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Editorial Board Member of *World Journal of Hepatology*, Kezhong Zhang, PhD, Assistant Professor, Center for Molecular Medicine and Genetics, Department of Immunology and Microbiology, Wayne State University School of Medicine, 3202 Scott Hall, 540 E. Canfield Avenue, Detroit, MI 48201, United States

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World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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EDITOR-IN-CHIEF
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World Journal of Hepatology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjh@wjgnet.com
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ORIGINAL ARTICLE

A study of the effects of saliva stimulation by nizatidine on dry mouth symptoms of primary biliary cirrhosis

Tetsu Kikuchi, Katsuharu Hirano, Takuya Genda, Hironori Tsuzura, Shunsuke Sato, Yoshio Kanemitsu, Yutaka Narita, Katsuyori Iijima, Takafumi Ichida

Tetsu Kikuchi, Katsuharu Hirano, Takuya Genda, Hironori Tsuzura, Shunsuke Sato, Yoshio Kanemitsu, Yutaka Narita, Katsuyori Iijima, Takafumi Ichida, Department of Gastroenterology and Hepatology, Juntendo University, Shizuoka Hospital, Shizuoka 410-2295, Japan
Author contributions: Kikuchi T wrote the paper; Hirano K, Genda T, Tsuzura H, Sato S, Kanemitsu Y, Narita Y, Iijima K and Ichida T designed the research.
Correspondence to: Katsuharu Hirano, MD,PhD, Department of Gastroenterology and Hepatology, Juntendo University, Shizuoka Hospital, 1129 Nagaoka, Izunokuni, Shizuoka 410-2295, Japan. khirano-tym@umin.ac.jp
Telephone: +81-55-9483111 Fax: +81-55-9485088
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Abstract

AIM: To elucidate the effect of saliva stimulation by nizatidine on oral symptoms of primary biliary cirrhosis (PBC) by administering it to PBC cases.

METHODS: From among 73 cases that had been definitively diagnosed as PBC at our hospital by February 2010, we selected 27 cases of PBC, 4 males and 23 females, as subjects. We obtained subjects' consent after giving them a full explanation of the administration of nizatidine. Nizatidine 150 mg was administered internally twice daily, after morning and evening meals. To observe changes in the quantity of saliva secreted, chewing gum tests were carried out four times: before the initial dose, and after 6 mo, 12 mo and 24 mo of administration. For subjective dry mouth symptoms, a visual analog scale (VAS) method was used to assess their feelings of oral dryness and eating difficulty, five times: before the initial dose, and after 1, 6, 12 and 24 mo of administration in 8 cases. The nutritional condition and the hepatic functional reserve were compared between before and after the nizatidine treatment.

RESULTS: The result of a chewing gum test on the subjects before the administration of nizatidine showed that 50% produced less than 10 mL of saliva, *i.e.*, the standard under which cases are considered to have hyposalivation. The results of these tests showed that the quantity of saliva secreted was 10.5 ± 6.8 mL before administration of nizatidine, 10.9 ± 6.0 mL after 6 mo, 10.6 ± 4.9 mL after 12 mo, and 11.8 ± 6.8 mL after 24 mo administration. Thus, there was a slowly increasing trend in the quantity of saliva in the whole group. The percentage of subjects with saliva production above 10 mL was 45.8% after 6 mo administration of nizatidine, that is, only a slight change from before its administration, but it was 64.3% after 12 mo, that is, a significant increase. The saliva secretion by subject patients was examined before the beginning of administration of nizatidine, 12 mo later, and 24 mo later, and Fisher's combined probability test was used to examine the results for increases in saliva secretion. The analysis yielded *P* values of 0.51 and 0.53 for 12 mo later and 24 mo later, respectively. Thus, although there was no statistically significant increase, it was confirmed that saliva secretion tended to increase. A VAS method was employed to study the intensities of subjective symptoms of oral dryness and eating difficulty. Almost every case indicated some improvement of subjective oral dryness on the VAS early in the administration, *i.e.*, one month after. We also studied the effects of the administration of nizatidine on nutritional condition, hepatic functional reserve, and long-term prognosis of PBC. No significant improvements in cholinesterase (ChE) level, albumin (Alb) level, or Child-Pugh score were found during the period of observation from the beginning to the end of administration of nizatidine, nor in comparison with the non-administration group. A comparative analysis between before administration and 24 mo later yielded *P* values of 0.41 for Alb, 0.56 for ChE, and 0.59 for the Child-Pugh scores.

CONCLUSION: It was confirmed that administering

nizatidine to cases of PBC with dry mouth increased the secretion of saliva and improved the symptoms.

Key words: Primary biliary cirrhosis; Nizatidine; Dry mouth; Sicca syndrome; Visual analog scale

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INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic hepatic disease, in the onset of which an autoimmune mechanism is involved. This disease is characterized serologically by anti-mitochondrial antibody positivity and high serum immunoglobulin M values, histologically by chronic non-suppurative destructive cholangitis of the hepatic lobules, and the presence of florid duct lesions that involve severe inflammation and biliary epithelial inflammation[1,2]. The condition develops gradually from the asymptomatic phase when no subjective symptoms are observed into the symptomatic phase when itching, jaundice, and other symptoms appear. A group of cases with poor prognosis may finally develop from cirrhosis to hepatic failure to death. There are no radical cures for this disease other than liver transplantation. Thus, it is one of a group of intractable liver diseases for which there are no established treatments[3].

PBC shows a high rate of complication by Hashimoto's disease or collagen diseases, such as Sjogren syndrome[4,5]. Sjogren syndrome includes dry mouth and dry eyes; dry mouth and/or dry eye symptoms of PBC are called sicca syndrome, and are found in approximately 70%, of patients with PBC[6-8]. However, only 20% to 30% of PBC cases with these symptoms meet the diagnostic criteria for Sjogren syndrome[9] and many such patients are not well treated for these symptoms.

It has been reported that nizatidine, an H2 blocker, has a saliva stimulation effect, in addition to its effects of accelerating gastric juice release and increasing the pressure of the lower esophageal sphincter which suppress the onset of gastroesophageal reflux disease[10].

We report here a study of whether administering nizatidine, which has a saliva stimulation effect, improves dry mouth as a subjective symptom of PBC.

MATERIALS AND METHODS

From among 73 cases that had been definitively diagnosed as PBC at our hospital by February 2010, we selected 27 cases of PBC as subjects, including 4 males and 23 females, giving them a full explanation of the administration of nizatidine and thereafter obtaining their consent. Nizatidine 150 mg was administered internally twice daily, after morning and evening meals. In order to observe changes in the quantity of saliva secreted, chewing gum tests were carried out four times: before the initial dose, and after 6, 12 and 24 mo of administration. For subjective dry mouth symptoms, a visual analog scale (VAS) method was administered five times to assess patients' feelings of oral dryness and eating difficulty: before the initial dose, and after 1, 6, 12 and 24 mo of administration in 8 cases. The VAS scale ranged from 0 to 10 (0: no subjective symptoms; 1-3: mild; 4-6: moderate; 7-9: severe; 10: very severe).

In addition, nutritional condition and hepatic functional reserve of patients were checked in terms of albumin (Alb) levels, cholinesterase (ChE) levels, as well as Child-Pugh scores before and after the nizatidine treatment. Data were compared between the nizatidine administration group and the nizatidine non-administration group.

Statistical analysis

The obtained data were statistically analyzed using SPSS v.17 to perform Willcoxon signed-rank tests or paired *t* tests with a level of significance of $P < 0.05$.

RESULTS

The average age of the subjects was 66.7, and the female subjects accounted for 85% of the subject group. The aspartate aminotransaminase level was 63.7 IU/L and the alanine aminotransaminase (ALT) level was 69.2 IU/L, indicating that liver function was mildly impaired. In comparison, the alkaline phosphatase level was 679.1 IU/L and the γ-glutamyl transpeptidase level was 242 IU/L, thus indicating high biliary enzyme values. In the phase before administering nizatidine, any significance differences, in addition to ALT value, were identified between the administered and non-administered group (ALT: $P = 0.04$). The 12 cases on which liver biopsies were performed were histologically classified according to Scheuer's classification as 11 cases in stage 1 and 1 case in stage 2, with no case in which there was a high level of fibrosis. The M-2 antibody-positive rate was 67%. In 14 cases, 54% of the subjects, collagen disease complications were found, such as Sjogren syndrome, chronic thyroiditis, and/or rheumatoid arthritis (Table 1).

The changes in the quantity of saliva secreted that were observed in the chewing gum tests were 10.5 ± 6.8 mL (2.2-30 mL) before the start of administration of nizatidine, 10.9 ± 6.0 mL after 6 mo, 10.6 ± 4.9 mL after 12 mo, and 11.8 ± 6.8 mL after 24 mo of administration. Thus, they showed a slowly increasing trend (Figure 1A). In order to further analyze the changes in the quantity of saliva secreted, we divided the subject group into one sub-group with less than 10 mL before the start of administration of nizatidine and another with 10 mL or more before administration of the drug. The sub-group with initial secretion of large quantities of saliva, of 10 mL or more, did not show a significant increase after 6 mo of administration. On the other hand, the sub-group

Table 1 Characteristics of the patients at baseline

	All PBC cases	Cases with nizatidine	Cases without nizatidine	*P* value
No. of cases	73	27	46	
Age (mean ± SD)	65.6 ± 12.2	68.2 ± 11.8	64.1 ± 12.3	0.32
Sex (male/female)	9 (18%)/64 (88%)	4(%)/23(%)	5 (18%)/41 (88%)	0.72
Histological classifications	24/8/3/2	11/1/0/0	13/7/3/2	0.12
Scheuer (1/2/3/4)	65%/22%/8%/5%	92%/8%/0%/0%	52%/26%/12%/8%	
Alb (g/dL)	4.0 ± 0.3	4.1 ± 0.3	4.0 ± 0.4	0.50
ChE (IU/L)	288.3 ± 66.4	302.2 ± 68.5	277.9 ± 64.0	0.24
AST (IU/L)	55.6 ± 47.3	64.6 ± 44.5	50.3 ± 48.5	0.10
ALT (IU/L)	58.2 ± 64.6	75.7 ± 67.0	48.0 ± 61.5	0.04
γGTP (IU/L)	183.8 ± 205.6	221.0 ± 254.7	162.0 ± 169.8	0.10
ALP (IU/L)	638.7 ± 446.8	661.0 ± 616.3	625.7 ± 315.5	0.12
T-Bil (mg/dL)	0.69 ± 0.37	0.71 ± 0.33	0.67 ± 0.39	0.33
Plt ($\times 10^4/\mu L$)	21.7 ± 8.8	23.2 ± 10.9	20.7 ± 7.3	0.74
M2 antibody (< 5/5)	17 (23%)/56 (77%)	9 (33%)/18 (67%)	8 (17%)/38 (83%)	0.15
ANA (< 40/40)	23 (32%)/50 (68%)	7 (26%)/20 (74%)	16 (35%)/30 (65%)	0.60
Collagen disease complication (presence/absence)	45 (62%)/28 (38%)	13 (48%)/14 (52%)	32 (70%)/14 (30%	0.08

PBC: Primary biliary cirrhosis; Alb: Albumin; ChE: Cholinesterase; AST: Aspartate aminotransaminase; ALT: Alanine aminotransaminase; γGTP: γ-glutamyltranspeptidase; ALP: Alkaline phosphatase; T-Bil: Total bilirubin; Plt: Platelet; ANA: Anti-nuclear antibody.

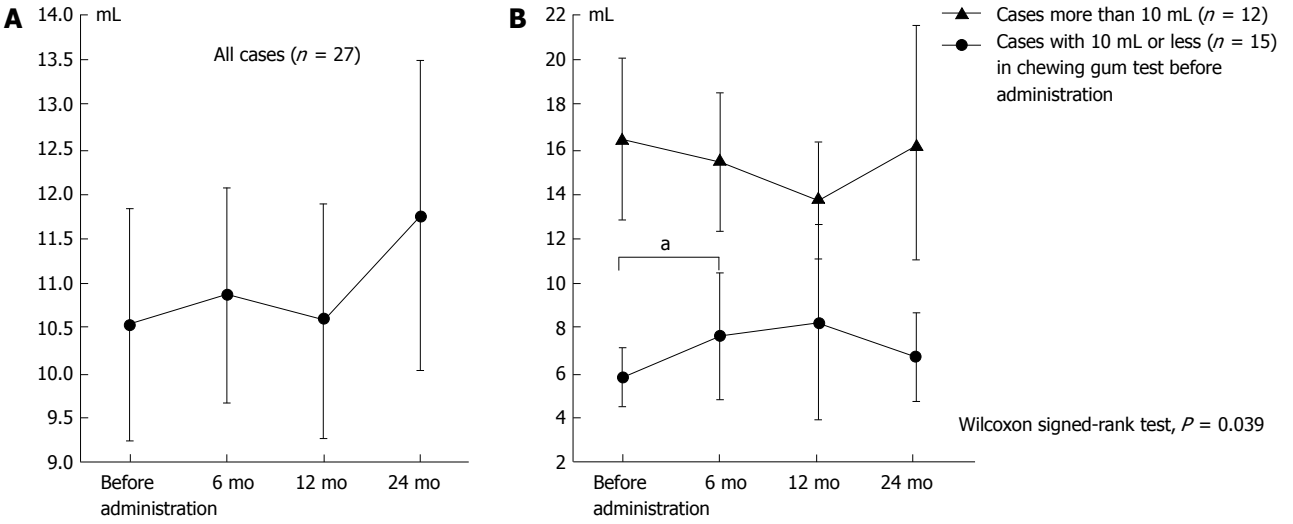

Figure 1 Course of saliva secretion before and after the administration of nizatidine in all cases (A) and in cases with 10 mL or less of saliva in chewing gum test before administration (B). [a]*P* < 0.05.

with initial secretion of small quantities of saliva, less than 10 mL, showed a statistically significant increase after six months of administration, with $P = 0.039$ in the Wilcoxon signed-rank test (Figure 1B). The percentage of all subject patients with saliva secretion of 10 mL or more was 48.1%, before the start of administration, and 45.8% after 6 mo of administration, thus indicating no large changes. However, this increased significantly to 64.2% after 12 mo of administration. The saliva secretion by patients was examined before the beginning of administration of nizatidine, 12 mo later, and 24 mo later, and Fisher's combined probability test was used to examine the results for increases in saliva secretion. The analysis yielded *P* values of 0.51 and 0.53 for 12 mo later and 24 mo later, respectively. Thus, although there was no statistically significant increase, it was confirmed that saliva secretion tended to increase (Figure 2).

A VAS method was employed to check the patients in terms of their subjective feelings of oral dryness and eating difficulty. In almost every case, feelings of oral dryness improved according of the VAS evaluation after 1 mo of administration of nizatidine (Figure 3A). In general, this showed a continuing modest increase after 12 and 24 mo of administration although, in some cases, it was seen to fall back to the level before the start of administration. Feelings of eating difficulty were also improved after one month of administration in some cases, according to the VAS evaluation. However, this parameter improved less and in a smaller number of cases than feelings of oral dryness. In addition, while the symptoms continued to improve in the long-term in some cases, no improvements were observed at all in other cases. Indeed, in some cases, even long-term administration of nizatidine not only failed to produce a significant positive

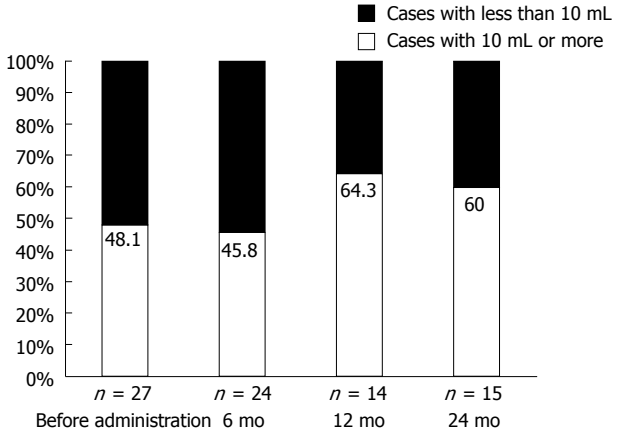

Figure 2 The percentage of all t patients with saliva secretion of 10 mL or more before and after the administration of nizatidine.

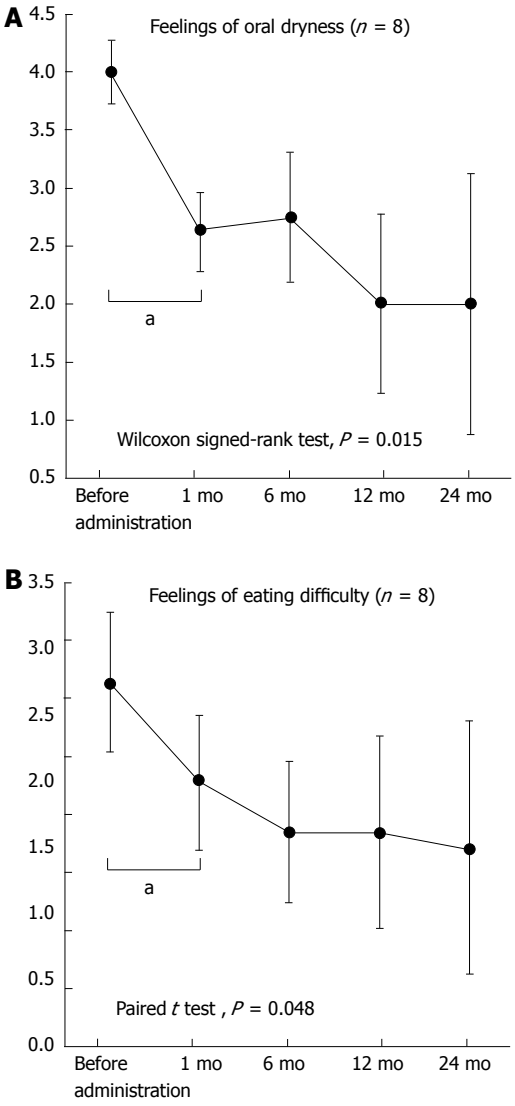

Figure 3 Visual analog scale method employed to check the subjects in terms of their feelings of oral dryness (A), and eating difficulty (B). [a]*P* < 0.05.

effect, but the symptoms actually worsened, back to the levels before the start of administration (Figure 3B). The VAS evaluation results showed that, over time, while both feelings of oral dryness and of eating difficulty improved with the administration of nizatidine by a statistically significant difference, feelings of oral dryness improved by a more significant amount.

The ChE and Alb values were compared between before and after the administration of nizatidine. ChE before administration was 302.2 IU/L, 297.5 IU/L after 1 mo of administration, 300.5 IU/L after 6 mo of administration, 304.0 IU/L after 12 mo of administration, and 314.0 IU/L after 24 mo of administration. No significant improvements in either ChE or Alb were found over the period of observation. A comparative analysis between values before administration and 24 mo later yielded *P* values of 0.41 for Alb, 0.56 for ChE, and 0.59 for the Child-Pugh scores. Thus, no significant improvements were found. These values were also compared between the nizatidine administration group and the nizatidine non-administration group of patients with PBC, yielding *P* values of 0.67 for Alb and 0.73 for ChE. Thus, no statistically significant differences were found (Table 2).

Furthermore, in order to study the effect of nizatidine on hepatic functional reserve, changes in Child-Pugh scores were checked and analyzed. Before the start of administration of nizatidine, one case in the subject group showed a Child-Pugh score of 6 points in class A, one case 7 points in class B, and the remaining 25 cases 5 points in class A. After an average of 58.5 mo (15-100 mo) of observation, the equivalent results were: one case with 7 points in class B; one case that developed into symptomatic PBC progressing to liver failure to death during the observation period; and the remaining 25 cases with 5 points in class A. In the 46 cases of PBC in the nizatidine non-administration group, 42 were checked in terms of Child-Pugh scores when their illness was diagnosed as PBC: 41 of them showed 5 points in class A, and one showed 6 points in class A. The Child-Pugh scores after an average of 46.3 mo (10-98 mo) of observation of this group were: one case with 6 points in class A; one case of death; and the remaining 40 cases with 5 points in class A. A comparative analysis between the nizatidine administration group and the nizatidine non-administration group indicated no significant differences (Table 2).

DISCUSSION

Dry mouth and dry eye symptoms are frequently found in cases of PBC. It is assumed that these common symptoms are associated with a high rate of autoimmune disease complications with PBC. The likelihood that PBC has at least one autoimmune disease complication is 84%, and is 41% for the presence of at least two complications[11-13]. Sjogren syndrome, rheumatoid arthritis, and scleroderma are the most frequent complications of PBC, with cross-sectional study on more than 5000 Japanese cases of PBC indicating that they were found in 13.5%, 7.3% and 2.0% of PBC cases, respectively[14-16]. Another report showed and an even higher frequency of Sjogren syndrome as a complication of PBC, at 26% to 72%[15,17-19]. PBC is char-

Table 2 The effects of the administration of nizatidine on the nutritional condition, the hepatic functional reserve, and the long-term prognosis of primary biliary cirrhosis

	Nizatidine administration group (n = 27)			Nizatidine non-administration group (n = 46)		
	Before administration	24 mo	P value	Before treatment	24 mo	P value
Alb	4.1 ± 0.3	4.2 ± 0.3	0.41	4.0 ± 0.4	4.1 ± 0.3	0.67
ChE	302.2 ± 68.5	314.0 ± 62.3	0.56	277.9 ± 64.0	286.5 ± 71.1	0.73
Child-Pugh score	5.1 ± 0.4	5.1 ± 0.4	0.59	5.0 ± 0.2	5.0 ± 0.2	0.99

Alb: Albumin; ChE: Cholinesterase.

acterized by high rates of complications of other autoimmune diseases and by slow progression of the disease in many cases[20,21]. In many cases of PBC, patients are in the asymptomatic phase, without subjective symptoms, even after onset, and complications in these cases are found only during detailed examinations after a definitive diagnosis of PBC is made, or after patients enter the symptomatic phase. The way in which PBC develops has yet to be well elucidated. Some reports indicate that PBC is associated with medical history of the patient and their family as well as their lifestyle. Various environmental factors such as infections including urinary tract infection as well as cigarette smoking, and a history of administration of estrogen, can cause immune tolerance failures, which lead to the onset of PBC[22-25]. It is well conceivable that immune tolerance failures due to such environmental factors may lead to the onset of not only PBC but also other autoimmune diseases. In this study, Sjogren syndrome was found as a complication in 25% of the cases, rheumatoid arthritis in 14.8%, and CREST syndrome in 7.4%. Thus, the frequency of Sjogren syndrome as a complication appeared to be relatively low. This may be partially because this study was based on chewing gum tests, which could not detect cases with dry eye symptoms but without oral symptoms, and partially because a definitive diagnosis of the syndrome might not have been made in some cases. In many cases, salivary secretion disorder due to secondary Sjogren syndrome as a PBC complication is milder than primary Sjogren syndrome as a clinical symptom, and there were many asymptomatic PBC cases in the subject group with a mild complaint of dryness. Another report indicated that hepatic dysfunctions were found in 38.2% of cases of collagen diseases, and that 15.9% of cases with such disorders were found to have PBC on diagnosis[26]. It is therefore necessary for various medical departments to cooperate closely to detect such complications of PBC early and exactly, in order to begin the most appropriate treatment.

Saliva has an essential role in functions such as cleaning the oral cavity, inhibiting the proliferation of bacteria and fungi in the mouth, protecting oral mucosa, and helping swallow food[27]. In PCB patients aged 60 or more, an age group with a predilection for PBC, subjective symptoms of oral dryness are found in 60%-70% of cases, and more than 20% of these cases have oral candidiasis, which is likely to damage their quality of life[28]. Sicca syndrome with PBC has been treated with immunomodulators, without substantial clinical effects. Our study demonstrated that administering nizatidine to cases of PBC with subjective symptoms of oral dryness and eating difficulty increased the quantity of saliva secreted, leading to improvements in the subjective symptoms.

Actions of both sympathetic and parasympathetic nerves are involved in the secretion of saliva[29,30]. Nizatidine simulates parasympathetic cholinergic nerves, to accelerate the gastrointestinal motility by inhibiting acetylcholine esterase. In addition, it increases the quantity of acetylcholine secreted by inhibiting acetylcholine esterase at the endings of cholinergic nerves, stimulating muscarinic receptors in the salivary glands to increase saliva secretion[30-32]. Other H2-receptor antagonists such as ranitidine and cimetidine cause increases in acetylcholine concentration at cholinergic nerve endings, but do not increase saliva secretion as a result. Cevimeline, an anticholinergic agent, was found to be effective for dry mouth symptoms in Sjogren syndrome but produced side effects and its administration is now limited[10,33-35]. In view of the foregoing, other H2-receptor antagonists are not expected to produce similar effects to those of nizatidine. In this study, nizatidine exhibited a stronger effect in accelerating saliva secretion in cases where saliva secretion in the chewing gum test was under 10 mL before the start of internal administration. The subjects were an average of 66.7 years old, and older people generally have reduced salivary gland secretion and more frequent occurrence of dry mouth symptom. However, the internal administration of nizatidine was thought to stimulate muscarinic receptors in the salivary glands and revitalize salivary gland cells, leading to increased saliva secretion.

Measured with the VAS method, both feelings of oral dryness and feelings of eating difficulty improved after only one month of internal administration of nizatidine. This may have been partially because the patients were sensitive to changes in the oral cavity and easily sensed such changes. The VAS results for both symptoms improved in almost every case after one month of administration, although in some cases they returned after 6 to 12 mo of admto where they were before the start of administration inistration. It is assumed that the cases involving worsening of symptoms may initially have felt subjective improvements in the symptoms because of psychological factors in addition to increased saliva secretion, but grew accustomed to the ongoing symptoms

over time. Another possibility is that nizatidine's salivary gland stimulation effect on the salivary secretion ability of older patients where salivary secretion had previously become poor may be only transient, finally resulting in the reduction in saliva secretion and the worsening of symptoms in some cases.

In this study, the H2-receptor antagonism of nizatidine stimulated appetite and increased saliva secretion, making the oral mucosa moister thus making it easier to masticate food. We studied how these effects of nizatidine improved the intake of food, and the effect on nutritional condition, hepatic functional reserve, and the long-term prognosis of PBC. The ChE and Alb values did not significantly improve after the administration of nizatidine, and the values in the nizatidine administration group, before or after administration, were not significantly different from the nizatidine non-administration group. The increased saliva secretion and improved dry mouth symptoms did not directly lead to improvements in nutritional condition in this short observation period. We also studied whether increased saliva secretion affects hepatic functional reserve by checking the Child-Pugh scores before and after the administration of nizatidine. The results showed no significant improvements in hepatic functions between before and after administration of the drug. Furthermore, no significant differences in hepatic functions were found between the nizatidine administration group and the nizatidine non-administration group. These results are partially because the cases of PBC in the control group were all in the asymptomatic phase, and partially because the general prognosis of PBC is relative good. PBC has a 5-year survival rate of 91% for men and 92% for women, and a ten-year survival rate of 81% for men and 85% for women, while the disease itself has an extremely long asymptomatic phase[36]. It is therefore possible that while this study found no significant difference made by nizatidine with respect to hepatic functional reserve, a longer observation period might reveal changes in hepatic functional reserve due to the administration of nizatidine. To determine whether increased saliva secretion caused by the administration of nizatidine may affect the long-term prognosis of PBC, it will be necessary to administer the drug for a longer time to a greater number of cases of PBC in both the symptomatic phase and in the asymptomatic phase, and to observe various changes in these cases.

In this study, we actually started to administer nizatidine to more than 27 of the 73 cases that had been definitively diagnosed as PBC at our hospital. Some of the patients, however, could not continue to come to the hospital for regular examinations. Only the 27 cases continued to undergo regular chewing gum tests and VAS interviews every six months for two years. In selecting patients for the nizatidine administration group and for the non-administration group, we did not take any particular action to avoid bias, but we determined that there was no bias between the two groups because there was no statistically significantly difference between the groups in terms of their hepatic functions (without ALT: $P = 0.04$).

Strictly speaking, as the control group, a placebo should have been administered to the nizatidine non-administration group. One reason this was not done was that many cases dropped out and could not continue to take nizatidine, or undergo VAS interviews or chewing gum tests. This aspect will be improved in any future study.

In this study, we confirmed that administering nizatidine to cases of PBC with dry mouth increased saliva secretion and improved dry mouth symptoms. However, we were unable to show that this improvement led to improvement in the nutritional condition and long-term prognosis of the patients. The prognosis of PBC is generally good. However, it can be inferred that there is a group of PBC cases with poor prognoses, and a further extensive study is needed to establish effective treatments for such a group.

COMMENTS

Background

Dry mouth and/or dry eye symptoms of primary biliary cirrhosis (PBC) are called sicca syndrome, and are found in approximately 70%, of patients with PBC. The authors have investigated whether administering nizatidine, which has a saliva stimulation effect, improves dry mouth as a subjective symptom of PBC.

