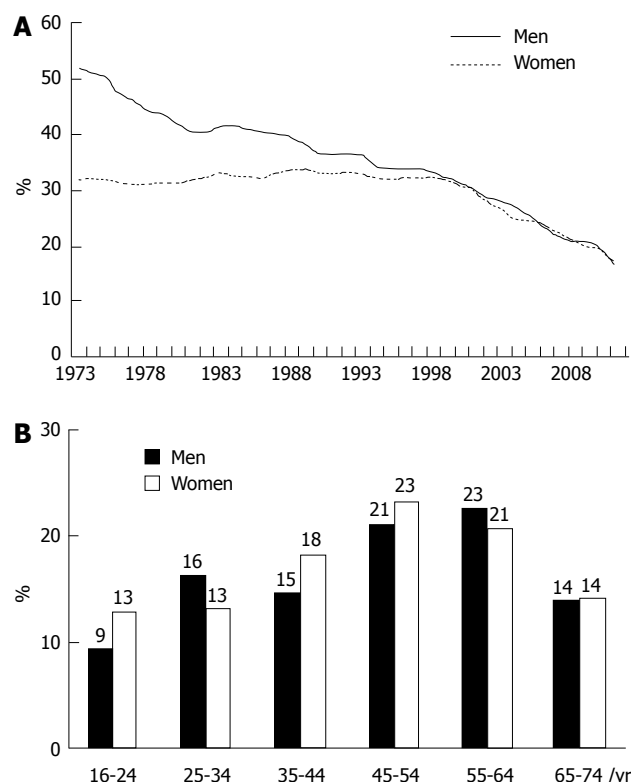


Figure 4 Change in prevalence of daily smokers in Norway. A: The time trend of daily smokers over time in the Norwegian population aged between 16-74 years, showing a decline over time for each gender; B: The distribution of smokers according to age groups and gender for the fiscal year 2011. Data are reproduced and presented from the Norwegian Directorate of Health (<http://www.helsedirektoratet.no>, accessed October 5, 2012).

years^[23]. As previously stated, PPU is much more common in older than younger people, and the declining number of smokers in the population should influence incidence rates of PPU with time. However, a decade of decline in smoking in the population may not be enough time to reveal this. Also, an increasing age-population and preserved smoking habits in the older may contribute to a stable incidence still.

Medications may influence PPU incidence and occurrence in several ways, both as risk factors and as preventive means. NSAID-use is associated to peptic ulcers and to peptic ulcer complications, however, the majority of PPUs are not related to NSAIDs^[24]. In recent years an increase of NSAID-use has been seen and according to numbers from the Norwegian pharmaceutical association: both acetylic acid (14% increase), diclofenac (35% increase) and ibuprofen (26% increase) usage increased from 2006 to 2010 (over the counter sales included), while the usage of the most common PPI (Esomeprazol) was stable^[25]. Previous data from Australia has also shown increasing use of NSAIDs in the population without increasing incidence rates of PPU^[26]. A study of peptic ulcer complications in the Swedish population over three decades up to 2002, found declining incidence rates after 1988, when PPI were introduced in Sweden. The authors concluded that the reasons for this most likely were multifactorial, but then

including an effect of PPI^[14]. However, few studies have found an effect of PPI or H2 blockers on the PPU incidence or mortality rates^[13].

The overall mortality at 16.3% is in comparison to other contemporary European studies from Denmark, the United Kingdom and the Netherlands ranging from 12% to 27%^[27-29], but differs from a recent South Korean study reporting mortality at 3%^[30]. The difference may be explained by methodology to retrieve data, as the European studies are hospital-based cohorts, and the South Korean study is based on a national health insurance claims database. In one Danish national cohort^[31], the overall mortality was 27%, and reduced to 17% for patients entered in a pre-specified perioperative protocol to reduce mortality^[31]. In the current population under study with an overall mortality of 16%, there was no pre-specified protocol in use, but institutional trend towards an increased use of pre-operative computed tomography for diagnosis and laparoscopy as mode of intervention during the study period^[6].

Ulcer site definition may be confusing, since some classify prepyloric and pyloric ulcers as duodenal^[10], while others classify those ulcers as part of the stomach and hence gastric ulcers^[13]. Further, extensive perioperative and inflammatory tissue changes may make it difficult to distinguish between the duodenum and the pyloric area during the operation, hence clinical misclassification can obviously occur. Nevertheless, gastric ulcers predominated in this study and this is in accordance to similar reports from Norway, The Netherlands and Iceland^[32-34]. However, a non-significant decline of perforated gastric ulcers was seen in the latter years of the decade, while the frequency of duodenal ulcers was stable. Decreasing incidence of gastric ulcers in those aged > 70 years have also been shown in a large study from England and Wales^[17]. We did not identify any associations according to ulcer site, but a trend towards younger men having more duodenal ulcers and older women having more gastric ulcers were seen. This has been shown in similar studies before and may represent a somewhat different aetiology, with older, female patients more often having NSAIDs exposure prior to PPU episodes^[17,35]. The latter was also seen in this study.

The off-hour admission rates were high with almost two of three patients admitted at either evening/night time shifts or during weekends (i.e., Saturday/Sunday). Consequently, a high proportion of potentially very sick patients requiring an acute operation will be seen when senior staff may not be immediately available or in-house, which may potentially delay a correct work-up and timely diagnosis and consequently operative intervention, than if admitted at office hours. However, we do not have data to substantiate this potential association in the current study.

Several studies have addressed seasonal variation of perforated peptic ulcer^[9,11,36,37]. In this study a peak of perforated peptic ulcers was observed during the four months of late spring/summer, but this pattern faded

when seasons were divided in three months. However a previous study from Western Norway over 5.5 decades found similar seasonal variations and those variations were consistent over time^[38]. However, no statistical significance was found for variations over time, and this is in line with previous reports that failed to find such patterns^[9,10].

The retrospective nature of this study limits the accuracy of data quality, which would have been obtained by a prospective manner for some variables, such as exposure to risk factors (i.e., smoking status; medications used, *etc.*) which may be subject to bias by underreporting of past history. However, with a population-based catchment area and a well-developed health system of general physicians and a universal health insurance program for all citizens in Norway, patients are usually admitted with admission notes containing past history, although this may obviously be more prone to failure outside office hours and for emergency referrals. Due to restrictions of the number of patients, we are cautious of performing unwarranted subgroup analysis, and may thus not have been able to confirm significant trends found in other studies.

In conclusion, the adjusted incidence rates in the first decade of the 21st century was stable and reflected the decline seen towards the end of the 20th century. Smoking cessation in the general population and a reduction in the population represented by birth cohorts at higher risk are two important factors that may influence the current low incidence of PPU, compared to that in past decades. Perforated peptic ulcer continues to present outside regular work-hours in over half the time and frequently during weekends, with little difference in seasonal distribution. Mortality is unchanged and stable and is most considerable in the aged population. This may be further subject to change with an increasingly elderly population and should be followed by population-based monitoring for this disease over time.

COMMENTS

Background

Perforated peptic ulcer (PPU) is a life threatening disease with historically reported high morbidity and mortality rates. Disease epidemiology has changed during the last century, but current data on epidemiological trends in PPU is lacking. The aim of this study is to investigate epidemiological trends in a well-defined Norwegian population over a decade.

Research frontiers

Central research in perforated peptic ulcer the latter years has focused on safety of laparoscopy as primary operation and regarding short term mortality after operation. Few studies have presented data regarding epidemiology over the last decade. This study demonstrates a stable incidence and mortality rate over a decade.

Innovations and breakthroughs

A change in peptic ulcer disease epidemiology the last decades came with the discovery of *Helicobacter pylori* with the subsequent eradication therapy with antibiotics, and the introduction of proton pump inhibitors. For perforated peptic ulcers little has changed except the use of laparoscopy as a surgical alternative for repair. The mortality remains high and the incidence has been stable.

Applications

The epidemiology of perforated peptic ulcer appears to be stable with few changes compared to the very recent past. A better understanding of the aetiol-

ogy may be warranted for better prevention and reduction of incidence. Understanding factors contributing to mortality will be important to further improve outcomes.

Peer review

The study is an interesting, well designed and well written one. It will help to shed light on the current situation regarding peptic ulcer disease.

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P- Reviewer Abdel-Salam OMEIS **S- Editor** Song XX
L- Editor A **E- Editor** Li JY



Biliary fistula after treatment for hydatid disease of the liver: When to intervene

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Received: May 6, 2012 Revised: November 12, 2012

Accepted: November 24, 2012

Published online: January 21, 2013

Abstract

AIM: To determine the outcome of patients with biliary fistula (BF) after treatment for hydatid disease of the liver.

METHODS: Between January 2000 and December 2010, out of 301 patients with a diagnosis of hydatid cyst of the liver, 282 patients who underwent treatment [either surgery or puncture, aspiration, injection and reaspiration (PAIR) procedure] were analysed. Patients were grouped according to the presence or absence of postoperative biliary fistula (PBF) (PBF vs no-PBF groups, respectively). Preoperative clinical, radiological and laboratory characteristics, operative characteristics including type of surgery, peroperative detection of BF, postoperative drain output, morbidity, mortality and length of hospital stays of patients were compared amongst groups. Multivariate analysis was performed to detect factors predictive of PBF. Receiver operative characteristics (ROC) curve analysis were used to determine ideal cutoff values for those variables found to be significant. A comparison was also made between patients whose fistula closed spontaneously (CS) and those with intervention in order to find predictive fac-

tors associated with spontaneous closure.

RESULTS: Among 282 patients [median (range) age, 23 (16-78) years; 77.0% male]; 210 (74.5%) were treated with conservative surgery, 33 (11.7%) radical surgery and 39 (13.8%) underwent percutaneous drainage with PAIR procedure. A PBF developed in 46 (16.3%) patients, all within 5 d after operation. The maximum cyst diameter and preoperative alkaline phosphatase levels (U/L) were significantly higher in the PBF group than in the no-PBF group [10.5 ± 3.7 U/L vs 8.4 ± 3.5 U/L ($P < 0.001$) and 40.0 ± 235.1 U/L vs 190.0 ± 167.3 U/L ($P = 0.02$), respectively]. Hospitalization time was also significantly longer in the PBF group than in the no-PBF group [37.4 ± 18.0 d vs 22.4 ± 17.9 d ($P < 0.001$)]. A preoperative high alanine aminotransferase level (> 40 U/L) and a peroperative attempt for fistula closure were significant predictors of PBF development ($P = 0.02$, 95%CI: -0.03-0.5 and $P = 0.001$, 95%CI: 0.1-0.4), respectively. Comparison of patients whose PBF CS or with biliary intervention (BI) revealed that the mean diameter of the cyst was not significantly different between CS and BI groups however maximum drain output was significantly higher in the BI group (81.6 ± 118.1 cm vs 423.9 ± 298.4 cm, $P < 0.001$). Time for fistula closure was significantly higher in the BI group (10.1 ± 3.7 d vs 30.7 ± 15.1 d, $P < 0.001$). The ROC curve analysis revealed cut-off values of a maximum bilious drainage < 102 mL and a waiting period of 5.5 postoperative days for spontaneous closure with the sensitivity and specificity values of (83.3%-91.1%, AUC: 0.90) and (97%-91%, AUC: 0.95), respectively. The multivariate analysis demonstrated a PBF drainage volume < 102 mL to be the only statistically significant predictor of spontaneous closure ($P < 0.001$, 95%CI: 0.5-1.0).

CONCLUSION: Patients with PBF after hydatid surgery often have complicated postoperative course with serious morbidity. Patients who develop PBF with an output < 102 mL might be managed expectantly.

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Key words: Hydatid disease; Biliary fistula; Postoperative complications; Surgery

Zeybek N, Dede H, Balci D, Coskun AK, Ozerhan IH, Peker S, Peker Y. Biliary fistula after treatment for hydatid disease of the liver: When to intervene. *World J Gastroenterol* 2013; 19(3): 355-361 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/355.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.355>

INTRODUCTION

Hydatid disease, which is caused by the larval form of *Echinococcus granulosus*, is endemic in Mediterranean countries, the Middle East, Australia and South America and has a worldwide distribution^[1]. Despite a decreasing incidence, Turkey is an endemic country according to the Ministry of Health database with more than 14 000 cases recorded from 2001-2005^[2]. Although this zoonosis is rare in the United States and Europe, it is a gradually increasing cause of morbidity and mortality in these regions, largely due to travel and ongoing immigration^[3].

As a definitive treatment, surgery was considered to be relatively safe based on over 4 decades of accumulated experience^[4], but a recent meta-analysis of 14 studies analyzing the outcomes of surgery in endemic areas reported minor and major complication rates of 33.0% and 25.1%, respectively, with a mortality rate of 0.7%^[5]. Intrahepatic rupture (IBR), which involves a communication of the cyst cavity, which is under high pressure, with the biliary system, is the most common and troublesome complication^[6]. IBR was reported to occur at a relatively high rate of 5%-30% of the cases, and it has a wide range of complications, such as biliary obstruction, fistula, infection and secondary biliary cirrhosis^[7,8]. If not promptly diagnosed and treated, these biliary complications may result in serious postoperative morbidity and mortality^[9]. However, most IBRs are occult in nature, and patients may remain clinically silent in most cases, without any specific radiological or laboratory findings being observed in the preoperative work-up and with the IBRs only being discovered during or after surgery. The presence of a postoperative biliary fistula (PBF) is believed to determine the prognosis in these patients^[10].

In fact, there is limited information regarding the natural course and outcome of those patients who had an IBR that was not treated successfully and developed a PBF. We previously reported our experience with the treatment of liver hydatid disease^[11]. In this study, we aimed to report the clinical significance of PBF after treatment for hydatid disease in a separate cohort of patients and to identify clinical predictors associated with the closure of PBF.

MATERIALS AND METHODS

Between January 2000 and December 2010, 301 patients

with a diagnosis of hydatid cyst of the liver were admitted to the Gulhane Military Medical Academy Hospital, Ankara, Turkey. The data were gathered from a prospectively collected database and the electronic medical records of the patients, including age, gender, medical history of hydatid disease, main symptoms and findings, abdominal ultrasonographic cyst characteristics (number of cysts, single, or multiple; presence or absence of other organs involved with the disease), chest radiography, presence of preoperative complications (jaundice, dilation of the biliary tree, intrahepatic, peritoneal or intrathoracic rupture), type of surgical procedure performed, postoperative daily follow-up of complications, duration of stay after the operation and mortality.

All of the patients underwent a complete blood cell count and liver function test determinations. An abdominal ultrasonographic examination (USG) was performed in all of the patients, but computed tomography (CT) was not used routinely. An indirect hemagglutination test was used for the serological confirmation of the diagnosis.

The liver cysts were classified into 5 types according to their ultrasonographic appearance^[12]: type I, a simple hydatid cyst with pure fluid collection; type II, a cyst containing undulated hyperechogenic membranes that float in the cystic fluid (the detached germinative layer of the endocyst); type III, a cyst containing secondary vesicles (daughter cysts); type IV, a cyst with a heterogeneous echopattern and filled with a matrix or amorphous mass; and type V, a cyst with a thick and calcified wall. The presence of irregular linear echogenic structures without acoustic shadowing in the bile duct and/or the dilated biliary tract was accepted as a suggestive USG finding of IBR^[13]. All of the patients with these USG findings were further evaluated with either abdominal CT or magnetic resonance cholangiopancreatography (MRCP).

In total, 19 patients were excluded from the analysis. This group included patients discharged without treatment (5 patients), patients with a preoperative diagnosis of cholangitis (the presence of 2 out of the following 3 symptoms or findings: fever > 38 °C, right upper abdominal pain and jaundice) or patients with suggestive findings of IBR on USG (9 patients) who underwent further evaluation with endoscopic retrograde cholangiography (ERCP) preoperatively. Four additional patients with perioperative common bile duct exploration plus T-tube placement and a patient who died of anaphylactic shock after the operation due to free rupture of the hydatid cyst into the peritoneal cavity were excluded.

The technique of the procedure was mainly selected according to the World Health Organization guidelines on the management of the echinococcal disease, with criteria including the size, type and location of the cyst and the general medical condition of the patient^[14]. In patients with significant comorbidities who refused surgery and had cysts > 5 cm that were Gharbi Types 1-2 on USG were treated with the puncture, aspiration, injection and reaspiration (PAIR) procedure^[15]. Open surgical

Table 1 Preoperative characteristics of patients with hydatid disease of the liver (mean \pm SD)

	Bile fistula		P value
	Absent	Present	
Age, yr	32.0	31.5	0.8
Gender (M/F)	179/57	38/8	0.3
Cholangitis	6 (3%)	24 (52%)	< 0.001
Albendazol treatment	118 (50%)	23 (50%)	0.7
AST (U/L)	221.0 \pm 32.2	44.0 \pm 36.0	0.5
ALT (U/L)	223.0 \pm 41.2	44.0 \pm 38.4	0.8
ALP (U/L)	40.0 \pm 235.1	190.0 \pm 167.3	0.02
GGT (U/L)	25.0 \pm 71.7	151.0 \pm 64.3	0.08
Total bilirubin (mg/dL)	0.9 \pm 1.44	1.5 \pm 3.0	0.05
White blood cell	8077.3 \pm 2945.0	8802.2 \pm 3222.7	0.1

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyltranspeptidase.

procedures were classified as radical for any type of liver resection and en-bloc cystectomy^[16]. A partial cystectomy with tube drainage, capitonnage or omentoplasty was considered to be a conservative procedure^[17]. In patients with multiple or communicating cysts, the same surgical procedures were used. The intraoperative detection of a biliary fistula (BF) was at the discretion of the attending surgeon. Uniformly, the cyst cavity was opened, explored for a BF or resection surface and carefully evaluated for a bile leak. Further measures included cholecystectomy and saline or methylene blue injection from the cystic duct stump. A PBF was defined as any drain output consistent with a biliary appearance and a bilirubin count higher than the synchronous serum bilirubin count within 5 d postoperatively.

The patients were grouped according to the presence or absence of PBF (PBF *vs* no-PBF groups, respectively). The preoperative clinical, radiological and laboratory characteristics, operative characteristics including the type of surgery, perioperative detection of BF, postoperative drain output, morbidity, mortality, and length of hospital stay of the patients were compared among the groups. A comparison was also performed between the patients whose fistula closed spontaneously and those who received intervention. The postoperative biliary interventions included ERCP with sphincterotomy and stent placement or nasobiliary tube drainage. Percutaneous drainage catheter insertions were performed under USG guidance.

The following variables were analyzed as potential predictors of PBF development: age, sex, findings on physical examination, leukocyte count, preoperative higher-than-normal liver function test results [alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyltranspeptidase, and total bilirubin levels], ultrasonographic cyst features (type, diameter, number, and localization), operation type and perioperative attempted fistula closure.

Statistical analysis

The quantitative data were compared using the *t* test, and

the categorical data were compared using the Pearson χ^2 test or Fisher's exact test for the presence of a PBF. Receiver operating characteristic (ROC) curve analysis with calculations of the area under the curve (AUC) was used to determine the ideal cutoff values for those variables that were found to be significant. Logistic regression analysis was used for the multivariate analysis, with a *P* < 0.20 being entered into the model, and a *P* < 0.05 was considered to be significant. All of the statistics were performed using the SPSS 18.0 software (SPSS, Inc., Chicago, IL, United States). The study was approved by the local Ethics Committee of the Gulhane Military Medical Academy.

RESULTS

Of the 282 patients, 217 (77%) were male, and 65 (23%) were female. The median age was 23 years (range 16-78 years). A preoperative diagnosis of cholangitis was found in 30 (10.6%) of the patients. The duration of the symptoms ranged from 1 mo to 7 years (median, 3 mo) (Table 1).

Overall, 178 patients (63.1%) had a single cyst, and 104 (36.9%) had multiple cysts. The cysts were localized only in the right lobe of the liver in 150 (53.2%) patients and in the left in 39 (13.8%), and bilobar involvement was observed in 93 (33%). A total of 484 hepatic cysts were treated in the 282 patients. Thirty-two patients (11.3%) had concomitant cysts in the lung, and 17(6%) had cysts elsewhere.

According to the largest cyst diameter in the USG evaluation, 79 (28%) were type I, 35 (12.4%) were type II, 127 (45%) were type III, 35 (12.4%) were type IV, and 3 (1.1%) were type V. The cyst diameter ranged from 2 cm to 20 cm (median, 8.0 cm) (Table 2).

Operations and perioperative interventions

Of the 282 patients, 210 (74.5%) were treated with conservative surgery, 33 (11.7%) were treated with radical surgery, and 39 (13.8%) underwent percutaneous drainage with the PAIR procedure (Table 2). Three patients (9%) treated with radical surgery, 6 (15%) treated with the PAIR procedure and 37 (17.6%) treated with conservative surgery developed a PBF (*P* = 0.44). An infected cyst cavity was found in 13 (4.6%) patients, with 1 developing a PBF. Perioperative suture ligation of the detected cyst-biliary communication was attempted in 39 (13.8%) patients, with 11 (4.2%) developing a PBF.

Outcome of patients with PBF

In total, PBF developed in 46 (16.3%) patients, all of whom were diagnosed within 5 d after the operation. The maximum cyst diameter (cm) and preoperative ALP levels (U/L) were significantly higher in the PBF group than in the no PBF group (10.5 \pm 3.7 U/L *vs* 8.4 \pm 3.5 U/L, *P* < 0.001; and 40.0 \pm 235.1 U/L *vs* 190.0 \pm 167.3 U/L, *P* = 0.02, respectively). The hospitalization time was also significantly longer in the PBF group (37.4 \pm 18.0 d *vs* 22.4 \pm 17.9 d, *P* < 0.001) (Table 3).

The multivariate analysis revealed that high preopera-

Table 2 Characteristics of hydatid cysts in patients with hydatid disease of the liver (mean \pm SD)

Cyst	Bile fistula		P value
	Absent <i>n</i> = 236	Present <i>n</i> = 46	
Size localisation (cm)			
Diameter	8.46 \pm 3.52	10.59 \pm 3.74	0.0001
Right lobe	127 \pm 45.9	23 \pm 8.2	0.2
Left lobe	36 \pm 12.8	3 \pm 1.1	
Bilobar	73 \pm 25.9	20 \pm 7.1	
Type			
I	68 \pm 24.4	11 \pm 3.9	0.7
II	30 \pm 10.8	5 \pm 1.8	
III	102 \pm 36.6	102 \pm 36.6	
IV	30 \pm 10.8	5 \pm 1.8	
V	3 \pm 1.1	0	
Operation type, <i>n</i> (%)			
Conservative surgery	173 (61.3)	37 (13.1)	0.4
Radical surgery	30 (10.6)	3 (1.1)	
PAIR	33 (11.7)	6 (2.1)	

PAIR: Puncture, aspiration, injection and reaspiration.

tive ALT levels (> 40 U/L) and a perioperative attempted fistula closure were significant predictors of PBF development ($P = 0.02$, 95%CI: -0.03-0.5 and $P = 0.001$, 95%CI: 0.1-0.4, respectively).

Factors related to spontaneous fistula closure

The patients with PBF were further divided into 2 groups regarding fistula closure-either spontaneous (CS group, 12 patients, 4.6%) or with biliary intervention (BI group, 34 patients, 12.1%). The mean diameter of the cyst was not significantly different between the CS and BI groups (9.9 ± 4.1 cm *vs* 10.8 ± 3.6 cm). The maximum drain output was significantly higher in the BI group (81.6 ± 118.1 mL *vs* 423.9 ± 298.4 mL, $P < 0.001$). The time for the fistula closure was significantly higher in the BI group (10.1 ± 3.7 d *vs* 30.7 ± 15.1 d, $P < 0.001$).

The ROC curve analysis revealed cut-off values of a maximum bilious drainage < 102 mL and a waiting period of 5.5 postoperative days for spontaneous closure with sensitivity and specificity values of 83.3%-91.1% (AUC: 0.90) and 91%-97% (AUC: 0.95), respectively.

The multivariate analysis demonstrated a postoperative biliary drainage volume < 102 mL to be the only significant predictor of spontaneous closure ($P < 0.001$, 95%CI: 0.5-1.0), and the presence of multiple cysts on preoperative imaging approached significance ($P = 0.06$, 95%CI: 0.2-0.5).

In the BI group, 15 (5.3%) patients had an ERCP with sphincterotomy, and 19 (6.7%) had an ERCP and NBD. Two patients required repeated ERCPs and plastic stent placement. In addition, 5 (10.8%) patients required catheter placement with USG guidance to control the biliary collections that were not adequately drained.

There were 3 (1.1%) deaths in our series: 2 due to spontaneous rupture of the cyst with subsequent anaphylactic shock and 1 who underwent multiple biliary inter-

Table 3 Postoperative characteristics of patients with postoperative biliary fistula (mean \pm SD)

	Biliary fistula closed		P value
	Spontaneous <i>n</i> = 12	Intervention <i>n</i> = 34	
Maximum output (mL)	81.6 \pm 118.1	423.9 \pm 298.4	< 0.001
Drain removal (d)	13.0 \pm 6.0	27.1 \pm 12.8	0.001
Maximum cyst diameter (cm)	9.9 \pm 4.1	10.8 \pm 3.6	0.4
Time to fistula closure (d)	10.1 \pm 3.7	30.7 \pm 15.1	< 0.001
Time to intervention (d)	10.1 \pm 3.7	11.4 \pm 6.7	0.5
Hospitalization (d)	22.4 \pm 17.9	11.4 \pm 6.7	< 0.001

ventions due to PBF and died due to myocardial infarction.

DISCUSSION

This study investigated the outcome of patients with PBF due to the IBR of a hepatic hydatid cyst. Whether minimally invasive techniques, conservative surgery or radical surgery were used, the goal of treatment was to eliminate the parasite completely and prevent complications, including pressure on adjacent structures, secondary infection or rupture of the cyst into the biliary system that results in PBF. In our series, PBF occurred in 46 (16%) patients, despite preoperative and operative efforts to prevent this complication. The fistula closed spontaneously in only 12 (26%) of the patients with PBF without further intervention, confirming that patients with PBF often have a complicated postoperative course, requiring multiple endoscopic and other interventional procedures with serious morbidity and mortality^[18,19].

Although there are several surgical techniques described to control cyst-biliary communications intraoperatively, 10%-32% of cases eventually develop a PBF^[20]. A cyst-biliary communication complicates the natural history of the disease and the treatment processes. Aktan *et al*^[21] previously reported a median intracystic pressure of 25 mmHg (range 5-55 mmHg) and a positive correlation between cyst size and pressure in viable cysts. Manometric studies indicate that the sphincter of Oddi pressure (basal 10 mmHg, peak 124 mmHg) is higher than the normal common bile duct pressure (10 mmHg). According to the LaPlace Law, the expansion of the cyst due to increased intracystic pressure results in increased cyst diameter, which causes increased tension on the cyst wall that serves as the pericyst containing the neighboring bile ducts. The pressure dynamics arising from higher intracystic pressures than the resting bile duct pressure may explain how the cyst communicates with the biliary system as the pericyst becomes thinner and thinner, eventually eroding into the bile ducts and leading to the development of an IBR^[22].

Several studies reported a history of cholangitis, high bilirubin, high ALP levels, a cyst larger than 10 cm and the presence of suggestive USG findings as clinical predictors of IBR, and an ERCP was suggested to delineate the presence of cyst-biliary communication in these cas-

es^[8,13]. Our analysis also showed that a high preoperative ALP, history of cholangitis and larger cyst diameter (> 10 cm) were significantly more common in patients that developed PBF. However, the multivariate analysis revealed that a perioperative attempt at suture ligation and a high preoperative ALT level were significant predictors of PBF. Perioperative suture ligation was performed in 39 patients in this cohort, and 11 (28%) patients developed a PBF. We have not encountered any reports on the success rate of perioperative suture ligation attempts in the literature. Our data indicate that there might be a significant failure rate for an attempt to control a detected BF intraoperatively if that was used as the only method to control the cyst-biliary communication.

Our analysis showed no significant difference in the development of PBF based on the type of procedure. During the hydatid surgery in a given patient with a cyst-biliary communication, regardless of whether a conservative or radical approach (total pericystectomy) is taken, the same biliary pedicles will be encountered, and the fistula risk should be the same unless each communicating pedicle is individually detected and effectively controlled. In our experience, PBF developed in 3 patients [3 (9%) out of 33 patients in the radical surgery group] despite at least 1 attempt to control the fistula with suture ligation, suggesting that controlling all of the detected fistulae with suture ligation may not even be possible, especially when the fistula orifices are deeply located in the cyst cavity and liver. Furthermore, our data indicate that certain fistulae may not even be detected intraoperatively. Other than technical failure, such misses may be partially explained by the abovementioned fluctuating pressure dynamics in the biliary system arising from intermittent Oddi sphincter contractions, which may cause a cyst-biliary communication that is not visible at the time of the surgery unless further manipulation is performed. We agree with others that all interventions on liver hydatidosis should be considered to be potentially major operations and that further intraoperative intervention searching for a cyst-biliary communication is warranted in patients with preoperative risk factors^[23]. Our results underline the importance of preoperative detection and effective treatment of the cyst-biliary communication. A preoperative MRCP for patients with clinical and laboratory risk factors could detect biliary complications, and an intervention with ERCP and sphincterotomy could decrease the pressure in the biliary tract, with the further benefit of providing useful anatomical information for the surgeon to select the best therapeutic approach^[24]. This information may enable the surgeon to safely perform a conservative approach and avoid a possible cholecystectomy and/or biliary exploration procedure that potentially confers further morbidity, especially in patients with previous symptoms or a history of cholangitis. These patients have a cyst that communicates with the biliary system, resulting in an ascendant infection of the cyst contents. Although bacterial superinfection may kill the parasite, this phenomenon

causes extensive inflammation with the subsequent adhesion of the duodenum and colon to the cyst, which renders biliary exploration potentially dangerous. There is a growing body of literature suggesting that it is not the type of surgery but rather the preoperative determination of cyst-biliary communication that is more important in avoiding PBF^[25,26].

In contrast to post-cholecystectomy fistulae, which close rapidly after ERCP and sphincterotomy, hydatid fistulae were reported to be more resistant and rarely closed spontaneously without decompression of the biliary tract^[27]. Endoscopic treatment has been advocated in high-output fistulae with a duration of more than 1 wk and no signs of reduction and in low-output fistulae with a duration of more than 3 wk and no signs of reduction^[28]. The ROC curve analysis enabled the detection of a maximum biliary drainage < 102 mL and a waiting period of 5.5 postoperative days for spontaneous closure (sensitivity and specificity values of 83.3%-91.1%, AUC: 0.90 and 91%-97%, AUC: 0.95, respectively) as significant cut-off levels, and multivariate analysis demonstrated a postoperative biliary drainage volume < 102 mL to be the only significant predictor of spontaneous closure ($P < 0.001$, 95%CI: 0.5-1.0). This information might help with the decision of when to attempt further intervention in a patient with PBF to prevent delays in treatment.

There are several limitations of this study. In addition to the study's retrospective nature and the lack of a uniform treatment protocol, our results may also be influenced by patient selection bias for each type of surgical or PAIR procedure. Furthermore, there was intersurgeon variability in terms of technical experience, which might have an effect on operative outcomes.

This study showed that an aggressive approach to the detection of cyst-biliary communications, both preoperatively and intraoperatively, is warranted to prevent the development of PBF and thus to avoid serious morbidity and mortality in patients with hydatid disease of the liver. Patients who develop PBF with a fistula output < 102 mL may be managed expectantly, but timely endoscopic intervention and aggressive monitoring is necessary to prevent further complications.

COMMENTS

Background

Hydatid disease, which is caused by *Echinococcus granulosus*, has a worldwide distribution, resulting with tissues developing cysts containing the parasite located mainly in the liver in humans. There are several treatment options including, medical, interventional and surgical modalities. Intrabiliary rupture (IBR), which involves a communication of the cyst cavity with the biliary system is the most common and troublesome complication resulting with a biliary fistula (BF). Development of BF complicates the natural history and outcomes of the disease treatment.

Research frontiers

To date, there is limited information regarding to the development of BF after hydatid disease treatment and its outcomes. Several risk factors including the size and location of the cyst in the liver have been documented however, very few groups reported the outcome of the patients with BF. Furthermore, treat-

ment of BF after treatment of hydatid disease is seldom reported. In order to prevent BF complication; developing multimodality approaches for detecting patients with risk factors preoperatively and developing guidelines for selecting the most suitable surgical or conservative management for these patients as well as best postoperative treatment for this complication is an active field of research.

Innovations and breakthroughs

The authors found certain risk factors in preoperative radiology and biochemistry tests of patients for development of a BF. However, this study is different from others in two aspects. The authors' analysis revealed that certain amount of patients undergoing treatment for hydatid disease of the liver would eventually develop a BF regardless of the method chosen to control the disease. Secondly, the authors documented that detecting a BF intraoperatively and attempting to control by the current standard surgical means may not be enough to control the development of BF hence emphasizing the importance of preoperative detection and aggressive intervention. The authors were also able to find a cut-off value for postoperative BF drainage output in order to choose between expectant or interventional treatment.

Applications

The study results suggest that an aggressive approach to the detection of IBR both preoperatively and intraoperatively is warranted to prevent the development of a postoperative BF and thus to avoid serious morbidity and mortality in patients with hydatid disease of the liver. Patients who develop postoperative BF with a fistula output < 102 mL may be managed expectantly.

Peer review

The manuscript is well designed, with clear objective. The manuscript aim to determine the outcome of patients with BF after treatment for hydatid disease of the liver. The authors found certain risk factors in preoperative radiology and biochemistry tests of patients for development of a BF.

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Right recumbent position on gastric emptying of water evidenced by ^{13}C breath testing

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Received: February 3, 2012 Revised: December 11, 2012

Accepted: December 15, 2012

Published online: January 21, 2013

Abstract

AIM: To compare the impact of the right recumbent position with the sitting position on gastric emptying of water.

METHODS: In eight healthy male volunteers, the ^{13}C acetate breath test was performed twice to assess gastric emptying of 100 mL tap water. Subjects were seated in one test and lying on their right side in the other. In both positions, pulmonary $^{13}\text{CO}_2$ exhalation curves were obtained by plotting breath data against time. Percent gastric retention curves were created by analyzing data using the Wagner-Nelson protocol.

RESULTS: No significant posture effect was found in pulmonary $^{13}\text{CO}_2$ output curves ($P = 0.2150$), whereas

a significant effect was seen in gastric retention curves ($P = 0.0315$). The percent retention values at 10 min and 15 min were significantly smaller when subjects were in the right recumbent position compared with the seated position ($P < 0.05$). Our results verified the accelerating effect of the right recumbent position on gastric emptying of non-nutritive solutions. Concerning clinical implications, this study suggests that placing patients with acute pain on their right side after oral administration of analgesic drugs in solution is justified as an effective practice for rapid pain relief. For patients with gastrointestinal reflux symptoms, sleeping in the right recumbent position may reduce nocturnal symptoms, as delayed gastric emptying can cause reflux symptoms.

CONCLUSION: Gastric emptying of water occurs more quickly when a subject lies on the right side compared with sitting.

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Key words: Gastric emptying; Breath test; Right recumbent position; Water

Sanaka M, Urita Y, Yamamoto T, Shirai T, Kimura S, Aoyagi H, Kuyama Y. Right recumbent position on gastric emptying of water evidenced by ^{13}C breath testing. *World J Gastroenterol* 2013; 19(3): 362-365 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/362.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.362>

INTRODUCTION

Gastric emptying of orally ingested contents is a great concern to clinicians because it is associated with gastrointestinal symptoms and can affect the efficacy of medications. Little absorption takes place in the stomach,

and any oral medication must therefore pass beyond the stomach before being absorbed within the small intestine. The rate of gastric emptying is determined by the balance between the propulsive force (fundic tonic pressure and antral contractions) and the outlet resistance (pyloric and duodenal contractions). For calorie-containing meals, the force-resistance balance is primarily regulated by the nutrient-evoked feedback loop that strictly controls duodenal entry of meals so as to optimize intestinal absorption of nutrients^[1]. On the other hand, for non-caloric fluids (e.g., water and normal saline), the feedback system is barely activated and consequently, the stomach behaves like “a motionless bag” in which the fluids passively flow back and forth depending on gravity^[2]. Considering this gravity-dependent flow, the idea has been proposed that, among the variety of body positions, the right lateral recumbent position makes emptying of non-nutritive fluids the fastest^[3].

Positioning a patient has traditionally been regarded as an effective maneuver to modulate the absorption of orally administered drugs in solution. In the case of oral overdosing, for which drug absorption should be delayed, being recumbent on the left side (“pylorus up” status) is advisable.; In the case of acute pain, for which the rapid onset of analgesic effect is favored, being recumbent on the right side (“pylorus down” status) is recommended. Indeed, the delaying effect of the left recumbent position on gastric emptying of non-caloric liquids has been well documented. However, the evidence that the right recumbent position enhances liquid emptying is much less certain^[4]. According to a recent review on the postural effect on gastric emptying^[4], only one trial supports the enhanced emptying of non-nutritive liquids in the right recumbent position, showing that emptying is significantly faster than in the left recumbent position^[2]. However, this result only suggests that emptying in the left recumbent position is slower than that in the right.

The present study was conducted to elucidate whether gastric emptying of water is faster when lying on the right side than when seated. The sitting position is regarded as “neutral” if the effect of the gravity over intragastric fluids is accounted for, and was thereby set as the reference posture. The ¹³C-acetate breath test with Wagner-Nelson analysis was used to measure gastric emptying accurately without any invasive procedures.

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers (age: 19-52 years, median 38 years; weight: 48.0-80.2 kg, median 64.0 kg) participated in this study. No subject had a history of, or symptoms referable to, gastrointestinal and pulmonary disease or chronic medical problems. Additionally, no subjects were receiving medication at the time of the study. Written informed consent was obtained from each volunteer. The study protocol was approved by the Ethics Committee of Toho University.

Protocol

The ¹³C-acetate breath test was used to assess gastric emptying^[5]. The assessment was carried out on two randomized occasions, at least 3 d apart, with subjects either seated or lying on their right side. On both occasions, subjects had fasted overnight, and they drank 100 mL tap water labeled with 100 mg ¹³C-acetate in the sitting position. Breath samples were collected at baseline and, following ingestion of the water, at 5-min intervals for the first 20 min and at 10-min intervals thereafter up to 120 min. On one occasion, the subjects remained seated throughout the 120-min period. On the other occasion, immediately after drinking the test solution, they lay down on their right side and maintained the position until they exhaled the 15-min breath sample; thereafter they remained seated.

Data analysis

Breath ¹³CO₂ isotopic enrichment was determined using non-dispersive infrared isotope spectrometry (UbiT-IR 300; Otsuka Electronics, Osaka, Japan). The pulmonary recovery of ¹³CO₂ was expressed in %dose/h based on the body surface area and the assumed CO₂ production. The %dose/h recovery values were plotted against time to create the time-breath ¹³CO₂ excretion curve, which indirectly reflects gastric emptying. The time to the maximal recovery (T_{max}), a semi-quantitative index of gastric emptying, was determined by visual inspection of the ¹³CO₂ output curve^[6]. The breath data were further analyzed using the Wagner-Nelson method, which enables creation of a percent gastric retention-time curve that is as accurate as the scintigraphic emptying curve^[7]. The Wagner-Nelson procedure has been detailed elsewhere^[7,8]. In short, estimated fraction of the labeled test meal that has been emptied from the stomach by time *t*, *F(t)*, is obtained from the conventional equation; *F(t)* = area under the concentration curve (AUC)(*t*)/AUC(∞), although the value can differ from those obtained using the scintigraphic method. Wagner-Nelson method allows accurate estimation of *F(t)* using the revised equation as follows: *F(t)* = [AUC(*t*) + *C(t)*/Kel]/AUC(∞). In the equation, Kel (1/h) is the first-order rate constant for total elimination of ¹³CO₂ from the human body, *C(t)* is the ¹³CO₂ excretion rate (%dose/h), AUC(*t*) is the area under the *C(t)* curve (%dose) (= the pulmonary recovery of ¹³CO₂), and AUC(∞) is the cumulative amount of ¹³CO₂ recovered in the breath at the infinite time (% dose).

The time by which half of the water has been emptied (*t*_{1/2}), which is used as the standard index in gastric emptying scintigraphy, was determined by interpolation from the retention-time curve^[7].

Statistical analysis

Differences in the pulmonary ¹³CO₂ output and the percent gastric retention curves between the two postures were assessed with repeated measures of analysis of variance (ANOVA) using “posture” and “time” as factors. When the ANOVA showed a significant difference, it was

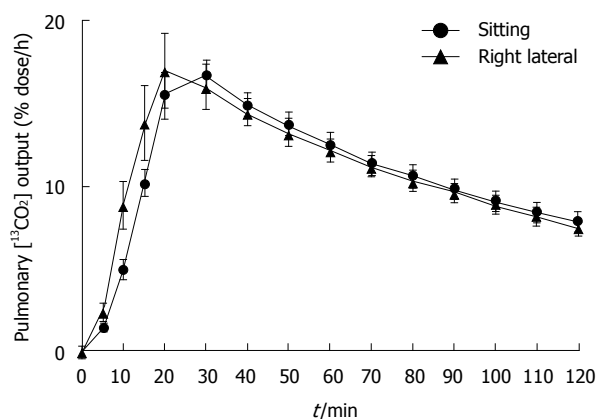


Figure 1 Pulmonary $^{13}\text{CO}_2$ recovery-time curves. Error bars indicate SE.

followed by *post hoc* comparisons at each measurement time with Student's paired *t* test with Bonferroni adjustment. The t_{\max} and $t_{1/2}$ values are presented as mean \pm SD. Differences in t_{\max} and $t_{1/2}$ between the two body positions were also assessed with Student's paired *t* test. The level of significance was set at $P = 0.05$ (two-sided probability).

RESULTS

No significant posture effect was found in pulmonary $^{13}\text{CO}_2$ output curves ($P = 0.2150$; Figure 1), whereas a significant effect was found in gastric retention curves ($P = 0.0315$; Figure 2). The *post hoc* comparisons revealed that the percent retention values were significantly smaller in the right recumbent position at 10 min ($P < 0.01$) and at 15 min ($P < 0.05$). On the other hand, neither T_{\max} nor $t_{1/2}$ was significantly different between the two positions (T_{\max} : 25.0 ± 5.35 min in the sitting position and 23.1 ± 9.23 min in the right recumbent position; $P = 0.644$, $t_{1/2}$: 13.7 ± 2.79 min in the sitting position and 11.6 ± 5.92 min in the right recumbent position; $P = 0.378$).

DISCUSSION

It seems natural that non-caloric fluids in the stomach should gravitate toward the duodenum in the right recumbent position. It is, therefore, reasonable to assume that the right recumbent position would hasten gastric emptying of fluids compared to a sitting position. However, this assumption has not been verified. The present results provided clear evidence that water is emptied significantly faster when a subject is in the right recumbent position than when seated.

Burn-Murdoch *et al.*^[2] showed that emptying of saline was significantly faster in the right recumbent position than in the left recumbent position whereas it was not significantly different between the right recumbent position and the seated position. We think that the lack of significant difference between the right recumbent position and the seated position in that study might be

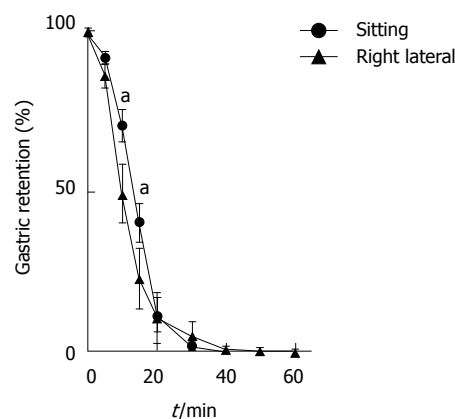


Figure 2 Percent gastric retention-time curves created by Wagner-Nelson analysis. Error bars indicate SE. The asterisk indicates a significant difference ($^*P < 0.05$ vs right lateral group).

due to the suboptimal protocol used to measure gastric emptying: the emptying was quantified as the volume aspirated *via* a nasogastric tube at 10 min after ingestion of 750-mL saline. The aspiration technique appears unreliable to measure residual gastric volume^[9].

In the present study, the gastric retention at 10 and 15 min indicated a significant positional effect (Figure 2), whereas the pulmonary $^{13}\text{CO}_2$ output curve did not (Figure 1). This is possibly because the Wagner-Nelson modification improved the accuracy of breath testing. In contrast to gastric scintigraphy, which allows direct visualization of duodenal transit of gastric contents^[10], the conventional ^{13}C -acetate breath test indirectly reflects gastric emptying and thus is less sensitive^[6]. The Wagner-Nelson analytical strategy has been introduced into the breath test system to overcome its indirect nature, making the ^{13}C -breath test as accurate as the “gold standard” of scintigraphy^[7]. Gastric emptying of non-nutrient fluids in the sitting position per se is so rapid that conventional breath testing would have overlooked a subtle, albeit real acceleration of emptying in the right recumbent position. This subtle difference would be detected only by the gastric retention curve created by Wagner-Nelson analysis. The $t_{1/2}$ values were not significantly different between the two body positions even though values were derived from the “accurate” Wagner-Nelson analysis. This is probably because the $t_{1/2}$ value itself would be a less accurate parameter than the percent gastric retention at a fixed time point, as shown in the expert recommended guidelines for scintigraphic gastric emptying studies^[10].

There are some limitations to the present study. First, we used only a small sample size. Second, only a small volume of tap water was used. A larger volume may influence the results.

In conclusion, our results verified the accelerating effect of the right recumbent position on gastric emptying of non-nutritive solutions. Concerning clinical implications, this study suggests that placing patients with acute pain on their right side after oral administration of analgesic drugs in solution is justified as an effective practice

for rapid pain relief. For patients with gastrointestinal reflux symptoms, sleeping in the right recumbent position may reduce nocturnal symptoms, as delayed gastric emptying can cause reflux symptoms.

COMMENTS

Background

Conventionally, gastric emptying of non-caloric fluids is thought to be accelerated when a person is in the right recumbent position. However, evidence supporting this hypothesis is weak. The present study compared the impact of the right recumbent position with the sitting position on gastric emptying of water.

Research frontiers

For calorie-containing meals, the force-resistance balance is primarily regulated by the nutrient-evoked feedback loop that strictly controls duodenal entry of meals so as to optimize intestinal absorption of nutrients. On the other hand, for non-caloric fluids, the feedback system is barely activated and consequently, the stomach behaves like "a motionless bag" in which the fluids passively flow back and forth depending on gravity.

Innovations and breakthroughs

The present study was conducted to elucidate whether gastric emptying of water is faster when lying on the right side than when seated. The sitting position is regarded as "neutral" if the effect of the gravity over intragastric fluids is accounted for, and was thereby set as the reference posture. The ^{13}C -acetate breath test with Wagner-Nelson analysis was used to measure gastric emptying accurately without any invasive procedures.

Applications

Their results verified the accelerating effect of the right recumbent position on gastric emptying of non-nutritive solutions. Concerning clinical implications, this study suggests that placing patients with acute pain on their right side after oral administration of analgesic drugs in solution is justified as an effective practice for rapid pain relief. For patients with gastrointestinal reflux symptoms, sleeping in the right recumbent position may reduce nocturnal symptoms, as delayed gastric emptying can cause reflux symptoms.

Peer review

This is a prospective study to evaluate the gastric emptying of non-caloric liquids in a seated position versus right lateral recumbence. A total of 8 healthy male volunteers were given 100 mL of tap water labeled with ^{13}C -acetate and evaluated in a seated position on one occasion and, on a separate day, evaluated in a right lateral recumbence position. Breath samples were collected at given intervals and tested for the pulmonary recovery of $^{13}\text{CO}_2$. The values were plotted against time to create a time-breath $^{13}\text{CO}_2$ excretion curve, which is an indirect measurement of the gastric emptying. The Wagner-Nelson procedure was then used to further analyze the data and extrapolate percent gastric retention-time curve.

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P- Reviewers Koch TR, Ghoshal UC S- Editor Gou SX
L- Editor A E- Editor Xiong L



Role of surgical resection for multiple hepatocellular carcinomas

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Received: May 13, 2012 Revised: December 3, 2012

Accepted: December 15, 2012

Published online: January 21, 2013

Abstract

AIM: To clarify the role of surgical resection for multiple hepatocellular carcinomas (HCCs) compared to transarterial chemoembolization (TACE) and liver transplantation (LT).

METHODS: Among the HCC patients who were managed at Yonsei University Health System between January 2003 and December 2008, 160 patients who met the following criteria were retrospectively enrolled: (1) two or three radiologically diagnosed HCCs; (2) no radiologic vascular invasion; (3) Child-Pugh class A; (4) main tumor smaller than 5 cm in diameter; and (5) platelet count greater than 50 000/mm³. Long-term outcomes were compared among the following three treatment modalities: surgical resection or combined radiofrequency ablation (RFA) ($n = 36$), TACE ($n = 107$), and LT ($n = 17$). The survival curves were computed using the Kaplan-Meier method and compared with

a log-rank test. To identify the patients who gained a survival benefit from surgical resection, we also investigated prognostic factors for survival following surgical resection. Multivariate analyses of the prognostic factors for survival were performed using the Cox proportional hazard model.

RESULTS: The overall survival (OS) rate was significantly higher in the surgical resection group than in the TACE group (48.1% vs 28.9% at 5 years, $P < 0.005$). LT had the best OS rate, which was better than that of the surgical resection group, although the difference was not statistically significant (80.2% vs 48.1% at 5 years, $P = 0.447$). The disease-free survival rates were also significantly higher in the LT group than in the surgical resection group (88.2% vs 11.2% at 5 years, $P < 0.001$). Liver cirrhosis was the only significant prognostic factor for poor OS after surgical resection. Clinical liver cirrhosis rates were 55.6% (20/36) in the resection group and 93.5% (100/107) in the TACE group. There were 19 major and 17 minor resections. *En bloc* resection was performed in 23 patients, multi-site resection was performed in 5 patients, and combined resection with RFA was performed in 8 patients. In the TACE group, only 34 patients (31.8%) were recorded as having complete remission after primary TACE. Seventy-two patients (67.3%) were retreated with repeated TACE combined with other therapies. In patients who underwent surgical resection, the 16 patients who did not have cirrhosis had higher 5-year OS and disease-free survival rates than the 20 patients who had cirrhosis (80.8% vs 25.5% 5-year OS rate, $P = 0.006$; 22.2% vs 0% 5-year disease-free survival rate, $P = 0.048$). Surgical resection in the 20 patients who had cirrhosis did not provide any survival benefit when compared with TACE (25.5% vs 24.7% 5-year OS rate, $P = 0.225$). Twenty-nine of the 36 patients who underwent surgical resection experienced recurrence. Of the patients with cirrhosis, 80% (16/20) were within the Milan criteria at the time of recurrence

after resection.

CONCLUSION: Among patients with two or three HCCs, no radiologic vascular invasion, and tumor diameters ≤ 5 cm, surgical resection is recommended only in those without cirrhosis.

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Key words: Hepatocellular carcinoma; Hepatectomy; Liver transplantation; Chemoembolization; Cirrhosis

Choi SH, Choi GH, Kim SU, Park JY, Joo DJ, Ju MK, Kim MS, Choi JS, Han KH, Kim SI. Role of surgical resection for multiple hepatocellular carcinomas. *World J Gastroenterol* 2013; 19(3): 366-374 Available from: URL: <http://www.wjg-net.com/1007-9327/full/v19/i3/366.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.366>

INTRODUCTION

Surgical resection is the established treatment modality for hepatocellular carcinoma (HCC) in patients with preserved liver function, and surgical outcomes have been greatly improved with mortality rates of 0%-6.4% and excellent 5-year survival rates of more than 50%^[1-3]. However, for patients with multiple HCCs, unfavorable disease-free and overall survival (OS) rates following surgical resection have led to the contraindication of surgical treatment. This contraindication is reflected in the guidelines suggested by the American Association for the Study of Liver Disease^[4] and the European Association for the Study of the Liver^[5] based on the Barcelona Clinic Liver Cancer (BCLC) staging system. Thus, liver transplantation is recommended as the best option for patients with multiple HCCs, but a worldwide shortage of donor organs greatly limits the application of this recommendation. Therefore, although multidisciplinary strategies are used in the treatment of multiple HCCs, multiple HCCs still pose a therapeutic challenge and are a matter of debate.

Currently, the main treatment modality for multiple HCCs is transarterial chemoembolization (TACE). Although several expert centers have recently reported encouraging durable long-term outcomes of surgical resection for multiple HCCs (50%-60% at 5 years) in patients with well-preserved liver function^[6,7], few comparative studies of surgical resection and liver transplantation (LT).

ACE have been reported^[8,9]. Therefore, we designed this study to clarify the role of surgery for multiple HCCs by comparing the long-term outcomes following surgical resection, TACE, and LT and investigating prognostic factors in patients who underwent surgical resection.

MATERIALS AND METHODS

Patient evaluation and follow-up

We analyzed a single-institution database of 3928 pa-

tients who received their initial treatments for HCCs at Yonsei University Health System, Seoul, South Korea between January 2003 and December 2008. The Institutional Review Board of Yonsei University Health System approved this study. Of the patients who underwent surgical resection ($n = 304$), TACE ($n = 854$), and LT ($n = 45$), the patients who met the following criteria were enrolled in this retrospective, single-cohort study: (1) two or three radiologically diagnosed HCCs; (2) no radiologic vascular invasion; (3) Child-Pugh class A; (4) main tumor smaller than 5 cm in diameter; and (5) a platelet count greater than 50 000/mm³. Small satellite nodules found in the resected specimen were not included in this study.

The cutoff value of tumor size for therapeutic decision making is a debated issue. The 7th edition of cancer staging of the American Joint Committee on Cancer describes main tumor size greater than 5 cm in multiple tumors as an independent prognostic factor for survival^[10]. Additionally, a study comparing the pathologically proven necrosis rate following TACE reported the following frequencies of complete necrosis according to tumor size: 66.7%, 30% and 0% for ≤ 3.0 cm, 3.1-5.0 cm, and > 5.0 cm, respectively^[11]. Although TACE has been performed as palliative care for larger HCCs, the OS rates of patients with tumors larger than 5 cm in diameter are very poor^[12,13]. In addition to tumor size, the presence of vascular involvement of the tumor is a significant prognostic factor for poor outcome. The 5-year survival rates among patients with tumors with and without macroscopic vascular invasion are significantly different ($48\% \pm 3\%$ vs $14\% \pm 5\%$, $P < 0.001$)^[14]. Therefore, patients with a tumor ≥ 5 cm in diameter or with macroscopic vascular involvement were excluded from this study because those patients were unsuitable for curative therapy.

Ultimately, we included 36 patients who underwent surgical resection or combined resection with radiofrequency ablation (RFA), 107 patients who received TACE, and 17 patients who underwent LT, and we compared long-term outcomes following the respective treatments.

Patients were evaluated preoperatively by abdominal ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and hepatic angiography, if indicated. All patients were assessed using alpha-feto-protein (AFP), protein induced by vitamin K absence or antagonist II (PIVKA II), hepatitis B surface antigen, anti-hepatitis C viral antibody, liver biochemistry, coagulation test, and indocyanine green retention rate at 15 min (ICG R15).

The selection of therapeutic options was determined by the anatomical locations of the tumors, liver functional reserve, and patient preference. TACE was generally considered when the tumor was ineligible for complete surgical removal, low remnant liver volume was expected after resection, or the patients declined operative inter-

vention. The patients who had an available liver donor underwent LT.

Eradicating all of the multifocal tumors required a multimodality approach using not only *en-bloc* resection but also separate multi-site resection or resection combined with RFA. All of the patients who underwent surgical resection were routinely assessed by intra-operative US. The effectiveness and safety of combined hepatectomy with RFA for multi-site HCCs were reported by Choi *et al.*^[15], and our previous work has also shown comparable results between *en-bloc* resection and multi-site resection or combination hepatectomy with RFA^[16]. Separate multi-site resection or resection plus RFA was performed for the multifocal tumors ineligible for *en-bloc* resection because of bilobar involvement or when there was not enough hepatic function reserve after *en-bloc* resection. Wedge resection was considered for superficial tumors, and RFA was performed for tumors less than 3 cm in diameter that were located deep in the liver.

The median follow-up period for the patients who underwent surgical resection was 38.6 mo (range: 1-94 mo). Surveillance after treatment was conducted with regular monitoring of AFP, PIVKA II, and US or CT every three to six months. Suspicious intrahepatic recurrence was confirmed by MRI, hepatic angiography, or image-guided fine-needle biopsy, if needed.

To identify the patients who gained a survival benefit from surgical resection, we also investigated prognostic factors for survival following surgical resection. Twelve clinical variables recorded at the time of diagnosis were analyzed. The variables included age at diagnosis; sex; serum albumin; alanine aminotransferase (ALT) and aspartate aminotransferase levels; ICG R15; serum AFP level; clinical liver cirrhosis; main tumor size and number of tumors on preoperative image studies; lobar distribution of the tumor; and type of operation.

Clinically diagnosed liver cirrhosis was defined as follows: (1) history of overt complications of liver cirrhosis, such as ascites, variceal bleeding, and hepatic encephalopathy; (2) evidence of clinical portal hypertension, including esophageal or gastric varices, or splenomegaly (maximal diameter > 12 cm) with platelet count < 100 000 mm³; and (3) liver morphology suggesting the presence of cirrhosis on preoperative image studies, including hypertrophy of the left lobe and/or caudate lobe, relative volume reduction of the right lobe, nodularity of the liver surface, presence of regenerative or dysplastic nodules, or the presence of a portosystemic shunt^[17-19]. All of the analysis in the current study were performed using a clinical diagnosis of liver cirrhosis to evaluate its clinical usefulness. Minor resection was defined as hepatectomy of two or fewer liver segments.

Statistical analysis

Continuous variables are presented as the mean \pm SD and were compared by Student's *t* test. Categorical vari-

ables are expressed as frequencies with percentages and were compared by the χ^2 test. Cumulative overall and disease-free survival rates were computed by the Kaplan-Meier method, and differences between the survival curves were compared using a log-rank test. Multivariate analyses of the prognostic factors for survival were performed using the Cox proportional hazard model and included the factors that had *P* values less than 0.1 upon univariate analysis. Statistical analyses were performed using SPSS 15 for Windows (SPSS Inc., Chicago, IL, United States). Statistical significance was set at a *P* value less than 0.05.

RESULTS

Clinical characteristics of the surgical resection group and TACE group

The patients who underwent TACE were older and had lower platelet counts, higher ALT levels, and a higher rate of clinical liver cirrhosis than those in the resection group. Clinical liver cirrhosis rates were 55.6% (20/36) in the resection group and 93.5% (100/107) in the TACE group. Surgical resection was performed more frequently in patients with larger diameter tumors (Table 1).

Operative procedures in the surgical resection group and additional treatments in the TACE group

Table 2 lists the operative procedures and combined treatments with RFA in the resection group. There were 19 and 17 major and minor resections, respectively. *En bloc* resection was performed in 23 patients, multi-site resection was performed in 5 patients, and combined resection with RFA was performed in 8 patients. In the TACE group, only 34 patients (31.8%) were recorded as having complete remission after primary TACE. Seventy-two patients (67.3%) were retreated with repeated TACE, one patient was retreated with repeated TACE and intra-arterial chemotherapy, two patients were retreated with RFA, two patients were retreated with radiation therapy, one patient was retreated with percutaneous ethanol injection, two patients were retreated with intra-arterial chemotherapy and systemic chemotherapy, two patients were retreated with intra-arterial chemotherapy and radiation therapy, and five patients were retreated with holmium therapy.

Long-term outcomes according to treatment modality

The OS rate was significantly higher in the surgical resection group than in the TACE group (48.1% *vs* 28.9% at 5 years, *P* < 0.005) (Figure 1A). LT had the best OS rate, which was better than that of the surgical resection group, although the difference was not statistically significant (80.2% *vs* 48.1% at 5 years, *P* = 0.447) (Figure 1A). The disease-free survival rates were also significantly higher in the LT group than in the surgical resection group (88.2% *vs* 11.2% at 5 years, *P* < 0.001) (Figure 1B).

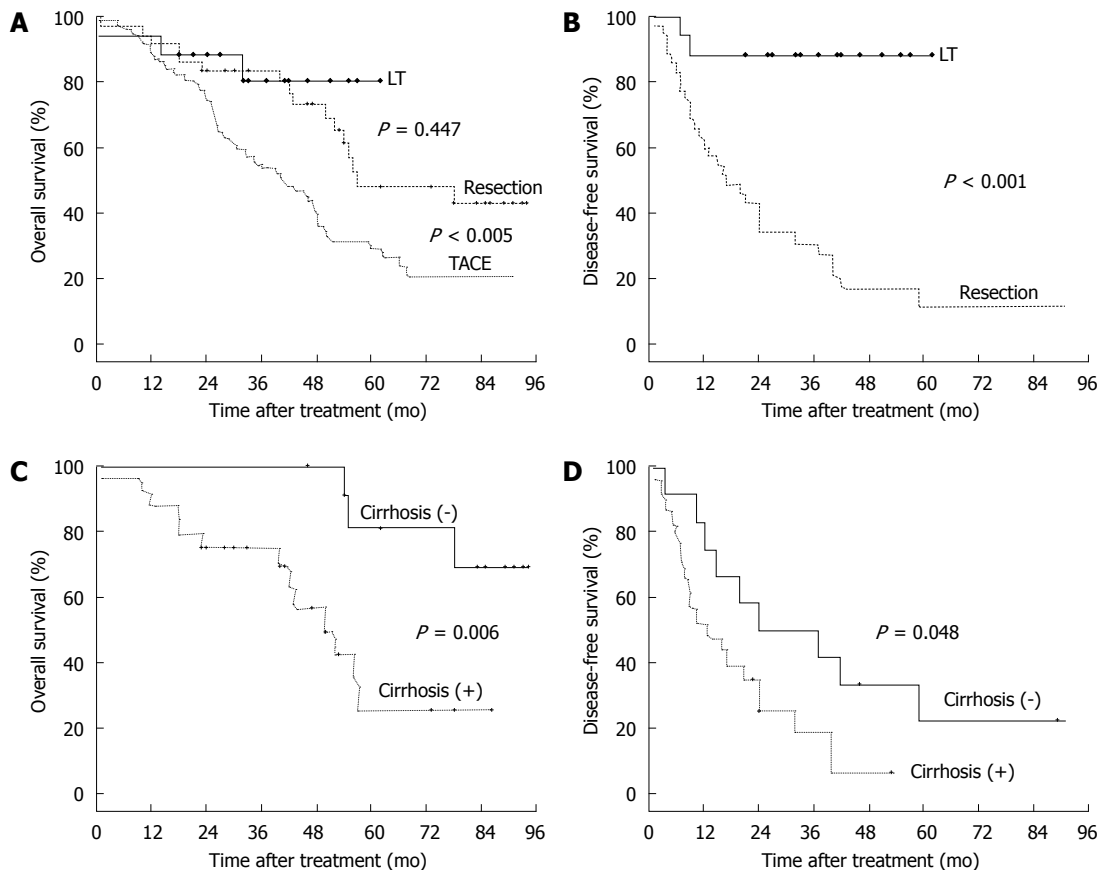


Figure 1 The overall and disease-free survival curves according to treatment modality and presence of liver cirrhosis in surgical resection patients. A: The 1-, 3- and 5-year overall survival (OS) rates were 94.1%, 80.2% and 80.2%, respectively, in the liver transplantation (LT) group; 91.7%, 83.3% and 48.1%, respectively, in the resection group; and 88.7%, 55.6% and 28.9%, respectively, in the transarterial chemoembolization (TACE) group. The OS rate was significantly higher in the surgical resection group than in the TACE group ($P < 0.005$). LT showed the best OS rate (better than the surgical resection group, but not statistically significant, $P = 0.447$); B: The 1-, 3- and 5-year disease-free survival rates were 88.2%, 88.2% and 88.2% in the LT group and 60%, 30.3% and 11.2% in the resection group, respectively. The disease-free survival rates were also significantly higher in the transplantation group than in the surgical resection group ($P < 0.001$); C: The 1-, 3- and 5-year OS rates were 100%, 100% and 80.8% in patients without cirrhosis (-) and 87.5%, 75% and 25.5% in patients with cirrhosis (+), respectively ($P = 0.006$); D: The 1-, 3- and 5-year disease-free survival rates were 75.0%, 50.0% and 22.2% in patients without cirrhosis (-) and 52.3%, 18.7% and 0% in patients with cirrhosis (+), respectively ($P = 0.048$).

Table 1 Clinical characteristics of the surgical resection patients vs the transarterial chemoembolization patients

Variable	Surgical resection (<i>n</i> = 36)	TACE (<i>n</i> = 107)	<i>P</i> value
Age (yr)	54.3 ± 8.6	61.2 ± 9.3	< 0.001
Gender (male:female)	34:2	86:21	0.047
Platelet (k/mm ³)	153.4 ± 53.9	121.0 ± 51.8	0.002
Albumin (g/dL)	4.05 ± 0.50	3.93 ± 0.48	0.203
ALT (IU/L)	36.4 ± 15.8	59.0 ± 40.4	0.001
AST (IU/L)	41.2 ± 25.8	53.2 ± 44.7	0.130
HBsAg	28 (77.8)	67 (62.6)	0.099
Clinical liver cirrhosis	20 (55.6)	100 (93.5)	< 0.001
Tumor number			0.549
2	30 (83.3)	90 (84.1)	
3	6 (16.7)	17 (15.9)	
Main tumor size (cm)			0.005
< 3	19 (52.8)	17 (47.2)	
≥ 3	29 (27.1)	78 (72.9)	
AFP > 1000 IU/mL	2 (5.6)	8 (7.5)	0.696

Data are expressed as absolute *n* (%) or mean ± SD. M: Male; F: Female; TACE: Transarterial chemoembolization; ALT: Alanine aminotransferase (reference range, 5–46 IU/L); AST: Aspartate aminotransferase (reference range, 13–34 IU/L); HBsAg: Hepatitis B surface antigen; AFP: α-fetoprotein.

Table 2 Operative procedures in the resection group

Degree of resection	Operative procedure	Number of patients (<i>n</i> = 36)
Major resection (<i>n</i> = 19)	Extended right hepatectomy	1
	Right hepatectomy only	12
	+ wedge resection	1
	+ RFA	1
	Left hepatectomy only	1
Minor resection (<i>n</i> = 17)	+ wedge resection	1
	Central bisectionectomy only	2
	Left lateral sectionectomy only	3
	+ RFA	1
	Sectionectomy only	2
	+ wedge resection	2
	+ RFA	2
	Bisegmentectomy only	1
	+ wedge resection	1
	+ RFA	2
	Segmentectomy only	1
	+ RFA	1
	Wedge resection + RFA	1

En bloc resection (*n* = 23); multi-site resection (*n* = 5); resection plus radio-frequency ablation (RFA) (*n* = 8).

Table 3 Prognostic factors for overall survival in surgical resection patients

Variable	Patients (n = 36)	1-yr OS	3-yr OS	5-yr OS	P value
Age (yr)					0.146
≤ 60	27	88.9%	81.5%	42.3%	
> 60	9	100%	88.9%	66.7%	
Gender					0.245
Male	34	91.2%	82.4%	45.1%	
Female	2	100%	100%	100%	
Serum albumin (g/dL)					0.642
≤ 3.5	10	90.0%	60.0%	48.0%	
> 3.5	26	92.3%	87.7%	46.1%	
ALT (IU/L)					0.593
≤ 50	27	92.6%	81.5%	53.7%	
> 50	9	88.9%	71.1%	23.7%	
AST (IU/L)					0.87
≤ 50	31	90.3%	80.6%	52.4%	
> 50	5	100%	100%	50.0%	
ICG R 15 (%)					0.992
≤ 14	25	92.0%	84.0%	47.1%	
> 14	9	88.9%	77.8%	58.3%	
α-fetoprotein					0.471
≤ 1000 IU/mL	32	90.6%	81.3%	47.3%	
> 1000 IU/mL	4	100%	100%	50.0%	
Cirrhosis					0.023
No	16	100%	93.8%	69.9%	
Yes	20	85.0%	75.0%	26.5%	
Main tumor size (cm)					0.629
< 3.0	17	94.1%	86.3%	43.1%	
≥ 3.0	19	89.5%	73.7%	52.6%	
Number of tumors					0.061
2	31	90.3%	80.6%	40.7%	
3	5	100%	100%	100%	
Lobar distribution of tumors					0.892
One lobes	24	91.7%	83.3%	49.4%	
Two lobe	12	91.7%	83.3%	48.6%	
Operation type					0.568
<i>En bloc</i> resection	23	91.3%	82.6%	43.4%	
Multiple resection or combined with RFA	13	92.3%	84.6%	57.1%	

OS: Overall survival; RFA: Radiofrequency ablation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ICG R 15: Indocyanine green retention rate at 15.