Research frontiers

It was confirmed that administering medicines for gastric ulcers to patients with hepatic problems improves their symptoms. Further, readily available medicines such as H2 Blockers were found to have a significant value in treatment if they are internally used.

Innovations and breakthroughs

It was noted that administering nizatidine not only increased the saliva secretion but also improved xerostomia, a subjective symptom.

Applications

When nizatidine are administered to PBC patients with PBC who have dietary intake difficulties, increase of saliva secretion and improvement of subjective symptoms are expected. However, the influence of dietary intake on the improvements of their liver function and their prognosis should be closely monitored.

Terminology

Sicca syndrome: Dry mouth and dry eye symptoms of PBC are called sicca syndrome, and are found in a high percentage of patients with PBC. Commonly, it is not diagnosed precisely and left without any effective medical treatment.

Peer review

The paper investigated the effects of saliva stimulation by nizatidine on dry mouth symptoms of PBC. It's well designed and written.

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ORIGINAL ARTICLE

Association between low molecular polypeptide 7 single nucleotide polymorphism and response to therapy in hepatitis C virus infection

Moataza H Omran, Basma E Fotouh, Samar S Youssef, Noha E Ibrahim, Wael Nabil, EL-Sayed M Mahdy, Wafaa G Shosha, Mostafa K El-Awady

Moataza H Omran, Basma E Fotouh, Samar S Youssef, Noha E Ibrahim, Wael Nabil, Mostafa K El-Awady, Microbial Biotechnology Department, Genetic Engineering Division, National Research Center, 12622 Giza, Egypt
EL-Sayed M Mahdy, Wafaa G Shosha, Chemistry Department Faculty of Science, Helwan University, 11795 Cairo, Egypt
Author contributions: Omran MH performed the majority of the experiments and wrote the manuscript; Fotoh BE performed the majority of molecular experiments; Youssef SS, Ibrahim NE and Nabil W coordinated some of the molecular experiments; Mahdy ESM and Shosha WG were involved in revising the manuscript; El-Awady MK was involved in writing and revising the manuscript.
Correspondence to: Dr. Moataza H Omran, Assistant Professor of Biomedical Biotechnology, Microbial Biotechnology Department, Genetic Engineering Division, National Research Centre, El-Tahrir Street, Dokki, 12622 Giza, Egypt. moataza13@yahoo.com
Telephone: +20-12-3735450 Fax: +20-23-3370931
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Abstract

AIM: To investigate the relationship between low molecular polypeptide-7 (LMP-7) gene polymorphism and response to interferon (IFN) therapy in chronic hepatitis C virus (HCV) patients.

METHODS: LMP-7 polymorphism at codon 49 with nucleotide substitution from A to C was amplified in 104 chronic HCV patients of genotype 4. The amplicons were digested with restriction endonuclease *Bsm*I and the produced restriction fragment length polymorphism was analyzed. Patients received IFN + regional blood volume therapy for 48 wk and the frequency of this single nucleotide polymorphism (SNP) was statistically correlated with treatment response. The exclusion criteria for these patients were stated by the national health program for treating viral hepatitis. Main exclusion criteria included co-infection with hepatitis B virus or schistosomiasis, thyroid dysfunction, uncontrolled diabetes mellitus, history of long term drug or alcohol intake and autoimmune hepatitis. Multivariate analyses were done to correlate LMP-7 SNP plus several factors such as age, gender, weight, serum alpha-fetoprotein (AFP) and alanine aminotransferase levels, liver activity, fibrosis score and viral load with response to therapy.

RESULTS: The data presented in this study clearly demonstrated statistically significant differences between sustained virological response (SVR) (defined as the absence of HCV RNA levels in the patient's sera at least 6 mo after discontinuation of treatment) and non-response (NR) (where HCV RNA levels in the patient's sera never become undetectable for 6 mo during or after treatment). Variables were described as odds ratio with 95%CI. The data were considered significant if P values were $\leqslant$ 0.05; highly significant if $P < 0.01$ and very highly significant if $P < 0.001$. Current data showed that 91.7% of patients carrying LMP-7 C/C allele were associated with SVR, while the other two genotypes C/A and A/A were associated with NR patients, 83.3% and 64.3% respectively, showing that genotype CC was strongly associated with response to interferon (95%CI: 12.0719-134.6572, $P = 0.0001$). The majority of parameters recorded in SVR and NR patients included higher values of mean age ($P = 0.004$), alanine aminotransferase ($P = 0.001$), AFP ($P = 0.001$), body weight ($P = 0.025$), viral load ($P = 0.025$), higher fibrosis and histological activity index indices among NR *vs* SVR patients. Also, the multivariate statistical analysis of the different factors of fibro-

sis score, liver activity grade, genotypes and alleles of LMP-7 gene polymorphism in responders and NRs of HCV patients in this study showed that HCV patients with A allele had a very highly significant association with the NRs, high fibrosis and higher liver activity, while the C allele had a very highly significant association with the responders, low fibrosis and lower liver activity (95%CI: 3.5800-13.2519, P = 0.0001).

CONCLUSION: LMP-7 SNP is a candidate gene that should be considered when designing a mathematical model for predicting response to therapy and disease progression in HCV patients.

Key words: Hepatitis C virus; Interferon therapy; Low molecular mass polypeptide; Host gene; Single nucleotide polymorphism

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INTRODUCTION

More than 170 million people are currently affected by chronic hepatitis C virus (HCV) infection worldwide, with the highest prevalence in Africa and Asia[1]. HCV cirrhosis, fibrosis and hepatocellular carcinoma are major sequelae of HCV chronic infection. The latter is one of the leading causes of mortality, morbidity and liver transplantation worldwide. Pegylated interferon (IFN) and ribavirin combined therapy is the standard approved treatment for HCV and is only effective in around 50% of patients[2]. Both virus and host-related elements have been reported as factors correlated to therapeutic effects of combination therapy, such as viral load, genotype and viral kinetics gender, age, ethnicity, obesity, degree of liver fibrosis and genetic polymorphisms[3]. The role of host genetic factors encoding gene products involved in the immune response following HCV infection are likely to influence the disease susceptibility and progression. As it has been shown that different host gene's single nucleotide polymorphisms (SNPs) were mediators of the antiviral and antiproliferative effects of IFN[4], it is plausible that host genetic factors control the immune responses and play a critical role in determining the outcome of HCV infection. Viral infection of host cells by HCV leads to the release of the INFs (α, β, γ) which induce the formation of immunoproteosomes [catalytic core that consists of low molecular polypeptide-2 (LMP-2), LMP-7 and LMP-10] that produce immunogenic epitopes that bind to major histocompatibility complex (MHC) I molecules and when presented to $CD8^+$ CTLs, enhances antigen presentation and triggers an antiviral response in the infected organism[5]. LMP-7 gene is a subunit of proteosomes encoded in the class II region of MHC on chromosome No. 6 adjacent to the TAP transporter genes[6]. The antigen recognition by cytotoxic $CD8^+$T cells is dependent upon a number of crucial steps in antigen processing, including LMP-7 that can alter the pool of peptides available for class I antigen presentation, improving the $CD8^+$T cells in response to viral antigens[7,8].

Previously, the relationship between LMP-7 genetic variation and some diseases has been reported. It has also been shown that LMP-7 might play an important role in the immunological reaction to HCV infection[7-9]. Thus, the aim of the current study is to determine the frequency of SNP at codon 49 of LMP-7 gene in chronic HCV patients receiving IFN therapy and to correlate the SNP frequency with clinical status of patients and the response to therapy.

MATERIALS AND METHODS

Subjects

Patients: The current study comprises of 104 patients (85 males and 19 females with an age range of 7-64 years old) infected with chronic HCV genotype 4. Only those patients fulfilling the criteria for being covered by the national health program for treating viral hepatitis were included. The criteria include absence of co-infection with hepatitis B virus (HBV), human immunodeficiency virus or schistosomiasis, patients with co morbid disorders such as thyroid dysfunction, uncontrolled diabetes mellitus, history of long term drug or alcohol intake and autoimmune hepatitis were excluded. Liver biopsy was carried out for histological examination and assessment of the stages of fibrosis and activity. Liver fibrosis score was divided into 5 stages from F0 to F4 and liver activity grade divided into 4 stages from A0 to A3. The number of patients belonging to each stage was detected in the two studied groups [responders and non-responders (NR)]. In NR, patient HCV viral loads persisted at or more than 2 logs of pre-treatment levels. Among those patients with an initial response to treatment, up to 50% will relapse after treatment is discontinued and are known as relapsed responders, whereas the rest will achieve a sustained virological response (SVR), as determined by the absence of detectable viremia six months after the end of treatment response. Patients received weekly *sc* injection of pegylated IFN α + daily oral ribavirin at a dose of 1000-1200 mg, depending on body weight, for 48 wk.

Controls: Healthy subjects (n = 33) with no history of acute or chronic disease and absence of any other viruses, diseases or disorders served as controls for LMP-7 typing.

Methods

HCV RNA tests: These include qualitative HCV nested

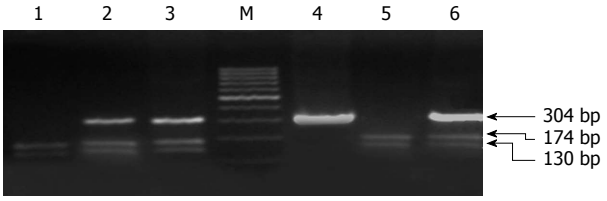

Figure 1 Agrose gel electrophoresis of digested low molecular polypeptide-7 amplicons. Purified genomic DNA from each patient was subjected to polymerase chain reaction amplification using specific primers bracketing the low molecular polypeptide-7 gene followed by *Bsm*I digestion and the products were resolved on 2% agarose gel. Lanes 2, 3 and 6 represent heterozygous C/A; Lanes 1 and 5 show C/C genotype; Lane 4 shows A/A genotype; M indicated molecular weight marker.

reverse transcription-polymerase chain reaction (RT-PCR), quantitative real-time PCR determination and genotyping of HCV RNA genome. Methods used for these assays were previously described as nested RT-PCR[10], quantitative test of HCV RNA (Copus Amplicore, HCV monitor test roche, United States) and HCV genotyping[11].

DNA extraction: A salting out technique was used to extract DNA from whole blood[12].

Genotyping of LMP-7 gene: The second exon of LMP-7 at codon 49 spanning the tested SNP was amplified by PCR using sequence specific primers as follows forward primer (F): 5'-CGGACAGATCTCTGGGT-GCT-3' and reverse primer (R): 5'-TCTCCGGGACT-GAAGGCT-3'. Reaction mix (50 μL) contained 2 units Taq polymerase (finnzyme, Finland), 5 μL of 10 × PCR buffer (supplied with the enzyme), 0.2 mmol/L dNTPs (Promega, Madison WI, United States), 1.5 mmol/L $MgCl_2$, 5 μmol/L from each primer, 3 μL of DNA and DDW to 50 μL. Thermal cycling protocol comprised of initial denaturation at 94 ℃ for 5 min, followed by 35 cycles, each includes 94 ℃ for 1 min, 57 ℃ for 1 min and 72 ℃ for 1 min and a final extension step at 72 ℃ for 10 min. Amplification product was resolved on 2% agarose gel electrophoresis. A fragment of 304 bp indicates successful amplification (Figure 1). Amplified products were subjected to digestion with 10 units of *Bsm*I restriction endonuclease (Amersham Pharmacia-Biotech, St Albans, United Kingdom) at 37 ℃ overnight. Restricted fragments were resolved on 2% agarose gel electrophoresis parallel with a DNA size marker (Amersham Pharmacia-Biotech) where C/C homozygotes appear as 2 bands at 174 and 130 bp, C/A heterozygotes appear as 3 bands of 174, 130 and 304 bp and uncut fragment at 304 bp represents A/A genotype (Figure 1).

Statistical analysis

Data were expressed as mean ± SD of percentage of each genotype (C/C, A/A or C/A) among the subject population whether SVR or NR. Comparison between mean values of different variables among SVR and NR patients was performed using unpaired student *t* test. Comparison between categorical data [*n* (%)] was done using χ^2 test.

Table 1 The demographic data of chronic hepatitis C virus patients infected with hepatitis C virus type 4a (sustained virological response *vs* non-response) after treatment

	NR (n = 48)	SVR (n = 56)	P value
Age (yr)			0.004
min-max	12-64	7-58	
mean ± SD	44.17 ± 10.97	37.91 ± 10.91	
Gender			0.906[1]
Female	9 (18.75)	10 (17.86)	
Male	39 (81.25)	46 (82.14)	
Weight (kg)	85.05 ± 13.98	78.54 ± 15.10	0.025
ALT	89.15 ± 62.70	48.39 ± 40.52	0.001
AFP	30.56 ± 22.58	11.28 ± 15.33	0.001
Viral load	686 150.38 ± 680 474.25	15 656.09 ± 452 782.09	0.001
Low fibrosis (F0-F1) (n = 39)	7 (17.95)	32 (82.05)	0.001
High fibrosis (F2-F4) (n = 65)	41 (63.08)	24 (36.92)	0.035
A0-A1 (n = 71)	24 (33.8)	47 (66.2)	0.006
A2-A3 (n = 33)	24 (72.73)	9 (27.27)	0.009

Data are expressed as mean ± SD or *n* (%). NR: Non-response; SVR: Sustained virological response; ALT: Alanine aminotransferase; AFP: Alpha-fetoprotein. [1]Not significant.

Multinomial logistic regression with forward stepwise variable selection was used to identify predictors associated with SVR rates. Variables were described as odds ratio with 95%CI. The data were considered significant if P values were $\leqslant 0.05$; highly significant if $P < 0.01$ and very highly significant if $P < 0.001$[13,14].

RESULTS

Demographic data

The data presented in Table 1 clearly demonstrate statistically significant differences between SVR and NR patients in the majority of parameters recorded, including higher values of mean age, alanine aminotransferase (ALT), alpha-fetoprotein (AFP), body weight, viral load, higher fibrosis and histological activity index (HAI) indices among NR *vs* SVR patients, whereas low fibrosis and HAI indices were statistically elevated in SVR patients compared with NR patients. All CHC patients in this study (n = 104) had genotype 4a of HCV RNA, using the method described previously[11].

Frequencies of LMP-7 genotypes

Current data showed that 91.7% of patients carrying LMP7 C/C allele were associated with SVR, showing that genotype CC was strongly associated with response to interferon and these data were very highly significant (P = 0.001) (Tables 2 and 3). However, the LMP-7 C/C allele constituted 54.5% of the healthy population which is slightly higher than the CHC patients 46% (Table 2, Figure 2), suggesting a possible protective role of C/C genotype. On the other hand, the genotypes C/A and A/A were more associated with NR, since 69% and 57% of NR patients were associated with C/A and A/A respectively, while 14.3% and 7.3% of relapsers carried C/A and A/

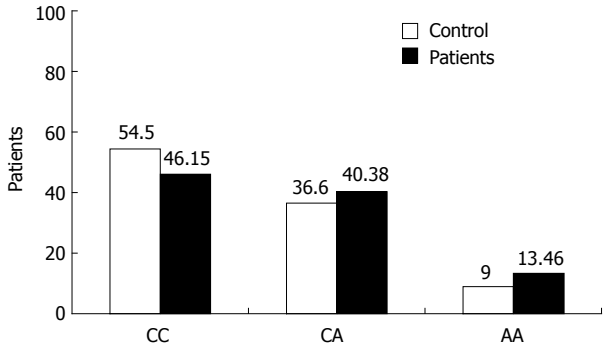

Figure 2 Frequencies of low molecular polypeptide-7 genotypes in control Egyptian subjects compared with chronic hepatitis C virus patients. Typing of low molecular polypeptide-7 (LMP-7) single nucleotide polymorphism was determined in 33 control subjects and 104 chronic hepatitis C virus patients. The frequency of each LMP-7 genotype in both patient groups was presented as columns.

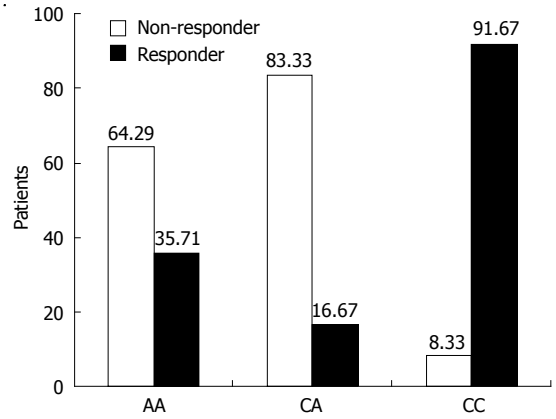

Figure 3 Rates of sustained virological response and non-response among the three genotypes of low molecular polypeptide-7 gene. Genotyping of low molecular polypeptide-7 (LMP-7) was determined in 56 sustained virological response (SVR) patients and in 48 non-response (NR) patients. Rates of SVR (black column) and NR (white column) were independently plotted as a function of each LMP-7 genotype, *i.e.*, CC, CA and AA.

A genotypes respectively, thus making a total of 83.3% C/A and 64.3% A/A as NR+ relapsed patients (Tables 2 and 3, Figure 3).

Genetic association of LMP-7 SNP with pathobiology of the liver in CHC patients

The strongest association between LMP-7 variations and pathological changes in the liver of chronic C patients was observed during stages of fibrosis where the percentage of C/C genotype was the highest in the lower fibrosis stage, *i.e.*, F0 and F1 (70% and 62.07%) respectively and these data were highly significant ($P = 0.045$ and $P = 0.004$, respectively) and decreases gradually till it reaches 0% at the severe fibrosis stage (F4). Also, C/C genotype was the highest at lower grades of liver activity, *i.e.*, A0-A1 (77.78% and 54.84% respectively) which was also highly significant ($P = 0.018$ and $P = 0.001$, respectively) and decreases gradually until it reaches its lowest value (16.67%) at (A3) (Table 4, Figures 4 and 5).

Table 2 The percentage of sustained virological response and non-response patients compared with control subjects in various genotypes of low molecular polypeptide-7 gene *n* (%)

	NR (*n* = 48)	SVR (*n* = 56)	All patients (*n* = 104)	Control (*n* = 33)
AA (*n* = 14)	9 (64.29)	5 (35.71)	14 (13.46)	3 (9)
CA (*n* = 42)	35 (83.33)	7 (16.67)	42 (40.38)	12 (36.5)
CC (*n* = 48)	4 (8.33)	44 (91.67)	48 (46.15)	18 (54.5)

NR: Non-response; SVR: Sustained virological response.

Table 3 Sustained virological response and non-response frequencies in various low molecular polypeptide-7 genotypes *n* (%)

	AA (*n* = 14)	CA (*n* = 42)	CC (*n* = 48)	*P* value
NR (*n* = 48)	9 (18.75)	35 (72.92)	4 (8.33)	0.001
SVR (*n* = 56)	5 (8.93)	7 (12.50)	44 (78.57)	0.001

Polymerase chain reaction-restriction fragment length polymorphism analysis were performed on chronic hepatitis C virus patients and the low molecular polypeptide-7 genotype frequencies were calculated as percentages of each group. NR: Non-response; SVR: Sustained virological response.

Table 4 The relationship of the genotypes of low molecular polypeptide-7 gene polymorphism within different liver fibrosis and activity stages in sustained virological response and non-response groups after treatment *n* (%)

	AA (*n* = 14)	CA (*n* = 42)	CC (*n* = 48)	*P* value
F0 (*n* = 10)	1 (10.00)	2 (20)	7 (70)	0.045
F1 (*n* = 29)	4 (13.79)	7 (24.14)	18 (62.07)	0.004
F2 (*n* = 36)	4 (11.11)	14 (38.89)	18 (50.00)	0.013
F3 (*n* = 23)	4 (17.39)	14 (60.87)	5 (21.74)	0.019
F4 (*n* = 6)	1 (16.67)	5 (83.33)	0 (0)	0.102[1]
A0 (*n* = 10)	1 (11.11)	1 (11.11)	7 (77.78)	0.018
A1 (*n* = 29)	9 (14.52)	19 (30.65)	34 (54.84)	0.001
A2 (*n* = 36)	3 (11.11)	18 (66.67)	6 (22.22)	0.001
A3 (*n* = 6)	1 (16.67)	4 (66.67)	1 (16.67)	0.023

[1]Not significant.

Multivariate analysis of different host factors, including LMP-7 variants in SVR and NR patients

The data presented in Table 5 summarize the role of several factors in achieving SVR to combined therapy. Patients with lower stages of fibrosis (F0-F1) are more associated with SVR than patients with advanced fibrosis (F2-F4) (95%CI: 0.0490-0.3346, $P = 0.0001$). Likewise, patients with higher liver activity (A2-A3) had lower chances of SVR compared to those with high activity (A0-A1) (95%CI: 0.0777-0.4759, $P = 0.0004$). Patients with higher levels of AFP (> 5 ng/mL) had less chance of SVR compared to those with AFP levels below 5 ng/mL (95%CI: 0.0871-0.5948, $P = 0.0002$). LMP-7 C/C genotype had significant association with SVR compared with C/A and A/A variants (95%CI: 12.0719-134.7572, $P = 0.0001$). In general, patients with A allele had significant association with NR, while C carriers had significant association with SVR (95%CI: 3.5800-13.2519, $P = 0.0001$).

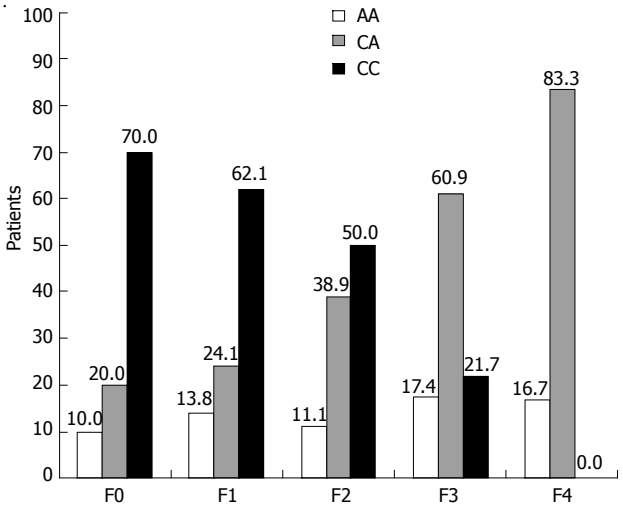

Figure 4 The fibrosis stages among low molecular polypeptide-7 genotypes polymorphism in patient groups (sustained virological response and non-response) after treatment. Frequencies of low molecular polypeptide-7 genotypes were determined in chronic hepatitis C virus patients after treatment with different fibrosis scores (F0-F4).

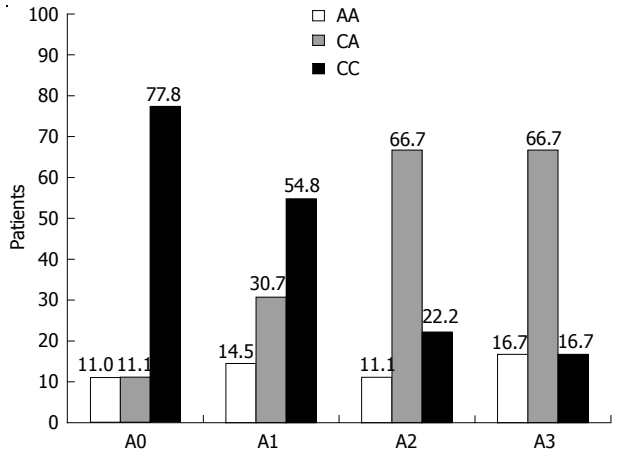

Figure 5 Distribution of low molecular polypeptide-7 genotypes in chronic hepatitis C virus patients after treatment with various hepatic activities (A0-A3). Frequencies of low molecular polypeptide-7 genotypes in chronic hepatitis C virus patients after treatment with various hepatic activities (A0-A3).

DISCUSSION

Recently, it has been shown that there is a complex relationship between HCV and immune responses of the host. MHC is a crucial molecule which initiates or regulates the immune response by presenting foreign or self-antigens to T lymphocytes that play essential roles in the HCV infection or even viral clearance[15]. It has been shown that LMP-7 is important in the MHC class I antigen presentation pathway. LMP-7 is a subunit of a multifunctional proteosome that plays a pivotal role in selective degradation of endogenous proteins into peptides suited for binding to human MHC class I molecule[9]. LMP-7 polymorphisms were found to be related to a number of immune diseases and HBV infection, while actually few studies have examined the relationship between LMP-7 and HCV infection[8,16,17]. The aim of the present study is to investigate the possible association between LMP-7 polymorphism and HCV infection in response to IFN therapy. Data of the current study show that demographic and clinical factors influence the likelihood of HCV viral clearance. Age is considered one of the contributing factors to treatment outcome. This was supported by earlier reports[18,19] that younger patients responded better to current HCV treatments than older ones. This is due to a couple of reasons: the immune system of someone who is younger is more intact and better able to help with the task of fighting HCV. Here, the longer that one has hepatitis C, the more the virus can replicate and possibly cause damage to the liver. This is why some medical providers now believe that people should be treated early before any liver damage has a chance to occur. The present work found no significant difference in gender distribution, similar to an earlier study[17]. However, others reported that females seem to respond better than males which is likely to be related to the positive effects of estrogens on immune responses that are influenced by IFN therapy[20,21]. Our data confirm the relationship between a patient's weight and response to interferon therapy, in agreement with other studies which showed that obesity is a predictor of disease progression in patients with chronic hepatitis C[22,23]. Serum ALT level is a marker of liver cell damage and the efficacy of antiviral treatment[24], as shown in our results where the mean value of ALT in SVR is much less than that in NR patients. Viral load is a key in predicting response to IFN. Many studies have shown that the lower the viral load, the more likely a patient is to eradicate the virus with IFN-based therapies[25,26]; these data support our results as the mean value in NR is much higher than in SVR patients. In general, lower viral loads are associated with less circulating viral quasispecies and also with a faster time towards HCV RNA negativity during treatment. A significant association was observed between LMP-7 polymorphism and viral clearance following IFN therapy. A strikingly higher frequency of the C/C genotype (91.67%) was observed in SVR *vs* 8.33% in NR cases ($P = 0.001$). Also, C allele was detected in 68.84% of SVR *vs* 31.16% in NR patients ($P = 0.001$). On the other hand, A allele frequency was 24.29% in SVR *vs* 75.71% in NR patients ($P = 0.001$). In addition to that, C/A genotype distribution was 16.67% in responders *vs* 83.33%

Table 5 Multivariate analysis of different clinical factors and alleles of low molecular polypeptide-7 gene polymorphism in responders and non-responders hepatitis C virus patients (%)

	Regression coefficient	SE	Odds ratio	95%CI	*P* value
F0-F1 *vs* F2-F4	2.0553	0.490	0.128	0.0490-0.3346	0.0001
A0-A1 *vs* A2-A4	-1.6529	0.465	0.192	0.0771-0.4759	0.0004
AFP (< 5 ng/mL *vs* > 5 ng/mL)	-1.4800	0.490	0.228	0.0871-0.5948	0.00025
CC *vs* CA + AA	3.6972	0.616	40.333	12.0719-134.7572	0.0001
C allele *vs* A allele	1.9298	0.334	6.888	3.5800-13.2519	0.0001

in non-responders, while that of A/A was 35.71% in responders *vs* 64.29% in non-responders. Earlier studies reported conflicting data where some reported that CA or AA genotypes LMP7 could increase the risk of HCV persistence compared with C/C genotype[21], while others showed that LMP-7 A/A genotype is higher in SVR patients than in non-responders[27].

The mechanism by which LMP-7 influences response to IFN remains unclear. Several reports have indicated that patients who display successful clearance of HCV either spontaneously or after IFN α therapy express strong T-cell reaction and increased IFN γ production in response to HCV antigens[28,29]. Since the expression of LMP-7 is up-regulated by IFN γ, the differences of LMP-7 phenotypes could alter the proteosome function of patients treated with IFN α. Moreover, an amino acid substitution [LMP-7-Q (Gln) of genotype (CC) and LMP-7-K (Lys)] of genotype (AA) alters its electric charge (from non-charged polar to charged polar) with possible functional consequences. This confirms the phenotypic effects of LMP-7 on IFN response in patients with chronic hepatitis[27,30].

The present work also shows a relationship between LMP-7 gene polymorphisms with different fibrosis stages and indicates that frequency of C/C genotype was the highest in lower fibrosis stage F0 and F1 (70% and 62.07%, respectively, $P = 0.045$ and $P = 0.004$). C/C genotype frequency decreases gradually until it reaches 0% during the severe fibrosis stage (F4). On the other hand, C/A genotype frequency was the highest in advanced fibrosis stages F3 and F4 (60.87% and 83.33%, respectively, $P = 0.019$) and decreases gradually until it reaches its lowest value (20%) at F0. In agreement with our data, other studies reported that LMP-7 activity influences the rate of progression of liver fibrosis and the increased risk of the clinical course of HBV and HCV infection[17,21,27]. Even further, impairment of LMP function may enhance human tumorigenesis[8,31].

In conclusion, the present findings implicate genetic variation of LMP-7 gene polymorphisms in the outcome of HCV infection and have a great association with response to IFN-therapy and could be used as a diagnostic marker for disease progression and prediction of drug response.

COMMENTS

Background

Hepatitis C virus (HCV) is a global health problem that infects more than 170 million people worldwide. HCV cirrhosis, fibrosis and hepatocellular carcinoma are major sequelae of HCV chronic infection and are the leading causes of liver transplantation, mortality and morbidity worldwide. Pegylated interferon (IFN) and ribavirin combined therapy is the standard approved treatment for HCV and is only effective in around 50% of patients. Host genetic markers are required to predict drug induced viral clearance.

Research frontiers

HCV results in chronic hepatitis in more than 70% of infected patients, while 20%-30% of patients recover spontaneously. This indicates the role of the host genetic factors that may influence the ability to clear the virus after infection and in response to antiviral therapy. In this study, the authors investigated the association between low molecular polypeptide-7 (LMP-7) single nucleotide polymorphism (SNP) and drug induced clearance of HCV as a possible host gene for pre treatment prognosis.

Innovations and breakthroughs

Predictors of response are very important for HCV treatment. There are various genetic predictors of response. In this study, LMP-7 is presented as a new novel SNP which predicts sustained virological response (SVR) in patients with chronic HCV (CHC). Indeed, the data of the current study indicate that LMP-7 gene polymorphism is a better prognostic marker for response to therapy than interleukin-28B (IL28B) SNP since both markers were examined in the same patient cohort with 91.7% in LMP-7 *vs* 67% in IL28B association of the protective allele with SVR.

Applications

The authors hereby introduce a novel predictor for response to therapy in CHC patients. This marker will be a candidate for designing a model for viral clearance, either drug induced or spontaneous, as well as for progression of chronic HCV disease.

Terminology

Host genes were shown to play important roles in the pathogenesis of chronic hepatitis C and serve to guide improvements to IFN-based therapy and the development of novel anti-HCV therapeutics. This led the authors to study one of these genetic variants as LMP-7 in the chronically infected to clarify the association of these variants with the spontaneous viral elimination, stage of fibrosis and response to antiviral therapy.

Peer review

This is a good study in which the authors investigated the relationship between LMP-7 gene polymorphism and response to IFN therapy in CHC for the importance of host genetic factors in either spontaneous or drug induced viral clearance. It revealed a novel SNP which predicts SVR in patients with CHC and could be used in the future as a useful marker for prediction of response to therapy.

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BRIEF ARTICLE

Low incidence of spontaneous bacterial peritonitis in asymptomatic cirrhotic outpatients

Jean-François Cadranel, Jean-Baptiste Nousbaum, Christophe Bessaguet, Pierre Nahon, Eric Nguyen-Khac, Richard Moreau, Thierry Thévenot, Christine Silvain, Christophe Bureau, Olivier Nouel, Christophe Pilette, Thierry Paupard, Arnaud Pauwels, Thierry Sapey, Jean-Didier Grangé, Albert Tran

Jean-François Cadranel, Liver, Gastroenterology and Nutrition Department, Centre Hospitalier Laennec, 60100 Creil, France
Jean-Baptiste Nousbaum, Department of Hepato-Gastroenterology, University Hospital La Cavale Blanche, 29609 Brest, France
Christophe Bessaguet, Department d'Information Médicale, Centre Hospitalier, 29000 Quimper, France
Pierre Nahon, Department of Hepato-Gastroenterology, Hôpital Jean Verdier, 93140 Bondy, France
Eric Nguyen Khac, Department of Hepato-Gastroenterology, Amiens University Hospital, 80054 Amiens, France
Richard Moreau, Department of Hepatology, Hôpital Beaujon, 92100 Clichy, France
Thierry Thévenot, Department of Hepatology, University Hospital Jean Minjoz, 25030 Besançon, France
Christine Silvain, Department of Hepato-Gastroenterology, Poitiers University Hospital, 86000 Poitiers, France
Christophe Bureau, Department of Hepato-Gastroenterology, Purpan Hospital, 31059 Toulouse, France
Olivier Nouel, Department of Hepato-Gastroenterology, Centre Hospitalier de Saint Brieuc, 22027 Saint Brieuc, France
Christophe Pilette, Department of Hepato-Gastroenterology, Centre Hospitalier, 72037 Le Mans, France
Thierry Paupard, Department of Hepato-Gastroenterology, Centre Hospitalier de Dunkerque, 59240 Dunkerque, France
Arnaud Pauwels, Department of Hepato-Gastroenterology, Centre Hospitalier de Gonesse, 95503 Gonesse, France
Thierry Sapey, Department of Hepato-Gastroenterology, Centre Hospitalier de Chateauroux, 36000 Chateauroux, France
Jean-Didier Grangé, Department of Hepatology, Hôpital Tenon, 75020 Paris, France
Albert Tran, Digestive Center, University Hospital of Nice, 06202 Nice, France
Club Francophone pour l'Etude de l'Hypertension Portale; Association Nationale des Hépato-Gastroentérologues des Hôpitaux Généraux de France

Author contributions: Cadranel JF and Nousbaum JB equally contibuted to this work; Cadranel JF, Nousbaum JB, Silvain C, Pauwels A and Tran A designed the study; Bessaguet C analyzed the data; Cadranel JF and Nousbaum JB wrote the paper; Cadranel JF, Nousbaum JB, Nahon P, Nguyen-Khac E, Moreau R, Thévenot T, Silvain C, Bureau C, Nouel O, Pilette C, Paupard T, Pauwels A, Sapey T, Grangé JD and Tran A collected the data and approved final version of the manuscript.
Correspondence to: Jean-François Cadranel, MD, Professor CMH, Liver, Gastroenterology and Nutrition Department, Centre Hospitalier Laennec, BP 72, 60100 Creil, France. jfrancois.cadranel@ch-creil.fr
Telephone: +33-3-44616443 Fax: +33-3-44616440
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Abstract

AIM: To compare the incidence of spontaneous bacterial peritonitis in cirrhotic outpatients and inpatients undergoing therapeutic paracentesis

METHODS: From January 1 to May 31, 2004, 1041 patients from 70 different hospitals underwent 2123 therapeutic abdominal paracentesis (AP) performed as a outpatient procedure in 355 and as inpatient procedure in 686 cases respectively. The following parameters were compared prospectively between outpatients and inpatients: spontaneous bacterial peritonitis (SBP) prevalence, age, gender, cause of cirrhosis, symptoms, score and grade according to Child-Pugh classification, cirrhosis complications, antibiotics treatment, serum creatinine, platelet count and ascitic protein concentration.

RESULTS: SBP was observed in 91 patients. In the whole population the SBP prevalence was 8.7% (95%CI: 7.2-10.6) it was 11.7% (95%CI: 9.5-14.3) in inpatients and 3.1% (95%CI: 1.7-5.5) in outpatients ($P < 0.00001$). SBP prevalence was 8.3% (95%CI: 4.3-15.6) in symptomatic outpatients *vs* 1.2% (95%CI: 0.4-3.4) in asymptomatic outpatients ($P < 0.002$). Patients undergoing outpatient AP were significantly different from

those undergoing inpatient AP; they were older (61.1 ± 11.1 years *vs* 59.4 ± 11.7 years; $P = 0.028$), cause of cirrhosis was less often alcohol (83 .7 *vs* 88.2%; $P < 0.001$), Child-Pugh score was lower (8.9 *vs* 10.1; $P < 0.001$) and more often B than C (63.7% *vs* 38%; $P < 0.001$). In addition, in outpatients the platelet count was higher (161 ± 93 Giga/L *vs* 143 ± 89 Giga/L; $P = 0.003$), serum total bilirubin concentration was lower (38.2 ± 60.7 μmol/L *vs* 96.3 ± 143.3 μmol/L; $P < 0.0001$), and ascitic protein concentration higher (17.9 ± 10.7 g/L *vs* 14.5 ± 10.9 g/L; $P < 0.001$) than in inpatients.

CONCLUSION: In asymptomatic cirrhotic outpatients, the incidence of spontaneous bacterial peritonitis is low thus exploratory paracentesis could be avoided in these patients without significant risk.

Key words: Liver cirrhosis; Ascites; Ascitic fluid; Bacterial infections; Paracentesis; Peritonitis

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INTRODUCTION

Therapeutic abdominal paracentesis (AP) is the recommended treatment for patients with ascites resistant or refractory to medical treatment[1]. Many cirrhotic patients with ascites are regularly hospitalized for AP. Good clinical practice guidelines of the American Association for the Study of Liver Diseases[2] recommend performing ascitic fluid analysis in association with AP in order to search for two serious complications: bacterial peritonitis and neutrocytic ascites[3,4]. However, except in cases where infection is suspected, the usefulness of this approach is not clearly established in outpatients. Indeed several retrospective and prospective studies have shown that spontaneous bacterial peritonitis (SBP) is very rare or even absent in outpatients[5-10]. These data are probably related to differences in demographic data and liver diseases severity and/or characteristics of ascitic fluid between inpatients and outpatients. However such probable differences have not been properly studied. The present study is ancillary to the prospective multicenter study of the usefulness of Multistix® strip for the detection of SBP already published[11]. The aims were to compare the incidence of SBP in outpatients and inpatients undergoing AP and to look for factors associated with low SBP prevalence in outpatients (if these data were confirmed).

MATERIALS AND METHODS

Patients

The present study is a retrospective analysis of data from a prospective study involving all consecutive cirrhotic patients with ascites, undergoing therapeutic abdominal paracentesis (AP) followed from January 1 to May 31, 2004 in 70 French participating centers under the authority of the "Club Francophone pour l'Etude de l'Hypertension Portale" (CFHTP) and the "Association Nationale des Hépato-Gastroentérologues des Hôpitaux Généraux de France" (ANGH). Centers affiliated with the CFHTP were academic hospitals and centers affiliated with the ANGH were primary referral hospitals.

Diagnosis of cirrhosis relied on clinical, biological and morphological criteria. Patients were admitted either for treatment of ascites or for complications of cirrhosis (*i.e.*, infection, gastrointestinal bleeding, hepatic encephalopathy, alcoholic hepatitis, acute renal failure, hepatocellular carcinoma), or were treated in an ambulatory setting (outpatients). These outpatients were eligible for inclusion whether they presented with or without signs of SBP, according to the following criteria defined by Jeffries *et al*[5] and Castellote *et al*[10]: temperature above 38 ℃ or below 36.5 ℃, chills, abdominal tenderness suggestive of peritonitis, developing or worsening hepatic encephalopathy, gastrointestinal bleeding within the last 15 d, acute renal failure (defined by an increase in the serum creatinine level to above 133 μmol/L) and arterial hypotension (systolic arterial pressure below 80 mmHg). These signs, suggestive of infection, were carefully searched for at admission by a senior physician. Exclusion criteria were: chylous ascites and ascites not related with portal hypertension (*i.e.*, pancreatic ascites, peritoneal tuberculosis, and peritoneal carcinomatosis). Patients with hemoperitoneum complicating hepatocellular carcinoma were also excluded[11].

Paracentesis

Ascitic fluid was examined for leukocyte counts and polymorphonuclear leukocytes counts/μL, total protein and bacteriological status. Ascitic fluid was centrifuged at 2500 *g* for 10 min. A smear was stained with Giemsa. Total and differential cell counts were made with an optical microscope. Bacterial cultures were obtained by bedside inoculation of 10 mL of ascitic fluid into aerobic and anaerobic bottles (BacT/ALERT, Biomérieux, France)[12].

Each sample of ascitic fluid was tested for urine using Multistix 8 SG® according to the technique described by the manufacturer. All reagent areas were immersed in ascitic fluid and the strip was removed immediately[11].

The strips were tested independently by each investigator and by a nurse, each of whom were unaware of the results of ascitic cytological examination at the time of reading. All strips were read manually[11]. AP was repeated during the period of the study for a maximum of 8 paracenteses for each patient. The study protocol was approved by the local ethics committee of the University Hospital of Brest, France.

Table 1 Characteristics of the 1041 patients who underwent diagnostic or therapeutic paracentesis

Characteristics	
Male, *n* (%)	748 (71.9)
Age (yr), mean (range)	60 (25-93)
Median number of paracenteses	2
Cause of cirrhosis, *n* (%)	
Alcohol	840 (80.7)
Alcohol + viral hepatitis (B or C)	52 (5)
Viral hepatitis	88 (8.4)
Hemochromatosis	6 (0.6)
Others	55 (5.3)
Child-Pugh score, *n* (%)	
A	0
B	470 (45.2)
C	571 (54.8)
Prothrombin time (%), median (range)	52 (20-85)
Serum bilirubin (μmol/L), median (range)	30 (10-812)
Albumin (g/L), median (range)	29 (16.5-53)
Platelet count (10^9 cells/mL), median (range)	130 (15-684)
Serum creatinine (μmol/L), median (range)	80 (22-474)

Table 2 Comparison between outpatients and inpatients

	Outpatients	Inpatients	*P* value
Age (yr)	61.1 ± 11.1	59.4 ± 11.7	< 0.03
Alcohol (%)	83.7	88.2	< 0.001
Child-Pugh score (mean)	8.9	10.1	< 0.001
Child-Pugh score B *vs* C (%)	63.7/36.3	38.0/62.0	< 0.001
Bilirubin (μmol/L)	38.2 ± 6.7	96.3 ± 143.3	< 0.0001
Platelets (10^9 cells/mL)	161 ± 93	143 ± 89	< 0.003
Ascitic protein concentration (g/L)	17.9 ± 10.9	14.5 ± 10.9	< 0.001

Statistical analysis

The following parameters were compared between outpatients and inpatients: SBP prevalence, age, gender, cause of cirrhosis, symptoms, score and grade according to Child-Pugh classification, cirrhosis complications, antibiotics treatment, serum creatinine, platelet count and ascitic protein concentration.

Statistical analysis was performed using the SPSS package (SPSS Inc., Chicago, United States). Results were expressed as mean and standard deviation with range unless specified otherwise. Quantitative characteristics (age, bilirubin, platelet count, *etc.*) were compared between the two patients populations by Student's *t* test. Depending on the qualitative characteristics (cause of cirrhosis, Child-Pugh score, *etc.*) proportional differences between the two populations were studied by χ^2 test.

RESULTS

One thousand and forty one cirrhotic patients with ascites, undergoing therapeutic paracentesis (AP) were studied. A total of 2123 paracenteses were performed. The procedure was performed as an outpatient procedure in 355 cases (34.1%) and in hospitalized patients (inpatients) in 686 cases (65.9%). The main characteristics of the patients are shown in Table 1; cirrhosis was related with alcohol in 80.6% of cases, to viral hepatitis in 8.5% patients and to several causes in 5% (Table 1).

Table 3 Prevalence of spontaneous bacterial peritonitis in asymptomatic outpatients in main published series

Ref.	No. of patients	No. of paracenteses	Prevalence of spontaneous bacterial peritonitis
Jeffries *et al*[5]	29	118	0 (0%)
Evans *et al*[8]	427	427	15 (3.5%)
Romney *et al*[9]	67	270	0 (0%)
Castellote *et al*[10]	40	204	1 (0.5%)
Present study	355	976	1.2

Data concerning Multistix® performance for the SBP diagnosis have been published elsewhere and will not be described here[11]. One hundred and seventeen SBP were observed in 91 out of 1041 patients undergoing 2123 paracenteses. Cultures were positive in 56 patients. SBP incidence was 8.7% (95%CI: 7.2-10.6) in the whole population, 11.7% (95%CI: 9.5-14.3) in inpatients and 3.1% (95%CI: 1.7-5.5) in outpatients ($P < 0.00001$). SBP incidence was 8.3% (95%CI: 4.3-15.6) in symptomatic outpatients *vs* 1.2% (95%CI: 0.4-3.4) in asymptomatic outpatients ($P < 0.002$).

Patients undergoing AP as an outpatient procedure differed significantly from those undergoing inpatient AP (Table 2); outpatients were older (61.1 ± 11.1 *vs* 59.4 ± 11.7 years, $P = 0.028$), cause of cirrhosis was less often alcohol (83.7% *vs* 88.2%; $P < 0.001$), Child-Pugh score was lower (mean score was 8.9 *vs* 10.1; $P < 0.001$) and more often B than C (63.7% *vs* 38%, $P < 0.001$). In addition, in outpatients the platelet count was higher [(161 ± 93) × 10^9 cells/mL *vs* (143 ± 89) × 10^9 cells/mL; $P = 0.003$], serum total bilirubin concentration was lower (38.2 ± 60.7 μmol/L *vs* 96.3 ± 143.3 μmol/L; $P < 0.0001$), and ascitic protein concentration was higher (17.9 ± 10.7 g/L *vs* 14.5 ± 10.9 g/L; $P < 0.001$) than in inpatients (Table 2).

DISCUSSION

In this study, 1041 cirrhotic patients underwent 2123 paracenteses with ascitic fluid analysis and two Multistix 8 SG strip determination[11]. Eighty percent of the patients had alcoholic cirrhosis and 44.2% were stage C cirrhosis according to the Child-Pugh classification. The incidence of SBP in asymptomatic outpatients was 1.2%. Our study thus confirms the low incidence of SBP in outpatients considered as asymptomatic according to well-defined clinical criteria[5,8,9]. In the two studies published in abstract form only, the incidence of SBP in outpatients was 0% in the first study (78 paracenteses performed in 26 patients)[6] and 0% in the second (173 paracenteses study performed in 51 patients)[7]. In the 4 studies published as peer-reviewed articles, including a paracenteses number ranging from 118 to 427, the incidence of SBP ranged from 0% to 3.5% (Table 3). In addition, Runyon[2] recently reported a 2% incidence of SBP in a series of 400 paracenteses performed in two years in an outpatient setting. In a study performed by one of the coauthors

of this study, including more than 500 paracenteses of ascitic fluid in cirrhotic ascitic patients on long-term ofloxacin treatment, the incidence of bacterial peritonitis was zero[9]. In their retrospective study of 427 cirrhotic patients seen in a single outpatient clinic, Evans *et al*[8] analyzed 427 exploratory paracenteses performed over a 6-year period. Their exclusion criteria were similar to those used by Jeffries *et al*[5]; however patients receiving primary or secondary prophylaxis with norfloxacin were excluded. The incidence of SBP in the study performed by Evans *et al*[8] was 1.4% and that of neutrocytic ascites was 2.1% (giving a combined incidence of 3.5%). In the prospective multicenter study of Romney *et al*[9] the incidence of SBP was nil and it was 0.5% in the recent study of Castellote *et al*[10] (Table 3). The incidence of SBP in asymptomatic outpatients found in our large prospective multicenter study was thus comparable to that of these 4 studies[5,8-10]. Our data confirm on a large scale that asymptomatic outpatients have a very low or even null risk of SBP. Although in our study the incidence of SBP in asymptomatic outpatients was less than 1.5%, it rose to more than 8% when symptoms and signs of infection were present underlining the validity of the criteria of Jeffries *et al*[5] and Romney *et al*[9]. When considering the whole population of outpatients, the incidence of SBP remained however significantly lower than that of inpatients ($P < 10^{-5}$). Although the reasons for such differences in SBP incidence between inpatients and outpatients are probably related to differences in demographic data and liver diseases severity and/or characteristics of ascitic fluid between inpatients and outpatients, these data had not been studied in previously published series. In the present study, outpatients differed significantly from inpatients: cause of cirrhosis was less often alcohol, Child-Pugh score was lower and more often B than C, platelet count was higher and serum total bilirubin concentration was lower; in addition, ascitic protein concentration was higher in outpatients. Several factors associated with SBP have been reported including: variceal bleeding, liver failure, low ascitic protein concentration and past history of SBP[13] leading to antibioprophylaxis in bleeding cirrhotic patients, in those with SBP history and/or low ascitic protein concentration[13]. It has been suggested, then shown, that cirrhotic patients with low platelet count, high serum bilirubin level[14], Child-Pugh C, hyponatremia or renal failure were at higher risk of SBP and that primary prophylaxis was particularly indicated in these settings[14,15]. It is noteworthy that outpatients in the present study presented with lower Child-Pugh score, higher serum bilirubin level; lower platelet count and higher ascitic protein concentration than inpatients accounting for the lower SBP incidence observed in this group.

In conclusion, the results of our study confirm that SBP is rare in outpatients undergoing exploratory paracentesis. The reason for this rarity is the lower frequency of SBP risk factors in these patients. Exploratory paracentesis could be avoided in most of the cases. However, we need to clarify the predictive factors associated with no risk of SBP in this setting.

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COMMENTS

Background

Therapeutic abdominal paracentesis is the recommended treatment for patients with ascites resistant or refractory to medical treatment. Good clinical practices guidelines recommend performing ascitic fluid analysis in association with abdominal paracentesis to search for bacterial peritonitis. However, except in cases where infection is suspected the usefulness of this approach is not clearly established in cirrhotic outpatients.

Innovations and breakthroughs

The results of this large scale study confirm that spontaneous bacterial peritonitis is rare in cirrhotic outpatients undergoing exploratory paracentesis. The reasons for this rarity are the lower frequency of spontaneous bacterial peritonitis risk factors in these patients. Patients undergoing abdominal paracentesis in an outpatient setting differed significantly from those undergoing inpatient abdominal paracentesis: they were significantly older, cause of cirrhosis was less often alcohol, Child-Pugh score was significantly lower and more often B than C, platelet count was higher, serum total bilirubin concentration was significantly lower and ascitic protein concentration was higher.

Applications

The study results suggest that exploratory paracentesis could be avoided in most cases of asymptomatic cirrhotic outpatients, according to well-defined clinical criteria.

Peer review

The question is not new and results were rather predictable, however few actual results were available in the literature. Statistical differences occur even in the presence of small numerical differences because of the large number of patients. Nevertheless the substantial sample is in itself a bonus and therefore findings should be accepted.

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BRIEF ARTICLE

Silymarin in non alcoholic fatty liver disease

Fulvio Cacciapuoti, Anna Scognamiglio, Rossella Palumbo, Raffaele Forte, Federico Cacciapuoti

Fulvio Cacciapuoti, Anna Scognamiglio, Rossella Palumbo, Raffaele Forte, Federico Cacciapuoti, Department of Internal Medicine and Geriatrics, Second University of Naples, 80138 Naples, Italy

Author contributions: Cacciapuoti F contributed to the conception and work design; Scognamiglio A and Palumbo R contributed to the statistical analysis and data interpretation; Forte R critically revised the paper; Cacciapuoti F critically revised and approved the final version.

Correspondence to: Federico Cacciapuoti, Associate Professor, Department of Internal Medicine and Geriatrics, Second University of Naples, Piazza L, Miraglia, 2, 80138 Naples, Italy. federico.cacciapuoti@unina2.it

Telephone: +39-8-1665022 Fax: +39-8-15665022
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Abstract

AIM: This study was undertaken to evaluate the hepatic effects of silybum marianum on non alcoholic fatty liver disease (NAFLD).

METHODS: In 72 patients affected by NAFLD, main metabolic, hepatic and anti-inflammatory parameters were assayed after 3 mo of a restricted diet and before silymarin treatment (twice a day orally). The brightness of liver echography texture (hepatorenal ratio brightness) was also defined at same time. These evaluations were repeated after 6 mo of treatment.

RESULTS: Serum levels of some metabolic and anti-inflammatory data nonsignificantly lowered after 6 mo of silymarin. On the contrary, Steato test, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase were significantly ($P < 0.001$) reduced. Instead, the AST/ALT ratio unchanged. Finally, the hepatorenal brightness ratio, as an index of hepatic steatosis, significantly ($P < 0.05$) dropped.

CONCLUSION: The obtained results indicate that silymarin appears to be effective to reduce the biochemical, inflammatory and ultrasonic indices of hepatic steatosis. Some parameters indicative of early stage of atherosclerosis were also lowered.

Key words: Alanine aminotransferase; Aspartate aminotransferase; Total cholesterol; Gamma-glutamyl transpeptidase; Non alcoholic fatty liver disease; Silymarin; Steato test; Hepatorenal ultrasonographic index; Fasting glucose level; High density lipoprotein and low density lipoprotein cholesterol; Homeostatic model assessment insulin resistance test

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INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) consists of a spectrum of conditions ranging from a simple fatty infiltration to steatohepatitis, fibrosis and cirrhosis. NAFLD is histologically similar to alcoholic liver disease, but without a history of ingesting significant amounts of ethanol. It produces few symptoms but can have evidence of fat in the liver on ultrasound[1-3] and hepatocellular fibrosis and/or inflammation evidenced by some biochemical tests. Obesity, type 2 diabetes mellitus and hyperlipidemia are often associated with NALFD[4-7]. These aspects may coexist with insulin resistance[8-10]. In turn, insulin resistance may lead to endothelial dysfunction and systemic hypertension and may induce an abnormal lipid profile. All these changes may promote the development of atherosclerotic vascular disease, even if the increased values of insulin resistance may be responsible for metabolic syndrome (MS) only.

Several medications have been proposed as NAFLD therapies, such as Metformin[11], Peroxisome proliferator-

activated receptor-γ agonists[12] and Ezetimibe[13]. Silymarin (a mixture of at least 4 closely related flavonolignans, 60% to 70% of which is a mixture of 2 diastereomers of silybin) also demonstrated antioxidant properties, through stimulation of polymerase and RNA transcription, by protecting the cell membrane from radical-induced damage and blocking the uptake of toxins. Studies performed in patients with liver disease have shown that silymarin increased superoxide dismutase activity of lymphocytes and erythrocytes. In addition, it has been shown to increase patient serum levels of glutathione and glutathione peroxidase[14]. In a previous report, silymarin treatment was associated with a reduction of insulin resistance and a significant decrease in fasting insulin levels, suggesting an improvement of the activity of endogenous and exogenous insulin[15]. In another study, silymarin extract caused an improvement in serum alanine aminotransferase (ALT) levels. Hajaghamohammadi *et al*[16] demonstrated that silymarin decreased serum aspartate aminotransferase (AST) levels. Federico *et al*[17] showed that a new silybin vitamin E complex improves insulin resistance and liver damage in patients with NAFLD. More recently, Hasjiani *et al*[18] found that in two patient groups receiving silymarin and Vitamin E, respectively, the AST and ALT serum levels significantly decreased.

In this study, we evaluated the effects of a mixture of silybum marianum (silymarin), vitamin B_{12}, vitamin E (Epaclin) and a restricted diet on NAFLD patients.

MATERIALS AND METHODS

From January 2010 to June 2011, 72 subjects (40 male and 32 female) with a mean age of 44 ± 3.2 years were selected. Exclusion criteria were ethanol intake > 20 g/d and the customary ingestion of drugs known to produce fatty liver disease, such as steroids, estrogens, amiodarone, tamoxifen or other chemotherapeutic agents. Viral hepatitis was excluded by the negative results of hepatitis B surface antigen and anti-hepatitis C virus tests. Hemochromatosis, autoimmune hepatitis, Wilson's disease and other hepatic diseases were also ruled out. After three months of a restricted diet, a food supplement containing Vitamin E, L-gluthatione, L-cysteine, L-methionine and silybum marianum (Epaclin 3.5 g) was given twice a day (as packet) in all selected subjects. Homeostatic model assessment insulin resistance (HOMA-IR)[19], total cholesterol (in mg/dL), high density lipoprotein (HDL)-C, low density lipoprotein (LDL)-C, triglycerides and fasting plasma glucose were evaluated at baseline and after six months of treatment. Steato test, ALT, AST with their ratio serum levels (ALT/AST) and γ-glutamyl peptidase (γ-GT) were also measured, both in basal conditions and after 6 mo. In addition, serum levels of tumor necrosis factor-α (TNF-α) were defined both at baseline and after silymarin treatment (6 mo). Finally, the hepatorenal brightness ratio was defined at the entry of the study and after 6 mo, using the sonographic brightness difference of the hepatorenal index[20].

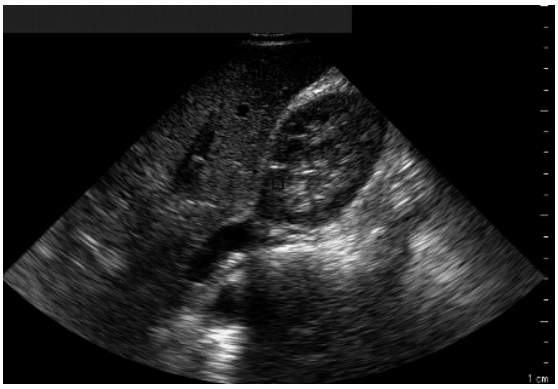

Figure 1 Echographic finding of liver and right kidney for calculation of the hepatorenal index brightness.