Prognostic factors for OS in patients who underwent surgical resection

Cirrhosis was the only significant prognostic factor for poor OS after resection in both the univariate ($P = 0.023$) and multivariate analyses ($P = 0.034$, odds ratio = 0.552, 95%CI: 0.105-0.915) (Table 3). Clinically diagnosed liver cirrhosis was correlated with pathological cirrhosis with a positive predictive value of 100%, a negative predictive value of 75%, a sensitivity of 83.3%, and a specificity of 100%.

Long-term outcomes of patients who underwent surgical resection according to the presence of cirrhosis

The 1-, 3- and 5-year OS rates were 100%, 100% and 80.8%, respectively, in 16 patients without cirrhosis, and 87.5%, 75% and 25.5%, respectively, in 20 patients with cirrhosis ($P = 0.006$) (Figure 1C). The disease-free 1-, 3-

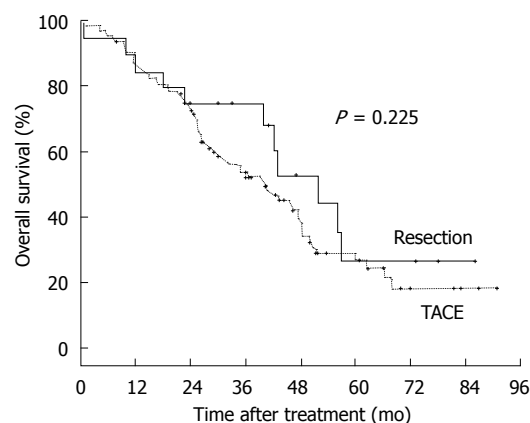


Figure 2 Overall survival curves of surgical resection and transarterial chemoembolization in patients with liver cirrhosis. Among patients with cirrhosis, the overall survival (OS) rates were not different between the surgical resection group and the transarterial chemoembolization (TACE) group (25.5% vs 24.7% 5-year OS rate, $P = 0.225$).

and 5-year survival rates were 75.0%, 50% and 22.2%, respectively, in patients without cirrhosis and 52.3%, 24.9% and 0%, respectively, in patients with cirrhosis ($P = 0.048$) (Figure 1D). The OS rates were not different between the surgical resection group and the TACE group among the patients with cirrhosis (87.5%, 75.0% and 25.5% vs 91.8%, 61.7% and 24.7% at 1-, 3- and 5-year, respectively, $P = 0.225$) (Figure 2).

Recurrence pattern after surgical resection

Twenty-nine of the 36 patients who underwent surgical resection experienced recurrence. Of the 29 patients with recurrence, 1 (4%) had a marginal recurrence on the resection margin, 21 (84%) had intra-hepatic recurrences, and 3 (12%) had extra-hepatic recurrences with one in the lung and two in bone. All of the patients with marginal and intra-hepatic recurrence were retreated by TACE. Chemotherapy, radiation therapy, and a clinical trial were used to treat extra-hepatic recurrences. Notably, of the patients with cirrhosis, 80% (16/20) were within the Milan criteria at the time of recurrence after resection.

DISCUSSION

Our study demonstrates that surgery for patients with multiple HCCs is recommended in patients without cirrhosis. Our data revealed that the survival of patients who underwent surgical resection was better than that of patients who received TACE. However, the survival of patients with HCCs was affected not only by HCC itself, but also by underlying liver disease. The majority of patients (93.5%, 100/107) who received TACE had liver cirrhosis. Therefore, when the survival of the patients in the surgical resection group was compared with the TACE group according to the presence of cirrhosis, surgical resection showed no survival benefit in cirrhotic patients.

To confirm the efficacy of surgical resection for mul-

multiple HCCs, its superiority over non-surgical treatment (TACE) should be proven. A recent retrospective cohort study by Ho *et al.*^[8] reported that hepatectomy yields better survival than TACE even in patients with multiple HCCs in various stages. The authors compared the prognosis of the patients according to stages in different staging systems; however, as the authors note, their study had several weaknesses. Although each staging system represents the prognosis of the patients who have HCC, and in particular, the BCLC system accounts for portal hypertension and the bilirubin level in addition to the Child-Pugh classification in staging, the liver function status in each stage encompasses a wide range and the prognosis in the subgroups of each stage differs significantly^[20]. Additionally, the patients in each stage of their study were heterogeneous. Therefore, it is unclear whether the degree of underlying liver disease was evenly distributed between the compared groups. In our study, we limited the inclusion criteria to patients with platelet counts greater than 50 000/mm³ in addition to a Child-Pugh A classification to exclude patients who had severely advanced liver cirrhosis, for whom surgery is contraindicated due to likely postoperative deterioration of liver function and poor prognosis^[17,21].

Liver cirrhosis is a well-known potent predictive factor for OS in patients with HCC^[14,22,23], as shown in our patient cohort. According to our analysis, the survival rates of cirrhotic patients with multiple HCCs who underwent surgical resection were extremely disappointing. The 5-year OS rate of the patients with cirrhosis was 25.5%, which was significantly worse than that of the patients without cirrhosis (69.3%, $P = 0.006$) (Figure 1C and D). Furthermore, the outcomes of surgical resection and TACE in the patients with cirrhosis were not different (Figure 2). Therefore, surgical resection for multiple HCCs would be beneficial in patients without cirrhosis, but it remains debatable in patients with cirrhosis.

Despite the fact that surgical resection for multiple HCCs showed acceptable OS rates in our study as well as in several other publications^[6-8,22,24], a high recurrence rate is a major drawback of surgical resection as a curative therapy. According to our data, the 5-year disease-free survival rates were 0% in patients with cirrhosis and 22.2% in non-cirrhotic patients. Thus, our single cohort study demonstrated that LT might be the preferred treatment option to offer the chance of a cure for multiple HCCs; the OS and disease-free survival rates were 80.2% and 88.2%, respectively (Figure 1A and B). However, in addition to inevitable immunosuppressive therapy, which has an adverse oncologic effect, the scarcity of liver donors is another great obstacle to the wide application of LT.

Which loco-regional therapy is superior as a bridge to LT is an issue because of the long waiting time on the transplant list, which results in patients progressing and falling outside the transplant criteria. According to Llovet *et al.*^[25], approximately 10% of patients are dropped

from the transplant list during the waiting period because of tumor progression or liver failure. TACE and RFA have been studied in detail and widely used as a bridge therapy in several transplant centers, but the efficacy of these modalities has not yet been established^[26-28].

Recently, Belghiti *et al.*^[29] proposed three different roles of resection for HCC prior to LT: (1) As a primary therapy, resection can delay or avoid transplantation and can be followed by salvage transplantation for recurrence and deteriorated liver function; (2) As an initial therapy, resection can provide pathologic information about the whole specimen, which enables selection of the best candidates for transplantation; and (3) As a bridge therapy, resection can offer the best control of HCC in patients listed for LT through the possibility of downstaging and providing detailed pathologic information. In addition to these benefits, liver resection can provide superior control and a good survival rate^[29,30]. Furthermore, several studies report that 60%-80% of patients who recur after resection for HCC are still amenable to transplantation, and these results are not different between patients with solitary and oligonodular primary HCCs^[22,31,32]. Our results also showed that 80% of the recurred patients with cirrhosis following resection were within the Milan criteria. Thus, surgical resection for cirrhotic patients with multiple HCCs might be performed as a bridge to LT. Surgical resection as a bridge is justified by the improved safety of liver surgery and no survival impairment in the event of subsequent LT^[31].

Recent studies demonstrated that salvage LT does not compromise the operative morbidity and mortality compared with primary LT^[31,33,34]. In contrast, Adam *et al.*^[35] reported that secondary LT is associated with a higher operative morbidity and mortality; they also argued that patients treated by resection when they were initially transplantable had a higher recurrence rate with more frequent extrahepatic metastasis and vascular invasion, which impair the transplantability and long-term survival of the patients. Therefore, it is too early to conclude whether resection can be performed as bridge therapy. Nevertheless, these efforts to use the limited number of donor organs effectively are necessary, as is further investigation of this issue.

We did not analyze the outcomes according to the types of multiple HCCs in the present study because our data included patients who received resection combined with RFA, which did not allow for pathologic analysis. Discrimination of intrahepatic metastasis (IM) and *de novo* multicentric (MC) HCCs may be important because generally, IMs that have acquired metastatic ability exhibit more aggressive biologic behavior^[36,37] and thus influence therapeutic strategy decisions. According to the guidelines of the Liver Cancer Study Group of Japan^[38], IM is diagnosed if the tumors definitely originated from portal vein tumor thrombi, if the tumors arose in multiple satellite nodules surrounding a main tumor, or if a satellite tumor near the main tumor shows similar or

poorer histological differentiation than the main tumor. Otherwise, multiple HCCs that do not meet these conditions are deemed *de novo* MC tumors. Although these conventional pathological criteria are convenient, they are relatively subjective. Currently, the most precise method to determine the origin of HCC is DNA clonal analysis^[39,40], and clinical differentiation between IM and MC is not possible preoperatively. If a credible diagnosis could be possible for preoperative distinction of the origin of multiple nodules, it might be helpful for the selection of therapy.

In conclusion, surgical resection for HCCs with two or three radiologically identified tumors, no radiologic vascular invasion, and diameters less than 5 cm is recommended for patients without cirrhosis but debatable for patients with cirrhosis. LT might be the best treatment option for patients with multiple HCCs. The retrospective design and the small number of cases are limitations of this-single cohort study. Therefore, further multi-center trials and randomized, controlled, prospective studies are needed, especially to examine the role of surgical resection in cirrhotic patients with multiple HCCs.

COMMENTS

Background

Although surgical resection is the established treatment modality for hepatocellular carcinoma (HCC) in patients with preserved liver function, however, for patients with multiple HCCs, unfavorable disease-free and overall survival (OS) rates following surgical resection have led to the contraindication of surgical treatment. Therefore, although multidisciplinary strategies are used in the treatment of multiple HCCs, multiple HCCs still pose a therapeutic challenge and are a matter of debate. There has been a lack of studies for the efficacy of surgical resection in patients with multiple HCCs.

Research frontiers

The survival of patients with HCCs was affected not only by HCC itself but also by underlying liver disease. Some studies have reported the superiority of surgical resection for multiple HCCs over non-surgical treatment. However, long-term outcomes of the patients according to not only stage but also the degree of underlying liver disease may help to select a proper treatment modality.

Innovations and breakthroughs

Their study demonstrates that surgery for patients with multiple HCCs is recommended in patients without clinical cirrhosis. When the survival of the patients in the surgical resection group was compared with the transarterial chemoembolization group according to the presence of cirrhosis, surgical resection showed no survival benefit in cirrhotic patients. This study is worthy because the surgical outcomes of the patients with multiple HCCs were compared according to the degree of underlying liver disease.

Applications

Liver cirrhosis is a well-known potent predictive factor for OS in patients with HCC. According to the analysis, the survival rates of cirrhotic patients with multiple HCCs who underwent surgical resection were extremely disappointing. Therefore, surgical resection for multiple HCCs is recommended for patients without cirrhosis but still debatable for patients with cirrhosis. Further multi-center trials and randomized, controlled, prospective studies are needed.

Peer review

This study provides clinical outcomes of surgical resection for multiple HCCs compared with the transarterial chemoembolization and liver transplantation. This issue is still debatable in patients with cirrhosis. The retrospective design and the small number of cases are limitations of this study.

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P- Reviewer Eghtesad B **S- Editor** Gou SX
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Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications

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Received: March 13, 2012 Revised: September 24, 2012

Accepted: September 29, 2012

Published online: January 21, 2013

Abstract

AIM: To compare the outcomes of concomitant cholangiocarcinoma (C-CCA) and subsequent cholangiocarcinoma (S-CCA) associated with hepatolithiasis.

METHODS: From December 1987 to December 2007, 276 patients underwent hepatic resection for hepatolithiasis in Changhua Christian Hospital. Sixty-five patients were excluded due to incomplete medical records and the remaining 211 patients constituted our study population base. Ten patients were diagnosed with C-CCA based on the preoperative biopsy or postoperative pathology. During the follow-up period, 12 patients developed S-CCA. The diagnosis of S-CCA was made by image-guided biopsy or by pathology if surgical intervention was carried out. Patient charts were reviewed to collect clinical information. Parameters such as CCA incidence, interval from operation to CCA diagnosis, interval from CCA diagnosis to disease-related death, follow-up time, and mortality rate were

calculated for both the C-CCA and S-CCA groups. The outcomes of the C-CCA and S-CCA groups were mathematically compared and analysed.

RESULTS: Our study demonstrates the clinical implications and the survival outcomes of C-CCA and S-CCA. Among the patients with unilateral hepatolithiasis, the incidence rates of C-CCA and S-CCA were fairly similar (4.8% vs 4.5%, respectively, $P = 0.906$). However, for the patients with bilateral hepatolithiasis, the incidence rate of S-CCA (12.2%) was higher than that of C-CCA (4.7%), although the sample size was limited and the difference between two groups was not statistically significant ($P = 0.211$). The average follow-up time was 56 mo for the C-CCA group and 71 mo for the S-CCA group. Regarding the average time intervals from operation to CCA diagnosis, S-CCA was diagnosed after 67 mo from the initial hepatectomy. The average time intervals from the diagnoses of CCA to disease-related death was 41 mo for the C-CCA group and 4 mo for the S-CCA group, this difference approached statistical significance ($P = 0.075$). Regarding the rates of overall and disease-related mortality, the C-CCA group had significantly lower overall mortality (70% vs 100%, $P = 0.041$) and disease-related mortality (60% vs 100%, $P = 0.015$) than the S-CCA group. For the survival outcomes of two groups, the Kaplan-Meier curves corresponding to each group also demonstrated better survival outcomes for the C-CCA group (log rank $P = 0.005$). In the C-CCA group, three patients were still alive at the time of data analysis, all of them had free surgical margins and did not have pathologically proven lymph node metastasis at the time of the initial hepatectomy. In the S-CCA group, only one patient had chance to undergo a second hepatectomy, and all 12 S-CCA patients had died at the time of data analysis.

CONCLUSION: C-CCA has better outcomes than S-CCA.

The first hepatectomy is crucial because most patients with recurrent CCA or S-CCA are not eligible for repeated surgical intervention.

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Key words: Hepatolithiasis; Intrahepatic duct stones; Recurrent pyogenic cholangitis; Cholangiocarcinoma; Concomitant cholangiocarcinoma; Subsequent cholangiocarcinoma

Lin CC, Lin PY, Chen YL. Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications. *World J Gastroenterol* 2013; 19(3): 375-380 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/375.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.375>

INTRODUCTION

Hepatolithiasis is the presence of stones in the intrahepatic duct (IHD) proximal to the confluence of the right and left main hepatic ducts. This disease is endemic in Southeast Asia and is also referred to as “IHD stones”, “recurrent pyogenic cholangitis”, “oriental cholangiohepatitis” and “Hong Kong disease” in the literature^[1-3]. Since Sanes and MacCallum pointed out the possible association between hepatolithiasis and cholangiocarcinoma (CCA) in 1942, numerous studies have reported similar observations and formulated theories about CCA pathogenesis^[4-7].

Currently, hepatic resection is frequently used for the definitive treatment of hepatolithiasis. Among patients who undergo hepatectomy for hepatolithiasis, approximately 5%-10% are incidentally found to have concomitant cholangiocarcinoma (C-CCA)^[8-10]. On the other hand, subsequent cholangiocarcinoma (S-CCA) may appear months to years after the initial hepatectomy. To the best of our knowledge, no previous study has reported on the clinical course and outcomes of S-CCA. During our daily practice, we observed that many patients with S-CCA progressed to death rather quickly. We wondered if patients with S-CCA really have worse outcomes than those with C-CCA. If this premise is true, it would be reasonable to treat hepatolithiasis more aggressively to minimize the deleterious consequences of S-CCA.

MATERIALS AND METHODS

Study design

From December 1987 to December 2007, a total of 276 patients underwent hepatic resection for hepatolithiasis in Changhua Christian Hospital. After excluding 65 patients with incomplete medical records, the remaining 211 patients constituted the base of our study. For each case, the diagnosis of hepatolithiasis was made by the means of image studies preoperatively (liver ultra-sonography, computed tomography, endoscopic retrograde cholangio-

pancreatography, or magnetic resonance cholangiopancreatography), which was then confirmed by postoperative pathologic examination. The C-CCA diagnosis was based on the preoperative biopsy or the postoperative pathologic examination. The S-CCA diagnosis was made by image-guided aspiration cytology, or by pathology if surgical intervention was carried out. By this assessment, 10 patients were diagnosed with C-CCA. During the follow-up period, 12 patients developed S-CCA (Figure 1).

Patient parameters evaluated

Patient charts were reviewed to collect clinical information. Parameters such as CCA incidence, interval from operation to CCA diagnosis, interval from CCA diagnosis to disease-related death, follow-up time, and mortality were calculated for both the C-CCA group and the S-CCA group. Finally, a mathematical comparison between the two groups was performed.

Statistical analysis

All data were recorded in a computerized database. Continuous variables are expressed as mean and range. A non-parametric test was used to examine the differences between the two groups. The Mann-Whitney *U* test was used for comparing continuous variables. Categorical variables were tested by the Person χ^2 test. Kaplan-Meier analysis with log rank test was used to compare survival between the two groups. *P* values less than 0.05 were considered statistically significant. The statistical analysis was performed with SPSS version 16.0 (Statistical Package for the Social Sciences; SPSS, Inc., Chicago, IL).

RESULTS

Patient clinical characteristics details are shown in Table 1. Among the patients with unilateral hepatolithiasis, the incidences of C-CCA and S-CCA were similar (4.8% *vs* 4.5%, respectively; *P* = 0.906). However, when we looked at bilateral hepatolithiasis, the C-CCA incidence (4.7%) was comparable with that for unilateral hepatolithiasis, but 12.2% of patients with bilateral hepatolithiasis developed S-CCA. However, the case number was too small to allow determination of a statistically significant difference (*P* = 0.211).

The average ages for the C-CCA group and the S-CCA group were 61 and 59 years old, respectively (*P* = 0.742). In both groups, the gender distribution were similar, and only approximately 10% of the patients were male (*P* = 0.892). After the patients were diagnosed with C-CCA, the average life span until disease-related death was 41 mo. On average, S-CCA is diagnosed after 67 mo from the initial hepatectomy. However, after the diagnosis is established, the average life span in these patients was only 4 mo. This difference between intervals from CCA to disease-related death approached statistical significance (*P* = 0.075).

The clinical outcomes of the C-CCA patients are summarized in Table 2. At the time of data analysis, 70% (7/10) of the C-CCA patients had died. Two of them

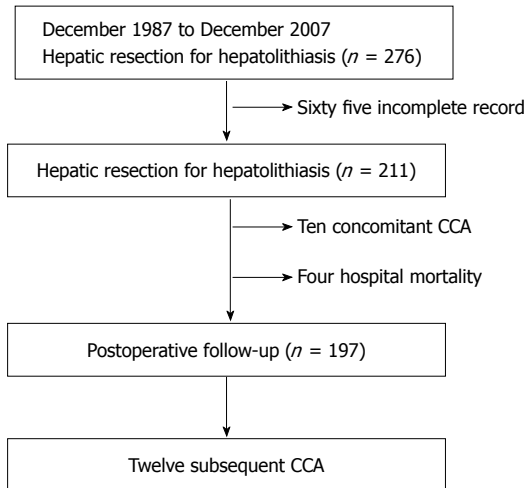


Figure 1 Schematic of the patient selection process. CCA: Cholangiocarcinoma.

Table 1 Clinical characteristics of patients

	C-CCA group	S-CCA group	P value
Incidence (%)	10/211 (4.7)	12/197 (6.1)	0.546
Unilateral stones	8/168 (4.8)	7/156 (4.5)	0.906
Bilateral stones	2/43 (4.7)	5/41 (12.2)	0.211
Age, yr (range)	61 (39-82)	59 (46-69)	0.742
Gender (%)			
Male	1 (10)	1 (8.3)	0.892
Female	9 (90)	11 (91.7)	
Mean interval from operation to CCA, mo (range)	-	67 (7-138)	-
Mean interval from CCA to disease-related death, mo (range)	41 (3-107)	4 (0-13)	0.075
Follow-up time, mo (range)	56 (2-140)	71 (7-144)	0.291
Mortality, n (%)			
Overall	7 (70)	12 (100)	0.041
Disease-related	6 (60)	12 (100)	0.015

C-CCA: Concomitant cholangiocarcinoma; S-CCA: Subsequent cholangiocarcinoma; CCA: Cholangiocarcinoma.

(cases 89 and 142) had regional lymph node metastasis at the time of hepatectomy. Three C-CCA patients (cases 119, 212 and 217) had positive surgical margins reported by pathology. One patient (case 227) had a free surgical margin and negative regional lymph nodes; however, she suffered from CCA recurrence at the common bile duct 4 years following the initial hepatectomy. After excision of the common bile duct (free surgical margin), she lived for 5 more years. One patient (case 128) had a free surgical margin and negative regional lymph nodes. However, a hypopharyngeal tumor was diagnosed 3 mo after the initial hepatectomy, and the patient finally expired due to acute respiratory failure. After excluding this last patient, the disease-related mortality was only 60% (6/10). The three patients (cases 90, 253 and 143) who were still alive share some common characteristics. They all had free surgical margins and did not have pathologically-proven lymph node metastasis at the time of the initial hepatectomy. Patients 90 and 253 had been completely disease-free for 140 and 49 mo, respectively. Patient 143 was a vic-

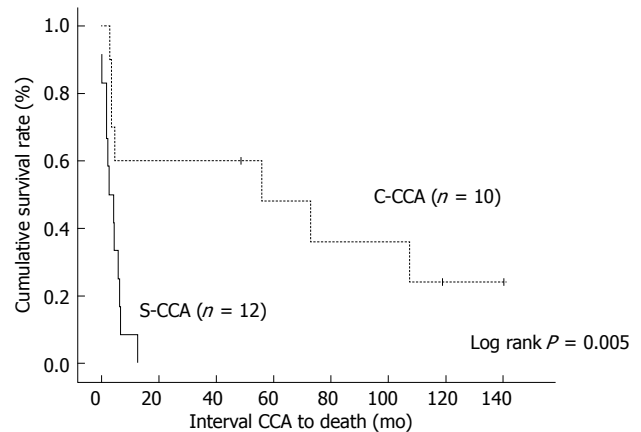


Figure 2 Comparison of survival rates between the concomitant cholangiocarcinoma group and the subsequent cholangiocarcinoma group. C-CCA: Concomitant cholangiocarcinoma; S-CCA: Subsequent cholangiocarcinoma; CCA: Cholangiocarcinoma.

tim of bilateral hepatolithiasis, and recurrent IHD stones were detected during the follow-up period. However, she refused treatment until recurrent CCA at the common bile duct was found 5 years following the initial hepatectomy. After excising the common bile duct (free surgical margin), she was disease-free for more than 43 mo. The C-CCA diagnosis was made pre-operatively in one patient (case 142), intra-operatively in four patients (cases 89, 128, 217 and 253), and post-operatively in five patients (cases 90, 119, 143, 212 and 227).

The clinical outcomes of S-CCA patients are summarized in Table 3. All 12 of the patients with S-CCA had died at the time of data analysis, resulting in a 100% overall, and disease-related, mortality. Among them, only one patient (case 11) had undergone a second hepatectomy. However, the surgical margin was positive for cancer and the patient expired 4 mo later.

Figure 2 shows the Kaplan-Meier curves corresponding to each group. The C-CCA group demonstrated clearly better survival outcomes than the S-CCA group ($P = 0.005$, log rank).

DISCUSSION

The S-CCA incidence in unilateral hepatolithiasis is 4.5%, which is comparable to that of C-CCA (4.8%). However, the S-CCA incidence in bilateral hepatolithiasis (12.2%) is more than double that of C-CCA incidence (4.7%). Our case number was too small for this difference to be reflected in statistical significance ($P = 0.211$). We believe, however, that this is quite possibly a true biological difference. This difference can be explained by recent studies that showed that hepatectomy for unilateral hepatolithiasis is associated with high stone clearance rates and low stone recurrence rates^[8,11-13]. However, with bilateral hepatolithiasis, there are still very few reports in the literature discussing the best management and long-term outcomes^[14]. According to a recent report and our unpublished data, treating bilateral hepatolithiasis with hepatic resection did

Table 2 Summary of patients with concomitant cholangiocarcinoma

Case	Age/sex	Stone location	Procedure	Residual/recurrent stones	Margin	pTNM	Interval from CCA to death (mo)
89	50/F	R	S5/6/7	-/-	Free	T1 N1 M0	3
90	68/F	L	LH	-/-	Free	T1 Nx M0	Alive (140)
119	82/F	L	LL	-/-	Atypical mucinous epithelium	T1 Nx M0	73
128	60/M	L	LH	-/-	Free	T1 N0 M0	3 (hypopharyngeal tumor)
142	39/F	L	LL	-/-	Free	T4 N1 M0	3
212	76/F	L	S2	-/-	Inadequate	T1 Nx M0	56
227	61/F	L	LH + RA	-/+	Free	T1 N0 M0	107 (re-operated 4 years later due to CCA recurrence at CBD)
253	72/F	L	LH	-/-	Free	T1 N0 M0	Alive (49)
143	50/F	BI	LH + S5	-/+	Free	T1 Nx M0	Alive (119) (re-operated 5 years later due to CCA recurrence at CBD)
217	56/F	BI	RH	+/-	Inadequate	T2a Nx M0	5

L: Left; R: Right; BI: Bilateral; LH: Left hemihepatectomy; LL: Left lateral sectionectomy; RH: Right hemihepatectomy; RA: Right anterior sectionectomy; S5: Segment 5; S2: Segment 2; CCA: Cholangiocarcinoma; CBD: Common bile duct; pTNM: Pathologic TNM stage for intrahepatic bile duct according to AJCC 7th edition; F: Female; M: Male.

Table 3 Summary of patients with subsequent cholangiocarcinoma

Case	Age/sex	Stone location	Procedure	Residual/recurrent stones	Interval from hepatectomy to S-CCA (mo)	Second hepatectomy for S-CCA	Interval S-CCA to death (mo)
1	56/F	L	LH	- / -	87	No	< 1
11	59/F	R	S5/6	- / -	91	Yes	4
						(with involved surgical margin)	
66	65/F	R	RH	- / +	114	No	6
69	61/F	R	RP	- / +	67	No	3
137	69/F	L	LL	- / -	95	No	2
203	60/F	L	LH	- / -	47	No	13
236	63/F	L	LL	- / -	7	No	< 1
37	46/F	BI	LL	- / -	138	No	6
96	65/F	BI	LL + S6	- / -	115	No	2
157	53/F	BI	LL + S6	- / -	19	No	4
159	63/F	BI	LL	+ / -	19	No	6
163	50/M	BI	LH	+ / -	10	No	2

L: Left; R: Right; BI: Bilateral; S6: Segment 6; LH: Left hemihepatectomy; LL: Left lateral sectionectomy; RH: Right hemihepatectomy; RP: Right posterior sectionectomy; S-CCA: Subsequent cholangiocarcinoma; F: Female; M: Male.

not show encouraging results as did resection in unilateral disease^[15]. We speculate that hepatic resection seems to be less effective in stopping the disease progression toward S-CCA in patients with bilateral hepatolithiasis.

This study showed clearly that the C-CCA group had lower overall mortality ($P = 0.041$) and disease-related mortality ($P = 0.015$) than the S-CCA group. This better survival outcome is demonstrated by the Kaplan-Meier curves (Figure 2, $P = 0.005$, log rank). Considering that the complete removal of the malignancy with a free surgical margin is essential for achieving good survival outcomes^[16-18], the role of the first hepatectomy is definitely crucial. Paradoxically, preoperative C-CCA diagnosis is sometimes difficult due to the presence of IHD stones^[19-21]. Among our 10 C-CCA patients, only one patient was diagnosed pre-operatively. In order to solve this problem, making good use of intraoperatively-collected frozen sections is recommended. In the case of a strongly suspected C-CCA in the absence of conclusive tissue proof, carrying out the hepatectomy aggressively can increase the likelihood of creating a safe surgical margin.

For the five patients (cases 90, 119, 143, 212 and 227) whose C-CCA diagnoses were made incidentally by post-operative pathology, the survival outcomes were good if they had safe surgical margins (cases 90, 143 and 227). The lymph node status is also an important prognostic factor for these patients; however, these data were incomplete because regional lymph node dissection was not routinely performed on these patients.

All 12 S-CCA patients died shortly after definitive S-CCA diagnosis. A possible explanation is that most patients at the time of S-CCA diagnosis are not eligible for a second hepatectomy due to distant metastasis, locally advanced disease, peritoneal seeding, or insufficient remaining liver volume. Only one S-CCA patient in our series (case 11) had the opportunity to undergo a second hepatectomy. However, the tumor could not be removed completely, and the patient died 4 mo after the operation.

The interval from the initial hepatectomy to S-CCA development ranged from 7 to 138 mo (average 67 mo). After hepatic resection for hepatolithiasis, we observed that in patients who presented residual or recurrent IHD

stones, the S-CCA incidence (11.8%, 4/34) was higher than in S-CCA patients who had achieved complete stone clearance and disease-free status after hepatectomy (4.9%, 8/163). However, 67% of S-CCA patients (8/12) had neither residual nor recurrent IHD stones. We can presume that undiagnosed CCA might be left in the liver remnant, resulting in S-CCA, especially for patients who developed S-CCA relatively soon after the initial hepatectomy (e.g., cases 157, 159, 163 and 236). Interestingly, however, one patient (case 37) had neither residual nor recurrent IHD stones but ultimately developed S-CCA after a follow-up period of 138 mo. Beyond the presence of residual and recurrent IHD stones, we should also consider other predisposing factors for the development of S-CCA such as cancer-related genes, female gender, choledochenterostomy, liver atrophy, smoking, family history, and viral hepatitis infection^[22-26].

This retrospective study is the first published investigation that subdivided and compared hepatolithiasis-related cholangiocarcinoma in concomitant and subsequent groups. The study design included patients who underwent hepatic resection for hepatolithiasis during the past two decades (1987 to 2007) in Changhua Christian Hospital. Unexpectedly, incomplete information in 65 patient medical records was noticed during data collection, forcing us to exclude these patients. The majority of the excluded patients were treated in the first decade (1987 to 1997). However, all CCA patients in our study were operated on after 1997. We believe that these missing records should cause minimal effect on our results, although the data collected in our study were skewed towards the second decade (1997 to 2007). Another limitation of this study could be the relatively small number of enrolled CCA patients. However, considering that the C-CCA incidence is only 5%-10% in our patient population, the difficulty in obtaining very large patient numbers is appreciable.

We want to reemphasize the importance of a successful first hepatectomy, because most patients with recurrent CCA or that develop S-CCA are not candidates for repeated surgical intervention.

COMMENTS

Background

Hepatolithiasis is the presence of stones in the intrahepatic duct (IHD) proximal to the confluence of the right and left main hepatic ducts. This disease is endemic in Southeast Asia and is also referred to as "IHD stones", "recurrent pyogenic cholangitis", "oriental cholangiohepatitis" and "Hong Kong disease" in the literature.

Research frontiers

Concomitant cholangiocarcinoma (C-CCA) has better outcomes than subsequent cholangiocarcinoma (S-CCA). The first hepatectomy is crucial because most patients with recurrent CCA or S-CCA are not eligible for repeated surgical intervention.

Innovations and breakthroughs

Parameters such as cholangiocarcinoma (CCA) incidence, interval from operation to CCA diagnosis, interval from CCA diagnosis to disease-related death, follow-up time, and mortality rate were calculated for both the C-CCA and S-CCA groups. The outcomes of the C-CCA and S-CCA groups were mathematically compared and analysed.

Peer review

C-CAs associated to hepatolithiasis is a crucial problem in hepatobiliary medicine. The authors can heal well unilateral hepatolithiasis today, but there are not effective methods in a treatment of bilateral hepatolithiasis. S-CAs associated to hepatolithiasis is a larger problem than unilateral hepatolithiasis. The long-term outcomes are bad. The authors show the average life span only 4 mo. Literature's data are similar. The subject is very interesting, rarely discussed in the literature, and the period of observation and follow-up are commendable. Manuscript is well written. Data table are well-documented. References is appropriately selected.

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P- Reviewers O'Dwyer P, Smigielski JA **S- Editor** Gou SX
L- Editor A **E- Editor** Lu YJ



Acupuncture transcutaneous electrical nerve stimulation reduces discomfort associated with barostat-induced rectal distension: A randomized-controlled study

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Received: May 29, 2012 Revised: September 5, 2012

Accepted: September 22, 2012

Published online: January 21, 2013

Abstract

AIM: To explore the effectiveness of acupuncture transcutaneous electrical nerve stimulation (Acu-TENS), a non-invasive modality in reduction of rectal discomfort during barostat-induced rectal distension.

METHODS: Forty healthy subjects were randomized to receive 45 min of either Acu-TENS or placebo-TENS (no electrical output) over acupuncture points *Hegu* (large-intestine 4), *Neiguan* (pericardium 6) and *Zusanli* (stomach 36). A balloon catheter attached to a dual-drive barostat machine was then inserted into the subjects' rectum. A step-wise (4 mmHg) increase in balloon pressure was induced until maximal tolerable or 48 mmHg. Visual analogue scale and a 5-point

subjective discomfort scale (no perception, first perception of distension, urge to defecate, discomfort/pain and extreme pain) were used to assess rectal discomfort at each distension pressure. Blood beta-endorphin levels were measured before, immediately after intervention, at 24 mmHg and at maximal tolerable distension pressure.

RESULTS: There was no difference in the demographic data and baseline plasma beta-endorphin levels between the two groups. Perception threshold levels were higher in the Acu-TENS group when compared to the placebo group, but the difference reached statistical significance only at the sensations "urge to defecate" and "pain". The distension pressures recorded at the "urge to defecate" sensation for the Acu-TENS and placebo-TENS groups were 28.0 ± 4.5 mmHg and 24.6 ± 5.7 mmHg, respectively ($P = 0.043$); and the pressures recorded for the "pain" sensation for these two groups were 36.0 ± 4.2 mmHg and 30.5 ± 4.3 mmHg respectively ($P = 0.002$). Compared to the placebo group, a higher number of participants in the Acu-TENS group tolerated higher distension pressures (> 40 mmHg) (65% in Acu-TENS vs 25% in placebo, $P = 0.02$). The plasma beta-endorphin levels of the Acu-TENS group were significantly higher than that of the placebo group at barostat inflation pressure of 24 mmHg (1.31 ± 0.40 ng/mL vs 1.04 ± 0.43 ng/mL, $P = 0.044$) and at maximal inflation pressure (1.46 ± 0.53 ng/mL vs 0.95 ± 0.38 ng/mL, $P = 0.003$).

CONCLUSION: Acu-TENS reduced rectal discomfort during barostat-induced rectal distension and concurrently associated with a rise in beta-endorphin level.

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Key words: Colonoscopy; Rectal discomfort; Transcutaneous electrical nerve stimulation; Acupuncture; Visceral pain

Leung WW, Jones AYM, Ng SSM, Wong CYN, Lee JFY. Acupuncture transcutaneous electrical nerve stimulation reduces discomfort associated with barostat-induced rectal distension: A randomized-controlled study. *World J Gastroenterol* 2012; 19(3): 381-388 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/381.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.381>

INTRODUCTION

Early colonoscopy screening is encouraged in prevention of development of colorectal cancer^[1]. However, the procedure is often associated with abdominal pain and discomfort^[2]. The unpleasant feeling during examination may affect the patient's overall tolerance and thereby jeopardizing the accuracy of outcome findings. A combination of narcotic analgesia and benzodiazepines is often used to decrease the discomfort during colonoscopy^[3,4]. However these medications are also associated with gastrointestinal upset and although uncommon, respiratory and cardiac arrests during colonoscopy were reported^[5,6]. Report of a European colorectal cancer screening project revealed that more than 50% of the subjects developed hypoxaemia and 6% had severe bradycardia after colonoscopy with sedation^[7].

Electroacupuncture (EA) is widely accepted in China and is considered a possible treatment option for acute and chronic pain of various origins^[8-10]. The role of acupuncture in managing pain and anxiety during colonoscopy however is unclear. A randomized sham-controlled study^[11] suggested that patients receiving EA to acupoints *Zusanli* (stomach meridian ST-36) and *Hegu* (large intestine meridian LI-4) experienced lower pain level during colonoscopy than those receiving sham acupuncture (SA), but the difference was non-significant statistically. Recently we have shown that application of EA to *Hegu* (large-intestine 4), *Neiguan* (pericardium 6) and *Zusanli* (stomach 36) was able to effectively reduce rectal discomfort during Barostat-induced rectal distension^[12]. Acupuncture however is invasive, and its application requires an experienced acupuncturist. Application of transcutaneous electrical nerve stimulation (TENS) over acupuncture points (Acu-TENS) is a non-invasive modality and a novel analgesic therapy that combines the advantages of acupuncture and TENS in management of painful conditions^[13,14]. Acu-TENS has been shown to be effective in reducing postoperative analgesic requirement^[14]. This pilot study aims to explore the potential of Acu-TENS in reduction of visceral pain. Prior to investigation of its role at the actual colonoscopy procedure, where the intraluminal pressures often varied in different subjects, it is necessary to first establish its role using a model which standardizes the intensity and duration of the stimulation. A dual drive barostat device was therefore used to mimic the discomfort caused by gaseous distension during colonoscopy, such model allows the nature of discomfort,

distension pressure and duration of stimulation to be standardized.

MATERIALS AND METHODS

Subjects

Hong Kong Chinese subjects with age between 18 to 65 years registered for a screening colonoscopy examination (a free health care service provided by the Hong Kong Hospital Authority for all local permanent residents with family history of colorectal cancer) were invited to participate in the study prior to the actual colonoscopy screening procedure. Exclusion criteria included known cardiovascular dysfunction (American Society of Anaesthesiologists grade III or above), irritable bowel syndrome (Rome II classification^[15]), renal impairment, previous abdominal surgery or experience of colonoscopy, gastrointestinal complaints, pregnancy or allergy to acupuncture needles. Subjects who met the inclusion criteria were randomized to either the Acu-TENS or placebo Acu-TENS (PA) group. The respective intervention code was generated randomly by a computer program (Random Allocation Software, Version 1.0, Isfahan University of Medical Sciences, Iran). The group allocation numbers were then placed in sealed opaque envelopes. An envelope was drawn and allocated to each subject by a secretary blinded to the groupings. The envelope was opened by the investigator who then applied the identified intervention accordingly. The subjects, surgeons and all other parties, including an independent outcome assessor, were blinded to the implementation of Acu-TENS or PA.

Bowel preparation

All subjects were instructed to have low fiber diet three days prior to the study. On the day before examination, all subjects underwent standard mechanical bowel preparation with 4 liters of polyethylene glycol (Klean-Prep, Norgine Ltd., Middlesex, United Kingdom) to induce bowel movement and to clear the bowel in preparation for the colonoscopy procedure. Participants were asked to empty their bowel at home before coming to the hospital for examination.

Equipment

A dual-lumen polyethylene catheter (Model CR3-0005, Mui Scientific, Mississauga, Ontario, Canada) was attached to a 10 cm long and 600 mL capacity polyethylene bag (Model CT-BP600R, Mui Scientific) using a sterile surgical silk suture (Ethicon, Mersilk Soie, Somerville, New Jersey, United States), with the surgical knots sealed by latex glue. One lumen of the catheter allowed passage of compressed air for bag inflation and the other was attached to a transducer for monitoring of the pressure inside the bag. The catheter was attached to the dual drive barostat (Distender Series II; G and J Electronics, Inc., Toronto, Canada) which controlled the rate of inflation and deflation of the bag electronically. To ensure

the bag attachment was airtight, the bag was inflated to 60 mmHg for 5 min and placed in sterile warm water to ensure there was no leakage of air from the system.

Intervention protocols

Acupuncture points *Hegu* (large-intestine 4), *Neiguan* (pericardium 6) and *Zusanli* (stomach 36) were first identified by the investigator before the procedure. LI 4 is located on the dorsum of the hand, between the 1st and 2nd metacarpal bones; Pericardium 6 is located on the palmar aspect of the forearm, 2 “cuns” above the transverse crease of the wrist between the flexor carpi radialis and palmaris longus tendons; ST 36 is located on the anterior aspect of the leg, at 3 cuns below the knee cap and one finger-breadth from the anterior crest of the tibia. One “cun” is the distance between the interphalangeal creases of the subject’s middle finger^[16]. These points were chosen because large-intestine 4 and pericardium 6 are reportedly used to reduce abdominal pain^[17,18] and ST36 was shown to regulate colorectal muscle contractility in conscious rats^[19,20]. All of the selected acupuncture points were swabbed with alcohol following a standard antiseptic process before the TENS electrodes were applied. Six gel pads were cut into size of 5 mm × 5 mm and placed over the subject’s acupuncture points (bilaterally). Each cleaned rubber electrode was placed over the small gel pad (HD-001, ITO Company Limited, Tokyo, Japan), this allowed electrical stimulation to be focused mainly over the acupuncture points. The electrodes were then attached to a dual channel TENS machine (Model 120Z, ITO Company Limited, Tokyo, Japan). Subjects in the Acu-TENS group received a constant mode of electrical stimulation at 2 Hz and pulse width at 200 μ s for 45 min. Intensity was set to just initiate muscle contraction. This stimulation protocol has been shown to be effective in inducing endorphin production^[21,22]. Electrode placements were similar for subjects in the PA group, except that the electrical output from the TENS unit was disconnected. The output light however remained active and subjects were told that they may or may not feel the current.

Experimental procedures

All subjects arrived at the laboratory at 9 am on the assessment day. They were instructed to rest in the supine position for 15 min and randomized to receive 45 min of either Acu-TENS or PA in the supine position. The subject then adopted a left side-lying position with both knees flexed to 90°. The polyethylene bag which attached to the barostat was slowly inserted into subject’s rectum to a distance 10 cm from the anal verge. The tube was taped to the buttock with micropores and the subject then resumed the supine position. After rested in this position for 15 min the bag was inflated for 60 s and then completely deflated in 60 s, a resting period of 1 minute then followed, this constituted one complete cycle of inflation. An incremental stepwise increase in rectal pressure at 4 mmHg was instilled at subsequent

cycles until a pressure of 48 mmHg or when the subject could not tolerate further discomfort. Acu-TENS or PA continued during the whole barostat procedure. When the maximal 48 mmHg or an intolerable pressure was reached, the stimulation was discontinued and all electrodes and gel pads removed.

Outcome measures

Subjective discomfort scale: During each 60-s phase of sustained incremental pressure, the subject was asked to rate their rectal sensation using an electronic panel attached to a computer. Ratings were “no perception”, “first perception of distension”, “urge to defecate”, “discomfort or pain” and “extreme pain”. Concurrently, each subject was also asked to rate the degree of rectal discomfort using a visual analogue scale (VAS)-a 10 cm ungraduated line, with words “no discomfort at all” anchored to “0” end and “discomfort cannot be tolerated” anchored to the number “10”. The VAS and subjective discomfort scales were recorded by an independent research assistant who was blinded to the intervention allocation.

Beta-endorphin measurement: Venous blood (3 mL) was drawn (from a cannula inserted into the cubital vein of each subject under aseptic technique) before the randomization process, immediately after the 45 min intervention, at a distension pressure of 24 mmHg and at highest tolerable pressure. The blood samples were transferred to the biochemistry laboratory of the involved hospital in EDTA tubes stored in a 4 °C ice box. The blood sample was then centrifuged and frozen until further assayed. Batch analyzed for beta-endorphin (Human) were analyzed using EIA kit (Phoenix Pharmaceuticals Inc, 330 Beach Road, Burlingame, CA94010, United States). All subjects then undertook the colonoscopy screening procedure in the same afternoon following the barostat study.

Sample size estimation

Sample size estimation was based on the assumption that Acu-TENS could increase the mean pain threshold by 8 mmHg (this was based on our previous work which explored the effect of acupuncture in relief of discomfort induced by the barostat^[12]). To yield a power of 80% with a significant level of 0.05, a sample size of at least 17 subjects in each group was required (sample size estimation was determined by SPSS Version 15.0 for Windows, SPSS Inc., Chicago, IL, United States).

Ethical approval

This study was conducted at a local district hospital from September 2009 to May 2010. The protocol was approved by the Ethics Review Committees of the involved hospital and ClinicalTrials.gov prior to data collection (Approval numbers: NCT01551654 and CRE-2008.546-T). Written informed consent was obtained from each subject prior to commencement of the study.

Table 1 Demographic data of subjects in the transcutaneous electrical nerve stimulation over acupuncture points and placebo transcutaneous electrical nerve stimulation over acupuncture points groups (mean \pm SD)

Item	Acu-TENS group (<i>n</i> = 20)	PA group (<i>n</i> = 20)	<i>P</i> value
Age (yr)	53.4 \pm 3.8	53.9 \pm 3.5	0.669
Gender (male/female)	8/12	8/12	1.000
Body weight (kg)	63.9 \pm 14.4	63.0 \pm 9.35	0.825
Body height (m)	1.62 \pm 0.07	1.61 \pm 0.07	0.692
BMI(kg/m ²)	23.9 \pm 3.61	24.0 \pm 2.53	0.899

Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points; PA: Placebo transcutaneous electrical nerve stimulation over acupuncture points; BMI: Body mass index.

Table 2 Number of subjects in the transcutaneous electrical nerve stimulation over acupuncture points and placebo transcutaneous electrical nerve stimulation over acupuncture points groups identified with colonic polyps during the colonoscopy screening procedure

Location of polyps	Acu-TENS group (<i>n</i> = 20)	PA group (<i>n</i> = 20)
Right side (caecum, ascending colon, hepatic flexure, transverse colon)	4	3
Left side (splenic flexure, descending colon, sigmoid)	1	1
Rectosigmoid junction	0	0
Rectum	0	0
No abnormality	15	16

Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points; PA: Placebo transcutaneous electrical nerve stimulation over acupuncture points.

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS Version 15.0 for Windows, SPSS Inc., Chicago, IL, United States). Repeated measures ANOVA were used to determine the change in sensation at different bag inflation pressures. Between-group sensation at each pressure was compared using independent sample *t* test for parametric data, and Pearson χ^2 test or Mann-Whitney *U* test for nonparametric data. A statistical significant level was set at *P* < 0.05.

RESULTS

A total of 40 healthy subjects (16 male, 24 female; mean age 53.7 \pm 3.62 years) were recruited to the study with 20 subjects randomized to either the Acu-TENS or PA group (Figure 1). The demographic data of all subjects were similar between the two groups (Table 1). All subjects completed the procedure and no adverse effects were observed during the intervention. Results of the colonoscopy screening procedure for this subject cohort are displayed in Table 2. While colonic polyps were found in some subjects, none were identified with rectal polyps or lesions. The pressures at which the subjects

Table 3 Perception threshold, distension pressures, levels of blood beta-endorphin in transcutaneous electrical nerve stimulation over acupuncture points and placebo transcutaneous electrical nerve stimulation over acupuncture points groups (mean \pm SD)

	Acu-TENS	PA	<i>P</i> value
Perception threshold (mmHg)			
First perception of distension	18.4 \pm 4.2	16.8 \pm 6.4	0.358
Urge to defecate	28.0 \pm 4.5	24.6 \pm 5.7	0.043
Discomfort or pain	36.0 \pm 4.2	30.5 \pm 4.3	0.002
Extreme pain	40.0 \pm 6.7	35.4 \pm 5.6	0.179
Pressure (<i>n</i> = 20)			
40 mmHg	17 (85)	10 (50)	0.020
44 mmHg	13 (65)	3 (15)	0.001
48 mmHg	11 (55)	2 (10)	0.022
Levels of blood beta-endorphin (ng/mL)			
Baseline	1.04 \pm 0.35	1.22 \pm 0.52	0.216
After 45 min of intervention	1.14 \pm 0.41	1.13 \pm 0.48	0.980
At 24 mmHg pressure	1.31 \pm 0.40	1.04 \pm 0.43	0.044
At maximal tolerable pressure or 48 mmHg	1.46 \pm 0.53	0.95 \pm 0.38	0.003

Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points; PA: Placebo transcutaneous electrical nerve stimulation over acupuncture points.

reported “urge to defecate” and “discomfort or pain” sensations were significantly higher in the Acu-TENS group compared to the PA group (Table 3). Furthermore, there were more subjects in the Acu-TENS group who tolerated a higher rectal distension pressures (at 40 mmHg, 44 mmHg and 48 mmHg) (*P* < 0.05) (Table 3). At each recorded pressure, the VAS score reported by the Acu-TENS group was lower (Figure 2A) although the differences did not reach a statistical significant level. The mean maximal tolerable pressure recorded during Acu-TENS (45.6 \pm 5.09 mmHg) was higher than that during PA (39.8 \pm 7.62 mmHg) (*P* = 0.007). The beta-endorphin level raised after 45 min Acu-TENS and continued to rise with increased inflation pressure (Table 3). Changes in beta-endorphin level in the PA group were not apparent. At 24 mmHg and maximal tolerable pressure, the beta-endorphin levels measured were significantly higher in the Acu-TENS when compared with the PA group (Figure 2B and Table 3).

DISCUSSION

To the best of the authors’ knowledge, this is the first report on the effect of Acu-TENS in reduction of rectal discomfort in human subjects. This study demonstrated Acu-TENS to large-intestine 4, pericardium 6 and stomach 36 reduced discomfort associated with rectal distension induced by a barostat. TENS is a non-invasive physical therapeutic modality commonly used for pain relief in conditions such as osteoarthritis and low back pain^[23]. Although widely used in clinical situations, the working mechanism of TENS remains unclear. The pain-gate hypothesis proposed by Melzack and Wall^[24] is considered a possible mechanism of control of stimulation

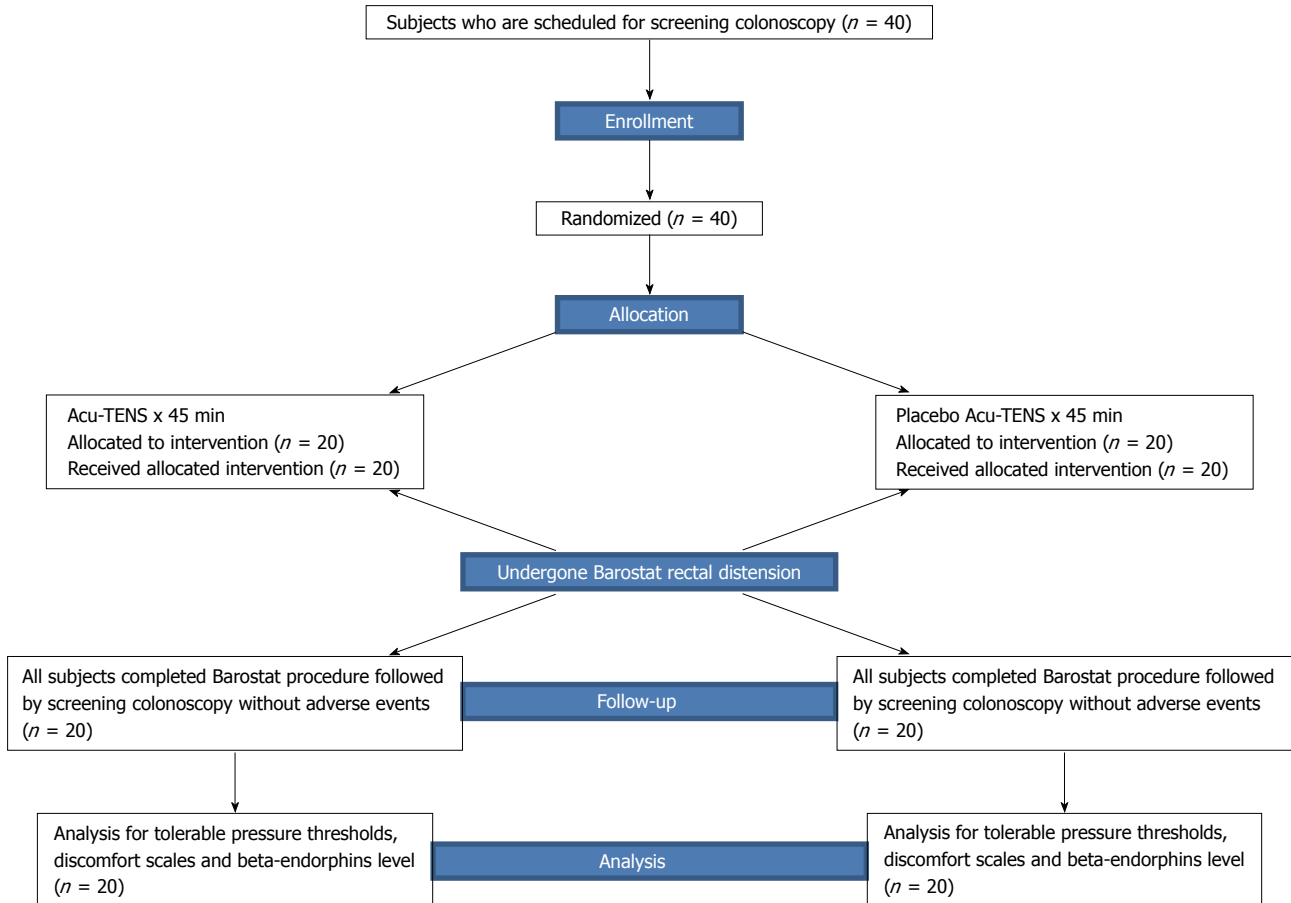


Figure 1 Flow diagram of the study. Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points.

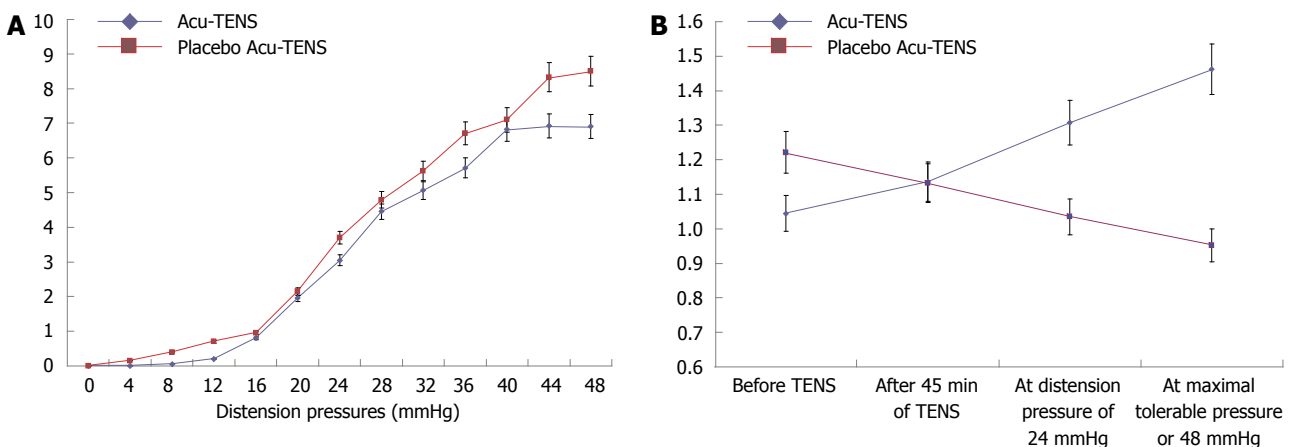


Figure 2 Visual analog discomfort score recorded at different distension pressures (A) and mean beta-endorphin levels recorded at different time-points (B). Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points.

pathways during TENS. It was also believed that TENS inhibits pain signals via the descending pathway^[25,26], the dorsal horn cell, and the spinothalamic tract^[27]. Another prevalent theory discussed in literature is the release of endogenous opioids such as beta-endorphins, enkephalins and dynorphin after TENS^[28-30]. Acu-TENS was previously shown to increase beta-endorphin levels in subjects with chronic respiratory disease^[31]; finding of this current study further confirms that application of 45 min Acu-

TENS induced the release of beta-endorphins in subjects with rectal discomfort. Our Acu-TENS protocol adopts a low stimulation frequency at 2 Hz. It was believed that low frequency TENS was able to trigger μ - and delta opioid receptors, the correspondents of beta-endorphin^[29,31]. On the other hand, high frequency (100 Hz) stimulation was believed to trigger κ -opioid receptor and results in the release of dynorphin^[29,31]. Irrespective of the working mechanisms, this study has shown that Acu-TENS

appears to be able to reduce the rectal distension pain induced by the barostat model and concurrently associated with an increase in beta-endorphin level. It was hypothesized that beta-endorphin possibly suppresses the action potential of neurons during neural transmission through the release of excess sodium molecules, consequently leading to inhibition of transmission of pain signals^[32]. This is the first study which reports the effect of Acu-TENS on visceral pain in humans. We have previously demonstrated that acupuncture was an effective modality in reducing rectal discomfort associated with barostat induced rectal distension^[12]. This study has shown that similar effect can be achieved non-invasively using Acu-TENS. The main difference in findings between our previous acupuncture study and this current one is that while the level of beta-endorphin increased immediately after 45 min of Acu-TENS, the increase reached a statistical significant level at 90 min after stimulation. However, in our previous study, the difference in change of beta-endorphin level between acupuncture and placebo acupuncture was significant at immediately after 45 min of acupuncture stimulation. This probably is associated with the fact that one of the acupoints adopted in our studies, ST36, is situated in a much deeper anatomical position^[33] and because Acu-TENS works in a “transcutaneous” manner, more time is therefore required for Acu-TENS to achieve the expected effect. Interestingly the level of beta-endorphin remained high for a longer period of time after Acu-TENS when compared with acupuncture stimulation. Acupuncture points large-intestine 4, pericardium 6 and stomach 36 were selected in this study because stimulation of *Hegu* (large-intestine 4) was associated with excitation of sensory nerve fibers in the pelvic complex and induced regulation of pelvic floor muscle contraction^[34]. Stimulation of large-intestine 4 was also shown to be effective in management of labor pain and plantar fasciitis^[34,35]. Stimulation of *Neiguan* (pericardium 6) and *Zusanli* (stomach 36) were reported to have significant clinical results in management of gastrointestinal symptoms^[36,37]. Apart from being a non-invasive modality, another advantage of employing Acu-TENS over acupuncture is the low costing involved in Acu-TENS. The TENS machine is inexpensive, the Acu-TENS procedure is simple and experienced acupuncturists are not required for its application. Furthermore, apart from allergy to the gel used in a very minority of subjects, no side effects were reported with the use of Acu-TENS.

This study showed that while a higher distention pressure was tolerated during Acu-TENS, the difference at the VAS scores at any pressure level however did not reach a statistical difference. This could possibly be a consequence of both the slow release of endorphin during Acu-TENS, as well as the small sample size of this study. However convincing subjects who signed up for colonoscopy screening to pay an extra visit to the theatre for experimental recording of discomfort induced by a barostat machine was deemed demanding by many subjects. Another limitation of this study is that only Acu-TENS and placebo groups were included in the study, the

strength of the placebo effect cannot be accurately evaluated without inclusion of a control group of no intervention. Inclusion of a control group however requires increasing the sample size, and as previously explained, this was difficult to comply. This study used the barostat model to mimic rectal discomfort. Previous reports on the use of the barostat model were mainly on patients with irritable bowel syndrome^[38]. The barostat-induced rectal discomfort cannot accurately reproduce the same painful sensation as the stretching of mesenteric tissues during actual colonoscopy procedure. However, gaseous distension during colonoscopy also creates visceral pain and discomfort^[39]. Our previous experience suggests that using the barostat model to mimic sensation of gaseous distension during colonoscopy appears to be a good model for evaluation of pain and distension discomfort under a standardized, controllable environment.

In conclusion, this study showed that 45 min of Acu-TENS over large-intestine 4, pericardium 6 and stomach 36, compared to placebo Acu-TENS, appeared to reduce the level of rectal discomfort induced by barostat distension and allowed the subjects to withstand a higher distension pressure. Subjective toleration of a higher inflation pressure was shown to be accompanied with a significant increase in beta-endorphin level. The role of Acu-TENS in application during clinical colonoscopy warrants further investigation.

COMMENTS

Background

Colonoscopy procedure is often associated with abdominal pain and discomfort. Application of transcutaneous electrical nerve stimulation over acupoints (Acu-TENS) is a non-invasive treatment modality for management of musculoskeletal pain, but its role for pain relief in endoscopic procedure has not been investigated.

Research frontiers

TENS has extensively been used as a physical modality for pain relief for musculoskeletal condition, however, the mechanism of action remains unclear. Beta-endorphin is a natural painkiller and largely found in the hypothalamus and pituitary gland. In this study, employing the barostat, an electronic device as an experimental pain model to standardize and mimic visceral pain caused by gaseous distension, the authors demonstrated that application of Acu-TENS is associated with the release of beta-endorphin effecting pain relief.

Innovations and breakthroughs

Endoscopic procedure is often associated with abdominal pain and discomfort, especially when there is increased gaseous distension. The unpleasant feeling during examination may affect the patient's overall tolerance and thereby jeopardizing the accuracy of outcome findings. Electroacupuncture is considered worldwide as a possible treatment option for acute and chronic pain of various origins, however, acupuncture is invasive and require an experienced acupuncturist. Acu-TENS, on the other hand, is a novel non-invasive modality that combines the advantage of TENS and acupuncture in the management of acute painful conditions.

Applications

Findings of this study suggest that Acu-TENS is potentially a therapeutic, non-invasive physical modality that could reduce rectal discomfort during an endoscopic procedure. The role of Acu-TENS during clinical colonoscopy warrants further investigation.

Terminology

Acu-TENS is the application of transcutaneous electrical nerve stimulation over acupuncture points. Visceral pain is an unpleasant sensation as a result of activation of nociceptors of the abdominal organs. Such type of pain is associated

with distension of the visceral tissue and often described as diffuse and difficult to localize. The electronic barostat served as an experimental pain model in this study to standardize and mimic visceral pain caused by gaseous distension.

Peer review

This is an interesting and well-designed study showing that Acu-TENS, a non-invasive analgesic therapy, reduced rectal discomfort during barostat-induced distensions. Moreover, reduction of pain was accompanied with a significant increase in beta-endorphin level, implying a key-role of endorphins in the mechanism of TENS.

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P- Reviewers Ladas S, Annese V **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



Preoperative carcinoembryonic antibody is predictive of distant metastasis in pathologically T1 colorectal cancer after radical surgery

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Supported by Changhai Hospital 1255 Project Fund, No. CH125542500

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Received: September 19, 2012 Revised: December 12, 2012

Accepted: December 22, 2012

Published online: January 21, 2013

Abstract

AIM: To identify the predictors of distant metastasis in pathologically T1 (pT1) colorectal cancer (CRC) after radical resection.

METHODS: Variables including age, gender, preoperative carcinoembryonic antibody (CEA) level, tumor location, tumor size, lymph node status, and histological grade were recorded. Patients with and without metastasis were compared with regard to age, gender, CEA level and pathologic tumor characteristics using the independent *t* test or χ^2 test, as appropriate. Risk factors were determined by logistic regression analysis.

RESULTS: Metastasis occurred in 6 (3.8%) of the 159 patients during a median follow-up of 67.0 (46.5%) mo. The rates of distant metastasis in patients with pT1 cancer of the colon and rectum were 6.7% and 2.9%, respectively ($P < 0.001$). The rates of distant metastasis between male and female patients with T1 CRC

were 6.25% and 1.27%, respectively ($P < 0.001$). The most frequent site of distant metastasis was the liver. Age ($P = 0.522$), gender ($P = 0.980$), tumor location ($P = 0.330$), tumor size ($P = 0.786$), histological grade ($P = 0.509$), and high serum CEA level ($P = 0.262$) were not prognostic factors for lymph node metastasis. Univariate analysis revealed that age ($P = 0.231$), gender ($P = 0.137$), tumor location ($P = 0.386$), and tumor size ($P = 0.514$) were not risk factors for distant metastasis after radical resection for T1 colorectal cancer. Postoperative metastasis was only significantly correlated with high preoperative serum CEA level ($P = 0.001$). Using multivariate logistic regression analysis, high preoperative serum CEA level ($P = 0.004$; odds ratio 15.341; 95%CI 2.371-99.275) was an independent predictor for postoperative distant metastasis.

CONCLUSION: The preoperative increased serum CEA level is a predictive risk factor for distant metastasis in CRC patients after radical resection. Adjuvant chemotherapy may be necessary in such patients, even if they have pT1 colorectal cancer.

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Key words: Colorectal cancer; Risk factor; Metastasis; Pathologically T1; Carcinoembryonic antigen

Lou Z, Meng RG, Zhang W, Yu ED, Fu CG. Preoperative carcinoembryonic antibody is predictive of distant metastasis in pathologically T1 colorectal cancer after radical surgery. *World J Gastroenterol* 2013; 19(3): 389-393 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/389.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.389>

INTRODUCTION

Colorectal cancer (CRC) is one of the most common ma-

lignancies and a leading cause of cancer-related deaths in Europe and the United States^[1-3]. Similarly, it is the fifth leading cause of cancer deaths in China and the incidence of CRC is rapidly increasing^[4,5].

The standard surgical treatment for CRC is radical resection. Recent advances in diagnostic methods have led to an increase in the detection of T1 CRC^[6,7]. Local excision has been substituted for radical resection in some patients with early CRC. However, local excision may result in a higher rate of local recurrence than radical resection in patients with T1 CRC. Local excision leaves metastatic lymph nodes in 8%-15% of pathologically T1 (pT1) CRC patients without definitively retrieving regional lymph nodes^[8], resulting in a recurrence rate of 4.1%-39%^[9]. However, the recurrence rate has been reported to be 1.3%-2.8% after radical resection of early CRC^[6,10]. To minimize recurrence, radical surgery has been performed in China for the treatment of most patients with pT1 CRC.

Previous studies have focused on evaluating the risk factors for lymph node metastasis^[10-13], and there is little evidence with regard to the risk factors for postoperative distant metastasis in patients with pT1 CRC. The aim of the present study was to identify the predictive risk factors that may suggest postoperative distant metastasis in patients with pT1 CRC after radical resection.

MATERIALS AND METHODS

One hundred fifty-nine patients with pT1 CRC who had undergone radical resection with lymph node dissection between January 2005 and June 2011 were enrolled. All patients were followed up until December 2011. The study was performed after approval by the Ethics Committee at the Second Military Medical University in Shanghai, China. None of these patients had received preoperative radiotherapy or neoadjuvant chemotherapy. Patients with pT1 CRC who were treated by endoscopic mucosal resection or transanal resection were excluded. Other exclusion criteria were recurrent CRC or cancer associated with familial adenomatous polyposis and inflammatory bowel disease.

Preoperative investigations included colonoscopy, chest X-rays, ultrasonography, computed tomography (CT) of the liver, and blood tests for carcinoembryonic antigen (CEA). A 3-mL peripheral blood sample from each patient was obtained. Serum CEA levels were determined using an enzyme immunoassay test kit (Beckman Coulter, Inc., Fullerton, CA, United States) with the upper limit of 5 ng/mL being defined as normal according to the kit manufacturer.

We established a 5- to 10-year follow-up period which included serum CEA measurements every 3 mo for the first 2 years and every 6 mo for the next 3 years, hepatic imaging (ultrasonography or CT) and chest X-rays every 3 mo, pelvic CT for rectal cancer every 6 mo, and colo-

noscopy every year.

Continuous variables were presented as means (standard deviation) and dichotomous variables were presented as number and percentage values. Patients with and without metastasis were compared with regard to age, gender, and clinicopathologic characteristics using the independent *t* test or χ^2 test, as appropriate. Logistic regression analysis was used to identify risk factors for distant metastasis. Variables significant at $P < 0.10$ by univariate analysis were subjected to stepwise logistic regression analysis to identify independent risk factors ($P < 0.05$) for distant metastasis. All analysis were performed with SPSS version 17 statistical software package (SPSS, Inc., Chicago, IL, United States).