Ultrasonography

An iE-33 ultrasonographic machine (Philips, Amsterdam) with a 3.5 MHz phased array convex transducer was used to verify the possible presence and the degree of hepatic steatosis. All subjects were evaluated in the left lateral recumbent position of 15°-20° to see the liver parenchyma and the right kidney cortex was seen contemporaneously. The brightness of both zones was examined. The liver was recorded from the intercostal space, posing the region of interest (ROI) (of 1.5 cm × 1.5 cm) in the mid or anterior axillary line (seventh or eighth intercostal space). The right kidney was evaluated, posing the ROI (0.5 cm × 0.5 cm) in the cortical zone[20]. Normal liver echo texture was considered as the absence of steatosis, as shown in Figure 1. In this case, the hepatorenal brightness ratio was 1. Mild steatosis was diagnosed for hyperechogenic liver tissue (compared with the kidney cortex) when the sonographic index results were between 1 and 2. Values between 2 and 2.5 were indicative of moderate liver steatosis. Finally, hepatic steatosis was judged as severe when the hepatorenal ratio was > 2.5. In each case, the calculation of the hepatorenal index was repeated at least twice. The main demographic, metabolic, serum liver indices and ultrasonographic data recorded at baseline and after 6 mo of treatment are reported in Table 1.

Statistical analysis

Calculations were performed as paired data, by comparing the biochemical (metabolic and serum liver-indices) values recorded at baseline and after 6 mo. Continuous variables are presented as mean ± SD. $P < 0.05$ was considered statistically significant for analysis. The degree of the liver echogenicity and the hepatorenal index recorded in basal conditions and at the end of treatment (6 mo) were also compared. All calculations were made using SPSS Version 13.0 for Microsoft Windows.

RESULTS

BMI was not significantly different before and after treatment in all subjects. Mean glucose fasting levels measured at baseline were 105 ± 0.7 mg/mL. This value fell to 101

Table 1 Demographic, metabolic, serum liver-indices and ultrasonic data obtained before and after treatment in enrolled subjects (mean ± SD)

Variables	Before	After	*P* value
Age (yr)	44 ± 3.2		
Sex (M/F)	40/32		
BMI (kg/m^2)	26.7 ± 1.67	26.4 ± 1.34	NS
Fasting plasma glucose (mg/mL)	105.7 ± 0.8	101 ± 0.5	NS
HOMA-IR	6.42 ± 0.4	5.27 ± 1.2	NS
Total Cholesterol (mg/dL)	205.7 ± 9.3	200.6 ± 8.1	NS
LDL-C (mg/mL)	157.4 ± 4.3	136 ± 1.8	NS
HDL-C (mg/mL)	43.6 ± 2.1	45.8 ± 1.1	NS
Triglycerides (mg/mL)	178.4 ± 4.1	155.7 ± 3.4	NS
Steato test	0.71 ± 0.07	0.40 ± 0.05	< 0.001
ALT (U/L)	109.48 ± 4.4	75.12 ± 3.3	< 0.01
AST (U/L)	72.39 ± 8.4	48.65 ± 3.2	< 0.01
AST/ALT ratio	0.66 ± 0.4	0.64 ± 0.9	NS
γ-GT (IU/L)	45.51 ± 1.2	29.33 ± 1.1	< 0.001
TNF-α (pg/mL)	16.2 ± 0.9	9.7 ± 0.7	< 0.001
Hepatorenal ratio	2.5 ± 0.3	1.8 ± 0.6	< 0.05

M/F: Male/female; BMI: Body mass index; HOMA-IR: Homeostatic model assessment insulin resistance; LDL: Low density lipoprotein; HDL: High density lipoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; γ-GT: Gamma glutamyl transpeptidase; TNF-α: Tumor necrosis factor-α; NS: Not significant.

± 0.5 mg/mL after a restricted diet and silymarin treatment. HOMA-IR results were 6.42 ± 0.4 *vs* 5.27 ± 1.2, without significant differences. Total cholesterol was 205.7 ± 9.3 mg/dL before and 200.6 ± 8.1 mg/dL after treatment. In agreement, both LDL-C and HDL-C were slightly and not significantly reduced after silymarin and a restricted diet (157.4 ± 4.3 and 43.6 ± 2.1 mg/mL in basal conditions and 136 ± 1.8 and 45.8 ± 1.1 mg/mL, respectively). Triglycerides levels were not significantly lower, going from 178.4 ± 4.1 to 155.7 ± 3.4 mg/mL. On the contrary, Steato test significantly ($P < 0.001$) reduced from baseline (0.71 ± 0.07) to the end of treatment (0.40 ± 0.05). ALT serum levels ($P < 0.01$) failed from a mean level of 109.48 ± 4.4 to 75.12 ± 3.3 U/L. AST recorded at baseline (72.39 ± 8.4 U/L) also significantly reduced ($P < 0.05$) after silymarin and diet (48.65 ± 3.2 U/L). Instead, AST/ALT ratio was not significantly decreased from basal conditions (0.66 ± 0.4) to the end of silymarin treatment (0.64 ± 0.9). γ-GT decreased from 45.51 ± 1.2 to 29.33 ± 1.1, with a significant difference ($P < 0.001$). TNF-α significantly fell ($P < 0.001$) from basal conditions (16.2 pg/mL) to post treatment phase (6 mo). Finally, the hepatorenal ratio dropped from 2.5 ± 0.3 to 1.8 ± 0.6. The reduction was also significant ($P < 0.05$) (Table 1).

DISCUSSION

NAFLD is the most common silent liver disease worldwide, marked by fat accumulation in the liver (steatosis)[21] and alterations in liver biochemical tests occurring in those who do not consume high amounts of alcohol. The prevalence of NAFLD in western countries is estimated to be 20%-30%[22,23]. Current guidelines recommended liver biopsy for diagnosis, that is the "gold standard" for quantification of hepatic steatosis associated with NAFLD[24]. However, it is hard to be accepted due to its invasiveness and a significant degree of sampling error. In addition, it is invasive, costly and prone to complications[25,26].

In our experience, NAFLD is associated with insulin resistance (HOMA-IR). Previous studies demonstrated that insulin resistance almost universally induces NAFLD[27,28]. It is known that this condition may precede the development of cardiovascular disease[29,30]. To confirm the connection between NAFLD and atherosclerosis, carotid atherosclerosis has recently been detected in patients with NAFLD[31]. Pathogenetic mechanisms responsible for that include an increased lipolysis and increased delivery of free fatty acids to the liver[32]. Other abnormalities that can contribute to fat accumulation in the liver include decreased synthesis of apolipoproteins and microsomal transfer protein gene polymorphism, both conditions that lead to decreased export of triglycerides out of the liver[33]. We also found an increased value of Steato test at baseline that significantly reduced after 6 mo of silymarin and a restricted diet. It is known that the test (score from 0 to 1) gives a quantitative estimation of steatosis of different origins. The behavior of the Steato test in patients with NAFLD is more important than ultrasonography for noninvasive diagnosis of steatosis and may reduce the need of liver biopsy, particularly in patients with other risk factors[34]. Mild to moderate elevation of serum aminotransferases (ALT and AST) found in our subjects at baseline represents the most common abnormality found in patients with NAFLD[1]. Their serum levels significantly reduced after diet and silymarin treatment. Unlike those with alcohol-induced steatohepatitis (who typically manifest disproportionate increases in the ALT level), patients with NAFLD usually have an AST/ALT ratio < 1 because the ALT level is higher than AST in NAFLD[35]. On the contrary, the AST/ALT ratio tends to increase with the development of cirrhosis, thus losing its diagnostic accuracy[36]. The reduction both of AST and ALT (with AST/ALT < 1) after silymarin treatment seems due to the antioxidant effect of silybum marianum that is also able to protect the liver against toxins. Previous studies also demonstrated that silymarin acts as a cytoprotectant, anticarcinogenic and supportive agent for liver damage from Amanita phalloides poisoning[37,38]. Mean serum levels of γ-GT were above the normal limits in selected subjects with NAFLD. That is due to obesity, hyperinsulinemia, oxidative inflammation and changes in hepatocyte membrane permeability[39]. In our study, silymarin was demonstrated to reduce its high serum levels, probably for stabilization of the hepatocyte membrane structure, thereby preventing toxins from entering the cells. This happens through enterohepatic recirculation and by promoting liver regeneration. A previous study demonstrated that this happens by stimulating nucleolar polymerase A and increasing ribosomal protein synthesis[40]. TNF-α was also reduced after silymarin therapy, as a consequence of the anti-inflammatory action of the drug. This clearly demonstrated a reduction of the hepatic inflammation. Finally, the changes of hepatorenal brightness index clearly dem-

onstrated the reduction of hepatic fat accumulation after silymarin therapy. From this point of view, although several techniques can be used to assess liver steatosis, ultrasonography represents the most commonly used method for this[20]. It can be assessed by hyperechogenicity of liver tissue ("bright liver") compared to the echogenicity of the kidney cortex (hepatorenal contrast). The ultrasound index is the main noninvasive imaging modality for the evaluation of hepatic steatosis[41-43]. The sensitivity of ultrasonography in detecting steatosis is between 60% and 90%[44], even if it is difficult to identify the inflammatory liver findings. It is also difficult to differentiate steatosis from steatohepatitis[45].

In conclusion, the results obtained indicate that silymarin seems to be effective in reducing the biochemical and ultrasonographic changes induced by NAFLD. These results are in agreement those obtained by other authors[46]. A pilot study performed in patients with NAFLD confirmed an improvement in liver enzymes and insulin resistance when a complex of silybin, vitamin E and phospholipid was given[47]. The effects of silybum marianum and vitamin E on some biochemical indices of atherosclerotic progression must be confirmed by other experiences performed on a wide range, even though these metabolic data may be reported, not only for atherosclerosis, but also for MS alone.

COMMENTS

Background

Studies performed in patients with liver disease have shown that silymarin increased superoxide dismutase activity of lymphocytes and erythrocytes. In addition, it has been shown to increase patient serum levels of glutathione and glutathione peroxidase.

Innovations and breakthroughs

The results obtained indicate that silymarin seems to be effective in reducing the biochemical and ultrasonographic changes induced by non alcoholic fatty liver disease (NAFLD). The effects of silybum marianum and vitamin E on some biochemical indices of atherosclerotic progression must be confirmed by other experiences performed on a wide range, even though these metabolic data may be reported, not only for atherosclerosis, but also for metabolic syndrome alone.

Applications

In this study, the authors evaluated the effects of a mixture of silybum marianum (silymarin), vitamin B12, vitamin E (Epaclin) and a restricted diet on NAFLD patients.

Peer review

This is a nice piece of work that addresses the effects of a mixture of vitamin B12, vitamin E and silybum marianum in improving the syndrome related to NAFLD and/or atherosclerosis. This manuscript is acceptable for publication.

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BRIEF ARTICLE

Percutaneous trans-hepatic bilateral biliary stenting in Bismuth Ⅳ malignant obstruction

Dimitrios Karnabatidis, Stavros Spiliopoulos, Paraskevi Katsakiori, Odissefs Romanos, Konstantinos Katsanos, Dimitrios Siablis

Dimitrios Karnabatidis, Stavros Spiliopoulos, Paraskevi Katsakiori, Odissefs Romanos, Konstantinos Katsanos, Dimitrios Siablis, Department of Interventional Radiology, Patras University Hospital, 26504 Patras, Greece
Author contributions: Karnabatidis D, Spiliopoulos S, Katsakiori P, Romanos O, Katsanos K and Siablis D contributed to conception and design, acquisition of data, analysis and interpretation of data; Karnabatidis D, Spiliopoulos S and Katsanos K contributed to drafting the article, revising it critically for important intellectual content; Karnabatidis D and Siablis D contributed to final approval of the version to be published.
Correspondence to: Dimitrios Karnabatidis, MD, PhD, EBIR, FCIRSE, Assistant Professor of Interventional Radiology, Department of Interventional Radiology, Patras University Hospital, 26500 Patras, Greece. karnaby@med.upatras.gr
Telephone: +30-26-23603218 Fax: +30-26-23603218
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Abstract

AIM: To investigate the clinical efficiency of percutaneous trans-hepatic bilateral biliary metallic stenting for the management of Bismuth Ⅳ malignant obstructive disease.

METHODS: Our hospital's database was searched for all patients suffering from the inoperable malignant biliary obstruction Bismuth Ⅳ, and treated with percutaneous bilateral trans-hepatic placement of self-expandable nitinol stents. The indication for percutaneous stenting was an inoperable, malignant, symptomatic, biliary obstruction. An un-correctable coagulation disorder was the only absolute contra-indication for treatment. Bismuth grading was performed using magnetic resonance cholangiopancreatography. Computed tomography evaluation of the lesion and the dilatation status of the biliary tree was always performed prior to the procedure. All procedures were performed under conscious sedation. A single trans-hepatic track technique was preferred (T-configuration stenting) and a second, contra-lateral trans-hepatic track (Y-configuration stenting) was used only in cases of inability to access the contra-lateral lobe using a single track technique. The study's primary endpoints were clinical success, defined as a decrease in bilirubin levels within 10 d and patient survival rates. Secondary endpoints included peri-procedural complications, primary and secondary patency rates.

RESULTS: A total of 35 patients (18 female, 51.4%) with a mean age 69 ± 13 years (range 33-88) were included in the study. The procedures were performed between March 2000 and June 2008 and mean time follow-up was 13.5 ± 22.0 mo (range 0-96). The underlying malignant disease was cholangiocarcinoma (*n* = 10), hepatocellular carcinoma (*n* = 9), pancreatic carcinoma (*n* = 5), gastric cancer (*n* = 2), bile duct tumor (*n* = 2), colorectal cancer (*n* = 2), gallbladder carcinoma (*n* = 2), lung cancer (*n* = 1), breast cancer (*n* = 1) or non-Hodgkin lymphoma (*n* = 1). In all cases, various self-expandable bare metal stents with diameters ranging from 7 to 10 mm were used. Stents were placed in Y-configuration in 24/35 cases (68.6%) using two stents in 12/24 patients and three stents in 12/24 cases (50%). A T-configuration stent placement was performed in 11/35 patients (31.4%), using two stents in 4/11 cases (36.4%) and three stents in 7/11 cases (63.6%). Follow-up was available in all patients (35/35). Patient survival ranged from 0 to 1763 d and the mean survival time was 168 d. Clinical success rate was 77.1% (27/35 cases), and peri-procedural mortality rate was 5.7% (2/35 patients). Biliary re-obstruction due to stent occlusion occurred in 25.7% of the cases (9/35 patients), while in 7/11 (63.6%) one additional percutaneous re-intervention due to stent occlusion resulting in clinical relapse of symptomatology was successfully performed. In the remaining

4/11 patients (36.4%) more than 1 additional reintervention was performed. The median decrease of total serum bilirubin was 60.5% and occurred in 81.8% of the cases (27/33 patients). The median primary and secondary patency was 105 (range 0-719) and 181 d (range 5-1763), respectively. According to the Kaplan-Meyer survival analysis, the estimated survival rate was 73.5%, 47.1% and 26.1% at 1, 6 and 12 mo respectively, while the 8-year survival rate was 4.9%. Major and minor complication rates were 5.7% (2/35 patients) and 17.1% (6/35 patients), respectively.

CONCLUSION: Percutaneous bilateral biliary stenting is a safe and clinically effective palliative approach in patients suffering from Bismuth Ⅳ malignant obstruction.

Key words: Bismuth Ⅳ; Malignant biliary obstruction; Percutaneous bilateral stenting; Nitinol stents; Palliative treatment; Fluoroscopically-guided

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INTRODUCTION

Malignant biliary tree obstruction occurs due to primary biliary or metastatic carcinomas. The most common causes of this condition are cholangiocarcinoma, gallbladder cancer and pancreatic carcinoma[1]. Although surgery is indicated in many cases for the management of malignant biliary obstruction, nearly 80% of the patients are inoperable by the time of diagnosis and the median survival ranges between 3 and 10 mo[2-4]. Minimally invasive decompression drainage modalities are established palliative methods for the management of inoperable hilar lesions and include percutaneous trans-hepatic biliary drainage, endoscopic stricture stenting and percutaneous trans-hepatic biliary stenting (PTBS)[4-7]. Malignant obstructions of the hepatic hilum are characterized by extremely poor prognosis resulting in a 5-year survival rate around 5%, and are also the most technically challenging biliary lesions for both endoscopic and interventional radiology management approaches[2]. Palliative decompression of the biliary tree using metallic stents focuses on achieving relief from obstruction-related pathology, in order to improve the patient's quality of life and survival. In Bismuth-Corlette Ⅲ and Ⅳ lesions the percutaneous trans-hepatic approach is the modality of choice[8,9].

To date, data regarding percutaneous trans-hepatic bilateral stenting in Bismuth Ⅳ lesions are scarce and the clinical decision whether to drain both hepatic lobes remains controversial[10-12]. The present study investigated the safety and clinical efficiency of palliative, percutaneous trans-hepatic bilateral metallic stenting, for the management of Bismuth Ⅳ malignant hilar obstructive disease.

MATERIALS AND METHODS

Board approval was obtained by the hospital's Scientific and Ethics Committee. Our hospital's databases were retrospectively searched for all patients suffering from Bismuth disease who were managed using percutaneous bilateral trans-hepatic placement of self-expandable nitinol stents in the Interventional Radiology Unit. The indication for percutaneous stenting was an inoperable, malignant, symptomatic, biliary obstruction. An uncorrectable coagulation disorder was the only absolute contra-indication for treatment. The international normalized ratio (INR) value required was < 1.5 and platelet count > 50×10^9/L. Bismuth grading was performed using magnetic resonance cholangiopancreatography. Computed tomography evaluation of the lesion and the dilatation status of the biliary tree were always performed prior to the procedure.

The patients were hospitalized at least 24 h before the drainage and were kept nil per mouth for at least 6 h prior to the procedure. Baseline pre-procedural laboratory tests included blood count, serum bilirubin, renal function and coagulation profile (INR, prothrombin time, partial thromboplastin time). Any clotting disorder was corrected appropriately. Prophylactic treatment with broad spectrum antibiotics (second-generation cephalosporin) was administrated 24 h prior to the procedure and continued for five days thereafter. Written informed consent was attained from all patients. All procedures were performed under conscious sedation using a combination of opioid analgesic (Fentanyl) and a sedative (Midazolam). Initial doses of 25-50 μg of Fentanyl and 1-2 mg of Midazolam were administrated and these were repeated when needed. The access site (right or left lobe puncture) was chosen according to the pre-procedural imaging. A fine 21 G × 15 cm Chiba needle was utilized and ultrasound-guidance was mainly used in left-lobe access. Following the initial diagnostic percutaneous trans-hepatic cholangiography a single trans-hepatic track technique was preferred (T-configuration stenting) and a second, contra-lateral trans-hepatic track (Y-configuration stenting) was used only in cases of inability to access the contra-lateral lobe using a single track technique. Access through the obstructed bile ducts to the small intestine was obtained using standard Interventional Radiology techniques and materials. In cases where bile tree infection was suspected, an external drainage catheter was positioned and stent placement was performed a few days later, following *iv* antibiotic therapy and bile tree decompression. Where no infection was suspected, moderate balloon pre-dilatation and self-expandable bare metal stenting was performed. The Kissing balloon technique was used for pre-dilatation prior to Y-configuration

Table 1 Patients' baseline demographics

Patients (*n*)	35
Female	18/35 (51.4)
Age (yr), mean ± SD	69 ± 13
Tumor type	
Cholangiocarcinoma	10/35 (28.5)
Hepatocellular carcinoma	9/35 (25.7)
Pancreatic carcinoma	5/35 (14.3)
Gastric cancer	2/35 (5.7)
Bile duct cancer	2/35 (5.7)
Colorectal cancer	2/35 (5.7)
Gallbladder carcinoma	2/35 (5.7)
Lung cancer	1/35 (2.8)
Breast cancer	1/35 (2.8)
Non-Hodgkin lymphoma	1/35 (2.8)

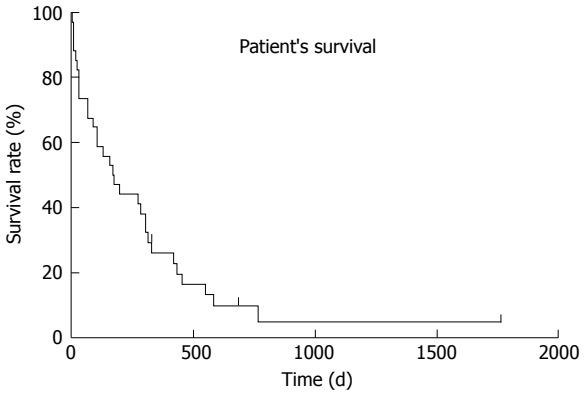

Figure 1 Overall patients' survival Kaplan-Meyer plot.

stenting. At the end of the procedure, a closed external-internal drainage catheter was left in place and was removed over-the-wire if good stent patency, without any signs of bleeding, was confirmed two days after. Post-procedural laboratory tests were repeated every day until the patient's discharge and monthly thereafter. In cases of significant bilirubin level increase and/or cholangitis, re-evaluation with ultrasound and revision was performed if needed.

The study's primary endpoint was clinical success defined as relief or improvement from the initial symptoms and survival rates. Secondary endpoints included peri-procedural 24 h major and minor complication rates, primary patency rates defined as the patency of the deployed stents without any additional intervention and secondary patency rates defined as the stent patency following one additional percutaneous or endoscopic re-intervention due to clinical relapse of the symptoms. Stents were considered patent in cases in which no increase in serum bilirubin levels or dilatation of the intrahepatic ducts was noted. Complications were divided into major and minor according to the standards of the Society of Interventional Radiology[13]. Statistical analysis was performed using dedicated statistical analysis software (GraphPad Prism version 5; San Diego, CA, United States).

RESULTS

In total, 35 patients (18 female, 51.4%) with a mean age 69 ± 13 years (range 33 to 88 years) were included in the study. All patients suffered from Bismuth IV type malignant disease and were considered unsuitable for operation due to tumour extension and/or poor physical conditions. The procedures were performed between March 2000 and June 2008 and mean time clinical follow-up was 13.5 ± 22.0 mo (range 0 to 96 mo). The underlying malignant disease was cholangio-carcinoma (n = 10), hepatocellular carcinoma (n = 9), pancreatic carcinoma (n = 5), gastric cancer (n = 2), bile duct tumour (n = 2), colorectal cancer (n = 2), gallbladder carcinoma (n = 2), lung cancer (n = 1), breast cancer (n = 1) or non-Hodgkin lymphoma (n = 1). Patient baseline demographics are analytically reported in Table 1. All procedures were performed by two senior interventional radiologists. In all cases various self-expandable bare metal stents with diameters ranging from 7 to 10 mm were used. Stents were placed in Y-configuration in 24/35 cases (68.6%) using two stents in 12/24 patients and three stents in 12/24 cases (50%). A T-configuration stent placement was performed in 11/35 patients (31.4%), using two stents in 4/11 cases (36.4%) and three stents in 7/11 cases (63.6%). Follow-up was available in all patients (35/35). Patient survival ranged from 0 to 1763 d and the mean survival time was 168 d. According to the Kaplan-Meyer survival analysis, the estimated survival rate was 73.5%, 47.1% and 26.1% at 1, 6 and 12 mo respectively, while the 8 year survival rate was 4.9% (Figure 1). The clinical success rate was 77.1% (27/35 cases), as 2/35 patients (5.7%) died within the first 24 h and in 6/33 patients (18.2%) a total serum bilirubin increase by 107% (range, 101%-112%), compared to baseline, was recorded. In 27/33 patients (81.8%) a decrease in total serum bilirubin level by 60.5% (range, 23%-92%) was noted, resulting in relief or improvement of symptoms.

The median primary and secondary patency were 105 d (range: 0 to 719 d) and 181 d (range: 100 to 1763 d), respectively. During the follow-up period biliary re-obstruction due to stent occlusion occurred in 25.7% of cases (9/35 patients). In 7/11 (63.6%) one additional percutaneous re-intervention because of stent occlusion resulting in clinical relapse of symptomatology was successfully performed. In the remaining 4/11 patients (36.4%) more than 1 additional reintervention was performed. In three cases, 2 additional successful re-interventions were performed, while only one patient underwent 3 additional re-interventions at 121, 305 and 526 d after the first stent insertion. The post-procedural, mean hospitalization period was of 10.2 ± 3.9 d. The peri-procedural mortality rate was 5.7% (2/35 patients) as one patient died during the procedure due to heart failure and a second patient died a day after the procedure due to aspiration. The major complications rate was 5.7% (2/35 cases) as one patient developed septicaemia, was further hospitalized and successfully treated, while one patient developed a right hepatic artery pseudoaneurysm causing haemobilia. The latter was successfully treated with percutaneous endovascular coil embolization. Mi-

Table 2 Procedural details and outcomes

Stents used (*n*)	48
Stent deployment	
Y-configuration	18/35 (51.4%)
T-configuration	17/35 (48.6%)
Technical success	35/35 (100%)
Clinical success	27/35 (77.1%)
Decreased bilirubin levels	27/33 (81.8%)
Serum bilirubin decrease	60.5% (23%-92%)
Survival interval (d), mean (range)	168 (0-1763)
6-mo survival rate	47.10%
Primary patency (d), mean (range)	105 (0-719)
Secondary patency (d), mean (range)	181 (100-1763)
Peri-procedural death	2/35 (5.7%)
Major complications	2/35 (5.7%)
Minor complications	6/35 (17.1%)
Hospitalization time (d), mean ± SD	10.2 ± 3.9

nor complications (cholangitis) were observed in 6/35 patients (17.1%) and were successfully managed using conservative antibiotic therapy. Procedural outcomes are analytically reported in Table 2.

DISCUSSION

The Bismuth-Corlette classification describes the extension of malignant disease into the intrahepatic bile ducts. In type Ⅳ disease the tumour extends from the confluence into both the right and left segmental hepatic ducts. Symptoms include severe jaundice, pruritus, cholangitis and right flank pain, rapidly leading to hepatic failure and death. Percutaneous biliary metallic stenting is the recommended palliative decompression method in patients suffering from Bismuth Ⅳ malignant obstructive jaundice, as it is characterized by lower technical failure and complication rates than the endoscopic approach[8,14]. Biliary drainage aims at the alleviation of symptoms and the amelioration of the patients' quality of life. In lesions involving both hepatic ducts a decision must made whether draining both hepatic lobes would improve palliation outcomes. In most cases the right hepatic duct drains 2/3 of the hepatic parenchyma and therefore unilateral right drainage should be sufficient to produce the desired palliative effect[15]. However, an un-drained left side could incite cholangitis and bilateral stenting offers the opportunity to choose the most appropriate lobe for drainage in cases where any repeat procedures may be necessary[16]. In very advanced disease the clinical effectiveness of single-lobe drainage remains controversial as some authors have reported superior patency rates and clinical outcomes in patients with Bismuth type Ⅳ obstruction when both biliary ducts were stented[12]. Furthermore, in cases of extensive right lobe tumour, unilateral right bile drainage could result in insufficient serum bilirubin level decrease, a clinical endpoint that has been identified as a main independent predictor of survival[17]. On the other hand, single left lobe drainage is rarely sufficient to eliminate or ease obstructive symptoms. For these reasons even multi-segmental biliary stenting should be performed in cases in which the tumoral obstruction extends beyond the segmental ducts and neither Y- nor T-configuration techniques can ensure the adequate decompression of the affected biliary tree (Figure 2). Few authors have investigated various techniques to achieve a bi-lobar hepatic drain in an attempt to increase clinical effectiveness[1,18-20]. To date, there are insufficient data to support either of the two percutaneous approaches in Bismuth Ⅳ lesions. This retrospective study was designed to investigate the clinical efficiency, as judged by survival and relief from jaundice symptoms, following percutaneous bi-lobar stenting for the management of Bismuth Ⅳ malignant disease. Bismuth Ⅳ disease has been identified as an independent predictor of almost 5-fold increased mortality following PTBS[17]. In this series the mean survival time of 169 d is comparable and even superior to previously reported results involving patients with less advanced, Bismuth I -Ⅳ, disease[2,3]. In addition, a very satisfactory 6- and 12 mo survival rate of 47.1% and 26.1% respectively was observed, while surprisingly 1 patient (4.9%) suffering from non-Hodgkin lymphoma was alive after 8 years of follow-up.