RESULTS

Demographic data of the 159 patients with pT1 CRC are shown in Table 1. Distant metastasis occurred in 6 (3.8%) of the 159 patients during a median follow-up of 67.0 (46.5%) mo. The rates of distant metastasis in patients with pT1 cancer of the colon and rectum were 6.7% and 2.9%, respectively ($P < 0.001$). The recurrence rates among male and female patients with pT1 CRC were 6.25% and 1.27%, respectively ($P < 0.001$). The most frequent site of metastasis was the liver in pT1 CRC. Preoperative serum CEA level was higher in patients with distant metastasis than in patients without distant metastasis (11.35 ng/mL *vs* 3.25 ng/mL). Comparisons of patients with and without distant metastasis are shown in Table 1. The distant metastasis negative and positive groups were similar with regard to patient demographics and clinicopathologic features.

Based on univariate analysis of the correlation between lymph node metastasis (LNM) and clinicopathologic features, we found that age ($P = 0.522$), gender ($P = 0.980$), tumor location ($P = 0.330$), tumor size ($P = 0.786$), histological grade ($P = 0.509$), and high serum CEA level ($P = 0.262$) were not predictive factors for LNM (Table 2).

Univariate analysis revealed that age ($P = 0.231$), gender ($P = 0.137$), tumor location ($P = 0.386$), and tumor size ($P = 0.514$) were not risk factors for distant metastasis after radical resection for pT1 CRC (Table 2). The patients with unfavorable histological grade [odds ratio (OR) 1.365] were more likely to have metastasis, although the difference did not reach statistical significance ($P = 0.086$). Postoperative metastasis was only significantly correlated with a high serum CEA level ($P = 0.001$, Table 2). Using multivariate logistic regression analysis, high serum CEA level (OR 15.341, 95%CI 2.371-99.275, $P = 0.004$) was an independent predictor for postoperative distant metastasis.

Details of patients with distant metastasis are shown in Table 3. All distant metastases were found less than 3 years after surgery. Two of the six patients died due to the metastases, and the remaining patients are still alive after hepatic resection.

Table 1 Characteristics of 159 patients *n* (%)

Characteristics	With T1 colorectal cancer	Without metastasis (<i>n</i> = 153)	With metastasis (<i>n</i> = 6)	<i>P</i> value
Age, yr, mean ± SD	60.7 ± 11.7	60.48 ± 11.83	66.33 ± 7.012	0.231
Gender				0.099
Male	80 (50.3)	75	5	
Female	79 (49.7)	78	1	
Primary site				0.647
Cecum	3 (1.9)	3	0	
Ascending colon	9 (5.7)	8	1	
Transverse colon	6 (3.8)	6	0	
Descending colon	6 (3.8)	5	1	
Sigmoid colon	21 (13.2)	20	1	
Rectosigmoid	8 (5.0)	8	0	
Rectum	104 (65.4)	101	3	
Anus canal	2 (1.3)	2	0	
Pathology				0.919
Well differentiated	11 (6.9)	11	0	
Moderately differentiated	83 (52.2)	79	4	
Poorly differentiated	2 (1.3)	2	0	
Mucinous	4 (2.5)	4	0	
Localized canceration	59 (37.1)	57	2	
Lymphnode metastasis				0.611
N0	146 (91.8)	141	5	
N1	11 (6.9)	10	1	
N2	2 (1.3)	2	0	
CEA, ng/mL, mean ± SD	3.60 ± 4.01	3.29 ± 3.50	11.35 ± 7.87	0.054

CEA: Carcinoembryonic antibody.

DISCUSSION

Patients with pT1 CRC have a favorable prognosis, however, some patients develop recurrence including local recurrence and distant metastasis after radical resection^[14,15]. Total recurrence rates have been reported to be as low as 0%-4% and as high as 17%-31% in T1 CRC^[16]. The rate of distant metastasis in the present study was 3.8% which was consistent with a previous report.

Various factors such as serum CEA level, histological grade, and LNM for distant metastasis in CRC have been identified in previous reports^[17-20], but most of these reports included pT2-T4 patients, and there is a paucity of evidence on the risk factors for distant metastasis in pT1 CRC^[21-25]. Following univariate analysis of our data, we found that preoperative serum CEA level (OR 18.400, 95%CI 3.106-109.006, *P* = 0.001) and histological grade (OR 1.365, 95%CI 0.957-1.945, *P* = 0.086) were risk factors for predicting postoperative distant metastasis in patients with pT1 CRC.

Previous studies have reported that the LNM rate is up to 21% for T1-T2 CRC^[21,22]. LNM is considered a risk factor for distant metastasis after radical resection for CRC^[22]. It is noteworthy that LNM did not reach statistical significance in our series, with a higher OR in univariate analysis (OR 2.154, 95%CI 0.234-19.850, *P* = 0.498). Although our results did not show the same conclusion as previous reports, it is difficult to confidently exclude a correlation between LNM and distant metastasis in T1

Table 2 Risk factors for lymph node metastasis and distant metastasis in T1 colorectal cancer

Parameter	Odds ratio (95%CI)	<i>P</i> value
Lymph node metastasis		
Age	1.015 (0.970, 1.062)	0.522
Gender	0.986 (0.329, 2.954)	0.980
Location	0.518 (0.138, 1.943)	0.330
Tumor size	0.859 (0.287, 2.574)	0.786
Histological grade	0.929 (0.748, 1.155)	0.509
CEA	0.445 (0.115, 1.805)	0.262
Distant metastasis		
Age	1.050 (0.969, 1.138)	0.231
Gender	0.192 (0.022, 1.685)	0.137
Location	0.485 (0.095, 2.491)	0.386
Tumor size	0.563 (0.100, 3.163)	0.514
Histological grade	1.365 (0.957, 1.945)	0.086
LNM	2.154 (0.234, 19.850)	0.498
CEA (preoperation)	18.400 (3.106, 109.006)	0.001

CEA: Carcinoembryonic antibody; LNM: Lymph node metastasis.

CRC, because most patients (5/6, 83.3%) had less than 12 lymph nodes investigated (mean 6.2 lymph nodes).

CEA has been proved to be important in the assessment of prognosis of advanced CRC. Koca *et al*^[11] conducted a study on 221 individuals, comprised of 69 (31.2%) patients with clinical stage II and 152 (68.8%) with clinical stage III, to evaluate potential predictors of recurrence and survival. They found that high serum CEA level was one of the risk factors for recurrence. Kim *et al*^[26] also found that elevation of serum CEA was an independent factor for pulmonary metastasis after curative resection in 105 patients with CRC. In multivariate analysis of our data, we found that preoperative serum CEA level was an independent risk factor (OR 15.341, 95%CI 2.371-99.275, *P* = 0.004) in the prediction of postoperative distant metastasis in patients with pT1 CRC. Adjuvant chemotherapy might be necessary for such patients. Regular surveillance after radical resection in CRC patients should be performed, even if they have pT1 CRC, especially in patients with an increased serum CEA level. To our knowledge, this is the first study demonstrating a predictive role for serum CEA level in distant metastasis after radical resection in patients with pT1 CRC.

Interestingly, in our study, with a median follow-up period of 67.0 mo, the rates of distant metastasis in patients with T1 cancer of the colon and rectum were 6.7% and 2.9%, respectively (*P* < 0.001). The rates of distant metastasis in male and female patients with T1 CRC were 6.25% and 1.27%, respectively (*P* < 0.001). It is necessary to accumulate evidence in further studies to confirm these differences, because there is a limit to the number of cases seen in a single institution.

On the basis of our study of 159 consecutive patients with pT1 CRC, we propose that the increased preoperative serum CEA level is the independent risk factor for distant metastasis after radical resection. Adjuvant chemotherapy and regular surveillance after radical resection

Table 3 Details of distant metastasis patients

No.	Age (yr)	Gender	Location	CEA (ng/mL)	Histological grade	LNM	DFS	Recurrent site	Prognosis
1	62	Male	Ascending colon	13.66	Moderately differentiated	0/3	8	Liver and spleen	Diseased
2	61	Male	Descending colon	24.00	Localized canceration	0/15	23	Liver	Survived
3	68	Male	Rectum	15.00	Moderately differentiated	1/1	10	Liver	Survived
4	63	Female	Sigmoid colon	7.91	Moderately differentiated	0/10	30	Liver and lung	Diseased
5	80	Male	Rectum	5.20	Localized canceration	0/2	11	Liver	Survived

CEA: Carcinoembryonic antibody; LNM: Lymph node metastasis; DFS: Disease-free survival.

for such patients should be performed.

COMMENTS

Background

Colorectal cancer (CRC) is one of the most common malignancies and a leading cause of cancer-related deaths. Previous studies have focused on evaluating the risk factors for lymph node metastasis, and there is little evidence with regard to the risk factors for postoperative distant metastasis in patients with pathologically T1 (pT1) CRC.

Research frontiers

Patients with pT1 CRC have a favorable prognosis, but some patients develop recurrence including local recurrence and distant metastasis after radical resection. In this study, the authors demonstrate that the preoperative carcinoembryonic antibody (CEA) level could be predictive of distant metastasis in pT1 CRC after radical surgery.

Innovations and breakthroughs

The authors described the relationship between preoperative CEA levels and distant metastasis in pT1 CRC after surgery. This is the first study to demonstrate a predictive role for serum CEA level in distant metastasis after radical resection in patients with pT1 CRC.

Applications

Preoperative CEA level could be predictive of distant metastasis in pT1 CRC patients after radical surgery. This study suggests that adjuvant chemotherapy might be necessary for such patients.

Terminology

Preoperative serum CEA level is a risk factor in the prediction of postoperative distant metastasis in patients with pT1 CRC.

Peer review

The authors present an interesting study on risk factors for the occurrence of distant metastasis in early pT1 colorectal carcinomas. The manuscript is well structured and the cited literature is comprehensive and up-to-date. This is a clinically very interesting topic. Their results are very valuable.

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P- Reviewers Linnebacher M, Tsuchida A **S- Editor** Huang XZ
L- Editor A **E- Editor** Li JY



Single-incision vs three-port laparoscopic cholecystectomy: Prospective randomized study

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Received: September 27, 2012 Revised: November 23, 2012

Accepted: December 15, 2012

Published online: January 21, 2013

Abstract

AIM: To compare the clinical outcome of single-incision laparoscopic cholecystectomy (SILC) with three-port laparoscopic cholecystectomy (TPLC).

METHODS: Between 2009 and 2011, one hundred and two patients with symptomatic benign gallbladder diseases were randomized to SILC ($n = 49$) or TPLC ($n = 53$). The primary end point was post operative pain score (at 6 h and 7 d). Secondary end points were blood loss, operation duration, overall complications, postoperative analgesic requirements, length of hospital stay, cosmetic result and total cost. Surgical techniques were standardized and all operations were performed by one experienced surgeon, who had performed more than 500 laparoscopic cholecystectomies.

RESULTS: One patient in the SILC group required conversion to two-port LC. There were no open conversions or major complications in either treatment

groups. There were no differences in terms of estimated blood loss (mean \pm SD, 14 ± 6.0 mL vs 15 ± 4.0 mL), operation duration (mean \pm SD, 41.8 ± 17.0 min vs 38.5 ± 22.0 min), port-site complications (contusion at incision: 5 cases vs 4 cases and hematoma at incision: 2 cases vs 1 case), total cost (mean \pm SD, $12\,075 \pm 1047$ RMB vs $11\,982 \pm 1153$ RMB) and hospital stay (mean \pm SD, 1.0 ± 0.5 d vs 1.0 ± 0.2 d), respectively. TPLC had a significantly worse visual analogue pain score at 8 h after surgery (mean \pm SD, 3.5 ± 1.6 vs 2.0 ± 1.5), however, the scores were similar on day 7 (mean \pm SD, 2.5 ± 1.4 vs 2.0 ± 1.3). Cosmetic satisfaction, as determined by a survey at 2 mo follow-up favored SILC (mean \pm SD, 8 ± 0.4 vs 6 ± 0.2).

CONCLUSION: SILC is a safe and feasible approach in selected patients. The main advantages are a better cosmetic result and less pain.

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Key words: Cholecystectomy; Laparoscopic; Single-incision; Randomized; Laparoscopic cholecystectomy

Pan MX, Jiang ZS, Cheng Y, Xu XP, Zhang Z, Qin JS, He GL, Xu TC, Zhou CJ, Liu HY, Gao Y. Single-incision vs three-port laparoscopic cholecystectomy: Prospective randomized study. *World J Gastroenterol* 2013; 19(3): 394-398 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/394.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.394>

INTRODUCTION

Laparoscopic cholecystectomy (LC) has become one of the most effective procedures for the treatment of benign gallbladder pathology since its introduction in 1985^[1]. However, surgical standards of practice continue to evolve toward less invasive approaches, therefore many researchers have

attempted to minimize the invasiveness by reducing the number and size of the ports. In this context, single-port laparoscopic cholecystectomy (SILC) emerged in 1997^[2]. The proposed advantages of SILC include fewer port sites with the potential for decreased pain, cosmetic benefit and faster recovery^[3-10]. Some investigators have predicted that it may become a standard approach to LC^[11,12]. However, it has also been suggested that SILC has many disadvantages, such as the technique is difficult to handle, prolonged operative time, cost of special instruments and increased risk of per-operative complications^[9,13]. It is difficult to make an unbiased comparison between SILC and multi-port LC because of lack of prospective, randomized controlled trials (RCT). Therefore, we designed and conducted this RCT to evaluate the advantages and disadvantages of SILC and three-port laparoscopic cholecystectomy (TPLC).

MATERIALS AND METHODS

Inclusion criteria were as follows: (1) patients aged between 18 and 70 years; (2) body mass index less than 40 kg/m²; (3) preoperative diagnosis of symptomatic gallstones or gallbladder polyps, and (4) willing to comply with the protocol requirements and signed written informed consent.

Exclusion criteria were as follows: (1) American Society of Anesthesiologists class IV and V; (2) Patients with contraindication to laparoscopy; (3) Patients with suspected Mirizzi syndrome, common duct stones or malignancy; (4) Patients with previous upper abdominal surgery; (5) Patients on long-term anticoagulant treatment; (6) Acute cholecystitis or choledocholithiasis; and (7) Gallstones > 3 cm in diameter.

Eligible patients were randomized into two groups (SILC and TPLC) using sealed opaque envelopes which contained a computer-generated random number. Before the trial, all patients underwent basic investigations such as blood tests, electrocardiogram, ultrasonography of the abdomen and radiologic imaging such as chest radiograph. All operations were performed by one experienced surgeon, who had performed more than 500 laparoscopic cholecystectomies.

Operative techniques

Surgical techniques were standardized. SILC was performed with the help of 2 slings of sutures, which included four steps: (1) Under general anesthesia, a 20 mm (approximately) bracket-shaped skin incision was made through the inner margin of the umbilicus and a pneumoperitoneum was set at 13 mmHg. One 10 mm trocar (Kanger, Tong Lu, China) to allow the insertion of a 30 degree laparoscope (Olympus, Tokyo, Japan) was inserted through the abdomen at the left side of the incision and a 5 mm trocar (Kanger, Tong Lu, China) was inserted at the right side for the harmonic scalpel. The tissues between the trocars were preserved to prevent air leakage; (2) The first suture using a straight needle was inserted



Figure 1 A sketch of the suspension procedure.



Figure 2 The incision is reconstructed after single-incision laparoscopic cholecystectomy.

through the right 7th intercostal space in the anterior axillary line, and the seromuscular layer of the gallbladder fundus was punctured and retracted toward the anterior abdominal wall. Hartmann's pouch was punctured and retracted using the second suture to expose Calot's triangle (Figure 1); (3) The harmonic scalpel (Ethicon Endo-Surgery, Cincinnati, OH, United States) was used to dissect Calot's triangle. Once the cystic artery and duct have been exposed, the cystic artery was cut using the harmonic scalpel, and the cystic duct was triple clipped and divided; and (4) The harmonic scalpel was used to dissect the gallbladder from the gallbladder fossa. When the gallbladder was free, the 5 mm trocar was exchanged for a 10 mm one, and through the 10 mm port, a specimen bag was inserted and the gallbladder was extracted after removal of the suspending sutures from the abdominal wall. The umbilical incision was closed and restored and no drainage tube was left in place (Figure 2).

In TPLC, a sub-umbilical incision, sub-xiphoid incision and right sub-costal incision were made. A 10 mm trocar placed in the sub-umbilical incision allowed the introduction of laparoscope and the other two trocars, a 10 mm and a 5 mm, respectively, were placed for the grasp and harmonic scalpel. The operation was performed following the routine TPLC procedure^[14], however, the cystic artery was divided and cut using the harmonic scalpel instead of being clipped and divided. The primary end-point of the

Table 1 Patients data and outcome

	SILC (<i>n</i> = 49)	TPLC (<i>n</i> = 53)	<i>P</i> value
Age (yr)	43.8 ± 14.0	45.2 ± 11.0	0.5493
Female	26	31	0.5810
WI (kg/m ²)	24.3 ± 6.0	25.1 ± 5.0	0.4649
ASA	1.5 ± 0.2	1.6 ± 0.3	0.0676
Clinical diagnosis			0.7790
Cholecystolithiasis	32	36	
Cystic polyps	17	17	
Operation duration (min)	41.8 ± 17.0	38.5 ± 22.0	0.4222
EBL (mL)	14 ± 6.0	15 ± 4.0	0.2643
VAS (1–10)			
8 h after surgery	2.0 ± 1.5	3.5 ± 1.6	0.0000
Day 7 after surgery	2.0 ± 1.3	2.5 ± 1.4	0.0651
Complications			
Contusion at incision	5	4	0.7350
Hematoma at incision	2	1	0.6070
Hospital stay (d)	1.0 ± 0.5	1.0 ± 0.2	1.0000
Cosmetic score	8 ± 0.4	6 ± 0.2	0.0000
Total cost (RMB)	12 075 ± 1047	11 982 ± 1153	0.6715

ASA: American Society of Anesthesiology; SILC: Single-incision laparoscopic cholecystectomy; TPLC: Three-port laparoscopic cholecystectomy; WI: Weight index; EBL: Estimated blood loss; VAS: Visual analog score; RMB: Renminbi yuan/Chinese yuan.

study was the postoperative pain score (at 6 h and 7 d). Secondary end-points included blood loss, operation duration, overall complications (intra- and post-operative complications), postoperative analgesic requirements, length of hospital stay, cosmetic result and total cost. A standard visual analog scale [range, 0 (no pain) to 10 (maximum pain)] was used for assessments at 8 h after surgery and on postoperative day 7. Cosmetic satisfaction of the surgical scar was rated on a scale [range, 0 (worst) to 10 (best)], and was evaluated at the 2-mo follow-up visit.

Statistical analysis

Statistical analysis was accomplished using the SPSS program for Windows 12.0 (SPSS, Chicago, IL, United States). The χ^2 test or the *t* test was used as indicated. All data were presented as mean ± SD. All *P* values were 2-sided. A *P* value of 0.05 was considered statistically significant.

RESULTS

From January 2009 to March 2011, 108 eligible patients were randomized to SILC (*n* = 51) or TPLC (*n* = 57). Two patients in the TPLC group and four patients in the SILC group refused to participate before surgery. In total, 102 patients (SILC, *n* = 49; TPLC, *n* = 53) were analyzed. Demographic variables in the two groups were similar (Table 1). All patients in the TPLC group successfully underwent three-port laparoscopic cholecystectomy without conversion to open surgery. Of 49 patients in the SILC group, one patient was converted to two-port LC due to anatomical difficulties and the operator felt it was unsafe to proceed with SILC. Overall, No major intra- or post-operative complications, such as biliary injury, abscess, bleeding, biliary collection or port-site hernia were

observed in the two treatment groups. Several patients experienced port-site complications such as contusion and hematoma, however, all recovered a few days after surgery. There were no significant differences in estimated blood loss (14 ± 6.0 mL *vs* 15 ± 4.0 mL; *P* = 0.2643), operation duration (41.8 ± 17.0 min *vs* 38.5 ± 22.0 min; *P* = 0.4222), port-site complications (contusion at incision: 5 cases *vs* 4 cases; *P* = 0.7350 and hematoma at incision: 2 *vs* 1; *P* = 0.6070), hospital stay (1.0 ± 0.5 d *vs* 1.0 ± 0.2 d; *P* = 1.0000) and total cost ($12\,075 \pm 1047$ RMB *vs* $11\,982 \pm 1153$ RMB; *P* = 0.6715) between the SILC and TPLC groups. However, the TPLC group had a significantly worse visual analogue pain score at 8 h after surgery (3.5 ± 1.6 *vs* 2.0 ± 1.5 ; *P* = 0.0000), but the score was similar on day 7 (2.5 ± 1.4 *vs* 2.0 ± 1.3 ; *P* = 0.0651). Cosmetic satisfaction as shown by the cosmetic score was significantly higher in the SILC group than in the TPLC group (8 ± 0.4 *vs* 6 ± 0.2 ; *P* = 0.0000) (Table 1).

DISCUSSION

SILC has attracted wide attention because of its potential cosmetic results. It may even be possible for this approach to become a gold standard for cholecystectomy^[11,12]. However, there is still a long way to go before this approach is a gold standard, as standardization, safety, and the cosmetic results of SILC require validation^[15]. Although recent reports have focused on comparisons between SILC and multi-port LC, the safety, better cosmetic results and faster recovery following SILC have been agreed, however, SILC has not been standardized and there is much technical variation. On the one hand, different surgeons have attempted SILC in different ways. For example, in exposing Calot's triangle, trials using sutures, Kirschner wires and loop retractors have been reported, in addition to different manipulative instruments, such as straight instruments and reticulating instruments^[7]. On the other hand, devices used to prevent air leakage vary from one surgeon to another: some use common trocars^[8], some tend to use SILS multi-port^[7] and others favor self-designed devices such as sterile gloves^[16]. In our center, we have tried many approaches in the initial stage. For example, to prevent air leakage, we have tried tri-port and gel-port, but due to their high cost in special ports and larger lesions of about 3 cm requiring a trans-umbilical incision these devices are no longer used. We decided to select routine trocars in our practice because they were sufficient to prevent air leakage and were more economical. With regard to the selection of surgical instruments, we use the suture suspension method in SILC, which requires only a 30 degree laparoscope and a manipulative instrument, eliminating the need for more instruments intra-operatively.

In practice, we focus on improving the auxiliary procedure with suspension sutures. To achieve ideal exposure of Calot's triangle, we select the puncture spot at the superior chest wall along the costal margin so that the suspension suture can draw the liver up a bit more than in other

places, different to Piskun *et al.*^[17] that the puncture spot should be selected at the inferior costal margin. In addition, the harmonic scalpel is effective for occluding 3 mm blood vessels and dissecting tissues^[18]. In our experience, cystic arteries in the trial were cut using the harmonic scalpel, indicating the safety of this scalpel. Intra-operative management of the gallbladder is critical for the safety in SILC. Initially, we were perplexed as to how to handle intra-operative bile leakage. First, we placed the suspension sutures into the seromuscular layer to avoid perforation of the cyst wall, but this was inevitable sometimes. Subsequently, we found that this technique was useful as long as evacuating and repeated rinsing were conducted.

There have been a few comparisons between SILC and conventional multi-port LC^[19-22]. These studies concluded that SILC is superior to multi-port LC in terms of cosmetic outcome, but not in terms of total cost, surgical time and postoperative pain. A similar cosmetic outcome in both treatment groups was observed in our study. However, there was no significant difference in the total cost between SILC and traditional LC when standard materials were selected. In addition, we found that SILC resulted in less postoperative pain than TPLC, and no significant difference in operation duration was observed between the two techniques. The higher pain score may be explained by the fact that there are more incisions in TPLC and the size of the incisions is larger than those in SILC. The different outcome of surgical time could be explained by difference in experience of the surgeon in SILC.

In conclusion, the results of this prospective trial demonstrate that SILC is a safe procedure when conducted by experienced surgeons, with some outcomes similar to that of TPLC, however, SILC was superior in terms of cosmetic outcome and postoperative pain. In the future, more high-powered randomized studies comparing SILC with conventional LC will be needed to validate its objective benefits and clinical role during the follow-up.

COMMENTS

Background

Laparoscopic cholecystectomy (LC) has been regarded as the "gold standard" for the management of benign gallbladder diseases since its introduction in 1985. However, surgical standards of practice continue to evolve toward less invasive approaches, therefore many researchers have attempted to minimize the invasiveness by reducing the number and size of the ports. In this context, single-incision laparoscopic cholecystectomy (SILC) emerged as a complement to standard LC. The proposed advantages of SILC include fewer port sites with the potential for decreased pain, cosmetic benefit and faster recovery. Some investigators have predicted that it may become a standard approach in LC. However, it has also been suggested that SILC has many disadvantages, such as the technique is difficult to handle, prolonged operative time, cost of special instruments and increased risk of per-operative complications. Therefore, it is difficult to make an unbiased comparison between SILC and multi-port LC due to the lack of prospective, randomized controlled trials.

Research frontiers

SILC has attracted wide attention because of its potential cosmetic results. However, it is unknown whether SILC could become a new standard procedure, and therefore it is necessary to compare the clinical outcome of SILC and multiple-port laparoscopic cholecystectomy.

Innovations and breakthroughs

This prospective randomized study was performed to explore the safety and

feasibility of SILC for the treatment of benign gallbladder diseases, and to compare the clinical outcomes with three-port laparoscopic cholecystectomy (TPLC).

Applications

In selected patients with benign gallbladder diseases, single-incision laparoscopic cholecystectomy is safe and feasible. The main advantages are that SILC result in a better cosmetic outcome and less pain compared with TPLC.

Terminology

SILC is a minimally invasive surgical procedure in which cholecystectomy is accomplished exclusively through a single 15-25 mm incision in the patient's navel. It is complementary to standard LC and an alternative to natural orifice transluminal endoscopic surgery. Unlike the traditional multi-port laparoscopic approach, SILC leaves only a single small scar in the navel.

Peer review

The authors have compared the clinical outcomes of SILC with TPLC and have concluded that SILC is a safe and feasible approach in selected patients. The main advantage is its better cosmetic result and less pain. The study is well organized and the content is adequately written, patient number is good and the authors have used good technique (harmonic for cystic artery).

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P- Reviewers De Nardi P, Shah OJ **S- Editor** Wen LL
L- Editor A **E- Editor** Li JY



***Helicobacter pylori* tumor necrosis factor- α inducing protein promotes cytokine expression *via* nuclear factor- κ B**

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Received: September 12, 2012 Revised: December 10, 2012

Accepted: December 20, 2012

Published online: January 21, 2013

However, the levels of cytokines (including IL-1 β , IL-8 and TNF- α) secreted by SGC-7901 cells were greater than those secreted by GES-1 cells following treatment with Tip- α at the same concentration and for the same duration ($P < 0.05$). After blocking NF- κ B with PDTC, the cells (GES-1 cells and SGC-7901 cells) underwent interference with Tip- α . We found that IL-1 β and TNF- α levels were significantly decreased compared to cells that only underwent Tip- α interference ($P < 0.05$).

CONCLUSION: Tip- α plays an important role in cytokine expression through NF- κ B.

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Key words: *Helicobacter pylori*; Tumor necrosis factor- α inducing protein; Interleukin-1 β ; Interleukin-8; Tumor necrosis factor- α ; Nuclear factor- κ B

Abstract

AIM: To study the effects of *Helicobacter pylori* (*H. pylori*) tumor necrosis factor- α (TNF) inducing protein (Tip- α) on cytokine expression and its mechanism.

METHODS: We cloned Tip- α from the *H. pylori* strain 26695, transformed *Escherichia coli* with an expression plasmid, and then confirmed the expression product by Western blotting. Using different concentrations of Tip- α that affected SGC7901 and GES-1 cells at different times, we assessed cytokine levels using enzyme-linked immunosorbent assay. We blocked SGC7901 cells with pyrrolidine dithiocarbamate (PDTC), a specific inhibitor of nuclear factor κ B (NF- κ B). We then detected interleukin (IL)-1 β and TNF- α levels in SGC7901 cells.

RESULTS: Western blot analysis using an anti-Tip- α antibody revealed a 23-kDa protein, which indicated that recombinant Tip- α protein was recombined successfully. The levels of IL-1 β , IL-8 and TNF- α were significantly higher following Tip- α interference, whether GES-1 cells or SGC-7901 cells were used ($P < 0.05$).

Tang CL, Hao B, Zhang GX, Shi RH, Cheng WF. *Helicobacter pylori* tumor necrosis factor- α inducing protein promotes cytokine expression *via* nuclear factor- κ B. *World J Gastroenterol* 2013; 19(3): 399-403 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/399.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.399>

INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) leads to chronic gastritis, peptic ulcer, and gastric lymphoma^[1-3]. *H. pylori* has also been associated with gastric cancer^[4], is classified as a class I carcinogen by the International Agency for Research on Cancer^[5], and *H. pylori* exerts its pathogenesis by secreting toxins, including hemolysin, lipopolysaccharides, CagA and VacA^[6-9]. CagA and VacA are the major virulence factors. Persistent infection by *H. pylori* enables these toxins to stimulate gastric epithelial cells to produce a large number of cytokines such as tumor necrosis factor (TNF- α) and interleukin 1, 6 and 8 (IL-1, IL-6 and

IL-8), thus generating an inflammatory reaction^[10-14]. Tumor necrosis factor- α inducing protein (Tip- α) is a new toxin discovered recently, and likely accelerates the inflammation and cancers caused by *H. pylori*^[15]. However, its function and the mechanism underlying these effects remain unclear. The present work was conducted to determine the effects of recombinant Tip- α (rTip- α) on human gastric epithelial cells and gastric cancer cytokine expression, as well as explore the mechanisms involved.

MATERIALS AND METHODS

Materials

H. pylori strain 26695 was obtained from the Shanghai Institute of Digestive Disease. The following reagents were used in this study: Dual Promoter TA Cloning[®] Kit pCR[®] II and pET28a vectors (Invitrogen); monoclonal rabbit anti-Tip- α antibody (Beijing Aviva Systems Biology); *Bam*H I, *Xho* I and Prestained Protein Molecular Weight Markers (Fermentas); DNA and gel extraction kit from Tiangen Biotech (Beijing) Co. Ltd.; DNA marker (TaKaRa); His Trap[™] *H. pylori* affinity chromatography column (GE Healthcare); and enhanced chemiluminescence kit (Pierce Protein Biology Products). The polymerase chain reaction primer sequences were 5'-TTGGATCCATGCTGCAGGCTTG-3', which contained an *Xho* I restriction site, and 5'-GGCTCGAGCTACATGGCTATAG-3', which contained a *Bam*H I restriction site. The primers were synthesized by Invitrogen. The human gastric epithelial cell line GES-1 and gastric cancer SGC7901 cells were purchased from the Shanghai Cancer Institute. Enzyme-linked immunosorbent assay (ELISA) kits were obtained from MultiSciences Biotech (Shanghai) Co., Ltd., while pyrrolidine dithiocarbamate (PDTC) was purchased from Jingmei Biotech Co., Ltd.

Methods

Expression, purification, and identification of Tip- α :

We cloned Tip- α from the genome of *H. pylori* strain 26695. The Tip- α gene and pET28a vector (His tag) were digested with *Bam*H I and *Xho* I, purified, and then ligated together to generate the ET28a-Tip- α plasmid expressing recombinant Tip- α . This plasmid was transformed into *Escherichia coli* and the resultant protein was purified by Ni-NTA affinity chromatography and verified by Western blotting.

Cell recovery, culture, and passage: Cryopreserved GES-1 and SGC-7901 cells were centrifuged at 1000 rpm for 5 min. After removal of the supernatant, these cells were cultured in 60 mm \times 60 mm dishes containing Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

IL-1 β , IL-8 and TNF- α levels at different times following interference by 12.5 μ g/mL rTip- α in GES-1 and SGC7901 cells: GES-1 and SGC7901 cells during their logarithmic growth phase underwent interference

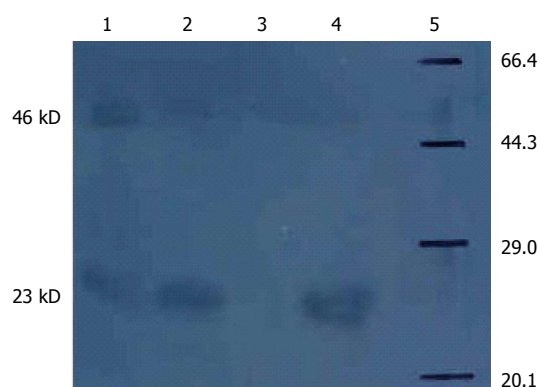


Figure 1 Western blotting identification of recombinant tumor necrosis factor- α inducing protein.

with 12.5 μ g/mL rTip- α after starvation in serum-free medium for 24 h. The levels of IL-1 β , IL-8 and TNF- α cytokines were then assessed at 0, 1, 2, 4 and 8 h post-interference using ELISA.

Levels of IL-1 β , IL-8 and TNF- α in GES-1 and SGC7901 cells following incubation with different concentrations of rTip- α : We incubated GES-1 and SGC7901 cells with the following concentrations of rTip- α : 0, 12.5, 25 and 50 μ g/mL. After 2 h, we examined the levels of IL-1 β , IL-8 and TNF- α using ELISA.

Effects of rTip- α on IL-1 β and TNF- α expression after PDTC-mediated inhibition of NF- κ B: Four groups consisting of the same number of GES-1 and SGC7901 cells were starved in serum-free medium for 24 h before undergoing different treatments. Group A was treated with 12.5 μ g/mL rTip- α for 2 h. Group B was treated similarly after PDTC blocking of NF- κ B for 4 h. Groups C and D were incubated with serum-free medium and dimethyl sulfoxide (the vehicle with which PDTC was diluted), respectively. ELISA was performed to detect the levels of IL-1 β and TNF- α in each group.

Statistical analysis

Data are presented as the mean \pm SD and analyzed using SPSS 17.0. The Student's *t* test was used to compare two groups, while one-way analysis of variance was used to compare among several groups. A *P* value < 0.05 was considered statistically significant.

RESULTS

Identification by Western blotting after rTip- α expression and purification

Western blotting analysis demonstrated that the Tip- α recombinant protein and anti-human Tip- α monoclonal antibody could be specifically bound; specific bands were found (Figure 1). Western blotting analysis by non-denaturing gel electrophoresis showed active dimer bands (46 kDa).