Furthermore, post-procedural clinical response was very encouraging. Serum total bilirubin level decrease was attained in almost 82% of the cases with a mean

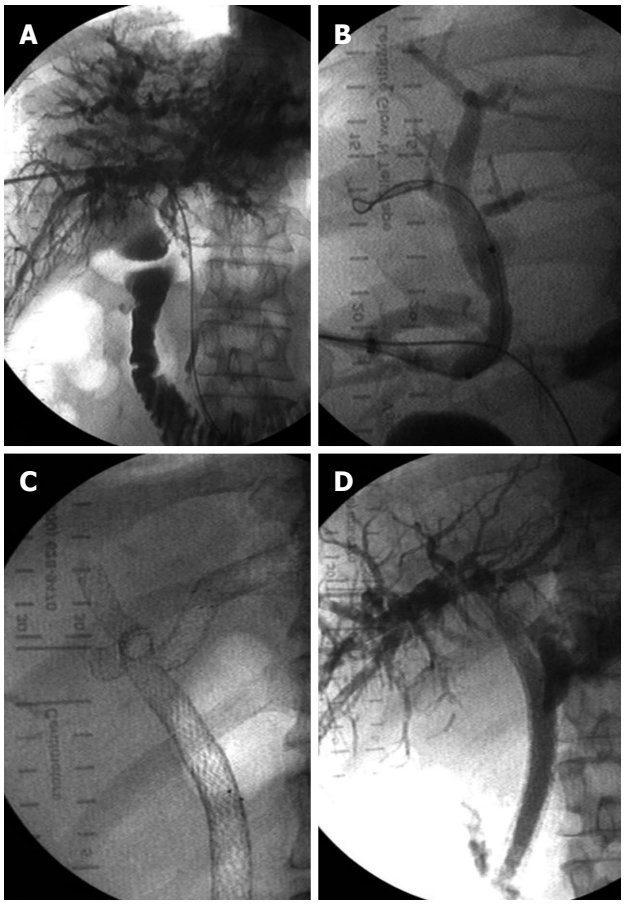

Figure 2 Bismuth Ⅳ lesions. A: Percutaneous trans-hepatic cholangiography image demonstrating a Bismuth Ⅳ biliary obstruction; B: Right-sided approach; C: Multiple (*n* = 4) bilateral stenting in segmental ducts communicating with the stented common bile duct; D: Final fluoroscopic image demonstrating the flow of contrast medium into the duodenum.

decrease of 61.3% ± 19.1%. The mean primary patency rate was 174.1 d, while biliary re-obstruction reoccurred in the 25.7% of our patients. This is in accordance with the 11%-29% reoccurrence reported by Inal *et al*[12], in a study investigating 32 patients suffering from Bismuth Ⅳ biliary obstructions. The fact that this satisfactory clinical success rate was coupled with very low re-obstruction rates, stresses the clinical significance of bilateral decompression.

Some issues regarding bilateral stenting still need to be addressed. For example, a higher risk of complications following the bilateral approach has been reported, and some authors advocate that this risk should not be taken as even 30% of functional liver parenchyma could be sufficient[1-6,20-25]. Nonetheless, although no control group was available, the authors support the notion that in the specific population of end-stage, advanced, hilar disease, bilateral decompression was vital for the clinical success and survival rates achieved. Recently published data coming from a single-center study comparing endoscopic bilateral versus unilateral stent deployment demonstrated the superior cumulative stent patency of the bilateral approach, further supporting this suggestion[26]. Moreover, in our study, the long-term clinical effectiveness was maintained by the successful revisions resulting in high secondary patency rates following stent occlusion. In detail, stent re-occlusion due to tumour overgrowth was detected in 27.3% (9/33 cases) that were subsequently managed successfully, using percutaneous balloon dilatation and additional stenting.

Only bare metal stents are used in our department as their superiority over plastic endoprosthesis has been widely reported[18]. Covered stents could also be used in hilar lesions in a Y-configuration. Recent data regarding covered metal stents have demonstrated significantly lower re-intervention rates. However, they are associated with complications such as cholecystitis, pancreatitis and stent migration[22,23,27].

The majority of the procedures were performed with a single right-sided access, following the pre-procedural imaging assessment. Major complication rates following biliary stenting ranges between 7% and 35%. In our study the safety of the bilateral approach was demonstrated by the low procedure-related major complication rate of 5.7%, considerably below the 10% threshold recommended for PTBS[7,23,28]. Peri-procedural mortality was 5.7%, while one patient died on the operating table from heart failure. This fact highlights the procedural risks, given that the majority of these drainages are performed in end-stage patients with poor general conditions.

The study's limitations include the lack of a predefined clinical and imaging follow-up, because of the retrospective design, as well as the inability to obtain detailed data regarding the patients' post-procedural quality of life using dedicated questionnaires. However, as quality of life in patients suffering from advanced cancer is mainly influenced by the frequency and length of hospitalization, as well as and the need for repeated procedures, it is the authors' belief that this technique contributed to the patient's quality of life overall. Finally, further limitations of this study were the possible bias generated by use of various metallic stents and the lack of a control group undergoing unilateral stenting procedures, which would allow direct comparison between the two methods.

In conclusion, in the present study, bilateral PTBS using self-expandable metallic stents was proven a safe and clinically effective, minimally invasive palliative method for the management of malignant, Bismuth Ⅳ, biliary obstruction.

COMMENTS

Background

Biliary drainage aims for the alleviation of symptoms and the amelioration of the patients' quality of life. In lesions involving both hepatic ducts a decision must be made whether draining both hepatic lobes would improve palliation outcomes.

Research frontiers

To date, data regarding percutaneous trans-hepatic bilateral stenting in Bismuth Ⅳ lesions are scarce and the clinical decision whether to drain both hepatic lobes remains controversial. The present study investigated the safety and clinical efficiency of palliative, percutaneous trans-hepatic bilateral metallic stenting, for the management of Bismuth Ⅳ malignant hilar obstructive disease. Both T-configuration and Y-configuration stenting were performed.

Innovations and breakthroughs

Bismuth Ⅳ disease has been identified as an independent predictor of almost 5-fold increased mortality following percutaneous trans-hepatic biliary stenting. In this series, the mean survival time of 169 d is comparable and even superior to previously reported results involving patients with less advanced, Bismuth Ⅰ-Ⅳ, disease. In addition, a very satisfactory 6 and 12 mo survival rate of 47.1% and 26.1% respectively was noted.

Applications

Biliary drainage aims for the alleviation of symptoms and the amelioration of the patients' quality of life. The authors support the notion that in lesions involving both hepatic ducts the decision to drain both hepatic lobes using nitinol bare stents would improve palliation outcomes.

Terminology

Bismuth Ⅳ obstructive disease: Malignant disease expanding in both left and right hepatic bile ducts; T-configuration biliary stenting: Bilateral biliary drainage achieved after single hepatic lobe access, using two stents one within the other; Y-configuration biliary stenting: Bilateral biliary drainage achieved after both right and left hepatic lobe access, using two stents side by side.

Peer review

The authors herein report the safety and long-term feasibility of bilateral self-expandable biliary stenting in advanced Bismuth Ⅳ biliary neoplastic disease. The article is very interesting and informative, and has sufficient value to be published.

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BRIEF ARTICLE

Severe adverse events during antiviral therapy in hepatitis C virus cirrhotic patients: A systematic review

Simona Bota, Ioan Sporea, Roxana Şirli, Alina Popescu, Adriana Maria Neghină, Mirela Dănilă, Mihnea Străin

Simona Bota, Ioan Sporea, Roxana Şirli, Alina Popescu, Mirela Dănilă, Mihnea Străin, Department of Gastroenterology and Hepatology, University of Medicine and Pharmacy, 300736 Timişoara, Romania
Adriana Maria Neghină, Department of Biochemistry and Pharmacology, University of Medicine and Pharmacy, 300736 Timişoara, Romania
Author contributions: Bota S, Sporea I and Şirli R designed the manuscript; Bota S wrote the manuscript; Bota S, Şirli R, Popescu A and Neghină AM acquired the data; Bota S and Neghină AM performed the statistical analysis; Bota S, Sporea I, Şirli R, Popescu A, Dănilă M and Strain M interpreted the data; Sporea I, Şirli R, Popescu A, Neghina AM, Dănilă M and Străin M revised the manuscript draft; Bota S, Sporea I, Şirli R, Popescu A, Neghină AM, Dănilă M and Străin M approved the final version of the article.
Correspondence to: Simona Bota, MD, Department of Gastroenterology, University of Medicine and Pharmacy, 300736 Timişoara, Romania. bota_simona1982@yahoo.com
Telephone: +40-721-656147 Fax: +40-256-488003
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Abstract

AIM: To identify severe adverse events (SAEs) leading to treatment discontinuation that occur during antiviral therapy in hepatitis C virus (HCV)-infected cirrhotic patients.

METHODS: We identified all the articles published prior to December 2011 in the PubMed, Medline, Lilacs, Scopus, Ovid, EMBASE, Cochrane and Medscape databases that presented these data in cirrhotic patients. These studies evaluated the rate of SAEs leading to discontinuation of standard care treatment: Pegylated interferon (PegIFN) alpha 2a (135-180 μg/wk) or PegIFN alpha 2b (1 or 1.5 μg/kg per week) and ribavirin (800-1200 mg/d). Patients with genotype 1 + 4 underwent treatment for 48 wk, whereas those with genotypes 2 + 3 were treated for 24 wk.

RESULTS: We included 17 papers in this review, comprising of 1133 patients. Treatment was discontinued due to SAEs in 14.5% of the patients. The most common SAEs were: severe thrombocytopenia and/or neutropenia (23.2%), psychiatric disorders (15.5%), decompensation of liver cirrhosis (12.1%) and severe anemia (11.2%). The proportion of patients who needed to discontinue their therapy due to SAEs was significantly higher in patients with Child-Pugh class B and C *vs* those with Child-Pugh class A: 22% *vs* 11.4% (P = 0.003). A similar discontinuation rate was found in cirrhotic patients treated with PegIFN alpha 2a and those treated with PegIFN alpha 2b, in combination with ribavirin: 14.2% *vs* 13.7% (P = 0.96). The overall sustained virological response rate in cirrhotic patients was 37% (95%CI: 33.5-43.1) but was significantly lower in patients with genotype 1 + 4 than in those with genotype 2 + 3: 20.5% (95%CI: 17.9-24.8) *vs* 56.5% (95%CI: 51.5-63.2), (P < 0.0001).

CONCLUSION: Fourteen point five percent of HCV cirrhotic patients treated with PegIFN and ribavirin needed early discontinuation of therapy due to SAEs, the most common cause being hematological disorders.

Key words: Liver cirrhosis; Hepatitis C virus; Adverse events; Sustained virological response

Bota S, Sporea I, Şirli R, Popescu A, Neghină AM, Dănilă M, Străin M. Severe adverse events during antiviral therapy in hepatitis C virus cirrhotic patients: A systematic review. *World J Hepatol* 2013; 5(3): 120-126 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i3/120.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i3.120

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a worldwide

public health concern, affecting approximately 170 million people[1]. This condition is responsible for 25%-30% of global cases of cirrhosis and is the most common cause for liver transplantation[2]. Cirrhotic patients infected with HCV develop hepatic decompensation at a rate of 30% over 10 years and hepatocellular carcinoma at annual rates ranging from 3% to 8%[3,4].

In the last 10 years, pegylated interferon (PegIFN) and ribavirin became the standard of care (SOC) treatment in chronic HCV infection. The sustained virological response (SVR) rates range from 42% to 46% in patients with genotype 1 or 4 infection and from 76% to 82% in patients with genotype 2 or 3 infection[5-7]. In patients with liver cirrhosis, the SVR rate is even lower, at approximately 20% in genotype 1 or 4 infection and 55% in patients with genotype 2 or 3 infection[8]. Also, cirrhotic patients have a reduced tolerance to therapy[9,10] but the risk of further complications is smaller in patients who achieve SVR[11].

This systematic review aims to identify and analyze the severe adverse events (SAE) that lead to treatment discontinuation during treatment with PegIFN and ribavirin in cirrhotic patients infected with HCV.

MATERIALS AND METHODS

Eligibility criteria

This review included all the studies published in English prior to December 2011 that evaluated SAEs in cirrhotic patients infected with HCV and treated with SOC therapy: PegIFN alpha 2a (dosage: 135-180 μg/wk) or PegIFN alpha 2b (dosage: 1 or 1.5 μg/kg per week) and ribavirin (dosage range: 800-1200 mg/d). Patients with genotype 1 + 4 underwent treatment for 48 wk, whereas those with genotypes 2 + 3 were treated for 24 wk. The diagnosis of cirrhosis was made either by liver biopsy or by clinical, ultrasonographic, endoscopic or laparoscopic signs of cirrhosis. Studies that included liver-transplanted patients or cases co-infected with hepatitis B virus or human immunodeficiency virus were excluded from the analysis.

Outcomes

The pre-specified primary outcome was the rate of SAEs (leading to treatment discontinuation) that occurred during treatment with PegIFN and ribavirin in cirrhotic patients infected with HCV.

The secondary outcomes were: description of SAEs; the possible relationships between SAE rates in cirrhotic patients and the following factors: decompensation of the disease (class Child-Pugh B or C), type of PegIFN (alpha 2a and alpha 2b) used in SOC therapy and HCV genotype; the proportion of patients in whom the medication dosage was reduced; and the SVR rate in cirrhotic patients, according to HCV genotype.

SVR was defined as undetectable HCV RNA in serum by real-time polymerase chain reaction 6 mo after discontinuation of therapy.

Data sources and searches

Relevant studies published prior to December 2011 were searched in PubMed, Medline, Lilacs, Scopus, Ovid, EMBASE, Cochrane and Medscape databases using the following keywords: liver cirrhosis, chronic hepatitis C, HCV, adverse events, sustained virological response, SVR.

Study selection and data collection

Two authors independently screened titles and abstracts for potential eligibility and the full texts for final eligibility. The following data were extracted: country of origin, year of publication, number of patients, age and weight of the patients, HCV genotype, the Child-Pugh class, the baseline treatment history (naive or previously treated), the treatment administered, the rate and description of SAEs that lead to treatment discontinuation, the proportion of patients in whom the doses of PegIFN and/or ribavirin were reduced, and the SVR rate according to HCV genotype.

Statistical analysis

Statistical analysis were carried out with the software package SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL). Descriptive statistics (percentage, 95%CI) were calculated for each variable as appropriate. Standard binomial tests for differences in proportions were used to compare patient subgroups ("*n*" designates the total number of patients included in a particular subgroup). A *P* value of less than 0.05 was regarded as statistically significant.

RESULTS

Of 8793 titles identified during the initial search, 8764 were excluded based on one of the following reasons: data published only in abstract, duplicated titles, data on cirrhotic patients not presented, the treatment regimen did not include PegIFN in combination with ribavirin or the same author had several similar articles, but with a different number of patients (we selected the article with the higher number of patients if we did not receive the information regarding the number of patients included in two or more studies from the author). Twelve articles which presented data on cirrhotic patients were excluded for the following reasons: the dosage or treatment duration with PegIFN and ribavirin were not standard; liver-transplanted patients were included; and the study presented the follow-up of patients after SOC therapy, but not the SAE leading to the therapy discontinuation. Finally, seventeen papers with 1133 patients with HCV liver cirrhosis were retrieved for analysis[12-28] (Figure 1). The main characteristics of the studies included in this systematic review are presented in Table 1.

In 165/1133 patients (14.5%), the antiviral treatment was stopped early due to SAEs. In 116/165 patients (70.3%), detailed information regarding the SAEs was presented. The most common SAEs leading to premature discontinuation of antiviral treatment in HCV cirrhotic patients were: severe thrombocytopenia and/or neutropenia: $n = 27$ (23.2%); psychiatric disorders: $n = 18$ (15.5%); decompensation of liver cirrhosis: $n = 14$ (12.1%); and severe anemia: $n = 13$ (11.2%) (Table 2). The mortality rate in the cohort was 0.3% (4/1133 pa-

Table 1 Characteristics of the studies included in the systematic analysis

Ref.	Study design	No. of patients	Age (yr)	Weight	HCV genotype	Baseline treatment history	Child-Pugh class	Treatment
Syed *et al*[12]	Retrospective cohort study	104	52 ± 7.6	82 ± 15 kg (mean weight)	1, 2, 3	Naive and previously treated	A	Pegylated interferon alpha 2a (180 μg/wk) or alpha 2b (1-1.5 μg/kg per week) Ribavirin (800-1200 mg/d)
Butt *et al*[13]	Prospective cohort study	66	46.2 ± 10.1	22.3 ± 3.1 kg/m² (mean BMI)	3	Naive and previously treated	A, B	Pegylated interferon alpha 2a (180 μg/wk) or alpha 2b (1 μg/kg per week) Ribavirin (10-12 mg/kg per day)
Giannini *et al*[14]	Retrospective cohort study	85	56 ± 9	Not specified	1, 2, 3, 4	Naive and previously treated	A, B	Pegylated interferon alpha 2a (180 μg/wk) or alpha 2b (1.5 μg/kg per week) Ribavirin (800-1200 mg/ d)
Helbling *et al*[15]	Randomized controlled trial (standard doses *vs* low doses)	64	47 (median age)	74 kg (median weight)	1, 2, 3, 4	Naive	A	Pegylated interferon alpha 2a (180 μg/wk) Ribavirin (1000-1200 mg/ d)
Iacobellis *et al*[16]	Prospective cohort study	94	Not specified	Not specified	1, 2, 3, 4	Naive	B	Pegylated interferon alpha 2b (1.5 μg/kg per week) Ribavirin (800-1200 mg/d)
Roffi *et al*[17]	Randomized controlled trial (pegylated interferon *vs* IFN standard)	57	56 (median age)	75 kg (median weight)	1, 2, 3	Naive	A	Pegylated interferon alpha 2b (1 μg/kg per week) Ribavirin (800-1200 mg/d)
Sood *et al*[18]	Retrospective cohort study	28	48.3 ± 7	73.9 ± 11.2 kg (mean weight)	3 (25/28 patients) and not specified for the other patients	Naive	A, B	Pegylated interferon alpha 2b (1 μg/kg per week) Ribavirin (10-12 mg/kg per day)
Tekin *et al*[19]	Cohort study	20	54.2 ± 5.9	Not specified	1	Not specified	A, B	Pegylated interferon alpha 2a (135 μg/wk) Ribavirin (1000-1200 mg/d)
Moreno Planas *et al*[20]	Cohort study	12	52 ± 8	Not specified	1, 3	Naive and previously treated	A, B	Pegylated interferon alpha 2b (1.5 μg/kg per week) Ribavirin (10.6 mg/kg per day)
Di Marco *et al*[21]	Randomized controlled trial (pegylated interferon alpha 2B + ribavirin *vs* pegylated interferon alpha 2b)	52	57 ± 6.6	71 ± 10.1 kg (mean weight)	1, 2, 3, 4	Naive and previously treated	A, B	Pegylated interferon alpha 2b (1 μg/kg per week) Ribavirin (800 mg/d)
Höroldt *et al*[22]	Retrospective cohort study	61	Not specified	Not specified	1, 2, 3	Naive	A, B	Pegylated interferon alpha 2a or alpha 2b + ribavirin
Bruno *et al*[23]	Randomized study	106	Not specified	Not specified	1, 2, 3, 4	Naive	A	Pegylated interferon alpha 2a (180 μg/wk) Ribavirin (1000-1200 mg/d)
Floreani *et al*[24]	Prospective cohort study	87	55.7 ± 9.1	25.3 ± 3.1 kg/m² (mean BMI)	1, 2, 3	Naive	A	Pegylated interferon alpha 2b (80-100 μg/wk) Ribavirin (1000-1200 mg/d)
Annicchiarico *et al*[25]	Prospective cohort study	15	51.5	Not specified	1, 2, 3	Naive and previously treated	B, C	Pegylated interferon alpha 2b (1.5 μg/kg per week) Ribavirin (800-1200 mg/d)
Aghemo *et al*[26]	Prospective cohort study	106	57 ± 9.3	72.5 ± 11.8 kg (mean weight)	1, 2, 3, 4	Naive	A	Pegylated interferon alpha 2b (1.5 μg/kg per week) Ribavirin (≥ 10.6 mg/kg per day)
Kim *et al*[27]	Cohort study	86	56.4 ± 9.6	Not specified	1 and non-1	Not specified	A	Pegylated interferon alpha 2b (1.5 μg/kg per week) or Pegylated interferon alpha 2a (180 μg/wk) Ribavirin (1000-1200 mg/d)
Reiberger *et al*[28]	Prospective cohort study	90	51 ± 8	26.6 ± 5 kg/m² (mean BMI)	1, 2, 3, 4	Not specified	A	Pegylated interferon alpha 2b (1.5 μg/kg per week) or pegylated interferon alpha 2a (180 μg/wk) Ribavirin (1000-1200 mg/d)

HCV: Hepatitis C virus; BMI: Body mass index; IFN: Interferon.

tients). The causes of death in the four patients were: severe sepsis, decompensation of heart disease, hepatocellular carcinoma and severe hepatic failure (this patient died 3 wk after stopping the treatment due to decompensation of liver cirrhosis).

Fifteen studies[12-24,27,28], including 154 patients in whom

Table 2 Description of severe adverse event leading to premature discontinuation of antiviral treatment in hepatitis C virus cirrhotic patients (%)

Name of severe adverse event	No. of patient discontinuities
Severe thrombocytopenia and/or neutropenia	27 (23.2)
Psychiatric disorders (depression, psychosis, confusion, lethargy)	18 (15.5)
Decompensation of liver cirrhosis (ascites with or without spontaneous bacterial peritonitis; jaundice; hepatic encephalopathy)	14 (12.1)
Severe anemia	13 (11.2)
Occurrence of malignancies	6 (5.1) - 4 cases of hepatocellular carcinoma, 1 case of tongue carcinoma and 1 case of Non-Hodgkin's lymphoma recurrence
Allergic reactions to medication	5 (4.3)
Severe infections	5 (4.3)
Severe fatigue	5 (4.3) - in 2 cases accompanied also by "flu-like" syndrome
Neurological disorders (stroke, polyneuropathy, hemiparesthesia)	5 (4.3)
Heart disease (heart failure or acute coronary syndrome)	3 (2.5)
Endocrinology disorders	3 (2.5)
Diabetes decompensation	2 (1.7)
Persistent fever	2 (1.7)
Severe denutrition	2 (1.7)
Aminotransferases flare	1 (0.8)
Severe decrease of vision	1 (0.8)
Upper gastrointestinal bleeding	1 (0.8) - the cause of bleeding was not specified
Acute pancreatitis	1 (0.8)
Severe flare of psoriasis	1 (0.8)

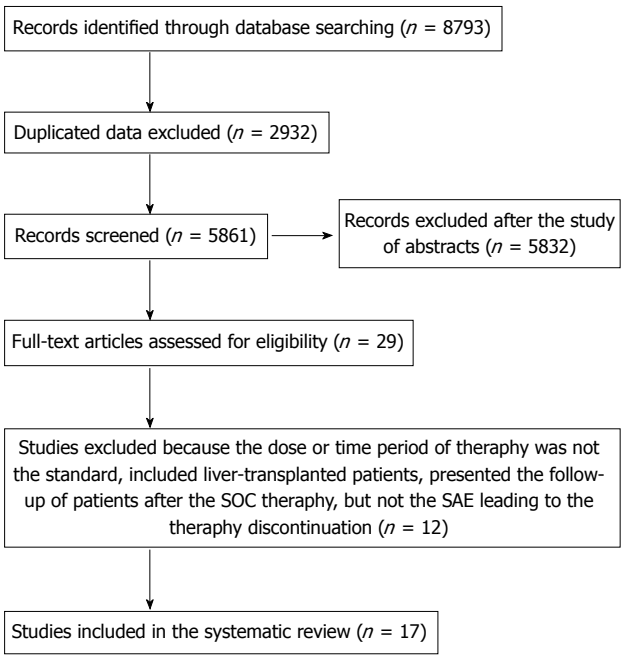

Figure 1 Flowchart of the selection of the studies. SOC: Standard of care; SAE: Severe adverse event.

the antiviral treatment was stopped because of SAEs, presented information regarding the incidence of hematological SAEs. In 49/154 patients (31.8%), the antiviral treatment with PegIFN and ribavirin was stopped as result of severe anemia, thrombocytopenia and/or neutropenia.

From ten studies[12,15-17,23-28], we extracted data regarding the SAEs leading to antiviral treatment discontinuation according to the Child-Pugh class. The SAEs rate was significantly higher in patients with Child-Pugh class B and C (n = 109) *vs* those with Child-Pugh class A (n = 700): 22% *vs* 11.4%, P = 0.003.

From eleven studies[15-21,23-26], we extracted data regarding the SAEs rate according to the type of PegIFN used in combination with ribavirin for treatment. The SAEs rate was similar for PegIFN alpha 2a (n = 190) and PegIFN alpha 2b (n = 451): 14.2% *vs* 13.7%, P = 0.96.

We were able to extract data regarding the rate of SAEs leading to early treatment discontinuation according to the HCV genotype from only three studies[13,18,29]. The SAEs rate was significantly higher in patients with genotype 1 (n = 20) *vs* those with genotype 3 (n = 94): 30% *vs* 8.5%, P = 0.02.

Five studies[13,15,16,18,25] presented data regarding the number of patients in whom the doses of PegIFN and/or ribavirin were reduced. From a total of 267 patients, the dosage for either drug was reduced in 87 (32.5%).

Eight studies[14,17,19,20,22,26-28] presented separately the number of patients in which the doses of antiviral medication were reduced: in 107/517 patients (20.6%) for PegIFN and for ribavirin in 141/517 patients (27.2%).

The overall SVR rate in the seventeen studies included in this systematic review was 37% (95%CI: 33.5-43.1). SVR rates were significantly higher in patients with genotype 2 + 3 (n = 495) compared to those with genotype 1 + 4 (n = 570): 56.5% (95%CI: 51.1-63.2) *vs* 20.5% (95%CI: 17.9-24.8), P < 0.0001.

DISCUSSION

Patients with HCV liver cirrhosis are a category of subjects difficult to treat due to the high risk of complications and the relatively low SVR rates. This systematic review summarizes and analyses the available data on the rates of SAEs leading to antiviral therapy discontinuation in cirrhotic patients infected with HCV. In 14.5% of the patients, treatment was discontinued due to SAEs, the most common of which were hematological disorders.

The proportion of cirrhotic patients presented in this systematic review in which the treatment was discontinued due to SAEs was higher than the proportion of patients with all stages of fibrosis in whom the treatment was discontinued due to SAEs presented in others studies. For example, in the study by Fried *et al*[29], only 32/453 patients (7%) discontinued their antiviral treatment due to SAEs. In the IDEAL study[7], 98/1016 patients (9.6%) treated with low doses of PegIFN alpha 2b (1 μg/kg per week) and ribavirin, 129/1019 patients (12.7%) treated with standard doses of PegIFN alpha 2b (1.5 μg/kg per week) and ribavirin and 135/1035 patients (13%) treated with standard doses of PegIFN alpha 2a (180 μg/wk) and ribavirin, had to discontinue their treatment as a result of SAEs developed during antiviral therapy.

In the present review, dose reduction of PegIFN was needed in 20.6% of patients and of ribavirin in 27.2%. These percentages were also higher than those presented in studies that included patients with all stages of fibrosis. In the study by Fried *et al*[29], the dose of PegIFN was reduced in 11% of patients and of ribavirin in 21%. In the IDEAL study[7], the dose of PegIFN was reduced in 6.9% of patients treated with low doses of PegIFN alpha 2b, in 10.1% of patients treated with standard doses of PegIFN alpha 2b and in 11.8% treated with standard doses of PegIFN alpha 2a. The dose of ribavirin was reduced in 16.7%, 18.4% and 17.4% of patients, respectively, included in the three arms of the IDEAL study.

In our review, the proportion of patients in whom the treatment had to be discontinued due to SAEs was significantly higher in patients with Child-Pugh class B and C *vs* those with Child-Pugh class A: 22% *vs* 11.4% ($P = 0.003$). The results are similar to those published in a review by Vezali *et al*[30], in which 20% of patients with decompensated liver disease and 12% with compensated cirrhosis needed to discontinue their therapy as result of SAEs. The proportion of drug discontinuation due to SAEs in cirrhotic patients with compensated liver disease *vs* those with less advanced liver disease was similar: 12% *vs* 13%. But the review by Vezali *et al*[30] also included decompensated cirrhotic patients treated with small doses of PegIFN[31], patients treated for a short period of time before or after liver transplantation[32,33], and patients with compensated liver cirrhosis treated only with PegIFN[34], while in our review, we included only cirrhotic patients in whom the dosage and the duration of therapy was standard.

The data analyzed in the present review showed a similar discontinuation rate due to SAEs in cirrhotic patients treated with PegIFN alpha 2a *vs* those treated with PegIFN alpha 2b (both in combination with ribavirin), similar to those obtained in the IDEAL study[7] (which included patients with all stages of fibrosis).

The discontinuation rate was significantly higher in patients with genotype 1 *vs* those with genotype 3, but this data could only be extracted from 3 studies. Also, in the only study which included genotype 1 patients[19], the majority of them were Child-Pugh class B (70%), while in one of the two studies which included only genotype 3 patients[15], most were Child-Pugh class A (92.4%). In the other study which includes only genotype 3 patients[18], data regarding the distribution of patients according to the Child-Pugh class is not presented. However, the discontinuation rate due to SAEs is probably higher in patients with genotype 1 + 4 than in those with genotype 2 + 3, in relationship to the longer period of time in which the patients could maintain the full dosing of antiviral medication. For example, in the study of Bruno *et al*[23], 86% of patients with genotype 2 + 3 maintained the full dosing and duration of therapy for PegIFN and 85% for ribavirin, while only 65% of patients with genotype 1 + 4 could maintain the full dosing of PegIFN and 56% for ribavirin. Another explanation is the longer duration of therapy for patients with genotype 1 + 4 (48 wk *vs* 24 wk).

Despite the low rate of SVR (especially in genotype 1 + 4), as well as the higher percentage of patients in whom the treatment is discontinued or the medication doses are reduced, Saab *et al*[35] demonstrated (using a Markov model) that treatment of patients with HCV genotype 1 liver cirrhosis (especially compensated) is cost effective. The study included approximately 4000 subjects followed over 17 years. Compared to the no-antiviral treatment strategy, treatment during compensated cirrhosis increased quality-adjusted life years by 0.950 and saved 55 314 dollars, while treatment during decompensated cirrhosis increased quality-adjusted life years by 0.044 and saved 5511 dollars. Also, treatment of patients with compensated cirrhosis resulted in 119 fewer deaths, 54 fewer hepatocellular carcinomas and 66 fewer transplants compared to the no-treatment strategy.

In recent years, several studies have used triple therapy (SOC therapy + direct antiviral agents) in patients with HCV genotype 1 infection; the most utilized direct antiviral agents are Telaprevir and Boceprevir[36-39]. This therapy could become the SOC in a short time.

There are few data regarding discontinuation rates of triple therapy as result of SAEs in cirrhotic patients. Only the RESPOND-2 trial[36] presented this kind of data. The percentage of patients in whom the antiviral therapy was discontinued due to SAEs was similar in SOC therapy *vs* triple therapy: 10% *vs* 15.3% ($P = 0.93$). Also, the proportion of patients in whom the doses had to be reduced was similar: 30% *vs* 33.3% ($P = 0.85$). But it should be noted that the number of cirrhotic patients was quite small: 10 patients treated with SOC therapy and 39 patients treated with triple therapy. If we consider all patients included in the RESPOND-2 trial[36], the percentage of patients in whom the treatment was stopped because of SAEs was much higher in patients treated with triple therapy than in those treated with SOC therapy: 8% and 12% (in the two arms which included patients treated with Boceprevir) *vs* 2%. Also, the proportion of patients in whom medication doses were reduced was higher in patients treated with triple therapy: 29% and 33% (in the two arms which included patients treated with Boceprevir) *vs* 14% (SOC therapy).

It is a known fact that one of the most common adverse events of Boceprevir treatment is anemia. In the SPRINT-2 trial[37], the proportion of patients in whom medication doses were reduced was much higher in pa-

tients treated with triple therapy *vs* those treated with SOC therapy: 21% *vs* 13%. It will be interesting to see the effect of triple therapy using Boceprevir as a direct antiviral agent in a large cohort of cirrhotic patients, knowing that SOC therapy needed to be discontinued due to severe hematological adverse events in 31.8% of patients in the present review. It should also be noted that erythropoietin was administered to correct anemia in the Boceprevir trials, while it was specified that the use of erythropoietin was allowed in only 5/17 of the studies included in the present review[12-14,16,18].

Also, the discontinuation rate as a result of SAEs was much higher in patients treated with triple therapy in the studies in which Telaprevir was used as a direct antiviral agent. In the REALIZE trial[38], the discontinuation rate due to SAEs was 3% for SOC therapy and 11% and 15%, respectively, in the two arms which used Telaprevir. In the PROVE 3 trial[39], 4% of patients treated by SOC therapy discontinued treatment as a result of SAEs, compared to 9%-26% of patients in whom Telaprevir was used as a direct antiviral agent.

In conclusion, 14.5% of cirrhotic patients treated with PegIFN and ribavirin needed early discontinuation of therapy because of SAEs, the most common cause being hematological disorders, while in approximately 30% of patients, the medication doses were reduced. Most likely these percentages will increase in the future with the use of direct antiviral agents. The overall SVR rate in cirrhotic patients included in this review was 37%; however, it was much lower in cases infected with HCV genotype 1 + 4 (20.5%).

COMMENTS

Background

Patients with hepatitis C virus (HCV) liver cirrhosis are difficult to treat. One of the reasons for this is the rate of severe adverse events (SAEs).

Research frontiers

In the last 10 years, pegylated interferon (PegIFN) and ribavirin have become the standard of care treatment in chronic HCV infection. In patients with liver cirrhosis, the sustained virological response (SVR) rate is even lower, at approximately 20% in genotype 1 or 4 infection and 55% in patients with genotype 2 or 3 infection. Also, cirrhotic patients have a reduced tolerance to therapy but the risk of further complications is smaller in patients who achieve SVR.

Innovations and breakthroughs

This systematic review aims to identify and analyze the SAEs that lead to treatment discontinuation during treatment with PegIFN and ribavirin in cirrhotic patients infected with HCV.

Peer review

The manuscript is very well written and makes clear conclusions about the risks of anti-viral treatment in patients with HCV related cirrhosis.

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BRIEF ARTICLE

Liver transplantation in Wilson's disease: Single center experience from Saudi Arabia

Musthafa Chalikandy Peedikayil, Hamad Ibrahim Al Ashgar, Abdullah Al Mousa, Mohammed Al Sebayel, Khalid Al Kahtani, Faisal Aba Alkhail

Musthafa Chalikandy Peedikayil, Hamad Ibrahim Al Ashgar, Abdullah Al Mousa, Khalid Al Kahtani, Department of Medicine, King Faisal Specialist, Hospital and Research Center, Pin 11211, Riyadh, Saudi Arabia
Mohammed Al Sebayel, Faisal Aba Alkhail, Liver Transplantation and Hepatobiliary Surgery, King Faisal Specialist Hospital and Research Center, Pin 11211, Riyadh, Saudi Arabia
Author contributions: Peedikayil MC designed the study, collected data and wrote the paper; Al Ashgar HI, Al Mousa A, Al Sebayel M, Al Kahtani K and Alkhail FA contributed in writing the project and preparation of manuscript.
Correspondence to: Hamad Ibrahim Al Ashgar, MD, Department of Medicine, King Faisal Specialist, Hospital and Research Center, PO Box 3354, Pin 11211, Riyadh, Saudi Arabia. alashgar@kfshrc.edu.sa
Telephone: +966-1-4424729 Fax: +966-1-4427499
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Abstract

AIM: To determine liver transplantation outcomes in Wilson's disease (WD) patients, focusing on neurological manifestations.

METHODS: This retrospective study assessed data from 16 WD patients (nine males, 56%) who had liver transplants between 1991 and 2007. Survival, graft function, and neurological complications were assessed during a follow-up period of up to 15 years. In addition, each patient's medical record was reviewed in detail to find the type of Wilson's disease (hepatic or hepatic plus neurological WD), indication for liver transplantation, use of chelating agents prior to transplantation, immediate and long term complications following transplantation, the donor details, and the pathology of explanted liver.

RESULTS: End-stage liver disease was the indication for transplantation in all 16 WD patients. Four patients displayed WD-related neurological symptoms in addition to liver disease. Living-related liver transplantation was done in three cases. One patient died on postoperative day 6 due to primary graft non-function. One-year post liver transplant survival was 94%. Neurological manifestations of all four patients disappeared during their follow-up. Four patients developed acute cellular rejection, but all responded to treatment. One patient developed chronic ductopenic rejection after 15 years post-transplantation and their graft failed; this patient is currently waiting for re-transplantation. Fourteen patients (88%) are still living. The long-term average survival is currently 10.5 years, with a current median survival of 8 years. Long-term graft survival is currently 81%.

CONCLUSION: Short- and long-term survival in WD patient liver transplantation was excellent, and neurological and psychological WD manifestations disappeared during long-term follow-up.

Key words: Wilson's disease; Liver transplantation; Neurological; Psychiatric; Penicillamine; Saudi Arabia; Transplantation

Peedikayil MC, Al Ashgar HI, Al Mousa A, Al Sebayel M, Al Kahtani K, Alkhail FA. Liver transplantation in Wilson's disease: Single center experience from Saudi Arabia. *World J Hepatol* 2013; 5(3): 127-132 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i3/127.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i3.127

INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive inborn

error of copper metabolism resulting from mutations of the *ATP7b* gene on chromosome 13[1]. The *ATP7b* gene product, a P-type ATPase transporter, is responsible for copper excretion into bile. In Wilson's disease, impaired biliary copper excretion leads to accumulation of this metal in the liver. When liver storage capacity is exceeded, hepatocyte death ensues with copper release into the plasma. Elevated circulating copper results in hemolysis and copper deposition in extra-hepatic tissues[2]. With treatment, hepatic, neurological and psychiatric manifestations gradually improve. Prognosis is generally poor without treatment, with advanced liver disease, acute liver failure, and haemolysis. Patients presenting with neurological symptoms fare better with life expectancy. But the neurological symptoms only partially reverse with medications and may worsen initially with treatment[3].

The recommended initial treatment of symptomatic WD patients or those with active disease is with chelating agents. Symptomatic liver disease generally shows signs of recovery within 2-6 mo of treatment, but further recovery can occur during the first year of treatment. Maintenance medical therapy is required for an indefinite period. Failure to comply with therapy may lead to significant progression of liver disease and liver failure within 1-12 mo following discontinuation of treatment, resulting in death or necessitating liver transplantation[2,4].

Liver transplantation is an effective option for WD patients with acute liver failure and for patients presenting with advance decompensated liver disease that is unresponsive to medical therapy or caused by interrupted medical therapy. However, the efficacy of transplantation in managing patients with neurological WD, in the absence of hepatic insufficiency, remains uncertain[5].

The worldwide prevalence of WD ranges from 10 to 30 cases per million persons[6]. The literature describing liver transplantation experience in WD is limited; the number of cases in each series ranges from 6 to 55[5,7]. None of the studies published from Saudi Arabia discuss the results of liver transplantation in WD[8-11]. A large cohort of WD patients are being treated at our center, and we identified 16 patients who had undergone liver transplantation. The objectives of our study were to assess the outcomes of WD patients after liver transplantation and study the role of liver transplantation in WD patients with neurological and psychiatric manifestations.

MATERIALS AND METHODS

The study was conducted in a tertiary care hospital in Riyadh. We included WD patients who had undergone liver transplantation either in our institute or other centers but with follow-up at our center. Between 1991 and 2007, 16 WD patients from a cohort of 71 WD patients treated in our center underwent liver transplantation. The Research Promotion Group of the Department of Medicine and the Research and Ethics Committee of our Institute approved the research protocol. A retrospective study was conducted and the data were statistically analyzed using SPSS version 16 software (IBM, Amonk, NY).

Patients were diagnosed with WD if they had evidence of liver and/or neuropsychiatric illness along with one or more of the following criteria: (1) Presence of Kayser-Fleischer (KF) rings by slit lamp examination; (2) Low serum ceruloplasmin level < 200 mg/L; and (3) Elevated pre-treatment 24-h urinary copper excretion > 0.6 μmol/d. A 24-h urine excretion after challenge with D-penicillamine was performed when required[2].

Wilson's patients were classified as having hepatic WD if they had asymptomatic hepatomegaly, isolated splenomegaly, persistently elevated serum aminotransferase activity (alanine transaminase/aspartate transaminase), fatty liver, acute hepatitis, apparent autoimmune hepatitis, cirrhosis (compensated or decompensated), or acute liver failure[2]. Patients were diagnosed with neurological WD if they had one or more of the following symptoms: movement disorders (tremor, involuntary movements), drooling, dysarthria, rigid dystonia, pseudobulbar palsy, dysautonomia, migraine headaches, insomnia, or seizures[2].

Liver disease presentation was classified as chronic (*i.e.*, slow deterioration of liver function in a patient with known WD and disease-related cirrhosis); fulminant hepatic failure (*i.e.*, occurrence of hepatic decompensation within six months from the diagnosis of liver disease in patients without known WD); acute-on-chronic presentation (*i.e.*, acute decompensation in the context of diagnosed WD)[12].

Statistical analysis

Categorical variables are presented as numbers and percentages, and continuous variables are given as mean and medians. The data were analyzed by Microsoft Office Excel 2007 and SPSS version 16 software (IBM, Amonk, NY).

RESULTS

Sixteen patients underwent liver transplantation for WD. Nine of these patients were males (56%). The mean age at the time of liver transplantation was 19.3 ± 9.6 years (range 8 to 40 years). The time interval from the diagnosis of WD to liver transplantation varied from 1 to 204 mo, with a mean of 67.6 ± 0.7 mo.

Ten patients underwent liver transplantation at our center in Riyadh, five in the United States and one in the United Kingdom. Three patients' donors were living-related and rest of the transplants were done with cadaveric livers. The indication for transplantation was chronic liver disease not responding to chelating agents (chronic). Four patients had neurological symptoms in addition to liver disease. Prior to transplantation, eight patients' Child-Turcotte-Pugh (CTP) scores were B, five patients' CTP scores were C, and three had a CTP Score of A. The median Model for End-Stage Liver Disease (MELD) score was 14 with mean MELD scores of 17.4 ± 7.7. Table 1 gives baseline characteristics of all 16 patients who had liver transplants.

Table 1 Baseline characteristics of 16 Wilson's disease patients' pre-transplant details

Case	Age (yr)	Gender	Nationality	Ascites	Jaundice	HE	Neuro	24-h Copper	Ceruloplasmin	Penicillamine	KF ring	CTP	MELD
1	14	F	Saudi Arabia	Yes	Yes	No	Yes	-	100	Yes	Yes	C	19
2	14	F	Saudi Arabia	Yes	Yes	Yes	No	-	155	-	No	C	31
3	3	M	Saudi Arabia	No	Yes	No	No	-	325	Yes	No	B	12
4	12	M	Saudi Arabia	No	Yes	No	No	14.1	395	Yes	No	B	11
5	8	M	Saudi Arabia	Yes	Yes	No	No	-	250	-	No	B	11
6	4	M	Saudi Arabia	Yes	Yes	Yes	No	4.5	< 71	Yes	No	C	18
7	12	F	Saudi Arabia	No	Yes	No	No	10.02	54	Yes	No	B	10
8	38	M	Saudi Arabia	Yes	Yes	No	No	14	71	-	No	B	19
9	13	M	Saudi Arabia	Yes	Yes	No	No	10.2	227	Yes	No	B	13
10	6	M	Syrian Arab	Yes	Yes	No	No	-	< 72	-	No	B	13
11	25	F	Saudi Arabia	Yes	Yes	Yes	Yes	6.84	< 71	Yes	Yes	A	12
12	23	F	Saudi Arabia	Yes	Yes	Yes	Yes	0.89	128	-	Yes	C	34
13	18	M	Saudi Arabia	Yes	Yes	No	No	37	346	Yes	No	C	27
14	7	F	Saudi Arabia	No	Yes	No	No	17.06	360	Yes	No	A	-
15	22	F	Saudi Arabia	Yes	Yes	Yes	Yes	1.86	< 71	Yes	Yes	A	14
16	5	M	Yemeni	No	Yes	No	No	0.88	373	-	No	B	-

Age: Age at the time of diagnosis of Wilson's disease; M: Male; F: Female; HE: Hepatic encephalopathy; KF ring: Kayser-Fleischer ring; CTP: Child-Turcotte-Pugh score; MELD: Model for end-stage liver disease; Penicillamine: These patients had received treatment with D-penicillamine prior to liver transplantation.

One patient died on postoperative day 6 following liver transplantation. The patient developed portal vein thrombosis and decreased flow in the right hepatic artery on postoperative day 1. The same day patient underwent portal vein thrombectomy, but developed primary graft non-function, acute liver failure, severe brain edema, and status-epilepticus, and died.

Three patients underwent living-related liver transplantation. All donors had uneventful postoperative courses. Case number 10, who died on postoperative day 6 following liver transplantation, had received the liver from his brother. All three cases of living-related transplantation were done in Riyadh. The other two patients who received living donors were case numbers 14 and 16; the recipients are healthy after liver transplantation (> 4 years).

Only one patient was lost from follow-up after 6 years post-transplantation. The patient had developed complications such as Castleman's disease, cardiomyopathy, and Kaposi's sarcoma and was not traceable for verification of survival.

Four patients developed acute cellular rejection, but all of them responded to treatment. One patient developed chronic ductopenic graft rejection after 15 years post-transplantation. This patient also developed chronic kidney disease and recovered from rapamycin-induced pancytopenia. The patient is being listed for liver re-transplantation. Thirteen patients are still being followed in our center; one patient's care was transferred to another center after 8 years post-transplantation. Table 2 describes post-transplantation details of the 16 patients.

Two of our patients were found to have small hepatocellular carcinoma (HCC) in the explanted liver; both of them are healthy and surviving after transplantation. Both cases were within Milan criteria; one had surgery in United States and the other in United Kingdom.

One-year post-liver transplant, graft survival, and patient survival were 94%. The long-term graft survival was 13/16 (81%) and patient survival 14/16 (88%). The long-term average survival was 10.5 years with a median survival of 8 years.

Four of our patients had neurological symptoms prior to transplantation, in addition to the liver disease. The neurological symptoms prior to transplantation in these patients are described in Table 3. The indication for transplantation for these four patients was decompensated end-stage liver disease. Their magnetic resonance imaging (MRI) brain findings, KF ring status, and improvement of neurological and psychological symptoms after liver transplantation is given in Table 3.

Case numbers 1, 11, 12 and 15 had neurological and or psychological symptoms and signs before liver transplantation. Case number 1 underwent liver transplantation in 1991 in the United States. The patient is 20 years post-transplantation now and free from neurological symptoms and signs.

Case number 11 had transplantation in our center. The patient's post-operative period was problematic, and included many complications including methicillin-resistant Staphylococcus aureus pneumonia, acute respiratory distress syndrome, central pontine myelinolysis, and major depression during the immediate postoperative period following transplantation, and required a prolonged hospital stay. After three months hospital stay, the patient went home and is currently doing fine. During post-transplant follow-up, the patient's neurological symptoms and depression disappeared.

Case number 12 was our third patient in the series with neurological symptoms. The neurological symptoms and psychiatric features disappeared during follow-up. Brain MRI 3 years post-transplantation was impressive; all of the findings (Table 3) that were there before transplantation had disappeared.

Case number 15 was our fourth patient with neurological symptoms. The patient is currently 5 years post-transplant, and not on any medication for bipolar depression. The KF ring that was there before transplantation

Table 2 Post-liver transplant details of 16 Wilson's disease patients

Case	Gender	Age	Year of TX	Indication for TX	Neurological WD	TX center	Donor	Rejection	Graft survival	Pt survival	Death or lost FU	HCC
1	F	14	1991	Chronic	Yes	United States	Cad	No	20 yr	20 yr	-	No
2	F	14	1994	Chronic	No	KFSHRC	Cad	Chronic	15 yr	17 yr	-	No
3	M	11	1995	Chronic	No	United States	Cad	No	6 yr	6 yr	Lost FU	No
4	M	12	1995	Chronic	No	KFSHRC	Cad	No	16 yr	16 yr	-	No
5	M	13	1995	Chronic	No	United States	Cad	Acute	16 yr	16 yr	-	Yes
6	M	13	1995	Chronic	No	KFSHRC	Cad	No	16 yr	16 yr	-	No
7	F	12	1996	Chronic	No	United Kingdom	Cad	No	15 yr	15 yr	-	Yes
8	M	40	1999	Chronic	No	United States	Cad	No	12 yr	12 yr	-	No
9	M	22	2003	Chronic	No	United States	Cad	No	8 yr	8 yr	-	No
10	M	23	2004	Chronic	No	KFSHRC	LR	No	6 d	6 d	Died	No
11	F	40	2004	Chronic	Yes	KFSHRC	Cad	No	7 yr	7 yr	-	No
12	F	23	2004	Chronic	Yes	KFSHRC	Cad	Acute	7 yr	7 yr	-	No
13	M	18	2006	Chronic	No	KFSHRC	Cad	Acute	5 yr	5 yr	-	No
14	F	9	2006	Chronic	No	KFSHRC	LR	No	5 yr	5 yr	-	No
15	F	25	2006	Chronic	Yes	KFSHRC	Cad	Acute	5 yr	5 yr	-	No
16	M	8	2007	Chronic	No	KFSHRC	LR	No	4 yr	4 yr	-	No

Age: Age at the time of transplantation; M: Male; F: Female; WD: Wilson's disease; HCC: Hepatocellular carcinoma; TX: Transplantation; FU: Follow-up; KFSHRC: King Faisal Specialist Hospital and Research Center.

Table 3 Neurological and psychiatric manifestations of Wilson's disease before and after liver transplantation

Characteristics	Case 1	Case 11	Case 12	Case 15
Neurological symptoms before transplantation	Akinesia dysarthria abnormal movement	Tremor dysarthria abnormal movement	Tremor nystagmus	Abnormal movement
Psychiatric abnormalities before transplantation	Negative	Negative	Depression restlessness	Delusion of persecution depression restlessness hallucination
MRI brain before transplantation	No available data	High signal intensities involving the thalami, mid brain, and pons	Bilateral and symmetrical abnormal hyper-intensity at level of basal ganglia	High signal intensity in the basal ganglia and substantia nigra with mild cerebral atrophy
KF ring before transplantation	Positive	Positive	Positive	Positive
Neurological symptoms after transplantation	Negative	Negative	Negative	Negative
Psychiatric abnormalities after transplantation	Negative	Negative	Negative	Negative
MRI brain after transplantation	No available data	Normal MRI of the brain	No available data	No available data
KF ring after transplantation	Negative	Negative	Negative	Negative

KF ring: Kayser-Fleischer ring; MRI: Magnetic resonance imaging.

has also disappeared.

DISCUSSION

The current study explored the results of liver transplantation in WD patients and the effect on neurological manifestations of WD. Our study proves excellent short- and long-term survival, and total recovery from the neurological manifestations of WD following liver transplantation.

In our series, 16 patients' data were analyzed. Almost equal number of males and females underwent liver transplantation. Many of them had surgery in the second or third decade of life. Transplantation was done after a mean of 5.5 years from the WD diagnosis. Many of our patients underwent liver transplantation for end-stage liver disease and few others for acute-on-chronic liver failure. Five of the transplanted patients had one or more affected siblings in the family. Ten patients were treated with chelating agents such as D penicillamine before transplantation. Ten patients underwent liver transplantation in our center, and fourteen patients were Saudi nationals.

The outcome of liver transplantation in our series was excellent; only one patient died and another lost from follow-up. One patient lost their liver graft after 15 years post-transplantation and developed decompensated liver disease.

In 1971, DuBois *et al*[13] demonstrated that liver transplantation is an effective treatment modality to reverse the metabolic effects of WD. In 1984, Sternlieb *et al*[14] described the indications for liver transplantation in WD as cirrhosis with severe hepatic decompensation not responding to medical therapy, fulminant hepatic failure, and hepatic decompensation after discontinuation of previously effective treatment. The role of liver transplantation in neurological WD is questioned by some experts in this field[15].

The various centers that perform liver transplantation have done transplants in only a small number of WD patients. The long-term survival after transplantation varies in different centers, from 74% to 100%. Our center's long-

term patient survival following transplantation is 14/16 (87%) (including one patient who was lost to follow-up). The long-term graft survival was 81% and median survival was 8 years. Our short- and long-term survival results are comparable to the results of other centers.

The improvement of neurological symptoms after transplantation was remarkable in our series. Four of our patients who had neurological symptoms related to WD are still alive and have no residual neurological symptoms or signs. One patient had bipolar mood disorder, which also disappeared after transplantation, and was able to discontinue all psychiatric medications.

The experiences of a few other transplant centers agree with our findings. In a series from Turkey that described 24 patients who had liver transplantation for WD, nine had neurological symptoms and seven patients improved symptomatically after transplant. A study by Eghtesad *et al*[16] from United States had 14 patients with neurological symptoms in addition to liver disease in a 45 WD patient series that had liver transplants. Ten of fourteen patients improved from neurological manifestations of WD after the liver transplantation. From our experience and other center's experiences, we believe that transplantation should not be denied to WD patients with neurological or psychiatric manifestations. However, the current study did not include patients with severe neurological/psychiatric WD. Hence we are not sure about the role of liver transplantation in disabling neurological/psychiatric WD.

Twelve patients' pathologies of explanted liver showed features of micro- or macronodular cirrhosis and some had varying cholestasis. Two patients' explanted livers had features of HCC, which is generally considered rare in WD[17]. One patient in the Turkey series who had transplantation for WD reported HCC in the hepatectomy specimen. This information suggests that HCC in WD is probably missed rather than rare.

The current study describes the largest number of WD patients who had liver transplantation from Middle East. The limitation of the current study is that it was a retrospective study. We did not face major hurdles in retrieving crucial information due to availability of excellent medical records, which we had for many years. Moreover, to study a rare genetic disease and procedure such as liver transplantation, we believe the retrospective study is still the best study design.

Liver transplantation for WD with end-stage liver disease is an effective treatment. Short- and long-term survival in our series was excellent; 94% of our patients survived at 1 year, with a median long-term survival of 8 years. Neurological and psychological manifestations of WD in our series disappeared during long-term follow-up.

COMMENTS

Background

The worldwide prevalence of Wilson's disease (WD) ranges from 10 to 30 cases per million persons. These patients generally present with acute or acute-on-chronic or chronic liver disease, and or neurological and psychiatric symptoms and signs. The disease is treated by chelating agents such as D Penicillamine or trientine. Liver transplantation is a treatment option for patients who are not responding to medical therapy, with acute liver failure or with advanced decompensated liver disease. Liver transplantation for severe neurological WD is controversial.

Research frontiers

The experience of liver transplantation for WD is limited. Over a period of 15 years, the authors gathered 16 WD patients who underwent liver transplantation and followed up in one single center. The authors studied short and long term survival following transplantation and the effect of liver transplantation on neurological and psychiatric manifestations.

Innovations and breakthroughs

The current study demonstrates that short and long term prognosis of WD patients with liver transplantation is excellent. The one year survival was 94%. The long-term average survival is currently 10.5 years, with a current median survival of 8 years. Long-term graft survival is currently 81%. Liver transplantation has a positive effect on neurological and psychiatric symptoms and signs. All of the patients with neurological and psychiatric symptoms and signs improved with liver transplantation.

Applications

The application of this study is that liver transplantation is an excellent method of treatment when it is really indicated for WD. Patients with mild to moderate degree of neuro-psychiatric symptoms and signs should not be denied chance for liver transplantation.

Terminology

WD is a rare autosomal recessive inborn error of copper metabolism resulting from mutations of the *ATP7b* gene on chromosome 13. The *ATP7b* gene product, a P-type ATPase transporter, is responsible for copper excretion into bile. In WD, impaired biliary copper excretion leads to accumulation of this metal in the liver. When liver storage capacity is exceeded, hepatocyte death ensues with copper release into the plasma. Elevated circulating copper results in hemolysis and copper deposition in extra-hepatic tissues such as brain, red blood cells, renal, *etc*.

Peer review

The current study provides valuable information of a rare inborn error of metabolism, and the short and long term results of liver transplantation. The drawback of the study is that the numbers represented in the study is small and it is a retrospective study. Only few centers have published papers relating this subject and the numbers from each center is similar to this one. Moreover, the knowledge of such rare metabolic disorders often comes from retrospective studies.

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CASE REPORT

Mastabol induced acute cholestasis: A case report

Brett M Hymel, David W Victor, Luis Alvarez, Nathan J Shores, Luis A Balart

Brett M Hymel, Department of Gastroenterology, Louisiana State University School of Medicine, New Orleans, LA 70112, United States
Brett M Hymel, David W Victor, Luis Alvarez, Nathan J Shores, Luis A Balart, Department of Gastroenterology and Hepatology, Tulane University School of Medicine, New Orleans, LA 70118, United States
Author contributions: Hymel BM, Victor DW, Alvarez L, Shores NJ and Balart LA contributed to this paper.
Correspondence to: Brett M Hymel, MD, Department of Gastroenterology, Louisiana State University School of Medicine, New Orleans, LA 70112, United States. bretthymel@aol.com
Telephone: +1-504-5684498 Fax: +1-504-5682127
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Abstract

A 26-year-old male presented with three weeks of jaundice after the self-initiation of the injectable anabolic steroid, Mastabol [Dromastanolone Di-Propionate (17 beta-Hydroxy-2alpha-methyl-5alpha-androstan-3-one propionate)]. He reported dark urine, light stools, and pruritus. He denied abdominal pain, intravenous drug use, intranasal cocaine, blood transfusions, newly placed tattoos, or sexually transmitted diseases. He used alcohol sparingly. Physical exam revealed jaundice with deep scleral icterus. The liver was palpable 2 cm below the right costal margin with no ascites. The peak bilirubin was 23.6 mg/dL, alkaline phosphatase was 441 units/L, and aspartate aminotransferase/alanine aminotransferase were 70 units/L and 117 units/L respectively. A working diagnosis of acute intrahepatic cholestasis was made. Liver biopsy revealed a centrilobular insult with neutrophilic infiltrates and Ito cell hyperplasia consistent with acute drug induced cholestasis. The patient's clinical symptoms resolved and his liver enzymes, bilirubin, and alkaline phosphatase normalized. Anabolic steroids with 17 alpha carbon substitutions have been associated with a bland variety of cholestatic injury with little hepatocellular injury. Cholestasis, under these circumstances, may be secondary to the binding of drugs to canalicular membrane transporters, accumulation of toxic bile acids from canalicular pump failure, or genetic defects in canalicular transport proteins. Mastabol is an injectable, 17 beta hydroxyl compound with no alpha alkyl groups at the 17 carbon position. As such, it has been reported to have little potential toxic effects on the liver. This is the first known reported case of Mastabol-induced cholestatic liver injury. It highlights the need for physicians to consider such widely available substances when faced with hepatic injury of unclear etiology.