Table 1 Cytokine levels at different times after interference of GES-1 and SGC7901 cells with 12.5 μ g/mL recombinant tumor necrosis factor- α inducing protein

Groups	0 h	1 h	2 h	4 h	8 h
GES-1 (IL-1 β)	0.34 \pm 0.04	0.88 \pm 0.09 ^a	2.07 \pm 0.30 ^a	1.35 \pm 0.20 ^a	1.41 \pm 0.15 ^a
SGC-7901 (IL-1 β)	0.22 \pm 0.04	0.35 \pm 0.05	2.07 \pm 0.10 ^a	1.11 \pm 0.04 ^a	1.14 \pm 0.04 ^a
GES-1 (IL-8)	0.35 \pm 0.05	0.60 \pm 0.12 ^a	0.84 \pm 0.11 ^a	0.64 \pm 0.06 ^a	0.50 \pm 0.07 ^a
SGC-7901 (IL-8)	0.70 \pm 0.02	0.78 \pm 0.19	2.53 \pm 0.50 ^a	2.26 \pm 0.24 ^a	2.14 \pm 0.68 ^a
GES-1 (TNF- α)	0.39 \pm 0.06	0.39 \pm 0.07	0.72 \pm 0.08 ^a	0.53 \pm 0.03 ^a	0.51 \pm 0.14 ^a
SGC-7901 (TNF- α)	0.33 \pm 0.09	1.02 \pm 0.09 ^a	1.41 \pm 0.10 ^a	0.86 \pm 0.07 ^a	0.47 \pm 0.03 ^a

^a*P* < 0.05 vs 0 h. IL-1 β : Interleukin-1 β ; TNF- α : Tumor necrosis factor- α .**Table 2** Cytokine levels in GES-1 and SGC7901 cells after interference with different concentrations of recombinant tumor necrosis factor- α inducing protein for 2 h

Groups	0	12.5 μ g/mL	25 μ g/mL	50 μ g/mL
GES-1 (IL-1 β)	0.59 \pm 0.11	2.07 \pm 0.30 ^a	2.20 \pm 0.09 ^a	1.23 \pm 0.13 ^a
SGC-7901 (IL-1 β)	0.36 \pm 0.01	2.07 \pm 0.10 ^a	1.22 \pm 0.03 ^a	1.02 \pm 0.04 ^a
GES-1 (IL-8)	0.39 \pm 0.08	0.84 \pm 0.11 ^a	0.75 \pm 0.09 ^a	0.61 \pm 0.15 ^a
SGC-7901 (IL-8)	0.78 \pm 0.09	2.53 \pm 0.50 ^a	1.50 \pm 0.16 ^a	1.41 \pm 0.14 ^a
GES-1 (TNF- α)	0.30 \pm 0.06	0.72 \pm 0.08 ^a	0.54 \pm 0.13 ^a	0.63 \pm 0.10 ^a
SGC-7901 (TNF- α)	0.26 \pm 0.18	1.41 \pm 0.10 ^a	0.62 \pm 0.07 ^a	0.62 \pm 0.02 ^a

^a*P* < 0.05 vs group 0. IL-1 β : Interleukin-1 β ; TNF- α : Tumor necrosis factor- α .**Table 3** Effects of recombinant tumor necrosis factor- α inducing protein on the levels of interleukin-1 β and tumor necrosis factor- α in SGC7901 cells under different conditions

Groups	A	B	C	D
SGC-7901 (IL-1 β)	2.32 \pm 0.25	1.05 \pm 0.75 ^a	0.84 \pm 0.08	0.57 \pm 0.09
SGC-7901 (TNF- α)	1.51 \pm 0.64	0.72 \pm 0.20 ^a	0.43 \pm 0.07	0.71 \pm 0.23
GES-1 (IL-1 β)	2.07 \pm 0.30	0.98 \pm 0.34 ^a	0.69 \pm 0.06	0.63 \pm 0.06
GES-1 (TNF- α)	0.81 \pm 0.08	0.36 \pm 0.03 ^a	0.27 \pm 0.03	0.29 \pm 0.04

^a*P* < 0.05 vs group A. IL-1 β : Interleukin-1 β ; TNF- α : Tumor necrosis factor- α .

IL-1 β , IL-8 and TNF- α levels at different times following interference by 12.5 μ g/mL rTip- α in GES-1 and SGC7901 cells

The levels of IL-1 β , IL-8 and TNF- α were significantly higher after GES-1 and SGC7901 cells underwent interference by 12.5 μ g/mL rTip- α for 1, 2, 4 and 8 h than those at 0 h. Cytokine secretion by GES-1 and SGC7901 cells peaked after rTip- α interference for 2 h, indicating no obvious dependence on time (Tables 1 and 2). However, after interference by rTip- α for 2 h, the levels of IL-8 (2.53 \pm 0.50) and TNF- α (1.41 \pm 0.10) in SGC7901 cells were significantly higher than those in GES-1 cells (0.84 \pm 0.11 for IL-8 and 0.72 \pm 0.08 for TNF- α). As shown in Table 1, the levels of IL-1 β in GES-1 and SGC7901 cells (2.07 \pm 0.10 and 2.07 \pm 0.30, respectively) were not statistically different after rTip- α interference for 2 h.

Levels of IL-1 β , IL-8 and TNF- α in GES-1 and SGC7901 cells following incubation with different concentrations of rTip- α

The levels of IL-1 β , IL-8 and TNF- α were significantly higher than those in the blank control in GES-1 and SGC7901 cells after rTip- α interference for 2 h. Cytokine secretion of GES-1 and SGC7901 cells peaked at 12.5 μ g/mL, suggesting that this effect was not concentration-dependent (Table 2).

Effects of rTip- α on IL-1 β and TNF- α expression after PDTC-mediated inhibition of NF- κ B

The levels of IL-1 β and TNF- α in SGC7901 cells in Group B (treated with PDTC + rTip- α) were higher than those in Groups C and D (no rTip- α and PDTC), but

markedly lower than those in Group A (only treated with rTip- α). As shown in Table 3, these differences were statistically significant (*P* < 0.05, *F* = 40.15).

DISCUSSION

Tip- α is a novel gene that was discovered recently in *H. pylori*. Located in the *H. pylori* 0596 open reading frame of the *H. pylori* 26695 strain, Tip- α is also called *H. pylori* 0596 protein. Its open reading frame is 519 bp in length and constitutes 173 amino acids. Tip- α has a molecular weight of 19 kDa and can form active homodimers with a molecular weight of 38 kDa through disulfide bonding^[15]. Recent studies have found that Tip- α is associated with the adsorption and colonization of *H. pylori* in gastric mucosa^[16]. Some studies have shown that the structure of Tip- α is different from penicillin binding proteins. Tip- α is composed of three closely linked domains that interact with other proteins and nucleic acids. In particular, the homodimer formed by cysteine C25 and C27 is essential for Tip- α to serve its role in the gastric mucosal acidic environment^[17]. Detected by gene chip technology, expression of the chemokines Cc 12 Cc17, Cc120, Cx11 and Cx15 was enhanced after Tip- α treatment in gastric cancer cells and gastric epithelial cells^[18]. These chemokines can induce chemotaxis of immune cells to local sites of infection, resulting in immune regulation and pathology, and ultimately the inflammatory response^[19].

Because our pET28a-Tip- α vector also expresses a 3.74 kDa His tag, the recombinant Tip- α protein we produced possesses a molecular weight of about 23 kDa and can form active homodimers with a molecular weight of 46 kDa through disulfide bonding. Our work indicates that after affecting gastric epithelial and cancer

cells, rTip- α can promote the expression of IL-1 β , IL-8 and TNF- α . These cytokines are important in promoting the inflammatory response, thus linking Tip- α to inflammation and the occurrence of *H. pylori*-related gastrointestinal diseases. After incubating the cells for 2 h with rTip- α , the levels of cytokine expression peaked at 12.5 μ g/mL, which was the best concentration for interference. The levels of cytokine expression induced by rTip- α were not time- or concentration-dependent. These results suggest that Tip- α affects the host by inducing toxin secretion into the exterior environment of the bacteria through the type II secretion system^[20]. The toxins then enter host cells *via* receptor-mediated endocytosis instead of through injection *via* the IV secretion system^[21-23]. Studies have shown that Tip- α possesses DNA binding activity. In particular, DNA affecting gastric mucosal cells can combine with some transcription factors to promote IL-1 β , IL-8 and TNF- α expression, leading to the occurrence and development of *H. pylori*-related gastrointestinal diseases^[24].

PDTC is a specific NF- κ B inhibitor that works by blocking degradation of the NF- κ B p65 subunit or I κ B, thereby reducing NF- κ B nuclear translocation^[25]. Our data demonstrated no significant increase in IL-1 β and TNF- α levels after pretreatment of SGC7901 cells with PDTC followed by rTip- α interference. This finding suggests that the promotion of cytokine expression by Tip- α may be regulated by NF- κ B. However, further study is required to determine the underlying mechanism.

In addition, we found that when gastric epithelial and cancer cells were treated with the same concentration of Tip- α for the same duration, the level of cytokine expression in gastric cancer SGC7901 cells was significantly higher than that in gastric epithelial GES-1 cells. This difference may be due to differential effects of rTip- α on NF- κ B expression in the cell types or may be related to variations in the DNA binding activity of Tip- α in the cells. Some studies suggest that the increased cytokine expression promoted by Tip- α may hasten the invasion and metastasis of gastric cancer^[26]. Overall, Tip- α can activate cytokine expression in an NF- κ B-dependent manner. Tip- α plays a major role in the pathogenesis of *H. pylori*, however, its mechanism requires further investigation.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) exerts its pathogenesis by secreting toxins. Tumor necrosis factor- α (TNF- α) inducing protein (Tip- α) is a new toxin discovered recently, however, its function and mechanism of pathogenesis remain unclear.

Research frontiers

The pathogenesis of *H. pylori* is partially clear, as *H. pylori* may secrete many types of toxins. With the exception of CagA and VacA, new *H. pylori* toxins have been discovered, such as Tip- α , mammalian, prokaryotic high-temperature requirement A, and the duodenal ulcer-promoting gene. Only the function of the toxins and their mechanism of pathogenesis are clear, as the mechanism of *H. pylori* pathogenesis is known.

Innovations and breakthroughs

Other studies discovered that Tip- α promoted the expression of cytokines, however, this article first showed the difference in the promotion of the expres-

sion of cytokines between gastric epithelial cells and cancer cells, and that Tip- α activates cytokine expression in a nuclear factor κ B (NF- κ B)-dependent manner.

Applications

Tip- α may become a marker of *H. pylori* virulence. The virulence of *H. pylori* may be determined by detecting Tip- α .

Terminology

Tip- α is the abbreviation for tumor necrosis factor- α inducing protein. It was first discovered that this new *H. pylori* toxin can promote the expression of TNF- α , therefore, it was called Tip- α .

Peer review

The authors certified that Tip- α -a new toxin of *H. pylori*, promoted the expression of cytokine, discovered the difference of this function between gastric epithelial cells and cancer cells, and in an NF- κ B-dependent manner, further interpreted the mechanism of pathogenesis of *H. pylori*.

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P- Reviewer Fukuhara K S- Editor Song XX

L- Editor Webster JR E- Editor Li JY



Magnifying chromoendoscopy combined with immunohistochemical staining for early diagnosis of gastric cancer

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Supported by Grant from the Medical and Health Research Program of Inner Mongolia Autonomous Region, No. 2010069

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Received: November 7, 2012 Revised: December 7, 2012

Accepted: December 15, 2012

Published online: January 21, 2013

Abstract

AIM: To assess the diagnostic value of using magnifying chromoendoscopy combined with immunohistochemical staining of proliferating cell nuclear antigen (PCNA) and p53 in the detection of gastric precancerous lesions.

METHODS: Ninety-five patients who were treated for abdominal discomfort, abdominal pain, bloating, and acid reflux at our hospital from January 2010 to December 2011 were included in the study. An ordinary

gastroscopic procedure was initially performed to select the lesions. All subjects underwent magnifying chromoendoscopy to observe morphological changes of gastric pits. Biopsies were then taken from each area of interest and sent for pathological examination and detection of PCNA and p53 expression by immunohistochemistry. An immunoreactivity score for each lesion was calculated. Based on immunoreactivity scores, immunohistochemical staining was then considered.

RESULTS: Compared to intestinal metaplasia, gastric pits were more diverse in size, more irregular in shape, and more disorderly in arrangement in moderate and severe dysplasia. PCNA and p53 expression was significantly higher in precancerous lesions (intestinal metaplasia and dysplasia) than in chronic gastritis. PCNA expression showed an upward trend in types A-F pits. The number of cases that showed strong PCNA positivity increased significantly with an increase in the severity of lesions. Rank sum test for independent samples showed that p53 expression was significantly higher in types E and F pits than in types A-D pits ($H = 33.068, P = 0.000$). Rank sum test for independent samples showed that PCNA expression was significantly higher in types E and F pits than in types A-D pits ($H = 31.791, P = 0.001$).

CONCLUSION: The presence of types E and F pits, in which p53 and PCNA are highly expressed, is highly suggestive of the occurrence of early cancer, and patients developing these changes should be closely followed.

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Key words: Magnifying chromoendoscopy; Gastric precancerous lesions; p53; Proliferating cell nuclear antigen; Early gastric cancer

Meng XM, Zhou Y, Dang T, Tian XY, Kong J. Magnifying chromoendoscopy combined with immunohistochemical stain-

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INTRODUCTION

Gastric cancer is one of the most common human malignancies, ranking first in terms of both incidence and mortality among gastrointestinal malignancies in China. Although great technological progress has been made in the diagnosis and treatment of gastric cancer, there is no significant improvement in its prognosis, and the 5-year survival rate of gastric cancer is only about 20%^[1]. This is because 67% of patients have already had local spread or distant metastasis at initial diagnosis^[1]. Therefore, early diagnosis and treatment are important for improving the prognosis of gastric cancer.

Early gastric cancer only invades the mucosal layer and submucosal layer and has a 5-year survival rate as high as 90% to 95%. Gastroscopy has the advantages of easy operation and low false-negative detection rate and has long been considered the preferred and most reliable diagnostic modality for gastric cancer. It allows detection of minute lesions and flat lesions easily, estimation of the depth of invasion^[2], and the performance of histological and molecular biological analysis of diseased tissue; it therefore has a higher detection rate of early cancer and precancerous lesions. Thus, it is important to carry out a regular follow-up of gastric precancerous lesions to increase the detection rate of early gastric cancer. However, the widespread use of gastroscopy does not significantly improve the detection rate of early gastric cancer. A recent study in Japan^[3] indicates that endoscopy has a sensitivity of only 81% in detecting early gastric cancer, and this means that the false-negative rate is 19%. Therefore, worldwide efforts are being made to improve the sensitivity of endoscopy in the detection of early cancer and precancerous lesions so that the tumor can be detected at an early treatable stage.

The human genome is composed of about 100 000 genes, and the selective expression of these genes determines the whole human life course. Changes in gene expression play a central role in biological regulatory mechanisms. Tumorigenesis is the result of multiple genetic changes. Activation of oncogenes and inactivation or deletion of tumor suppressor genes are considered closely related to tumorigenesis. The tumor suppressor gene *p53* is well known as the guardian of the cell. To date, extensive and in-depth research on *p53* has been conducted. Wild-type *p53* is an important negative regulator of cell growth and can antagonize oncogenic functions *in vivo* to maintain the relative stability between positive and negative proliferation signals. Wild-type *p53* is involved in many important biological processes such as cell cycle, DNA repair, cell differentiation, and apoptosis.

Proliferating cell nuclear antigen (PCNA) is an acidic

nuclear protein that is involved in eukaryotic DNA synthesis and plays an important role in the regulation of the cell cycle. PCNA expression is significantly up-regulated in the process of malignant transformation of normal epithelium and is deemed a marker reflecting cell proliferation activity in most tumor types.

The aim of this study was to assess the diagnostic value of magnifying chromoendoscopy combined with detection of PCNA and *p53* in the detection of gastric precancerous lesions.

MATERIALS AND METHODS

Patients

Ninety-five patients who were treated for abdominal discomfort, abdominal pain, bloating, and acid reflux at our hospital from January 2010 to December 2011 were selected. There were 60 males and 35 females. Their ages ranged from 17 to 78 years, with a median age of 52 years.

Methods

Endoscopic examination: The procedure was performed using a Fujinon (EG-590) gastroscope and EPX 4400 processor. An ordinary gastroscopic procedure was initially performed to select the lesions, and magnifying chromoendoscopy was then performed to observe morphological changes of gastric pits. Three to four biopsies were taken from each area of interest and sent for pathological examination and detection of PCNA and *p53* expression.

Criteria for morphological classification: Based on morphological classification criteria proposed by Yang *et al.*^[4], gastric pits were divided into six fundamental types: A (round spot-like pits), B (short rod-like pits, arranged regularly and tightly, with elongations, branches and curvatures), C (linear pits, more sparse and thick compared to type B), D (patchy pits, elongated and tortuous pits connected to form a reticular appearance), E (villous pits, with finger-like tubers similar to enteric villus-like changes) and F (the pits have obscure or disappearing structures and extremely irregular arrangement). The boundaries between bowl-shaped defects on the erosion surface and neighboring structures are unclear. Defect areas may show granular protrusions and irregular thickened capillaries). When two or more types of gastric pits were present in a lesion, the lesion was defined as having the severe type of pits.

Immunohistochemical staining for PCNA and *p53*:

Immunohistochemistry: Immunohistochemical analysis was performed using the streptavidin-peroxidase method to determine the expression of *p53* and PCNA in the gastric mucosa. Briefly, tissue samples were fixed in 10% neutral buffered formalin, dehydrated in a graded series of ethanol, cleared, paraffinized, and cut into 4 μ m sections. The sections were deparaffinized, hydrated

Table 1 Relation between gastric pit patterns and histopathological phenotypes

Pit type	Case	CSG	CAG	IM	Dysplasia	
					Mild	Severe
A	9	8	1	0	0	0
B	38	32	6	0	0	0
C	42	35	7	0	0	0
D	67	9	56	7	0	0
E	62	2	6	50	3	1
F	30	0	1	3	20	6
Total	248	74	84	60	23	7

CSG: Chronic superficial gastritis; CAG: Chronic atrophic gastritis; IM: Intestinal metaplasia.

in a graded series of ethanol, incubated in a microwave to heat and high pressure cook for 10 min for antigen retrieval, and washed in tris-buffered saline (TBS) (3×5 min). After treatment with 3% H_2O_2 for 10 min, slides were washed again in TBS (3×5 min). The slides were then incubated with primary anti-PCNA (1:50; Maxim, Fuzhou, China) or anti-p53 (1:100; Maxim) antibody for 30 min at room temperature and washed in TBS (3×5 min). Immunolabeling was visualized by reaction with DAB for 10 min at room temperature. The sections were counterstained with hematoxylin, dehydrated and mounted. This experiment was performed by an experienced technician.

Evaluation of immunohistochemical staining: Scoring of immunohistochemical results was performed according to the semi-quantitative method. The percentage of immunopositive cells was scored using a four-point system as follows: 0 point, < 5% of positive cells; 1 point, 5%-25% of positive cells; 2 points, 26%-50% of positive cells; 3 points, 51%-75% of positive cells; 4 points, > 75% of positive cells. The staining intensity was scored similarly, with 0 point for negative staining, 1 point for weak staining (light yellow), 2 points for moderate staining (brown), and 3 points for strong staining (dark brown). Immunoreactivity score for each lesion was calculated as (the score for the percentage of immunopositivity cells + the score for the staining intensity)/2. Based on immunoreactivity scores, immunohistochemical staining was considered negative (< 0.5), weakly positive (0.5-1.5), or strongly positive (> 1.5). Estimation of scores for all lesions was performed by the same pathologist.

Statistical analysis

All statistical analysis were performed using SPSS 11.5 software. P values ≤ 0.05 were considered statistically significant.

RESULTS

Magnifying chromoendoscopic findings

Relation between gastric pit patterns and histopathologi-

Table 2 Expression of p53 and proliferating cell nuclear antigen in various groups n (%)

Group	Cases	p53 expression			Positive cases	PCNA expression			Positive cases
		-	+	++		-	+	++	
A	9	7	2	0	2 (22.2)	6	3	0	3 (33.3)
B	38	21	14	3	17 (44.7)	23	12	3	15 (39.7)
C	42	21	18	3	21 (50.0)	16	21	5	26 (61.9)
D	67	28	35	4	39 (58.2)	22	35	10	45 (67.2)
E	62	18	38	6	44 (71.0)	14	35	13	48 (77.1)
F	30	4	19	7	26 (86.7)	2	13	15	28 (93.3)

$P < 0.05$ between-group. PCNA: Proliferating cell nuclear antigen.

cal phenotypes: A total of 248 biopsies were taken under a magnifying chromoendoscope. Of these biopsies, 9 had type A gastric pits, 38 had type B, 42 had type C, 67 had type D, 62 had type E, and 30 had type F (Table 1).

Under a magnifying chromoendoscope, intestinal metaplasia was characterized by the presence of type E gastric pits (villous pits). Dysplasia also showed changes similar to those of intestinal metaplasia (e.g., the presence of villous, linear or patchy pits); however, some characteristic changes were observed in some cases of dysplasia. Compared to intestinal metaplasia, gastric pits were more diverse in size, more irregular in shape, and more disorderly in arrangement in moderate and severe dysplasia. Type F gastric pits in the 30 cases of dysplasia showed varying degrees of these changes (Figure 1).

Immunohistochemical results

p53 expression: p53 expression was lowest in type A gastric pits and highest in type F pits, showing an upward trend from types A to F pits. p53 expression intensity also showed an upward trend (Figure 2A and B). Rank sum test for independent samples showed that p53 expression was significantly higher in types E and F pits than in types A-D pits ($H = 33.068$, $P = 0.000$). Between-group comparisons showed that p53 expression was significantly higher in types E and F pits than in type A pits (Table 2).

PCNA expression: PCNA expression was lowest in type A gastric pits and highest in type F pits, showing an upward trend from types A to F pits (Figure 2C and D). The number of cases that showed strong PCNA positivity increased significantly with an increase in the severity of lesions. Rank sum test for independent samples showed that PCNA expression was significantly higher in types E and F pits than in types A-D pits ($H = 31.791$, $P = 0.001$). Between-group comparisons showed that PCNA expression was significantly higher in types E and F pits than in type A pits (Table 2).

DISCUSSION

The normal gastric mucosa surface is divided by criss-cross small grooves into many lesser gastric areas, in

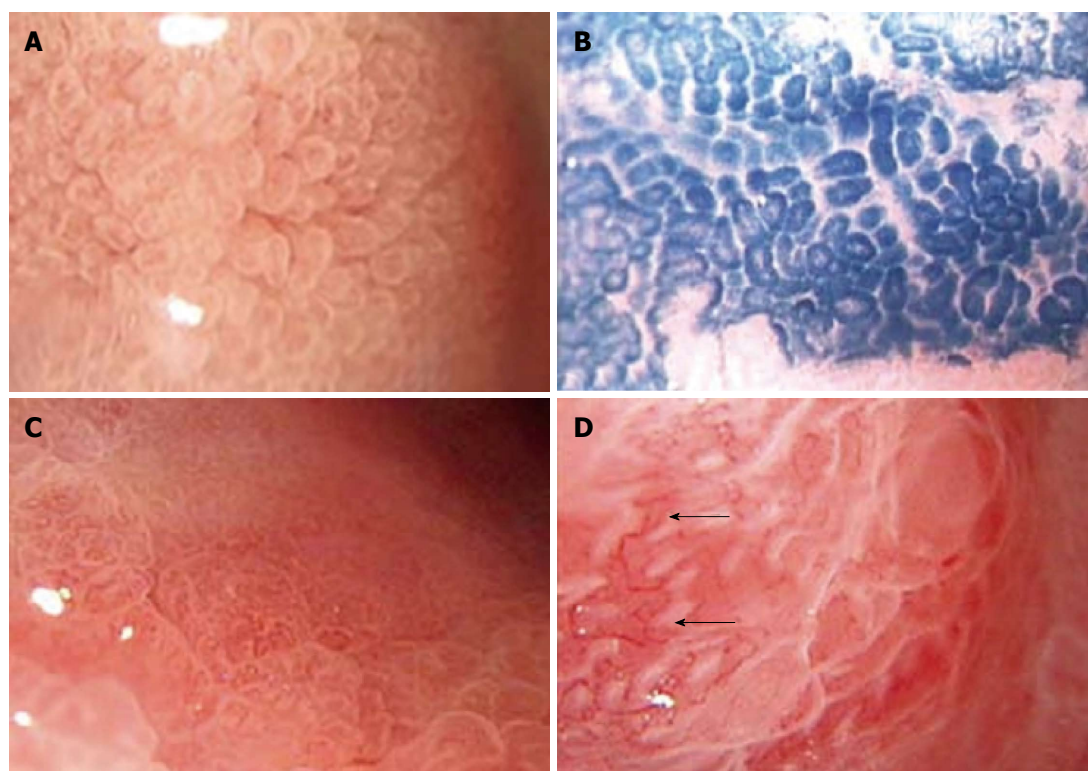


Figure 1 Magnifying chromoendoscopic findings. A: Type E pits; B: Type E pits after dye staining; C: Type F pits; D: Type F pits after dye staining, arrows show disorderly arranged thickened capillaries. Magnification, $\times 100$. Type E: Villous pits, with finger-like tubers similar to enteral villus-like changes; Type F: The pits have obscure or disappearing structures and extremely irregular arrangement.

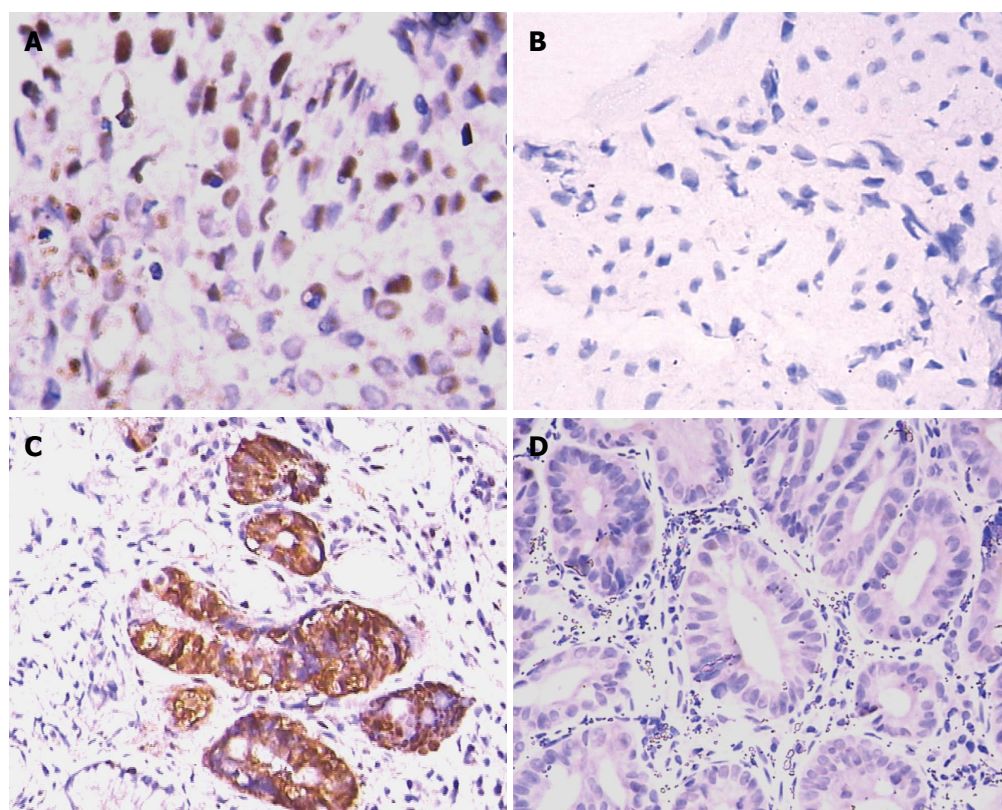


Figure 2 Immunohistochemical results. A: Expression of p53 in gastric pits in chronic atrophic gastritis, and nuclei were stained dark brown; B: p53 expression was undetectable in gastric pits in chronic superficial gastritis; C: Expression of proliferating cell nuclear antigen (PCNA) was detected in gastric pits in chronic atrophic gastritis, and nuclei were stained dark brown; D: PCNA expression was undetectable in gastric pits in chronic superficial gastritis.

which there are many spot- or rod-like gastric pits. When gastric mucosal lesions occur, morphological changes of gastric pits often appear first. Under an ordinary microscope, it is difficult to observe the morphological changes of gastric pits. In contrast, a magnifying endoscope has a magnification comparable to that of a stereomicroscope and allows observing gastric pit patterns clearly. Dye staining is an important auxiliary method for magnifying endoscopy. Magnifying chromoendoscopy can more clearly show the extent and surface conditions of lesions, which is conducive to clearer observation of lesions and more accurate biopsy. In recent years, domestic and foreign scholars have divided gastric mucosal pit patterns into four fundamental types: spot-like, reticular, granular, and villous^[4,5]. Yang *et al*^[4] and Huang *et al*^[6] discovered that gastric pit morphology correlates with the severity of mucosal inflammation. They further divided gastric pits into five fundamental types: A (round spot-like pits), B (short rod-like pits), C (elongated and tortuous pits), D (reticular pits), and E (villus-like pits). This classification can more accurately reflect the evolution from the normal mucosa to superficial gastritis and atrophic gastritis. The presence of type E pits is a characteristic change of intestinal metaplasia. Wang *et al*^[7] believe that epithelial and gland hyperplasia and intestinal metaplasia are main factors leading to morphological changes of gastric pits in atrophic gastritis. Endo *et al*^[8] examined intestinal metaplasia of Barrett's esophagus and gastric cardia and found that villus-like pits are a characteristic change of intestinal metaplasia.

Gastric mucosal erosions are common lesions that show a special pattern during endoscopy, and some of them have malignant potential with the progression of inflammation. Therefore, it is particularly important to detect and determine the nature of mucosal erosions by endoscopy. By carefully observing gastric erosions, we found that the evolution of six fundamental types of gastric pits reflects the evolution from the normal mucosa to superficial gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia. Type F pits evolve gradually from type E pits. They have obscure or disappearing structures and an extremely irregular arrangement. The boundaries between bowl-shaped defects on the erosion surface and neighboring structures are unclear. The erosive areas may show granular protrusions and irregular thickened capillaries. Histopathological analysis indicates that gastric mucosal lesions that have these subtle structural features are closely related to dysplasia or even early gastric cancer. Tajiri *et al*^[9] observed the lesions of depressed early gastric cancer and divided the patterns of microvessels on the lesion surface into six types. They found that microvessels with branches of different calibers, spiral microvessels, and microvessels of different calibers were more commonly seen in undifferentiated carcinoma. Liu *et al*^[10] applied magnifying endoscopy to the diagnosis of gastric mucosal erosions and found that there was an indirect or direct causal relationship between gastric mucosal erosions and gastric cancer. The

presence of mucosal protrusions on the erosion surface is an endoscopic feature of malignant erosions, and this is initially caused by the replacement of cancerous tissue defects by regenerative non-cancerous mucosa. As time goes on, regenerative mucosa can be replaced by cancerous tissue to form pink mucosal protrusions^[11]. In our study, about 80.6% of cases of type E mucosa showed intestinal metaplasia. Under a magnifying endoscope, type F mucosa usually contains no bowl-shaped defects on the erosion surface, which are commonly found in types C-E mucosa. However, the erosion surface has become rough, and the mucosa shows granular hyperplasia or irregular changes. The boundaries between the erosion surface and surrounding structures are unclear, and gastric pits in the periphery of the lesion are sparse, irregularly arranged type E pits. Type F mucosa often suggests varying degrees of dysplasia, which has a coincidence rate of 86.7% compared with pathological results. In addition, some cases of type F mucosa that is proved to have dysplasia by histopathology show varying degrees of capillary dysplasia, which manifests as the presence of irregular, thick, disordered capillaries on the erosive mucosal surface. This characteristic change is similar to tumor vascular change described by Otsuka *et al*^[12] and Tajiri *et al*^[9] in early gastric cancer. Thus, the presence of types E and F mucosa can be regarded as a subtle structural feature of gastric precancerous lesions. In this study, comparison of the findings of magnifying chromoendoscopy and histopathological analysis results indicates that the presence of types E and F mucosa suggests the emergence of intestinal metaplasia and dysplasia.

The p53 gene is a tumor suppressor gene that is located on the short arm of human chromosome 17. It encodes a 53-kDa nuclear phosphoprotein that has transcription factor activity and plays an important role in the control of the cell cycle and apoptosis^[13]. p53 mediates G1 arrest in response to DNA damage for DNA excision and repair to maintain the stability of the genome^[14]. Wild-type p53 acts as not only a transcriptional activator but also a transcription suppressor^[15]. Mutant-type p53 (mt-p53) can interfere with intracellular growth factor signaling to promote cell proliferation, suppress apoptosis, and eventually lead to cell transformation and tumor development and progression. In addition, mt-p53 can confer cell resistance to radiotherapy and chemotherapy by inhibiting apoptosis. Therefore, the biological behavior of tumors expressing mt-p53 is worse than that of tumors not expressing mt-p53^[16].

Wild-type p53 induces cell differentiation. After transformed cells or cancer cells that do not express wild-type p53 were transfected with wild-type p53, the malignant phenotypes of cells were suppressed, cell growth and division were inhibited, cells were arrested at G1 phase, and *in vivo* tumorigenicity was decreased after inoculation into nude mice. On the other hand, introduction of p53 antisense RNA into the colon cancer cell line SW1116 significantly decreased proliferation rate and blocked cells in G0/1 phase, as revealed by flow cytometry^[17].

Wild-type p53 induces apoptosis. After myeloid leukemia cell lines that do not express wild-type p53 were transfected with wild-type p53, typical apoptosis occurred, and cells lost viability. Treatment of tumors with radiation or anticancer agents not only induces DNA damage, blocks DNA metabolism, and leads to necrosis, but also induces apoptosis. The expression of transfected wild-type p53 gene can increase anticancer treatment-induced apoptosis. In contrast, tumor cells transfected with the *mt-p53* gene or tumor cells not expressing wild-type p53 gene are resistant to anticancer treatment-induced apoptosis. A study has proved that p53 induces apoptosis *via* the mitochondrial pathway in activated thymocytes^[18].