Key words: Cholestatic; Mastabol; Hepatic; Anabolic; Steroids

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INTRODUCTION

Mastabol is a readily available, injectable, synthetic dihydrotestosterone with no aromatization potential which limits its estrogen effects. This is a particularly attractive feature to the bodybuilding community. Mastabol is not regulated, in any way, by the Food and Drug Administration (FDA). There are very few medical indications for the use of anabolic steroids; one of which is the treatment of certain rare forms of aplastic anemia[1].

The adverse hepatic effects of anabolic steroids have largely focused on cholestatic effects, but there are several other types of hepatic injury which have been linked to these compounds. Case reports have suggested that anabolic steroids may play a role in the so called toxicant-associated steatohepatitis[2]. In 2005, Socas *et al*[3] reported anabolic-androgenic induced hepatocellular adenoma occurance in two bodybuilders. Typically, anabolic androgens result in cholestatic liver injury, but in 2002,

Stimac *et al*[4] reported a case of anabolic steroid induced hepatocellular necrosis. A case report was published by Patil *et al*[5] describing a spontaneous hepatic rupture with hemorrhage and shock in a male with a history of anabolic steroid use.

Anabolic steroids with substitution groups at the 17 carbon position can have particularly undesirable effects on the liver. In particular, 17 alpha alkyl substitutions result in a decreased first-pass hepatic metabolism and, therefore, can allow for a greater oral bioavailability. These compounds are known to provoke a highly characteristic intrahepatic cholestasis *via* their direct toxic effects[6]. In 2007, Kafrouni *et al*[7] reported on a case of cholestatasis in two male bodybuilders, ages 40 and 31, who were utilizing the anabolic steroids superdrol and superdrol/holodrol, respectively, as part of their training regimens. Within weeks of ingestion, they both presented with symptoms of weight loss, jaundice, and pruritus. The symptoms resolved and liver panels normalized upon discontinuation of the substances. Ursodeoxycholic acid for pruritus, and in one of the cases, a short course of prednisone were also utilized. The use of methandienone by mouth daily and stanozolol by intramuscular injection resulted in severe cholestatic liver injury in a 28 year-old-male, as reported by Habscheid *et al*[8] in 1999. In this case, the patient's condition deteriorated over a seven week course after the steroids were discontinued and improvement in jaundice and liver chemistries corresponded with the initiation of ursodiol[8].

Like other anabolic steroids, the mass building effects of Mastabol are thought to be secondary to enhanced protein synthesis and positive nitrogen balance. Other possible suggested uses for anabolic steroids include the stimulation of red blood cells, enhancement of male sexual characteristics, and palliation of androgen responsive, recurrent breast cancers in postmenopausal females. The 17 alpha substituted anabolic steroid, oxymetholone, has been FDA approved for treatment of anemia in patients with deficient red blood cell production[9]. Contraindications, as per the manufacturer, include drug hypersensitivity, development of male breast cancer, prostate cancer, and serious cardiac, liver, and renal impairment. The vast majority of anabolic steroids are not regulated or endosed by the FDA, and as they are available over-the-counter, are often assumed to be generally safe. Further, searching the term, Mastabol, in any internet search engine can produce up to 14 000 results detailing how it can be accessed. However, a pubmed search of "Mastabol" revealed no results, which further highlights the significance of this case report.

Mastabol (17 beta-Hydroxy-2alpha-methyl-5alpha-androstan-3-one propionate) is a common anabolic steroid used by bodybuilders as an over-the-counter supplement to augment their training efforts. Its chemical structure does not include a 17 alpha alkyl group, and as such, has been marketed on the internet as having little to no potential hepatotoxic affects. Here, we present the case of a 26-year-old male with biopsy proven drug induced cholestasis seven to ten days following the initiation of the injectable body building supplement, Mastabol. To our knowledge, this is the first reported case of adverse hepatobiliary effects associated with Mastabol.

CASE REPORT

A 26-year-old male presented with three weeks of jaundice beginning ten days after the self- initiation of the injectable bodybuilding supplement, Mastabol. He had previously been healthy and was working full time as an offshore oil field wire operator. Upon the onset of jaundice, he also reported associated dark colored urine and light colored stools. Days later, he developed generalized pruritus that progressively worsened such that he was unable to sleep at night. He reported losing 40 pounds over a three month period, roughly two thirds of which was through diet and exercise prior to the onset of illness. He denied nausea, emesis, abdominal pain, diarrhea, constipation, fever, chills, or night sweats. He reportedly followed the dosing instructions on the label. Further, he denied any exposure to known hepatitis carriers, exposure to intravenous drugs, intranasal cocaine, blood transfusions, newly placed tattoos, and sexually transmitted diseases, such as herpes. He reported a monogamous relationship with his wife.

Further social history was significant for drinking alcohol sparingly, one to two drinks per month, and smoked one pack of cigarettes daily for approximately ten years. He denied known toxic exposure at work. Past medical history included asthma and bronchitis as a child with no adult episodes and reported peptic ulcer disease four years ago. The patient's family history was negative for liver disease, including hepatitis, jaundice, cirrhosis, or malignancy. He was taking no prescribed medications and no herbal or over-the-counter medications except the previously mentioned Mastabol.

Physical exam revealed a jaundiced, oriented male with normal vital signs. Deep scleral icterus was noted. There was no stigmata of cirrhosis or portal hypertension. Several excoriated lesions were noted on the lower extremities. The liver was palpable 2 cm below the right costal margin in the midaxillary line on inspiration, the spleen was not palpable, and there was no evidence of ascites. No joint tenderness or effusion was noted. Neurological exam was non-focal with no asterixis.

On data evaluation, peak bilirubin was 23.6 mg/dL with a direct fraction of 20.5 mg/dL, alkaline phosphatase was 441 units/L, and aspartate aminotransferase/alanine aminotransferase were 70 units/L and 117 units/L respectively. The albumin was 3.1 and international normalized ratio 0.98 with all other chemistries and blood counts unremarkable. All other causes of hepatitis, including viral, autoimmune, and genetic, were ruled out by appropriate testing.

A working diagnosis of acute, nonobstructive, intrahepatic cholestatic hepatitis secondary to Mastabol was made. The patient was placed on phenobarbitol for management of pruritus, and a liver biopsy was scheduled. He was also

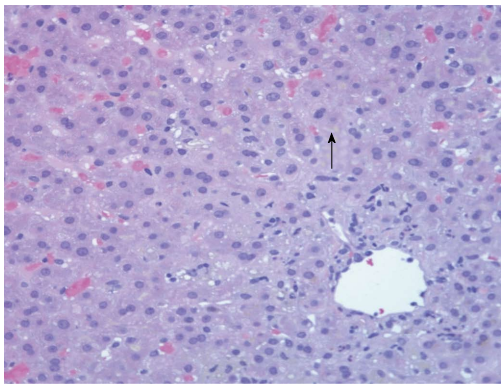

Figure 1 Liver biopsy revealing lobular cholestatic injury (arrow), ito (stellate) cell. Hyperplasia (small white circular areas resembling microsteatosis) and a neutrophilic infiltrate consistent with drug induced cholestatic injury.

urged to avoid any other medications in the interim.

The biopsy revealed a centrilobular insult with neutrophilic infiltrates and mild Ito (stellate) cell hyperplasia consistent with severe acute cholestatic hepatitis consistent with a drug induced liver injury (Figure 1). The hepatic stellate cell is normally a quiescent cell situated in the perisinusoidal space. However, with liver injury, they are activated resulting in hyperplasia and can contribute to hepatic fibrosis over time by secreting collagen.

Eventually, the patient's clinical symptoms resolved and his liver enzymes, bilirubin, and alkaline phosphatase trended toward normal.

DISCUSSION

This patient's presentation, physical examination, laboratory data, histology, and recovery were all consistent with severe cholestasis secondary to the anabolic steroid, Mastabol. In particular, this case was illustrative of the, so called, bland type of cholestatic injury with significant bilirubin and alkaline phosphatase elevation and only mild aminotransferase elevation, thus indicating minimal hepatocellular injury.

Drug-induced adverse reactions on the liver can occur through direct effects on the hepatocytes, effects on bilirubin metabolism/binding, cholestatic mechanisms, viral mechanisms, or nonspecific/mixed effects[10]. The addition of alkyl groups to the 17 alpha position ties up the bonds needed to change the molecule to the less active keto form, and therefore, results in a greater half life and risk of toxicity. Here, we will focus our discussion on the proposed cholestatic mechanisms of liver injury.

Drug-induced cholestasis can be of several varieties: bland, meaning that there is limited injury to hepatocytes, inflammatory, sclerosing, or ductopenic (disappearance of bile ducts)[6]. Seventeen alpha substituted anabolic steroids appear to lead to the bland cholestatic variety with little hepatocellular injury and aminotransferases often elevated less than five-fold[11]. Although the exact mechanism of cholestasis under these circumstances is not known, it may be secondary to the binding of the drugs to canalicular membrane transporters or accumulation of toxic bile acids due to canalicular pump failure. Genetic defects in canalicular transport proteins may also play a role[6]. Other possible mechanisms include decreased permeability of the biliary epithelium to water, decreases in the bile salt-independent fraction of bile secretion, and/or the interference with the hepatic disposal of bile salts[12].

In support of the theory of injury at the level of the canaliculi, electron microscopy of rat livers a following 17 carbon substituted anabolic steroid administration confirmed canalicular changes of dilation and loss of microvilli[10]. Further, cholestatic effects have been attributed to interference with bile flow with potential sites of anabolic injury at the canalicular, pericanalicular microfibrillar network, and the basolateral plasma membrane all resulting in canalicular contraction[13]. The steroid-like agent, icterogenin, also leads to cholestasis and canalicular distortion, lending further support to this theory[13].

In addition, biliary excretion has been found to be significantly decreased in the presence of 17 alkylated anabolic steroids[13]. The lesion produced by the androgens to result in poor excretion has been compared to that causing Dubin-Johnson Syndrome, whereby there is a mutation in the canalicular multidrug resistance protein resulting in poor conjugated bilirubin excretion[13].

Whatever the mechanism of injury, these patients often present with mild jaundice which is usually reversible on drug discontinuation. The reaction appears to be dose related and predictable. Hepatic dysfunction often resolves quickly with the discontinuation of the anabolic steroid in anicteric cases and within months in patients presenting with icterus[11]. In patients without jaundice, continuation of the offending agent has been noted to induce a tolerance to the adverse effects of anabolic steroids with amelioration of hepatic enzymes levels[11].

In conclusion, supplemental anabolic steroids are often poorly regulated but are commonly used by resistance trainers to augment their training programs. Seventeen alpha alkylated anabolic steroids have been historically associated with hepatic cholestasis. This is the first described case of the highly available steroid, Mastabol, causing severe cholestasis in the absence of alkyl groups at the 17 alpha position.

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CASE REPORT

Portal hypertensive biliopathy: A single center experience and literature review

Vanessa Suárez, Andrés Puerta, Luisa Fernanda Santos, Juan Manuel Pérez, Adriana Varón, Rafael Claudino Botero

Vanessa Suárez, Luisa Fernanda Santos, Adriana Varón, Rafael Claudino Botero, Gastroenterology and Hepatology Service, Department of Internal Medicine, Fundación Cardioinfantil, Bogotá 110131, Colombia
Andrés Puerta, Juan Manuel Pérez, Department of Diagnostic Imaging, Fundación Cardioinfantil, Bogotá 110131, Colombia
Author contributions: Suárez V contributed to acquisition of data, initial drafting of the paper, final approval of the paper; Puerta A contributed to acquisition of imaging, initial drafting of the imaging literature, final approval of the article; Santos LF contributed to acquisition of patients' data, initial revision of the data, initial editing of the draft, final correction and approval of the paper; Pérez JM contributed to initial design of the draft, selection and description of imaging, revising the imaging literature, final approval of the article; Varón A contributed to acquisition of data, initial drafting the paper, final approval of paper; Botero RC contributed to conception of the article and design, critical revision of the content, final correction and approval of the article.
Correspondence to: Rafael Claudino Botero, MD, Gastroenterology and Hepatology Service, Department of Internal Medicine, Fundación Cardioinfantil, Carrera 13B 161-85, Consultorio 17, Bogotá 110131, Colombia. claudino48@mac.com
Telephone: +57-1-2167813 Fax: +57-1-6791147
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Abstract

Portal hypertensive biliopathy (PHB) is characterized by anatomical and functional abnormalities of the intrahepatic, extrahepatic and pancreatic ducts, in patients with portal hypertension associated to extrahepatic portal vein obstruction and less frequently to cirrhosis. These morphological changes, consisting in dilatation and stenosis of the biliary tree, are due to extensive venous collaterals occurring in an attempt to decompress the portal venous blockage. It is usually asymptomatic until it progresses to more advanced stages with cholestasis, jaundice, biliary sludge, gallstones, cholangitis and finally biliary cirrhosis. Imaging modalities of the biliary tree such as Doppler ultrasound, computed tomography, magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography are essential to establish the diagnosis and the need of therapeutical interventions. Once the diagnosis is established, treatment with ursodesoxycholic acid seems to be beneficial. Decompression of the biliary tree to dilate, remove stones or implant biliary prosthesis by endoscopic or surgical procedures (hepato-yeyunostomy) usually resolves the cholestatic picture and prevents septic complications. The ideal treatment is the decompression of the portal system, with transjugular intrahepatic porto-systemic shunt or a surgical porto-systemic shunt. Unfortunately, few patients will be candidates for these procedures due to the extension of the thrombotic process. The purpose of this paper is to report the first 3 cases of PHB seen in a Colombian center and to review the literature.

Key words: Bile duct diseases; Biliary obstruction; Banti's syndrome; Cholestasis in children; Portal vein obstruction; Interventional endoscopy

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INTRODUCTION

Portal hypertensive biliopathy (PHB) is defined as the set of anatomical and functional alterations of the intra- and extrahepatic bile ducts in patients with portal hypertension due to extrahepatic portal vein obstruction (EHPVO). These changes include dilatation and stenosis of the bile ducts, common hepatic duct, gallblad-

der and intrahepatic ducts and they are due to extrinsic compression of these pathways by paracholecystic and paracholedochal venous plexuses that expand and compress in an attempt to decompress the venous blockage generated by the portal vein thrombosis[1].

Initially the process is silent and without any specific symptoms. However, as it progresses to more advanced stages, the patient presents with cholestasis, jaundice, biliary sludge, gallstones and finally secondary biliary cirrhosis. Some researchers have reported the presence of these symptoms in 70%-100% in patients from India with EHPVO[2].

It is a relatively new disease, and according to Löhr *et al*[3] early associations between jaundice and EHPVO were reported in 1944 by Fraser *et al*, in 1965 by Gibson *et al*[4], and it was finally Dhiman *et al*[5], who in 1999 proposed the term "portal hypertensive biliopathy"; as a newly described disease it is expected to be underdiagnosed. The incidence of PHB in patients with EHPVO (81%-100%)[6] is much higher than in patients with liver cirrhosis (0%-33%) or idiopathic portal hypertension (9%-40%)[1]. To our knowledge, there have been no cases of PHB reported in Colombia.

The aim of this paper is to present the first three cases documented in Colombia and to review the literature on the subject.

CASE REPORT

Case 1

This male patient first consulted at 7 years of age for upper gastrointestinal bleeding due to esophageal varices secondary to portal vein thrombosis. Endoscopic sclerotherapy and band ligation were performed on several occasions and treatment with oral propranolol was started. Liver function tests were normal and diagnostic imaging [Doppler ultrasound, computed tomography (CT)] showed cavernomatous degeneration of the portal vein and collateral circulation through spontaneous splenorenal shunts. A percutaneous liver biopsy showed non-cirrhotic liver with minimal nonspecific changes and portal fibrosis. Over the next 10 years he had 3 episodes of variceal bleeding controlled by endoscopic ligation.

At the age of 21 (14 years later), he was readmitted to the hospital due to diffuse abdominal pain, cholestatic liver test pattern and diagnostic images compatible with thrombosis and cavernomatous degeneration of the portal vein, associated with spleno-mesenteric thrombosis and diffuse intrahepatic dilatation of the biliary tree (Figure 1). A magnetic resonance cholangiopancreatography (MRCP) confirmed the findings and showed extrinsic compression of the bile duct by venous collaterals and distal common bile duct stenosis not passable endoscopically (Figures 2 and 3). All laboratory tests to rule out other liver diseases were negative including viral and autoimmune serologies, tumor markers and testing for procoagulant processes.

With this information a diagnosis of PHB was established and treatment with ursodeoxycholic acid and low-

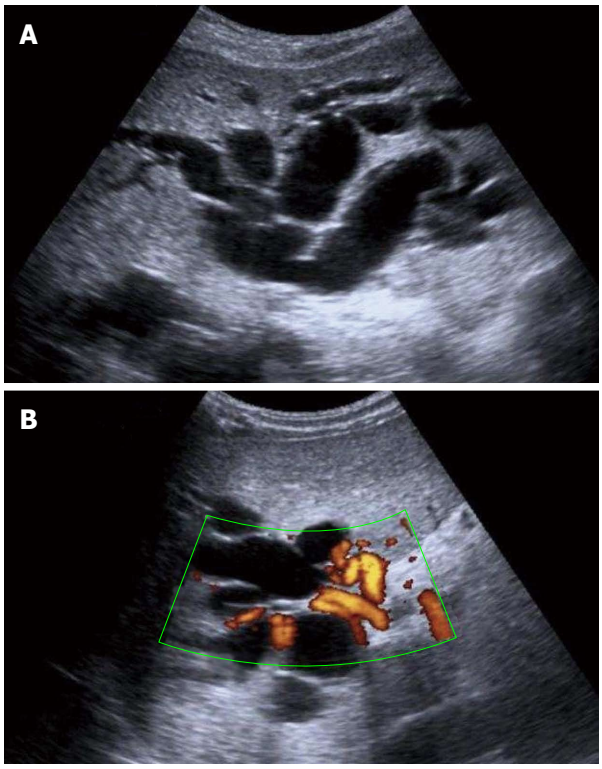

Figure 1 Ultrasound examination. A: Ultrasound examination showing dilatation of the intrahepatic bile ducts; B: Doppler ultrasound examination demonstrating cavernomatous degeneration of the portal vein with hilar collateral circulation.

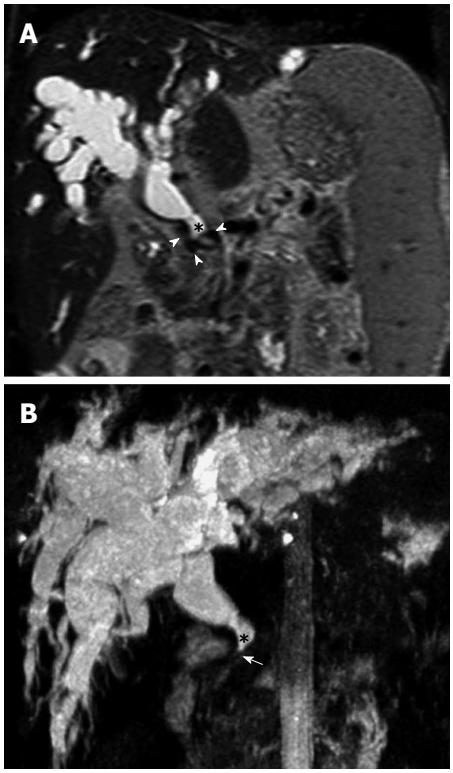

Figure 2 Magnetic resonance cholangiopancreatography. A: Coronal T2 weight Magnetic resonance indicates marked dilatation of the intra-and extra-hepatic bile duct (*), with presence of vascular structures with void flow that exert extrinsic compression (arrowheads), corresponding to the cavernoma. B: Coronal maximum intensity reconstruction shows the severity of the dilatation of the intra- and extrahepatic bile duct (*) with notable narrowing of the distal common bile duct (arrow).

Table 1 Liver function tests

Liver function test	Case 1		Case 2		Case 3	
	At diagnosis	Follow up	At diagnosis	Follow up	At diagnosis	Follow up
Alkaline phosphatase (U/L)	424	347	437	207	936	311
GGT (U/L)	131	128	201	99	241	111
Total bilirubin (mg/dL)	16.1	5.9	6.62	3.05	17.7	9.28
Direct bilirubin (mg/dL)	12.8	4.9	4.61	1.99	13.5	7.7
ALT (U/L)	114	63	125	68	235	24
AST (U/L)	159	83	151	59	131	33
INR		1.17		1.06		1.66
Albumin (g/dL)	2.6	2.8	3.68	4.13	1.5	2.4

GGT: γ glutamyl transferase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; INR: International normalized ratio. Normal values: Alkaline phosphatase 35-104 U/L; GGT 0-38 U/L; Total bilirubin 0.3-1.2 mg/dL; Direct bilirubin 0-0.3 mg/dL; ALT 10-49 U/L; AST 0-34 U/L; Albumin 3.4-5.0 g/dL.

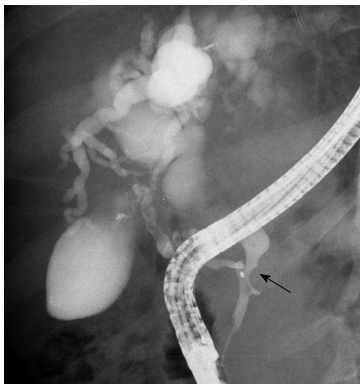

Figure 3 Pancreatic endoscopic retrograde cholangiopancreatography image indicates the site of stenosis of the bile duct (arrow) and a marked dilation of the biliary radicals.

molecular-weight heparin was started. He was discharged after establishing that he was not a candidate for a portal-systemic shunt or liver transplantation and improvement in his cholestatic pattern was confirmed (Table 1).

Case 2

A 20-year-old male presented with a history of gastrointestinal bleeding on several occasions, and esophageal varices secondary to portal hypertension, noncirrhotic, massive splenomegaly, hypersplenism and intermittent abdominal pain. Past medical history included umbilical vein catheterization in the neonatal period secondary to indirect hyperbilirubinemia due to ABO/Rh incompatibility. Esophagogastric devascularization, splenectomy, vagotomy and pyloroplasty were performed. His chief complaints were abdominal pain, jaundice with slight tinge in the sclera. Liver function tests were performed, CT of the abdomen showed dilatation of the intrahepatic bile duct (Figure 4), endoscopic retrograde cholangiopancreatography (ERCP) showed extrinsic compression of the distal common bile duct. In August 2011, he required hospitalization following another episode of jaundice, MRCP showed dilatation of the intrahepatic and extrahepatic bile ducts and cavernomatous degeneration of the portal vein (Figure 5). Due to extensive vascular thrombosis of the portal venous system, a liver transplant was not feasible and the risk of hepatoyeyunostomy was unacceptably high. Ursodeoxycholic acid treatment was started with apparent benefit (Table 1). Etiological tests to rule out other causes of liver disease were negative including viral, autoimmunity, tumor markers and procoagulant processes.

Case 3

A 30-year-old male with a past medical history of several episodes of upper gastrointestinal bleeding that started in 2005. Cavernomatous degeneration of the portal vein, non-cirrhotic portal hypertension with complications of hypersplenism and esophagogastric varices were documented. All etiological laboratory tests to rule out other liver diseases were negative, including viral and autoimmune serologies, tumor markers and procoagulant processes. Liver biopsy was reported as normal. In 2006, a meso-caval shunt was performed which, in the immediate postoperative period, showed signs of dysfunction and occlusion.

The case was presented to the liver transplant committee and it concluded that, given the patient's history and the characteristics of bleeding, esophagogastric devascularization surgery was performed, as there was no other possibility of a surgical shunt or transjugular intrahepatic porto-systemic shunts (TIPS). In August 2011, he presented to the emergency room with abdominal pain over 2 mo associated with jaundice and cholestasis. A hepatobiliary scan (up to 6 h) indicated signs of cholestasis without passage of tracer into the intestine or bile duct. The inability to perform an ERCP due to complete biliary obstruction resulted in him undergoing a hepato-yeyunostomy with an adequate postoperative course (Table 1).

DISCUSSION

Literature review and as subtitle definition

Portal hypertensive biliopathy is defined as changes in the biliary tract in patients with portal hypertension due to EHPVO[7]. These include stenosis and dilatation of

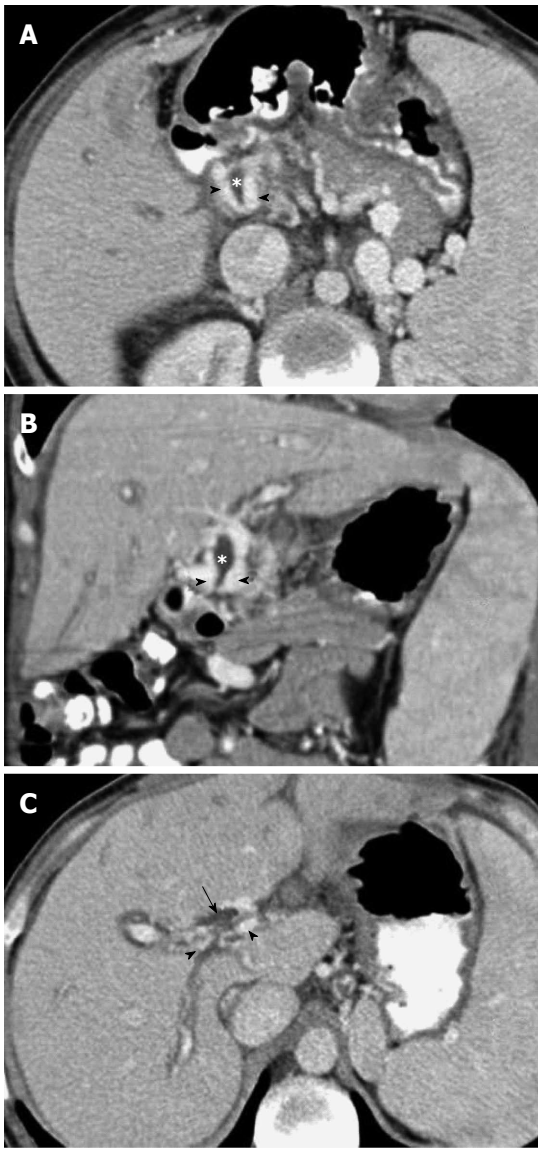

Figure 4 Portal phase abdominal computed tomography. A, B: Axial (A) and coronal planes (B) show multiple dilated collateral representing the paracholedochal plexus (arrowheads) with reduced diameter of the bile duct (*), secondary to extrinsic compression by the cavernoma; C: The superior axial image shows dilatation of the bile ducts (arrow).

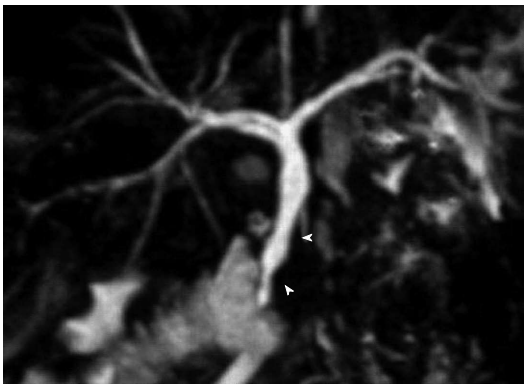

Figure 5 Magnetic resonance cholangiopancreatography. Coronal maximum intensity reconstruction identifies irregularities and extrinsic compression of the distal common bile duct (arrowheads).

the intrahepatic and extrahepatic bile ducts and secondary varicose veins surrounding the common bile duct and the gallbladder wall. When these changes evolve, the individual presents with cholestasis and jaundice, and choledocholithiasis is a common sequela[2,8]. In general, this disease has been reported in 70%-100% of patients with extrahepatic obstruction of the portal vein[8]. It is much less common in cirrhotic portal hypertension, and it is speculated that the reason is that in cirrhotic patients blocking of the portal circulation occurs at the level of hepatic sinusoid, giving origin to collateral circulation far from the vein complexes around the extrahepatic bile ducts.