The expression of p53 protein is detectable in almost all types of somatic cells. In normal cells, wild-type p53 protein has a half-life of about 20-30 min and shows little accumulation. Finlay *et al*^[19] found that, in actively growing tumor cells, mt-p53 protein, but not wild-type p53 protein, can form a complex with heat shock protein 70. This complex extends the half-life of mt-p53 protein, which significantly increases the accumulation of mt-p53 protein in the nucleus^[20]. The p53 gene is an important tumor suppressor gene, and many human tumors, such as breast cancer, lung cancer, colorectal cancer, and gastric cancer, carry various p53 gene mutations. High expression of the *mt-p53* gene is often detectable in many types of tumors and transformed cells. This study found that the expression of p53 protein in precancerous lesions (intestinal metaplasia and dysplasia) was higher than that in chronic gastritis.

PCNA was first discovered by Miyachi *et al*^[21] in 1978 in the sera of patients with systemic lupus erythematosus. It was named as such because it is only expressed in normal proliferating cells and tumor cells. PCNA is a 36-kDa nuclear protein that is the auxiliary protein of DNA polymerase delta. Two types of nuclear PCNA exist, soluble and insoluble. Soluble PCNA is expressed in various phases of the cell cycle, and its quantity does not change significantly in the process of DNA synthesis. It is susceptible to detergent extraction and destruction by methanol. Insoluble PCNA is more stable and insensitive to detergent elution and destruction by methanol. No obvious expression of soluble PCNA is detected in G0-G1 phase. However, its expression shows a significant increase in the late G1 phase, peaks in S phase, and decreases significantly in G2-M phase. Since the expression of soluble PCNA correlates well with DNA synthesis, it can be used as a marker for evaluation of cell proliferation. Given that cancer cells have a strong proliferative activity and that PCNA can be used as a cell proliferation marker, many domestic and foreign studies have investigated the relationship between PCNA expression and tumor development, grading, staging, radiation sensitivity, prognosis, recurrence and metastasis, death causes, and tumor markers in a variety of tumor types, and drawn many conclusions, although some of them are still controversial among different studies. In

addition, some conclusions were drawn from analyses of one or several tumor types, and it remains to be studied whether they are applicable to all tumor types.

PCNA, as the auxiliary protein of DNA polymerase δ , plays an important role in the regulation of DNA replication and is closely related to the proliferative state of cells. Wu *et al*^[22] examined the expression of PCNA in gastric cancer and precancerous lesions and found that the positive rate of PCNA in the cancer group was significantly higher than that in the control group (80.77% *vs* 55.36%, $P < 0.01$), suggesting that high PCNA expression correlates positively with cancerous transformation. These authors therefore believe that cell proliferation activity significantly increases in gastric dysplasia and intestinal metaplasia. In a study of 133 patients with early gastric cancer, Noda *et al*^[23] found that high expression of PCNA was significantly associated with a higher rate of lymph node metastasis and a lower 5-year survival rate, indicating that PCNA expression correlates with the degree of tumor differentiation and distant metastasis. PCNA expression was significantly higher in poorly differentiated gastric cancer or gastric cancer with distant metastasis than in well differentiated gastric cancer or gastric cancer without distant metastasis. In this study, we found that PCNA expression showed an upward trend from types A to F pits. The number of cases that showed strong PCNA positivity increased significantly with the increase in the severity of lesions.

In conclusion, this study demonstrates that, compared with ordinary endoscopy, magnifying chromoendoscopy can provide more detailed information about fine mucosal morphology and has a significant advantage in the diagnosis of minute lesions. Magnifying chromoendoscopy in combination with p53 and PCNA detection is particularly helpful for the diagnosis of gastric precancerous lesions. The presence of types E and F pits, in which p53 and PCNA are highly expressed, is highly suggestive of the occurrence of early cancer, and patients developing these changes should be closely followed. If necessary, surgery or endoscopy can be considered.

COMMENTS

Background

Early diagnosis and treatment are important for improving the prognosis of gastric cancer. However, the widespread use of gastroscopy does not significantly improve the detection rate of early gastric cancer. Worldwide efforts are being made to improve the sensitivity of endoscopy in the detection of early cancer and precancerous lesions.

Research frontiers

This study assessed the diagnostic value of magnifying chromoendoscopy combined with detection of proliferating cell nuclear antigen (PCNA) and p53 in the detection of gastric precancerous lesions.

Innovations and breakthroughs

Compared with ordinary endoscopy, magnifying chromoendoscopy can provide more detailed information about fine mucosal morphology and has a significant advantage in the diagnosis of minute lesions.

Applications

Magnifying chromoendoscopy is better than ordinary endoscopy; it can provide more information about mucosal morphology and has a significant advantage in

the diagnosis of minute lesions. Magnifying chromoendoscopy in combination with p53 and PCNA detection is particularly helpful for the diagnosis of gastric precancerous lesions.

Peer review

An interesting manuscript assessing the diagnostic value of magnifying chromoendoscopy combined with detection of PCNA and p53 in gastric precancerous lesions to increase the detection rate of early gastric cancer.

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P- Reviewers Pereiralima JG, Benz C **S- Editor** Gou SX

L- Editor Logan S **E- Editor** Xiong L



Gastric ischemia after epinephrine injection in a patient with liver cirrhosis

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Received: September 13, 2012 **Revised:** October 15, 2012

Accepted: November 11, 2012

Published online: January 21, 2013

Kim SY, Han SH, Kim KH, Kim SO, Han SY, Lee SW, Baek YH. Gastric ischemia after epinephrine injection in a patient with liver cirrhosis. *World J Gastroenterol* 2013; 19(3): 411-414 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/411.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.411>

INTRODUCTION

Acute gastric ischemia rarely occurs due to the rich vascular supply of the stomach and the vascular reserve of the intramural anastomosis^[1]. Therefore, there have been few case reports of gastric ischemia worldwide^[2,3]. In the rare cases that have been reported, the risk factors for gastric ischemia were smoking, hypertension, atherosclerosis and endoscopic injection therapy^[3]. Endoscopic epinephrine injection is a widely used therapy for the management of nonvariceal gastrointestinal bleeding, and it promotes initial hemostasis to stop the bleeding^[4].

We report a case of gastric ischemia and necrosis after endoscopic epinephrine injection at the site of the lesion of a biopsy in a patient with liver cirrhosis and hypertension

CASE REPORT

A 51-year-old woman with hypertension and liver cirrhosis was admitted with hematemesis and hematochezia. One day prior to admission, an upper gastrointestinal endoscopy was performed, revealing an elevated lesion with a central depression of the gastric antrum. A variceal lesion was not observed. She underwent an endoscopic biopsy of the lesion of the antrum (Figure 1A), and submucosal injection was performed with a 1:10 000 solution of epinephrine due to bleeding after the endoscopic biopsy. Injection boluses of 2 mL were used for a total of 6 mL. The patient returned home safely after hemostasis. However, she presented to the emergency

Abstract

Endoscopic epinephrine injection is relatively easy, quick and inexpensive. Furthermore, it has a low rate of complications, and it is widely used for the management of nonvariceal upper gastrointestinal bleeding. There have been several case reports of gastric ischemia after endoscopic injection therapy. Inadvertent intra-arterial injection may result in either spasm or thrombosis, leading to subsequent tissue ischemia or necrosis, although the stomach has a rich vascular supply and the vascular reserve of the intramural anastomosis. In addition to endoscopic injection therapy, smoking, hypertension and atherosclerosis are risk factors of gastric ischemia. We report a case of gastric ischemia after submucosal epinephrine injection in a 51-year-old woman with hypertension and liver cirrhosis.

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Key words: Hematemesis; Epinephrine; Gastric ischemia; Liver cirrhosis; Hypertension

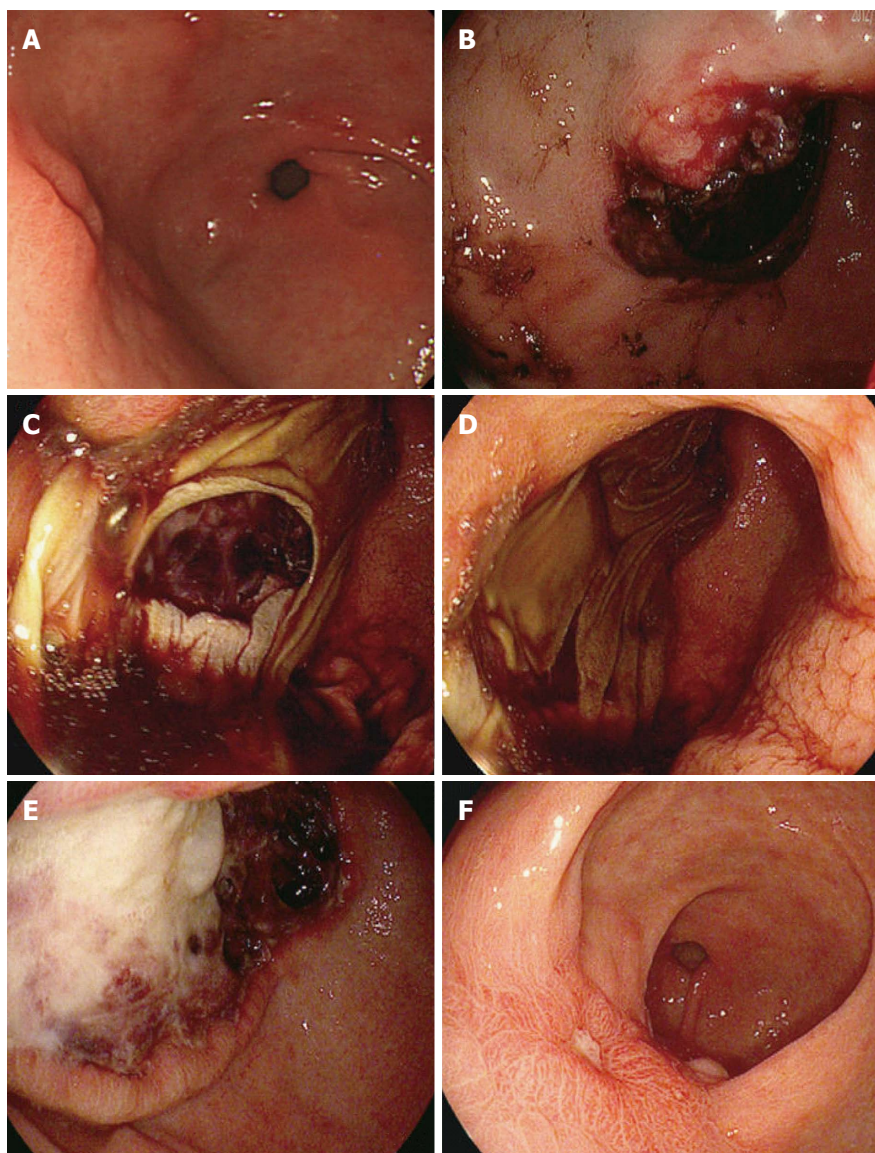


Figure 1 Upper gastrointestinal endoscopy. A: Mucosal elevated lesion with a central depression of the gastric antrum anterior wall. We took an endoscopic biopsy of the elevated lesion; B: Endoscopy revealed the oozing bleeding covered by a hemorrhagic clot in the distal antrum after the endoscopic biopsy. We tried the hemostasis by submucosal injection of a 1:10 000 solution of epinephrine; C: Mucosal dissection with extensive ulcerated; D: Necrotic areas from the distal to the proximal antrum. We did the conservative treatment with proton pump inhibitor, wide spectrum antibiotics and parenteral nutrition; E: Three days after the conservative treatment, a large extensive ulcer with fibrinous and hemorrhagic exudates surrounded by a clear elevated margin. The conservative care was continued; F: Two months later, the decreased size of the ulcer was surrounded by regenerative epithelia with a clear margin.

department reporting massive hematemesis and hematochezia the next day.

In the emergency room, she was in a severe distress with a heart rate of 90 beats/min, an arterial blood pressure of 100/70 mmHg and a respiratory rate of 20 breaths/min. Her abdomen was tender to palpation without guarding. A digital rectal examination was positive for bloody stools. The chest X-ray film and electrocardiogram were normal, as was the plain film of the abdomen. A complete blood count revealed anemia (hemoglobin level, 10.4 g/100 mL; hematocrit level, 29.7%) leukopenia (2360 cells/mm^3 with 56.3% polymorphonuclear cells) and thrombocytopenia ($53\,000 \text{ cells/mm}^3$). The Child-Pugh classification was A with a mildly prolonged

prothrombin time of 14.4 s and hypoalbuminemia (3.0 g/dL). The upper endoscopy revealed oozing bleeding on the site of a previous biopsy, and the lesion was covered by a hemorrhagic clot (Figure 1B). We considered hemoclipping at first, but it was difficult to target the site of clipping because the hemorrhagic clot blocked our view, although vigorous irrigation and precise vessel exposure were not observed. Therefore, we again attempted hemostasis by submucosal injection of a 1:10 000 solution of epinephrine (a total dose of 8 mL). We were not concerned that epinephrine injection would result in gastric ischemia or necrosis at that time. Two days later, the patient presented with severe epigastric pain and melena. The hemoglobin level was 7.7 g/dL. An upper endoscopy

was performed. The endoscopy revealed a mucosal dissection with extensive ulcerated and necrotic areas from the distal to the proximal antrum (Figure 1C and D). We believed that this condition represented mucosal necrosis and severe ischemia. Emergency abdominal computed tomography ruled out arterial thrombosis and perforation, and it revealed an edematous mural thickening with mild mucosal enhancement in the antrum of the stomach. We decided to use conservative treatment because the signs of perforation or sepsis were not observed and the patient had underlying liver cirrhosis. The patient was treated with proton pump inhibitor perfusion, wide spectrum antibiotics and parenteral nutrition. Three days later, endoscopy was performed again to identify changes in the lesion. Endoscopy showed a large extensive ulcer with fibrinous and hemorrhagic exudates surrounded by a clear elevated margin (Figure 1E). We thought that the ischemic ulcer was in a healing state, and conservative care was continued. Her condition was gradually improved, and there was no sign of bleeding. A liquid diet was started, and the patient was discharged from the hospital after 2 wk. A follow-up endoscopy 2 mo later showed the decreased size of the ulcer surrounded by regenerative epithelia with a clear margin (Figure 1F).

DISCUSSION

The rich blood supply of the stomach protects the stomach from ischemia and necrosis^[1]. Acute gastric ischemia, an emergency condition associated with high mortality, is rare^[3]. Possible risk factors of gastric ischemia include smoking, hypertensive and atherosclerotic vascular disease, trauma, infection, and epinephrine injection^[3]. We performed epinephrine injection to control the bleeding after a biopsy. Most cases of post-biopsy bleeding stop on their own without endoscopic therapy. However, we performed the injection therapy because of underlying cirrhosis and the strength of the injection procedure. Injection of epinephrine is effective for controlling upper gastrointestinal bleeding^[5]. This technique is relatively easy, quick, and inexpensive. The complication rate associated with epinephrine injection was minimal^[2].

However, a few cases have been reported of ischemic necrosis of the stomach and/or duodenum after injection of epinephrine (with or without a sclerosant) to control ulcer bleeding^[2,4]. There are multiple mechanisms of action invoked to explain the efficacy of epinephrine, including vasoconstriction, a vascular tamponade effect, and enhanced platelet aggregation^[5]. We postulated that gastric ischemia in this case resulted from the injection of a 1:10 000 solution of epinephrine as a vasoconstrictive drug. First, the epinephrine injection might have caused vascular contraction and opening of the intramural arteriovenous shunts, which could have contributed to gastric ischemia. Second, direct mechanical distress of the gastric artery caused by the needle followed by immediate obliteration of the arterial lumen was most

likely the cause of the ischemia. The double injection of epinephrine most likely aggravated the gastric ischemia in this patient. Furthermore, morphological alterations in the gastric microcirculation in cirrhosis might be the cause of ischemia. One study reported that increased arteriovenous anastomoses in the gastric wall were related to the occurrence of acute gastric mucosal lesions in patients with liver cirrhosis^[6]. Another study showed arteriovenous anastomoses of 50% in the gastric wall and a straight pattern of the arterioles with dilation of the precapillaries, capillaries, and submucosal and subserosal veins in cirrhotic patients, although the sample size was small^[7]. In addition, the long-term history of hypertension, might have caused atherosclerotic vascular changes. Therefore, the abnormal mucosal structure resulting from liver cirrhosis and hypertension was an important risk factor, in addition to the injection of epinephrine, for gastric ischemia in this patient.

Clinically, gastric infarction presents as an acute abdominal emergency with diarrhea or hematemesis that rapidly progresses to acute peritonitis, irreversible septic shock, and death if untreated^[8]. The treatment of acute necrotizing ischemic gastritis is emergency laparotomy with resection when necessary. In most cases, gastric ischemic necrosis has been treated with surgery^[3,4,9]. In our case, the patient was only treated with conservative therapy with total parenteral nutrition, intravenous antibiotics, and a proton pump inhibitor. The results of this conservative treatment were favorable.

This is first report of the occurrence of gastric ischemia after epinephrine injection in a patient with liver cirrhosis. We report a case of fatal gastric ischemia and postulate that accidental intra-arterial injection may be responsible for this event. Inadvertent intra-arterial injection may result in either spasm or thrombosis and lead to subsequent tissue ischemia or necrosis, and underlying liver cirrhosis might influence this rare complication. Therefore, we should pay more attention to the risk of bleeding in underlying vascular disease or cirrhosis during endoscopic hemostasis.

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P- Reviewers Atta HM, De Gottardi A **S- Editor** Gou SX
L- Editor A **E- Editor** Li JY



Primary aortoduodenal fistula: A case report

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Received: July 26, 2012 Revised: September 12, 2012

Accepted: October 19, 2012

Published online: January 21, 2013

from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/415.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.415>

INTRODUCTION

Primary aortoduodenal fistula (PADF) is a rare, challenging complication of abdominal aortic aneurysm (AAA). PADF is an abnormal communication between the infrarenal aorta and duodenum, whereas secondary aortoduodenal fistula usually results from a previously implanted endovascular stent-graft procedure^[1]. Since their first description about 100 years ago, more than 200 PADFs have been reported in the world literature^[2-4]. They are usually found between the 3rd part of the duodenum and infrarenal aorta, caused by evolutionary complication of an aortic aneurysm^[5]. Although less frequent, this abnormal communication also occurs between the aorta and other parts of the gastrointestinal (GI) tract, namely the esophagus, jejunum, ileum, or colon. The rarity of PADFs poses major diagnostic and therapeutic difficulties to the treating physicians.

The present case report describes a PADF between the infrarenal AAA and fourth part of the duodenum, which was successfully managed at King Khalid Hospital, Najran, Saudi Arabia.

CASE REPORT

A 47-year-old Saudi male presented to the emergency room of King Khalid Hospital Najran, Saudi Arabia with a 6-h history of massive fresh upper GI bleeding. He was a heavy smoker with no notable medical history. On examination, he had normal sensorium, pulse 113 beats/min, blood pressure 90/70 mmHg, and a cold sweaty skin. The laboratory investigations showed hemoglobin (Hb) 9.3 g/dL, white cell count 7100/mm³, while electrolytes, renal and coagulation profiles, serum amylase and liver function testes were normal. The abdominal examination was unremarkable except for tenderness in the epigastrium. After initial resuscitation,

Abstract

Primary abdomino-aortic fistula is an extremely rare cause of upper gastrointestinal (GI) bleeding. The diagnosis is frequently delayed due to the rarity of the disease and low index of suspicion by physicians. A range of invasive and non-invasive diagnostic tools are available, but helical computer tomography (CT) remains the mainstay. Surgery offers the only hope for survival. This case report presents a 47-year-old male with massive upper GI bleeding. Various diagnostic tests and an exploratory laparotomy failed to identify the diagnosis. Later, a primary aortoduodenal fistula was confirmed by CT scan which necessitated surgical repair of the fistula and a Goretex graft for the abdominal aortic aneurysm. The patient made an uneventful recovery and remained well to the first postoperative visit in the clinic 2 wk after surgery.

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Key words: Abdominal aortic aneurysm; Aortoduodenal fistula; Computer tomography; Mycotic aneurysm

Alzobydi AH, Guraya SS. Primary aortoduodenal fistula: A case report. *World J Gastroenterol* 2013; 19(3): 415-417 Available

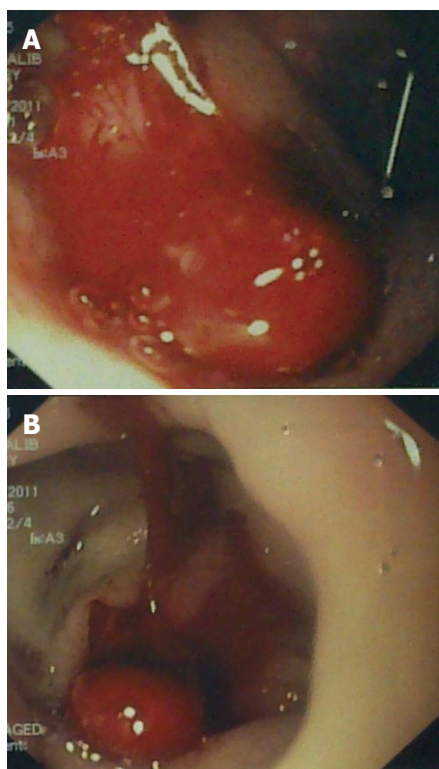


Figure 1 Endoscopic views of the stomach (A) and duodenum (B) showing a huge amount of fresh blood emerging from a concealed source.

upper GI endoscopy was performed and showed a huge amount of fresh blood emanating from the duodenum (Figure 1). No definite source of the bleeding could be identified. The patient was admitted in the intensive care unit, where, despite massive blood transfusion and active fluid resuscitation, the patient remained hemodynamically unstable, fresh bleeding continued in the nasogastric tube, and Hb dropped to 6.7 g/dL. On the 2nd post-admission day, the responsible consultant surgeon performed an exploratory laparotomy which revealed a distended duodenum and no blood in the peritoneum. A duodenotomy was performed which drained fresh and clotted blood, but no active source of the bleeding could be delineated. Perioperative upper GI endoscopy could not provide any additional information. Duodenotomy was closed over a tube drain followed by mass closure of the abdomen. Postoperatively, the patient continued to have fresh bleeding in the nasogastric tube. A computer tomography (CT) scan at that stage showed a PADF between the infrarenal aorta and fourth part of the duodenum (Figure 2). The vascular team was consulted and a second exploratory laparotomy was undertaken where the AAA was repaired with a prosthetic Goretex tube graft, and the duodenal fistula was closed in two layers. An omentopexy was performed between the aorta-graft anastomosis and duodenum. The culture and sensitivity report of the aortic wall reported the growth of *Klebsiella*. The patient made an uneventful recovery and was discharged home in a stable condition. His first follow-

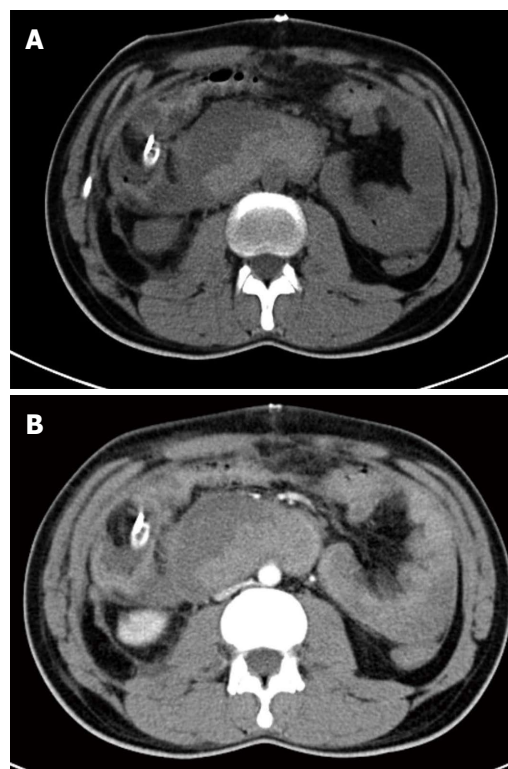


Figure 2 Computer tomography scan showing a primary aortoduodenal fistula between the infrarenal aorta and 4th part of the duodenum. A: Computer tomography (CT) scan without contrast showing a ruptured aortic aneurysm; B: Arterial phase of the CT scan showing the partially thrombosed abdominal aortic aneurysm.

up visit in the surgical outpatient clinic, 2 wk after discharge, showed normal parameters.

DISCUSSION

The term PADF was first coined by Cooper^[6], and the first case report was described by Salman^[7]. The incidence of primary aorto-enteric fistulas is very low and, according to autopsy findings, ranges between 0.04% and 0.07%^[8]. Up to December 2006, 366 cases of primary aorto-enteric fistulae have been reported^[9]. Regarding the anatomical distribution, the 3rd part of the duodenum is involved in two-thirds of cases while the 4th part is affected in one-third of cases. Because of the close approximation and fixed nature of the duodenum, the expanding nature of the AAA causes irritation and inflammation, resulting in eventual fistulization over the passage of time.

The proposed mechanisms for the development of PADF are direct wear and inflammatory destruction triggered by infection, foreign bodies, or erosion^[5]. Eighty percent of PADFs have been estimated to arise from an AAA. Gad reported 73% of PADFs developed from atherosclerotic aneurysms and 26% from traumatic or mycotic aneurysms^[10]. The remaining 1% are caused by radiation, pancreatic carcinoma, metastases, ulcers, gallstones, diverticulitis, and appendicitis. The classical presentations

of PADFs are upper GI bleeding (64%), abdominal pain (32%), and a pulsatile abdominal mass (25%)^[11,12]. Other rare symptoms include intermittent back pain, fever, and sepsis, melena, and syncope. Quite frequently an expansile abdominal mass and a bruit is noticed on abdominal examination.

The most valuable diagnostic tool for the diagnosis of PADF is considered to be helical CT scan with intravenous contrast^[13]. Rapid scanning, high resolution, non-invasive procedure, image quality, and data processing are the outright benefits of CT scanning. Loss of the aneurysmal wall, air in the retroperitoneum or thrombus, or destruction of the fat plane between the aneurysm and duodenum strongly suggest a PADF. In addition, visualization of the contrast within the GI tract is a striking CT finding. Other diagnostic modalities include upper GI endoscopy and angiography. Endoscopy is useful in diagnosing upper GI bleeding, although a negative report does not exclude the possibility of a PADF. The visualization of the fistula below the 3rd part of the duodenum is quite difficult due to acute angulation between 3rd and 4th parts of the duodenum^[12]. Angiography is not reliable in diagnosing PADF. In a retrospective study, 13 out of 36 aorto-enteric fistulas were diagnosed by angiography before surgery^[14]. Magnetic resonance imaging, tagged white blood cell scans, colonoscopy, and ultrasound are of limited value^[15].

The mortality from untreated PADF is almost 100%. The survival after surgery ranges from 18% to 93%^[16]. The recommended surgical procedure consists of closure of the duodenal fistula, aortic ligation to exclude the aneurysm, and an extraluminal bypass. In the case of an infected AAA, antibiotics should be given for 4-6 wk after surgery, if a positive culture is obtained^[17]. The most commonly encountered cultured organisms in primary mycotic PADF are *Salmonella* and *Klebsiella*. In our case, third generation cephalosporins were administered for 1 mo with promising results.

To conclude, PADF are an extremely uncommon cause of massive upper GI bleeding and abdominal mass. A CT scan is the gold standard for diagnosis of this entity, and surgical intervention offers the only treatment for survival.

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P- Reviewers Karmy-Jones R, Sun ZH S- Editor Jiang L
L- Editor Cant MR E- Editor Xiong L



Emergency caudate lobectomy for ruptured hepatocellular carcinoma with multiple primary cancers

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Received: November 2, 2012 Revised: December 17, 2012

Accepted: December 22, 2012

Published online: January 21, 2013

Multiple primary malignancies; Rectal adenocarcinoma; Hepatocellular carcinoma; Hematogenous metastasis

Sun LH, Han HQ, Wang PZ, Tian WJ. Emergency caudate lobectomy for ruptured hepatocellular carcinoma with multiple primary cancers. *World J Gastroenterol* 2013; 19(3): 418-421 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/418.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.418>

Abstract

We report a case of metachronous multiple primary malignancies involving both rectum and liver with colonic metastasis from hepatocellular carcinoma (HCC) through hematogenous pathway. A 72-year-old woman was admitted to the emergency department with right upper abdominal pain for 4 h. Considering her surgical history of Mile's procedure plus liver resection for rectal cancer with liver metastasis three years ago and the finding of urgent computed tomography scan on admission, the preoperative diagnosis was spontaneous rupture of rectal liver metastasis located in caudate lobe and colonic metastasis from rectal cancer. The patient underwent an emergency isolated caudate lobectomy at a hemorrhagic shock status. Pathology reported a primary HCC in the caudate lobe and colonic metastasis of HCC with tumor embolus in the surrounding vessels of the intestine. No regional lymph node involvement was found. It is hypothesized that HCC may disseminate hematogenously to the ascending colon, thus making it a rare case.

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Key words: Emergency isolated caudate lobectomy;

INTRODUCTION

The caudate lobe of the liver is located deep in the hepatic parenchyma and surrounded by some major vessels. Because of its surgically difficult-to-approach anatomic location and perception of early involvement of the inferior vena cava (IVC) or portal vein by tumors, caudate lobectomy was once used infrequently. With more precise anatomical knowledge, development of surgical technique, and improvement in perioperative care, it has become more common recently. But in order to succeed in the procedure, the optimal operative approach should be planned and determined carefully before operation. Emergency isolated caudate lobectomy was rarely reported worldwide, especially for the ruptured primary hepatocellular carcinoma (HCC). The incidence of colorectal cancers has increased in recent years. As the overall survival rate has improved due to the development of multiple therapies, it is not surprising to see more patients with multiple primary malignancies involving the colorectum and other organs. For colorectal cancer, stomach was the most common extracolonic site of both the synchronous and metachronous multiple primary malignancies, but liver is very rarely involved. Extrahepatic metastasis of HCC occurs occasionally and increases with prolonged survival. However, HCC with gastrointestinal (GI) tract metastasis is seldom reported, and it is only found in less than 6% of the cases in autopsy series. GI metastasis through hematogenous pathway is especially rare.

CASE REPORT

A 72-year-old woman was admitted with right upper abdominal pain. The pain started in the epigastrium. A few hours before admission, the pain disseminated to the upper right abdominal quadrant and right back region and became severe.

The patient had a previous history of rectal adenocarcinoma with liver metastasis. Three years earlier she underwent the Miles procedure and partial hepatectomy. Following the operation, she received combined chemotherapy with 5-fluorouracil, folinic acid and oxaliplatin. Six months ago, computerized tomography (CT) scan of the abdomen showed a 5 cm × 5 cm × 5 cm, diverticulum like, exophytic mass located in the ascending colon near the ileocecus, without lymph node enlargement. It was considered a metastasis of rectal cancer. The mass showed no obvious response to systemic adjuvant chemotherapy.

On admission, her temperature was 36.9 °C, pulse was 112 beats/min and blood pressure was 90/50 mmHg. The oxygen saturation was 95% while the patient was breathing ambient air. On physical examination, the patient was ill-looking, conscious with skin and conjunctival pallor. The abdomen was distended, and bowel sounds were absent. There was tenderness in the right upper abdominal quadrant, with guarding and rebound tenderness. Lungs and heart sounds were normal.

Her blood work-up on admission showed a hemoglobin level of 6.8 g/L, white cell count of 8.9×10^9 /L and neutrophils of 72%. Coagulation tests, liver function, renal function and electrolytes were all within the normal range. Urgent CT scan of the abdomen showed multiple masses localized in the liver, a 6 cm × 6 cm × 6 cm mass in the caudate lobe of the liver (Couinaud's segment 1), and signs of abdominal haemorrhage (Figure 1). The pre-operative diagnoses were hemorrhagic shock, spontaneous rupture of rectal liver metastasis, colonic metastasis of rectal cancer or multiple primary cancers.

Hemodynamic stability was achieved following rehydration with intravenous fluid and multiple blood transfusions. At the same time, the patient was promptly sent to the operating room. Open exploratory laparotomy was performed *via* a J-shaped skin incision, with more than 2 liters of blood and clots and intra-abdominal adhesions due to the last operation visualized in the peritoneum. Inspection of the liver revealed a ruptured mass of 6 cm × 6 cm × 6 cm in the hepatic segment I with active bleeding. A 5 cm × 5 cm × 5 cm erosive tumor lesion was localized in the beginning of the ascending colon and nearly occupied the whole lumen. Isolated caudate lobectomy was performed *via* bilateral approach, in addition to the resection of ileocecum. The operation time was 290 min and blood loss was 2000 mL. Ten units of packed red blood cells and 2000 mL of fresh frozen plasma were transfused during the perioperative period. Histological examination demonstrated a primary HCC located in the caudate lobe and a colonic metastasis of HCC with tumor embolus in the surrounding vessels of

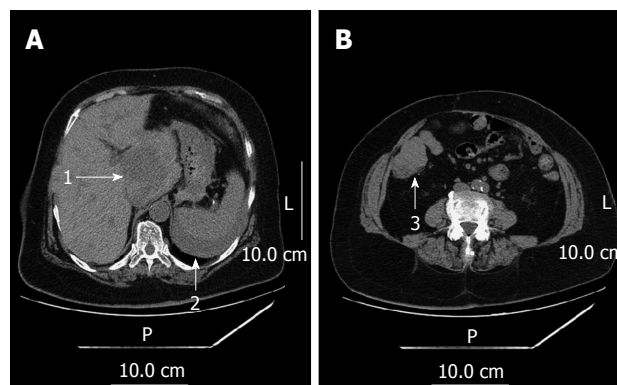


Figure 1 Urgent computed tomography. A: Sagittal view; B: Transverse view. 1: A ruptured mass of 6 cm × 6 cm × 6 cm localized in the I hepatic segment; 2: Perisplenic hemoperitoneum; 3: A 5 cm × 5 cm × 5 cm mass abutting the ileocecum without lymph node enlargement. L: Left; P: Posterior.

the intestine (Figure 2). No regional lymph node metastasis was found. Immunohistochemical findings revealed that polyclonal alpha fetoprotein and hepatocyte antigen were positive and carcinoembryonic antigen was negative. The patient made a full recovery from the surgery and was subsequently discharged 10 d after admission. Eight months later, the patient died of cachexia.

DISCUSSION

The caudate lobe of the liver is located deep in the hepatic parenchyma behind both major lobes and between the confluence of the three main hepatic veins, IVC, and hepatic hilum. It is generally divided into three parts, as described by Kumon: the left Spiegel lobe (Couinaud's segment 1), the caudate process, and the paracaval portion (Couinaud's segment 9). Because of its surgically difficult-to-approach anatomic location and perception of early involvement of the IVC or portal vein by tumors, caudate lobectomy was once called a forbidden zone of hepatic surgery and not performed frequently until it was first described by Lerut. In the early days, patients underwent resection of the caudate lobe along with removal of adjacent portions of the liver, i.e., right or left lobectomy, in order to get a better exposure. But for patients who concurrently had chronic liver disease, removal of too much normal hepatic parenchyma would delay recovery or even cause liver failure. With more precise anatomical knowledge, development of surgical technique, and improvement in perioperative care, isolated caudate lobectomy^[1] has become common recently. Nevertheless, clinical assessment should be conducted strictly before operative period^[2]. Laboratory investigations, including tests of complete blood count, coagulation, liver function, renal function, electrolytes, indocyanine green retention rate at 15 min, and virology, should be carried out to evaluate the patient's surgical fitness; and radiological studies, including ultrasonography, CT scan, and three dimensional reconstructive CT, should be undertaken to evaluate the hepatic lobe size, extent and location of the tumor, vascular involvement, lymph node affection, and the severity

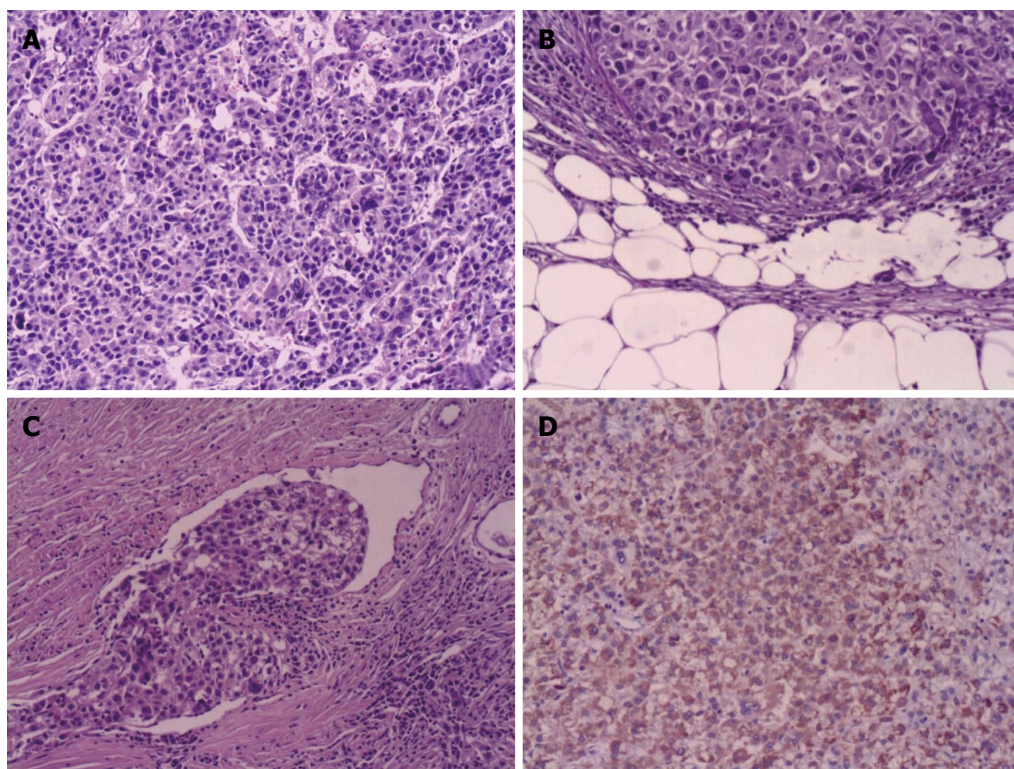


Figure 2 Histopathology. A: Hepatocellular carcinoma (HCC) located in the caudate lobe [hematoxylin and eosin (HE), $\times 100$]; B: Colonic metastasis of HCC (HE, $\times 100$); C: Cancer embolus in the vessels surrounding the intestine (HE, $\times 100$); D: Polyclonal hepatocyte antigen was positive by immunohistochemistry, $\times 100$.

of cirrhosis, and to rule out intra-abdominal metastasis. The most appropriate operative procedure should be planned and determined preoperatively^[3]. Consequently, emergency isolated caudate lobectomy was rarely reported worldwide, especially for the ruptured primary HCC. For these patients, routine treatments included compression and packing hemostasis, transcatheter arterial chemoembolization (TACE), and partial hepatectomy. Because the caudate lobe requires the arterial blood supply from the two major lobes of the liver, TACE cannot achieve the goal of hemostasis. The necrotic tumor tissue and important vessels surrounding the caudate lobe also affect the compression and packing hemostasis. As a result, emergency isolated caudate lobectomy is considered to be the best choice for these patients. There are four approaches for the resection of the caudate lobe of the liver, left side approach, right side approach^[4], bilateral approach, and anterior transhepatic approach by splitting parenchyma of the liver. The approach to the caudate lobe depends on the location of the tumor and the expertise of the surgeon. Hepatic surgery is a very bloody procedure, especially the caudate lobectomy, because of the unique anatomical relationship between the caudate lobe and IVC and the portal triad. Vascular control combined with positioning and central venous pressure control is recommended to minimize intraoperative blood loss. Full mobilization of liver is indicated before the dissection of the caudate lobe. Dividing the fibrous retrocaval ligament is extremely important in fully mobilizing the caudate lobe to expose the hepatic veins along the anterior sur-

face of the IVC. Once the left lateral edge of the caudate lobe is free, ligation of the short hepatic veins draining directly into IVC is performed. Our patient underwent a partial hepatectomy three years ago for rectal liver metastasis resulting in complicated intra-abdominal adhesions, which further increased the difficulties of the operative procedure^[5]. Likewise, the pathophysiological changes of hemorrhagic shock also make it more difficult to keep the stability of the haemodynamics of the patient.

Considering the history of rectal cancer three years ago in this case, the preoperative diagnosis was spontaneous rupture of rectal liver metastasis and colonic metastasis of rectal cancer. But histological examination demonstrated a primary HCC located in the caudate lobe and a colonic metastasis of HCC. Multiple primary cancers are relatively rare^[6]. The overall incidence rate is between 0.73% and 11.7%. However, more multiple primary cancers have been encountered due to an increase in the number of elderly patients and advancement in diagnostic techniques. In addition, cancer patients are assumed to be at an increased risk of developing cancers in other organs due to genetic alterations or exposure to the same environmental carcinogens^[7]. It is generally defined according to the criteria of Warren and Gates: (1) each tumor must be clearly malignant on histologic examination; (2) each tumor must be distinct; and (3) the possibility that the second tumor represents a metastasis must be excluded. Multiple primary cancers can be synchronous or metachronous depending on the interval between their diagnosis. Synchronous malignancies are secondary

cancers occurring simultaneously or within 6 mo after the diagnosis of primary cancers, while metachronous malignancies are secondary cancers that developed more than 6 mo after the diagnosis of primary cancers. The incidence of colorectal cancers has increased in recent years. As the overall survival rate has improved due to the development of multiple therapies, it is not surprising to see more patients with multiple primary malignancies involving the colorectum and other organs^[8]. For colorectal cancer, stomach is the most common extracolonic site of the synchronous multiple primary cancers. The thyroid gland is the second organ involved. The stomach is also the most common extracolonic site of the metachronous multiple primary malignancies, followed by the cervix, lung, and skin. The metachronous multiple primary malignancies involving both rectum and liver are very rare. Although the mechanisms underlying the development of multiple primary cancers are not fully understood, some factors such as sustaining environmental carcinogens, susceptibility to carcinogen, cancer cell immunodeficiency, and immunosuppression caused by radiotherapy and chemotherapy have been implicated. Under these circumstances, it is expected that as people live longer, they have more chances to develop multiple primary malignancies. The prognosis of the patients with multiple primary cancers can be predicted independently by the stage of each tumor.

HCC is one of the most common malignancies worldwide^[9]. Extrahepatic metastasis of HCC occurs occasionally and increases with prolonged survival. The most frequent sites of metastasis are lungs, bones, regional lymph nodes, and adrenal glands. However, HCC with GI tract metastasis is very rare, being found in only less than 6% of cases in autopsy series^[10]. The metastasis sites only reported in some case reports included duodenum, stomach, colon and jejunum. The major pathway of the metastasis is direct invasion to the contiguous GI tract via adhesion to the serosal side by a bulky tumor mass. However, the frequency of metastatic colon cancer arising from HCC is low. In this case, the solitary metastasis from HCC appeared in ileocecus, separated from other liver tissues. The mode of the metastasis to the colon is still unclear. It is hypothesized that HCC may disseminate hematogenously to the distant GI tracts. Tumor embolus in the vessels surrounding the intestine may be the key evidence of hematogenous metastasis. Absence of regional lymph node metastasis may indicate low possibility of lymphatic metastasis. The prognosis of GI metastasis from HCC is known to be poor with a median survival period of seven months.

In conclusion, we report a rare case of metachronous

multiple primary malignancies involving both rectum and liver with colonic metastasis through hematogenous pathway. This patient was manifested by spontaneous rupture of HCC located in the caudate lobe, and underwent an emergency isolated caudate lobectomy, with a full recovery after surgery.

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P- Reviewer Wong GLH S- Editor Gou SX L- Editor A
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Primary histiocytic sarcoma of the stomach: A case report with imaging findings

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Received: September 11, 2012 Revised: November 30, 2012

Accepted: December 15, 2012

Published online: January 21, 2013

To the best of our knowledge, this is the first report in English language literature that emphasizes the imaging findings of human gastric HS.

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Key words: Histiocytic sarcoma; Stomach; Primary; Endoscopic ultrasonography; Gastroscopy; Double contrast examination; Computed tomography

Shen XZ, Liu F, Ni RJ, Wang BY. Primary histiocytic sarcoma of the stomach: A case report with imaging findings. *World J Gastroenterol* 2013; 19(3): 422-425 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/422.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.422>

Abstract

Histiocytic sarcoma (HS) is a rare malignant neoplasm that originates from a histiocytic hematopoietic lineage characterized by histiocytic differentiation and its corresponding immunophenotypic features. We herein reported a case of primary HS of the stomach which was confirmed through histopathologic examination and immunohistochemical staining. A 52-year-old woman presented with progressive difficulty in feeding and dull pain in the epigastric region. Gastroscopy, endoscopic ultrasonography, double contrast examination, and computed tomography revealed a mass located on the posterior wall of fundus and lesser curvature of the stomach. Microscopically, the cytoplasm of the tumor cells was abundant and eosinophilic. Immunohistochemical staining revealed that the tumor cells were positive for CD45RO and CD68. It is difficult to differentiate HS of stomach from other gastric malignancies by radiological evaluation alone. However, HS may be considered when a protruding and ulcerated mass in stomach shows heterogeneous hypervascular features.

INTRODUCTION

Histiocytic sarcoma (HS) is an exceedingly rare lymphohematopoietic malignant neoplasm composed of tumor cells showing morphologic and immunophenotypic features of mature histiocytes^[1,2]. Most patients are adults (median age 46 years). Male predilection is found in some studies. About one-third of cases present in lymph nodes, about one-third in skin, and about one-third in a variety of other extranodal sites, most commonly the intestinal tract^[3].

HS of the stomach is extremely rare and it has rarely been described in the English literature^[3,4]. The imaging findings of gastric HS are not well known. Thus, it is still indistinguishable from other gastric neoplasms both clinically and radiographically^[5]. To the best of our knowledge, only ten cases of gastric HS have been reported in the international medical literature^[3-8].

We report herein a case of primary HS of the stomach with an emphasis on the imaging findings of endoscopic ultrasonography (EUS), gastroscopy, double contrast examination, and computed tomography (CT).



Figure 1 Gastroscopic examination revealed a large irregular ulcer with apophysis and erosive hemorrhage located on the posterior wall of fundus and lesser curvature.

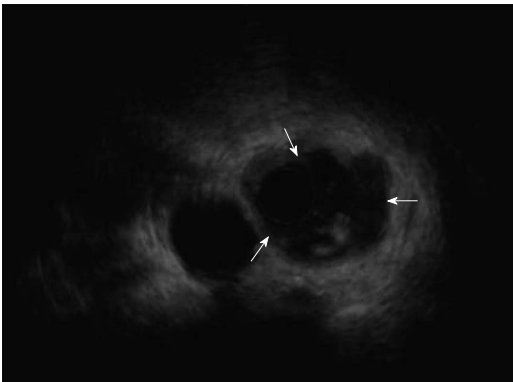


Figure 2 Endoscopic ultrasonography showed thickening about the semi-perimeter of the gastric fundal mucosa and a heterogeneous hypoechoic mass, the whole layer of the local wall involved (arrows).

CASE REPORT

A 52-year-old woman presented with a one-month history of progressive difficulty in feeding and dull pain in the epigastric region for 3 d, without haematemesis or black defecation. At physical examination, she was slightly tender in the middle of the upper abdomen, without abdominal distension. There was no history of previous known stomach disease.

Gastroscopic examination revealed a large irregular ulcer with apophysis and erosive hemorrhage located on the posterior wall of fundus and lesser curvature of the stomach (Figure 1).

EUS detected thickening about the semi-perimeter of the gastric fundal mucosa, and a heterogeneous hypoechoic mass, and the whole layer of the local gastric wall was infiltrated (Figure 2).

The upper digestive pneumobarium double imaging demonstrated a filling defect and a shadow of soft tissue mass around the cardia and lesser curvature of the stomach, and the abdominal part of esophagus was involved. There was destruction of the mucosa, rigidity of the gastric wall, and intracavitary niches in the lesion area (Figure 3).

Plain and contrast-enhanced CT scans of the abdo-

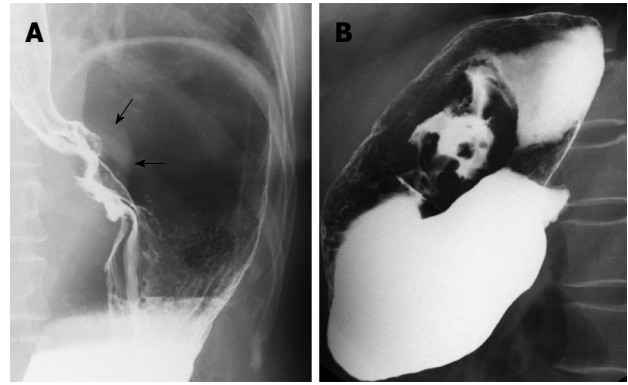


Figure 3 Double contrast examination demonstrated the shadow of soft tissue mass around the cardia and lesser curvature of stomach (arrows) (A), and filling defect, intracavitary niches, destruction of mucosa, as well as rigidity of the local wall were seen (B). The abdominal part of esophagus was involved.

men showed a focal irregular thickened gastric wall and a poorly margined, ulcerated soft tissue mass formation with heterogeneously obvious enhancement in the lesser gastric curvature and fundus area (Figure 4). Swelling of the adjacent regional lymph nodes was also found.

Radical total gastrectomy was performed, and a 7 cm × 6 cm irregularly protruded tumor in the gastric curvature and fundus with the distal esophagus involved was found during the operation, associated with enlargement of the 9th and 11th groups of perigastric lymph nodes.

The surgical specimens were sent to histopathologic examination. The greater omentum did not show any tumor invasion, and tumor metastasis was not found in any of the 22 examined perigastric lymph nodes. The tumors showed infiltrative borders and areas of ulceration, with invasion into the serosa. The tumor cells were monomorphic to pleomorphic in shape. The cytoplasm was abundant and eosinophilic. The nuclei were large, and round to oval. Bizarre multinuclear giant cells were scattered. Immunohistochemical analysis indicated that the tumor cells expressed CD68, CD45RO, CD31 and Cyclin D1, and approximately 90% of the cells stained for Ki-67, but Bcl-2, Bcl-6, CD10, CD23, CD35, CD56, EMA, CK, Mum-1 and Pax-5 were negative (Figure 5). The histological and immunohistochemical diagnosis for the malignant tumor of the stomach was histiocytic sarcoma.

The patient received three cycles of chemotherapy after radical surgery. She has been free of recurrence or metastasis for 4 mo.

DISCUSSION

Histiocytic sarcoma, also known as true histiocytic lymphoma, is an exceedingly rare lymphohematopoietic malignant neoplasm composed of tumor cells showing morphologic and immunophenotypic features of mature histiocytes^[2]. These malignant tumors most commonly present in lymph nodes or at extranodal sites, such as the gastrointestinal (GI) system, bone marrow and skin. The vast majority of GI HS are located in the intestinal tract; only few cases are found in the stomach. Hornick *et al.*^[3]

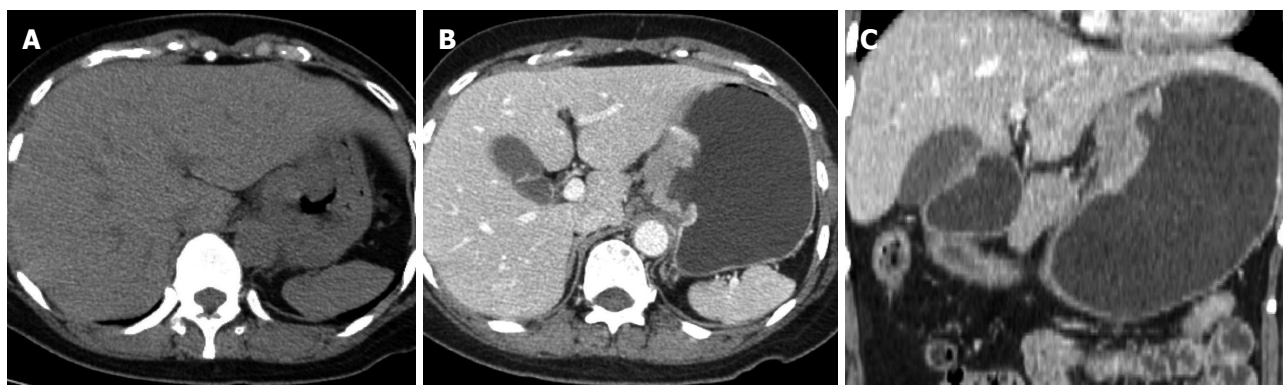


Figure 4 Abdominal computed tomography scan showed focal irregular thickened gastric wall and a ulcerated soft tissue mass formation with heterogeneously obvious enhancement in the lesser gastric curvature and fundus area. A: Plain scan; B: Contrast-enhanced scan; C: Coronal multiplanar reconstruction of enhancement scan.

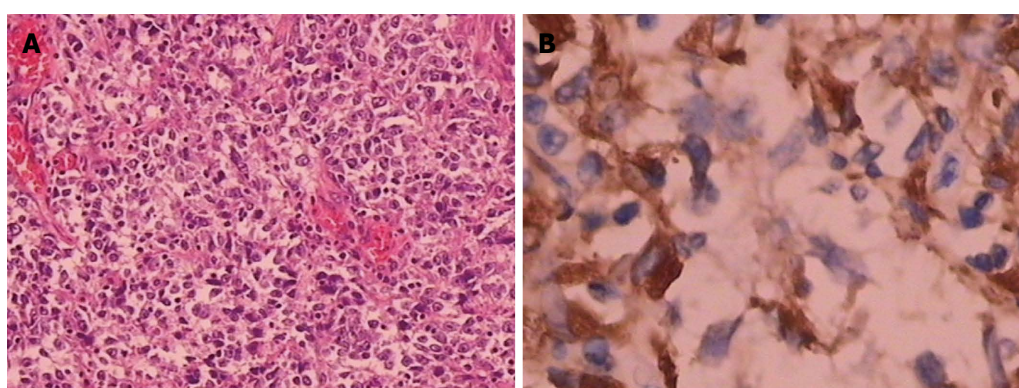


Figure 5 Histopathologic exam showed the tumor cells were marked inequality in the sizes, the cytoplasm was abundant and eosinophilic, the nuclei were large, round to oval, bizarre multinuclear giant cells were scattered. The tumor cells expressed CD68. A: Hematoxylin and eosin $\times 100$; B: Immunohistochemical staining for CD68 $\times 400$.

and Copie-Bergman *et al*^[4] once described 14 cases of extranodal HS and 13 cases of true histiocytic lymphomas, respectively; among these cases, a total of 12 lesions in 11 patients were located in the GI tract, but only one lesion arose in the stomach. Gastric HS can be located anywhere in the stomach: the cardia, the fundus, the antrum, the lesser curvature, or the greater curvature, but most arise in the lesser curvature and involve the adjacent area. There were ulcers in almost all lesions, and perigastric regional lymphadenopathies were also the common associated findings in the patients with gastric HS^[5-8].

Sundersingh *et al*^[7] reported a multifocal HS of the gastrointestinal tract, and a CT scan revealed multifocal, circumferential gastrointestinal wall thickening involving the stomach and jejunal loops. Gastric HS can also exist together with HS of the colon^[2]. In our case, the distal esophagus had also been infiltrated. Akiba *et al*^[9] reported a case of HS arising in the parotid gland region; the patient had recurrent lesions in the pelvis and stomach 5 mo after parotidectomy.

On CT or magnetic resonance imaging enhanced scans, extranodal HS lesions often present with notably heterogeneous contrast enhancement, because generally the neoplasm has a plentiful blood supply^[10,11]. Fant

et al^[12] reported one case of primary gastric HS in a dog, a large, protruding and ulcerated mass was observed on tomographic and endoscopic examinations, moreover, the lesion was also apparently strengthened on enhanced CT scan. The CT manifestations in our case were exceedingly similar with those of the above case.

The imaging features of gastric HS are nonspecific. Abdominal radiographs may show a soft tissue mass with an irregular ulcer and heterogeneous hypervascular features, mostly arising in the lesser curvature and involving the adjacent area.

In conclusion, HS of the stomach is extremely rare, and, to our knowledge, its imaging features have not been previously detailed and summarized in the English-language literature in humans^[5-8]. The gastric HS can be primary or secondary, but the primary tumor seems to be more prevalent, and it can also coexist with other sites of HS. It is difficult to differentiate HS of the stomach from other gastric malignancies by radiological evaluation alone. However, HS may be considered when a protruding and ulcerated mass in the stomach shows heterogeneous hypervascular features, particularly when it arises in the lesser curvature.

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P- Reviewers Greenawalt DM, Okita NT, Polymeros D

S- Editor Song XX **L- Editor** A **E- Editor** Li JY



Inflammatory bowel disease: A proposal to facilitate the achievement of an unequivocal diagnosis

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Supported by Fondazione Malattie Infiammatorie Intestinali (IBD) Onlus - Torino; Compagnia San Paolo, Torino

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Received: September 25, 2012 Revised: December 18, 2012

Accepted: December 22, 2012

Published online: January 21, 2013

ber of endoscopic biopsies obtained from IBD patients do not meet the above-mentioned requirements. The aim of the present proposal is to introduce a binary system of evaluation in the "diagnostic conclusion" of the histopathological report that will help to simplify the clinical decisions and consequent patient management. In patients with no history of disease, the pathologist should classify the biopsies in "Diagnostic", when the criteria established by the international guidelines are satisfied and "not diagnostic" when one or more of the above-mentioned criteria are not met. The term "not diagnostic" should replace "highly suggestive" and "probable". This new terminology could avoid ambiguous expressions that encourage the clinician to classify the patient as affected by IBD without fulfilling all of the requirements for an accurate diagnostic approach.

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Key words: Inflammatory bowel disease; Diagnosis; Biopsy

Canavese G, Bassotti G, Astegiano M, Castellano I, Cassoni P, Sapino A, Villanacci V. Inflammatory bowel disease: A proposal to facilitate the achievement of an unequivocal diagnosis. *World J Gastroenterol* 2013; 19(3): 426-428 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/426.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.426>

Abstract

Following the international guidelines criteria an adequate "diagnostic conclusion" of inflammatory bowel disease (IBD) can be achieved only if clinical, endoscopic and laboratory findings, together with sample technical adequacy and unequivocal histomorphological signs of the disease are available. Thus, a conclusive diagnosis requires a complex combination of clinical, endoscopic and histological data. A considerable num-

TO THE EDITOR

Various guidelines have defined the criteria used for the diagnostic evaluation of endoscopic biopsies as chronic idiopathic inflammatory bowel disease (IBD)^[1-5].

Most of these guidelines include a "preliminary section" of the histological report that includes the evaluation of the different morphological parameters of the

biopsy specimen and a final section with the “diagnostic conclusion” derived from the previous synoptic analyses. In practice, the adequate diagnosis of colon biopsy specimens for non-neoplastic disease requires the following parameters: (1) clinical, endoscopic, and laboratory findings; and (2) technical adequacy of endoscopic sampling and histological procedures, particularly a proper orientation of the biopsies^[3]; and unequivocal evidence of specific morphological signs of the disease in histological analysis^[5-7].

Thus, a conclusive diagnosis requires a complex combination of clinical, endoscopic and histological data. Consequently, a considerable number of endoscopic biopsies obtained from IBD patients do not meet the above-mentioned requirements and cannot receive a definite histological diagnosis. In the more recent guidelines, the categories “highly suggestive”^[1] and “probable”^[6] in the final section of the diagnostic report encompass all of these cases. Although these terms are ambiguous, they are often interpreted as a definitive diagnosis for the clinical management of patients with endoscopy suggestive for IBD.

The aim of the present proposal is to introduce a binary system of evaluation in the “diagnostic conclusion” section that will help to simplify the clinical decisions and consequent patient management. For this purpose, patients without a previous histological diagnosis of IBD and patients with a previous unequivocal diagnosis of IBD should be approached differently.

In patients with no history of disease, the pathologist should use the following terms to classify the biopsies.

Diagnostic, when the criteria established by the international guidelines^[1,2] for the diagnosis of IBD are satisfied: (1) complete or extensive mapping of ileo-colonic segments; (2) adequate specimens, correctly oriented; (3) adequate information, including clinical history, laboratory data, and previous histological and/or endoscopic reports; and (4) histological examination of specimens shows unequivocal microscopic signs of the disease (diagnostic for IBD) or definitely rules out the diagnosis of IBD (negative for IBD). If possible, the pathologist should specify the type of the non-IBD colitis (infective, drug-induced, lymphocytic, collagenous, *etc.*).

Not diagnostic, when one or more of the above-mentioned criteria are not met. The term “not diagnostic” should replace “highly suggestive”^[1] and “probable”^[6]. After discussion with the gastroenterologist and the endoscopist, these patients should be re-evaluated. In cases with persistent symptoms, the patient should be sent back to endoscopy with the goal of achieving diagnostic biopsies. IBD shows a distinctive microscopic morphology only after 2-3 wk^[8], which should be considered in the timing of subsequent biopsies.

In cases of a previous unequivocal diagnosis of IBD performed in the same institution (or in a different center, if specimens are available for revision), the sampling requirements are less stringent and more dependent on endoscopy. The categories “diagnostic/not diagnostic” in the final section of the report are not required, but the

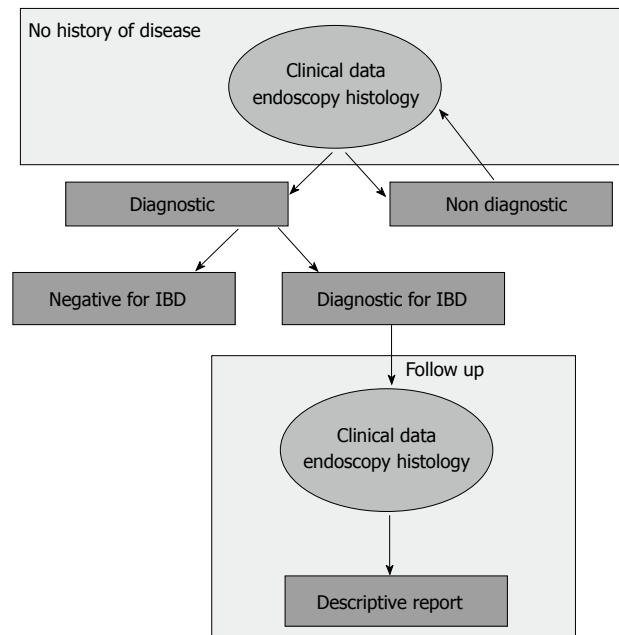


Figure 1 A graphic representation of the described terms (boxes) with suggestions for clinical management. IBD: Inflammatory bowel disease.

report should include an evaluation of disease activity, the presence or absence of dysplasia, and the presence or absence of Cytomegalovirus (follow-up evaluation). Figure 1 provides an example of an algorithm used for this classification.

In our opinion, the advantages of using this binary system to approach endoscopic biopsies in IBD are the following: (1) this terminology could avoid ambiguous expressions that encourage the clinician to classify the patient as affected by IBD without fulfilling all of the requirements for an accurate diagnostic approach; (2) a second set of biopsies after a “not diagnostic” result should simplify the differential diagnosis from non-IBD colitis. Moreover, after the first set of biopsies, the clinician may be able to collect other data (laboratory data, response to therapy, *etc.*) that are useful for diagnosis; (3) this approach will reduce the use of inappropriate treatments that might cause mucosal changes, which can complicate the histological evaluation and compromise the correct assessment of the patient^[8]; and (4) last, but not least, the quality of clinical and biological studies should be improved by including only case series with a definitive diagnosis.

The final goal of our proposal is to seek comments and suggestions about this topic and share this approach with other authors.

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P- Reviewers Rocha R, Tanaka T, Ukleja A, Wirth S

S- Editor Song XX **L- Editor** A **E- Editor** Li JY



Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design

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Received: September 27, 2012 Revised: December 11, 2012

Accepted: December 20, 2012

Published online: January 21, 2013

Abstract

Rafiei *et al* recently described an association between the presence of the C150T polymorphism of the inducible nitric oxide synthase (*iNOS*) gene and *Helicobacter pylori* (*H. pylori*) induced gastric cancer. When we used primer-BLAST to find the polymerase chain reaction (PCR) product that would be generated by the primers used by these authors no products against any of the sequences present in the GenBank database were found. Further analysis of the *iNOS* sequences present in the GenBank suggest that the result from their study might come from a faulty primer design and may thus represent an artifact. Alternatively they may be correct and have identified a truly interesting explanation for the mechanism whereby *H. pylori* induces gastric cancer but some additional experiments would be in order to exclude the possibility of a PCR artifact.

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Key words: *Helicobacter pylori*; Polymorphism; Inducible nitric oxide synthase

Abadi ATB, Kusters JG. Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design. *World J Gastroenterol* 2013; 19(3): 429-430 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i3/429.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i3.429>

TO THE EDITOR

Rafiei *et al*^[1] recently published an interesting article in the *World Journal of Gastroenterology* entitled “Inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection affect gastric cancer risk”. In this study, they investigated the possible association between inducible nitric oxide synthase (*iNOS*) genotype and gastric cancer among the 329 patients from northern Iran. They found a clear association between the presence of C150T polymorphism of the *iNOS* gene and the presence of gastric cancer in *Helicobacter pylori* (*H. pylori*) infected patients. Strikingly they found an overall frequency of the 150T allele of approximately 25% of the individuals (both controls and cases) tested^[1], which came as a surprise to us for the original description by Shen *et al*^[2] mentions a frequency of 13%. This discrepancy could obviously be explained by genetic differences in the ethnic populations but is not discussed by Rafiei *et al*^[1] in their paper and this prompted us to carefully study the methods used to establish the C150T polymorphism. In order to establish the polymorphism the authors used the method that was described by Shen *et al*^[3]. Briefly, a polymerase chain reaction (PCR) was used to produce a 255 bp amplicon of the *iNOS* gene that was subsequently digested with *Tsp* 509 I for determination of the C150T polymorphism. When we used primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>) to find the PCR product that would be generated by these primers this program was unable to find any products against

any of the sequences present in the GenBank database. Performing a manual search against the Assembled RefSeq human Genomes database (Build 37.3) did only result in two *iNOS* hit for the forward primer (database entry ref NT 010799.15 and NW 001838430.2) and no hits for the reverse primer. When we subsequently performed a basic BLAST search with the separate primers we only found hits with the forward primer in 7/9 *iNOS* sequences in the Genbank database version 25 September 2012 and no hits with the reverse primer. Further analysis of the 9 *iNOS* sequences present in the GenBank revealed that a *Tsp* 509 I site at the expected distance from the forward primer in all but one sequence (GenBank accession no DQ149843.1). This sequence (DQ149843.1) was submitted by Shen *et al.*^[3], the group who originally identified the C150T polymorphism. Interestingly the 3' end of this 288 bp sequence (supposedly containing the binding site for the reverse primer) differs considerably from all but one of the other *iNOS* sequences in the database; i.e., an 288 *iNOS* sequence with accession no X85772.1. This closely homologous sequence (X85772.1) differs only in the C150T position and was submitted to the database by Xu *et al.*^[4]. A possible explanation for the aberrant 3' end of this sequence is that the sequence from Xu *et al.*^[4] is assembled from two cosmid clones and as the sequence differences seem to be at the link region of these two cosmids it may represent a cloning artifact. Careful analysis of these two sequences in fact does show some similarity

to the reverse primer at the expected position, but the two 3' bases clearly differ, making it almost impossible to produce a PCR product with this primer. Conclusively, it looks like the unique results of these two studies^[1,2] might come from a faulty primer design and may thus represent an artifact. Alternatively they may be correct and present a truly interesting clue for the mechanism whereby *H. pylori* induces gastric cancer but we feel that some additional experiments would be in order to exclude the possible artifact as mentioned above.

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P- Reviewers Day AS, Tosetti C **S- Editor** Song XX
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GENERAL INFORMATION

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Aims and scope

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

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World Journal of Gastroenterology

ISSN

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Launch date

October 1, 1995

Frequency

Weekly

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- 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

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- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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- 5 **Vallancien G**, Emberton M, Harving N, van Moorseelaar RJ, Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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- 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]

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- 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]

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- 9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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- 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

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- 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/index.htm>

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- 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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ISSN 1007-9327