Prevalence

Liver cirrhosis is the most common cause of portal hypertension in the Western world and a rare cause of PHB. However, non-cirrhotic portal hypertension constitutes 40% of all cases of portal hypertension in developing countries[9]. Other causes in non-cirrhotic patients include: non-cirrhotic portal fibrosis, schistosomiasis, EHPVO, the idiopathic Budd Chiari syndrome, congenital hepatic fibrosis, nodular regenerative hyperplasia and sinusoidal obstruction syndrome (veno-occlusive disease of the liver). Although there are no population studies and its frequency is much higher in India, South Korea and Turkey[10,11], PHB is an increasingly important entity that seems to impact the natural history of EHPVO. We found proof of this in a literature review we carried out in PubMed using the term "portal biliopathy" in which we found 70 English-language publications worldwide, most of them consisting of reports of a few patients and review articles with 9 publications reporting more than 10 cases[10-18]. We found a total of 223 cases most in Asian countries and to a lesser extent in Europe and North America. In South America, PHB has been reported in Mexico, Chile and Brazil[19-21]. To the best of our knowledge, no cases have ever been reported in Colombia.

PVT presents frequently during childhood and adolescence[22], but PHB usually presents later in life.

Pathophysiology

It is well known that, to maintain hepatic blood flow, the development of multiple collateral veins occurs in response to obstruction of the extrahepatic portal vein. One study showed that the time between complete acute thrombosis and the formation of the cavernoma is 6 wk[23]. These collateral veins, called portal cavernoma, form a dense vascular pattern and fibrous stroma in the peripancreatic region along the portal vein occluded, and provide an alternative route around the thrombosed segment of the portal vein[4]. In normal conditions, the venous drainage of the bile duct is divided into two special plexuses. The first is formed by the pericholedochal Saint's venous plexus which extends as a fine grid around the bile duct and main hepatic ducts. The second is formed by the pericholedochal Petren's venous plexus that is parallel

to the bile duct and is connected to the gastric vein, the pancreatic-duodenal and portal vein. Its conversion into collateral veins causing pressure and bulging of the thin and flexible bile duct walls is called portal biliopathy[24,25]. In addition to the hypothesis of compression of the varices around the bile duct, there is an ischemic hypothesis that implies that the vascularization of the bile ducts is compromised, leading to scarring of the lining of the ducts, resulting in biliary strictures and cholangiectasias[2].

Differential diagnosis

The differential diagnosis is extensive and includes: primary and secondary sclerosing cholangitis, gallstone disease, ischemic cholangiopathy, acute and chronic rejection, primary biliary cirrhosis, cystic fibrosis and autoimmune pancreatitis. In tropical areas, parasitic diseases that compromise the bile duct should be considered[26].

Symptomatology

Although morphological changes have been reported in 80%-100% of patients with EHPVO[1,6], the majority remains asymptomatic for many years and is rare in children. Several investigators have reported frequencies of 5%-17%[1,4,6,27], depending on the duration and frequency of clinical follow-up especially in the adult population. The most common clinical presentation is recurrent abdominal pain, fever, jaundice and cholangitis with partial or, in some cases complete, biliary obstruction[14]. Alkaline phosphatase is elevated in 80% of cases[28], aminotransferases are normal until advanced stages of disease, and coagulation tests, and albumin levels may be abnormal in cases of complete obstruction with secondary biliary cirrhosis.

Diagnostic imaging

Typical indications of cavernomatous degeneration of the portal vein are visible by ultrasound: a decrease in its diameter, increased echogenicity of the tissue in the hilum, associated with multiple anechoic tubular structures, corresponding to distended paracholedochal veins, and it is technically difficult to identify the common bile duct. The indentations visible by ultrasound on the common bile duct are secondary to extrinsic compression by the enlarged paracholedochal venous plexus; these are larger and are connected with the gastric vein, pancreaticoduodenal and portal vein, while the irregularities caused by dilation of pericholedochal varices may not be observed by ultrasound, since their size is less than 1 mm[29]. Gallbladder varices, present in between 30%-55% of cases[29,30], are visualized as anechoic tubular structures of 1-5 mm in diameter in the external refractive surface of the gallbladder, outside the muscular layer. These varicose veins correlate with ERCP findings[29].

Evaluation of the extrahepatic bile duct by endoscopic Doppler ultrasound, shows compression of the biliary tree by the collateral circulation in patients with cavernomatous degeneration of the portal vein. This allows the exclusion of other causes such as stones, biliary sludge and tumors are not vizualised in other imaging modalities[30,31].

Multi-detector CT (MDCT), using narrower collimation and lower acquisition time, gives high quality images for visualizing the collateral circulation, the product of portal vein obstruction[32]. MDCT angiography techniques and post-processing, clearly demarcate the signs of cavernous transformation of portal vein, the compressive effect of collateral circulation around the bile duct, and gallbladder varices[15,29]. It has been claimed that 3D portography using MDCT has an accuracy similar to conventional portal angiography in demonstrating the characteristics of porto-systemic collaterals[33].

MRCP is currently the noninvasive diagnostic modality of choice, allowing an adequate characterization of the intra-and extrahepatic bile duct and with a capacity similar to ERCP for visualization of changes in the bile duct[34]. Condat *et al*[35] studied 25 patients with cavernous transformation of portal vein by crisis resource management (CRM), excluding those with malignancies and/or cirrhosis. Stenosis of the common bile ducts was seen in 16 cases while 5 had intra- and extrahepatic bile duct abnormalities, most consisting of short length stenosis (13 of 21) associated with retrograde dilatation in 16 cases. Using portography sequences with gadolinium, it was demonstrated in all cases that bile duct alterations corresponded with the mass effect of the cavernoma. Thus, CRM sequences with contrast portography are superior to CRM alone for detecting alterations of the intrahepatic bile duct and common bile duct stones and for recognizing and differentiating them from collateral circulation[35].

ERCP features described most often in the literature, include biliary segments with narrowing of variable length and degree, indentations and irregularity of the contours of the bile duct and the presence of angles, ectasia and calculi. An absence of branching can be seen in the intrahepatic bile ducts and in some dilated ducts[35]. Cholangiographic features are not specific and similar to other entities, explaining the previous name of "pseudo-cholangiocarcinoma"[36] and "pseudo-sclerosing cholangitis"[6]. Patients with extrahepatic occlusion of the portal vein have abnormal ERCP in 81% to 100% of cases, with involvement of the extrahepatic bile duct in 60%-97%, right hepatic duct in 40% to 56% and left hepatic duct in 55% to 100%[35]. One study identified alterations in the intra-and extrahepatic bile duct in 85% (17 of 20) of patients with extrahepatic obstruction of the portal vein, whereas cirrhosis without extrahepatic portal obstruction were found only in 27% (3 of 11) of cases[30]. Chandra *et al*[2] have proposed a morphological classification based on the topography of the cholangiography findings (Table 2), although its usefulness for management remains to be demonstrated.

Some reports on patients with cavernous transformation of the portal vein, especially those with extension to the superior mesenteric or splenic vein, have shown pancreatic head enlargement and images of a pseudo-mass at this location, with heterogeneous signal intensity in the

Table 2 Portal hypertensive biliopathy: Morphological classification[2]

Type	Findings
I	Involvement of extrahepatic bile duct
II	Involvement of intrahepatic bile ducts only
IIIa	Involvement of extrahepatic bile duct and unilateral intrahepatic bile duct
IIIb	Involvement of extrahepatic bile duct and bilateral intrahepatic ducts

simple phase in relation to the cavernoma, constituting intra and/or peripancreatic collateral circulation. Dynamic sequences with contrast allow an adequate assessment of intrapancreatic cavernoma, differentiating it from neoplastic lesions. In contrast to the high frequency of alterations in the biliary tract, altered pancreatic ducts have been reported in only a minority of cases[37]. Furthermore, a greater number of cases of extrahepatic portal biliopathy have been reported in cirrhotic patients with hypercoagulable states when the portal thrombosis extends to the superior mesenteric vein[18].

Histopathological changes

The portal vein is replaced by an extensive vascular network in a stromal support with multiple anastomoses that eventually achieve the passage of blood to the liver. This vascular structure called a "cavernoma" extends along the entire porta and in some cases to the intrahepatic portal branches[38]. The liver has a generally smooth or finely granular aspect with some fibrous septa that project into the parenchyma, a consequence of thrombosis of intrahepatic portal vessels[39]. Nodular regenerative hyperplasia has been reported, especially in cases of EHPVO associated with human immunodeficiency virus[40]. It is exceptional to find cirrhotic changes and liver function tests are normal in most cases. In clinically manifest biliopathy the spectrum of changes ranges from intrahepatic cholestasis, ductal proliferation and acute cholangitis. In cases of complete biliary obstruction secondary biliary cirrhosis usually occurs with time.

Treatment

In asymptomatic patients with EHPVO, specific treatment is not recommended to improve bile flow. However, the identification of early bile duct morphological alterations by MRCP, makes it possible to design a management program and to intervene early when symptoms begin. The use of therapeutic doses of ursodeoxycholic acid (10-15 mg/kg per day) is the first choice. This should always be associated with a detailed examination of the bile duct by ERCP, CRM and intervention if necessary to dilate, remove stones or implant prosthesis (14). Clearly there is a risk of injuring the dilated venous complex, causing hemobilia and worsening cholestasis[41,42]. The ideal treatment for EHPVO associated with biliopathy is to decompress the portal system with a surgical porto-systemic shunt or interventional radiology with a TIPS. There is regression of ectopic varices and improvement of cholestasis in most patients, although a proportion of them continue with significant changes in bile ducts. Unfortunately, in most cases this treatment is not possible due to the extension of thrombosis to most vascular territories that drain into the portal vein.

In a recent study, Agarwal *et al*[10] reported on 39 patients who underwent porto-systemic shunt (34) and 2 bilio-digestive anastomosis, finding improvement of biliary symptoms in more than 60% of cases and an acceptable complications rate. However, the vast surgical experience of this group is not easy to reproduce in other centers. Liver transplantation is not generally indicated although there are some reports of liver and intestinal transplants when any of the above mentioned procedures have not been successful[43]. When the patient does not benefit from medical or endoscopic treatment and a porto-systemic shunt is not possible, hepaticojejunostomy performed without decompressing the portal system is a high risk option because multiple venous collaterals can generate uncontrollable bleeding.

In conclusion, portal hypertensive biliopathy is a newly recognized complication that occurs primarily in patients with EHPVO and less frequently in cases of cirrhosis. Modern imaging technology allows identification of morphological abnormalities even before symptoms occur. The most appropriate treatment sequence is to start ursodeoxycholic acid administration, combinatined with instrumentation of the bile duct when necessary. It is imperative to decompress the portal system with a porto-systemic shunt or TIPS when anatomy permits, and is not advisable to perform bilio-digestive shunts without previous decompression of the portal vein because of the high risk of bleeding. In cases of procoagulant phenomena associated with EHPVO, anticoagulation with low molecular weight heparins can lead to recanalization of the portal vein with significant clinical improvement.

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CASE REPORT

Liver transplantation for recurrent hepatic adenoma

Giovanni Vennarecci, Roberto Santoro, Mario Antonini, Cecilia Ceribelli, Andrea Laurenzi, Enrico Moroni, Mirco Burocchi, Pasquale Lepiane, Giuseppe Maria Ettorre

Giovanni Vennarecci, Roberto Santoro, Cecilia Ceribelli, Andrea Laurenzi, Enrico Moroni, Mirco Burocchi, Pasquale Lepiane, Giuseppe Maria Ettorre, Division of Liver Transplantation, San Camillo Hospital, 00151 Rome, Italy
Mario Antonini, Intensive Care Unit, National Institute for Infectious Disease, "Lazzaro Spallanzani", 00151 Rome, Italy
Author contributions: Vennarecci G designed the study and wrote the paper; Santoro R analyzed the data; Antonini M and Lepiane P revised the article; Ceribelli C, Laurenzi A, Moroni E and Burocchi M collected the data; Ettorre GM designed study and final approval.
Correspondence to: Giovanni Vennarecci, MD, Division of Liver Transplantation, San Camillo Hospital, circ.ne Gianicolense 87, 00151 Rome, Italy. gvennarecci@scamilloforlanini.rm.it
Telephone: +39-338-4661336 Fax: +39-6-58704719
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Abstract

Hepatic adenoma (HA) is a rare indication for liver transplantation (LTx). So far 20 cases of LTx for HA are reported in PubMed. In rare cases HA presents as multiple hepatic adenomas or recurrent adenoma after initial liver resection and in such cases LTx is the only potential cure and prevents the risk of bleeding or cancer transformation into hepatocellular carcinoma. We report the case of a 56 years old lady who underwent a left hepatectomy for giant adenoma in 2005 and resection of segment V-VI for recurrence of liver adenoma in 2007. She developed a second recurrence of HA with 3 new lesions in the right liver in 2008. The patient underwent LTx. After 3 years the patient is alive with no evidence of disease. LTx is indicated in patients with HA in which resection is not technically feasible.

Key words: Liver transplantation; Liver adenoma; Liver resection; Recurrent disease; Immunosuppression

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INTRODUCTION

Hepatic adenoma (HA) is a benign tumor of the liver, which is mainly related to the estrogen-based oral contraception in women. It is often diagnosed, in asymptomatic patients, as an incidental finding during radiological procedures. Symptomatic patients usually present vague abdominal pain with normal hepatic function. HA greater than 5 cm in diameter are suitable of liver resection to prevent the risks of spontaneous rupture, bleeding and malignant transformation into hepatocellular carcinoma (HCC). Liver cell adenomatosis is a different clinical entity, that can occur in 10%-24% of the patients with HA. The peculiarity of liver cell adenomatosis is the presence of multiple HA, the association with oral contraceptive use is not as high as in solitary liver cell adenomas. These lesions can recur even after a complete surgical resection. HA and liver cell adenomatosis are a rare indication for liver transplantation (LTx). So far 20 cases of LTx for HA are reported in PubMed[1-6]. Here we report a case of recurrent HA, that underwent LTx after two previous hepatic resections.

CASE REPORT

A 56 years old lady presented in 2005 with a huge palpable mass in the upper abdomen. A computed tomography (CT) scan showed a 20 cm mass occupying the all left hemi-liver, this was compatible with a giant HCC, the right lobe was normal without signs of dysmorphism (Figure 1). Her past medical history was relevant for arterial hypertension, meningioma surgically removed in 2001 and Danazol assumption for 1 year. Laboratory

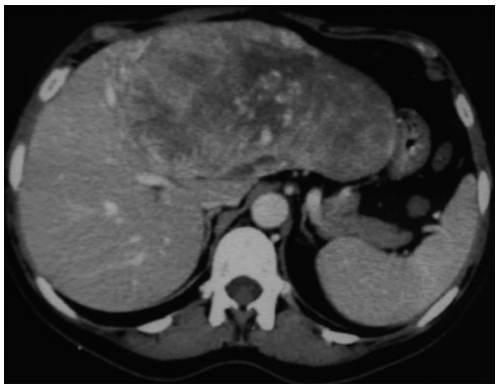

Figure 1 Time of presentation. Giant hepatic adenoma of the left liver measuring 20 cm.

tests did not show hepatitis C virus and hepatitis B virus infection, but a slight elevation of gamma-glutamyltransferase. Alpha-fetoprotein was normal. There was no evidence of alcohol or drug abuse. She underwent a left hepatectomy and the pathological examination revealed a giant adenoma.

During follow-up, 18 mo later, an ultrasound of the liver showed a small nodule 3 cm in diameter located in the sixth segment and a CT scan confirmed this nodule as a recurrent adenoma. The nodule was followed-up but imaging radiology showed an increase of its dimension. Therefore it was decided to remove it by an atypical resection of segment V and VI. Pathological examination revealed again a liver adenoma.

The patient underwent close postoperative follow-up. Nine months later, 3 new lesions were discovered in the liver: the largest, measuring 5-6 cm in diameter, was located close to the inferior vena cava and the right hepatic vein; the other two lesions of 2.5 cm and 1.8 cm in diameter were deeply located in the right lobe of liver (Figure 2). Considering this second recurrence after two hepatic resections and the size and location of nodules a surgical resection and less invasive treatment such as radio frequency ablation (RFA) and transarterial embolization (TAE) were judged not feasible and the option of LTx was recommended. After a deep medical examination the patient was listed. One month later a CT scan of the liver showed an increase in size of the three lesions. Two months later she underwent a LTx. The donor was 16-year old boy died in a car accident. The transplant procedure took 5 h and was performed with the so called "piggy-back" technique and caval flow preservation without veno-venous by-pass, cold ischaemic time was 5 h and 45 min. During the procedure, several technical problems were met and they were related to the two previous hepatic resections: division of adhesions, mobilization of the right lobe adherent to the right diaphragm, exposure of the supra hepatic vein and dissection of the hepatic pedicle with left branch of hepatic artery and portal vein absent. Immunosuppression was based on a triple association of steroids, prograf and mycophenolic acid. The postoperative course was uneventful and the patient was discharged after 10 d. Pathological examination of the explanted right lobe showed an healthy liver with 3 HA measuring 6, 3.5 and 2.2 cm respectively. Three years after LTx the patient is carrying on a normal life.

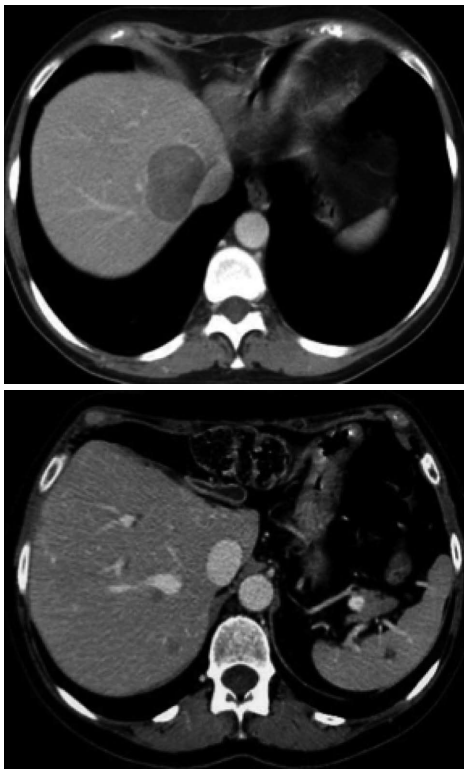

Figure 2 Time of second recurrence. Three hepatic adenomas ill located that could not be resected.

DISCUSSION

HA is a rare benign epithelial monoclonal tumor of the liver with an incidence of 3/1000.000 per year in Europe and North America[1]. It is frequently seen in clinical practice due to diffuse use of imaging radiology performed for unrelated reasons and consequently it is often discovered incidentally[1]. Its natural history has been better defined recently. HA are more frequent in women and are usually solitary lesions. Oral contraception is a classical cause of HA in women while the metabolic syndrome appears as an emerging condition associated with malignant transformation of HA in men[7,8]. The risk of bleeding varies from 21% to 50% and the risk of malignancy is around 8%-10%[9,10]. It seems that the risk of complication is unrelated to the number of HA but associated with a size > 5 cm particularly in telangiectatic and unclassified subtypes[8]. HA have been recently classified into four different groups due to the expression of three gene mutations: hepatocyte nuclear factor 1-alpha (35%-50%), β-catenin (15%-18%), serum amyloid A and C reactive protein (40%-55%) and unclassified type (< 10%)[11,12]. HA with aberrant nuclear β-catenin expression have higher risk of HCC development[13]. The risk of cancer degeneration is superior in men (47%) and the prevalence of malignancy is ten times more frequent in

men than in women[9] and in lesions larger than 4-5 cm.

Although several strategies are available for HA management, there is still a debate on the best treatment. Clinical observation is indicated in small adenomas less than 4 cm in diameter and for lesions that show a regression after stopping oral contraceptives (OC). Surgical resection should be taken into consideration in cases in which the risk of complication (bleeding and malignant transformation) is increased, in female patients with a wish for pregnancy with HA $\geqslant$ 5 cm, in post-menopausal women with HA > 5 cm after 6 mo of stop OC and in males regardless of HA size due to the high risk of β-catenin mutation[9,14]. It is more controversial the management of patients with multiple HA. Surveillance, hepatic resection or non-surgical treatments such as RFA and TAE are all valid options[15,16].

After resection a close monitoring is mandatory even if HA are benign tumors. HA have the tendency to recur (8%) as was highlighted even by our case[8]. The peculiar aspect of our case is that despite a complete resection of the nodule the patient had two local recurrences over a period of three years and at the time of the second recurrence the lesions were ill located and the patient was no more suitable for hepatic resection. LT appeared to be the best treatment of choice.

In such situation of recurrent HA the management is initially non-operative and based on strict radiological follow-up. Hepatic liver resection is again indicated when HA reaches 5 cm with an increased risk for complications. In patients who are not eligible for surgical resection or RFA and TAE, liver transplantation may be a valid option in highly selected cases.

Hepatic resection for HA should be always attempted when technically feasible. The postoperative results are excellent even in cases of extended liver resections. Depending on center experience, the morbidity associated to liver resections for benign tumors ranges from 10% to 25% and mortality from 0% to 3%[17]. In such situations surgery is often curative, avoids the risk of a liver transplant procedure, delays the exposition to immunosuppressor. Moreover the pathological examination excludes the possibility of malignant transformation, that in case of giant adenoma is often not possible through radiology or core needle biopsy.

Indications for LTx in patients with HA are very limited to cases of HA (giant, multiple or recurrent and progressive adenoma) in which hepatic resection is not technically feasible. In PubMed literature only 20 cases of LTx for HA are reported[1-6]. However up to 2008, United Network for Organ Sharing and European Liver Transplant Registry count 103 cases of LTx for HA which represent about 6%-11% all LTx performed for benign liver conditions and 0.085%-0.094% of all indications[18]. Literature highlights the risk of HCC development in the transplanted liver or lung metastasis[1]. This review of HA may rise some concerns particularly with regard to patient's follow-up and management of immunosuppression after transplantation.

LTx for recurrent HA is a technically demanding procedure as showed by our case and shows some different technical aspects if compared to a LTx performed for chronic liver disease. First, surgeons must take into consideration that in such situation a previous hepatectomy is responsible for the presence of adhesions that need to be divided before reaching the liver and the hepatic pedicle. Furthermore, after a previous major hepatic resection at least one of major hepatic veins is missing and the hepatic pedicle at bifurcation misses one major branch of portal vein and hepatic artery. These anatomical changes may render the liver implantation more difficult. On the contrary, the absence of portal hypertension could be an advantage when the adhesions are divided since they do not show an intrinsic vascular pattern.

HA and liver cell adenomatosis are benign tumors of the liver and they are often diagnosed incidentally. Nevertheless these clinical conditions can be complicated by bleeding and a malignant transformation can occur in about 10% of the cases with a prevalence in men with an associated metabolic syndrome. Liver resection is considered to be the treatment of choice in case of single lesions that can be completely resected. In case of recurrence liver transplantation represents a definitive and curative treatment but it should be taken into consideration as the last therapeutic option in patients in whom hepatic resection is indicated but not technically feasible. Moreover, the donor grafts shortage and the high risk of morbidity and mortality related to liver transplantation, let this treatment be not a standard option but an extraordinary choice in a few particular cases.

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CASE REPORT

Extrahepatic aneurysm of the portal venous system and portal hypertension

Wilma Debernardi-Venon, Davide Stradella, Greta Ferruzzi, Filippo Marchisio, Chiara Elia, Mario Rizzetto

Wilma Debernardi-Venon, Davide Stradella, Greta Ferruzzi, Chiara Elia, Mario Rizzetto, Gastro-Hepatology Unit, San Giovanni Battista Hospital, University of Turin, 10126 Turin, Italy
Filippo Marchisio, Diagnostic and Interventional Radiology Unit, San Giovanni Battista Hospital, University of Turin, 10126 Turin, Italy
Author contributions: Debernardi-Venon W and Stradella D contributed equally to designing the study, performing the research, analyzing the data and writing the paper; Ferruzzi G performed the research and wrote the paper; Elia C analyzed the data; Marchisio F reconstructed and analyzed the computed tomography images; Debernardi-Venon W and Rizzetto M reviewed the manuscript for intellectual content; and all authors approved the final version of the manuscript.
Correspondence to: Wilma Debernardi-Venon, MD, Gastro-Hepatology Unit, San Giovanni Battista Hospital, University of Turin, Corso Bramante 88, 10126 Turin, Italy. wilmadebernardi@yahoo.it
Telephone: +39-11-6335558 Fax: +39-11-6335927
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Abstract

Portal venous aneurysm (PVA) is a rare condition characterized by dilatation of the portal venous system. PVA manifestation of symptoms is varied and depends on the aneurysm size, location and related-complications, such as thrombosis. While the majority of reported cases of PVA are attributed to portal hypertension, very little is known about the condition's pathophysiology and clinical management remains a challenge. Here, we describe a 67-year-old woman who presented with complaint of dyspepsia and without a significant medical history, for whom PVA was incidentally diagnosed. The initial upper abdominal ultrasound revealed marked dilatation of the main portal vein, and subsequent contrast-enhanced computed tomography with angiography revealed a large aneurysm arising from the extrahepatic troncus portion of the portal vein, as well as gastroesophageal varices. A conservative approach using beta-blocker therapy was chosen. The patient was followed-up for 60 mo, during which time the asymptomatic status was unaltered and the PVA remained stable.

Key words: Portal vein aneurysm; Portal hypertension; Gastroesophageal varices; Hepatic venous pressure gradient

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INTRODUCTION

Portal vein aneurysm (PVA) is a disorder of hepatic vascularization that is clinically diagnosed upon imaging findings of venous dilatation larger than 2 cm[1]. The majority of reported PVAs are localized to the main portal vein. Moreover, an appreciable amount of PVAs are associated with artero-venous fistula or vascular malformations, such as hereditary teleangectasia[2]. PVAs are classified as congenital or acquired, and etiologies of the acquired cases include chronic liver disease, pancreatitis, abdominal surgery, or trauma[3]. The manifestation of PVA symptomatology is various and can depend upon the size and related complications, such as thrombosis. However, many cases are asymptomatic and are detected as an incidental finding in routine radiological examinations performed for other indications[2,4-6].

PVA, including asymptomatic types left undiagnosed or untreated, are associated with several severe complications with high mortality rates, including rupture, local compression, or portal hypertension[7-10]. While recent

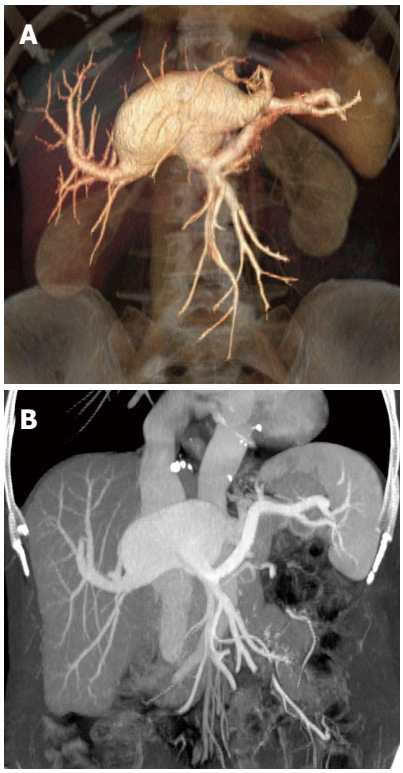

Figure 1 Computed tomography. A: Volume rendering reconstruction of the aneurysm; B: Maximum intensity projection of the aneurysm.

advances in imaging technologies have increased the number of PVA routinely discovered, our knowledge of the underlying pathophysiology remains limited and clinical management remains a challenge.

CASE REPORT

A 67-year-old Caucasian woman was referred to our gastroenterological unit for evaluation of dyspeptic complaint. The patient's past medical history was unremarkable, and she denied experiencing recent weight change or melena, or abusing alcohol. Initial physical examination revealed no evidence of abdominal pain, jaundice, or hepatomegaly. Results of standard blood tests, including the hepatic function marker profile panel, were within reference ranges. An upper abdominal ultrasound revealed marked dilatation of the main portal vein (diameter: 4 cm; length: 8.5 cm). However, the superior mesenteric vein, splenic vein, and other extrahepatic segments of the portal vein appeared normal. No features of thrombosis were present. The liver itself showed homogeneous parenchyma and normal size. The spleen and pancreas also appeared grossly normal. Subsequent contrast-enhanced computed tomography with angiography (CTA) was performed. The aneurysm was found to arise at the troncus of the extrahepatic portion of the portal vein, proximal to its bifurcation (Figure 1A). The overall size was measured as 8 cm × 4.5 cm, and the neck was 0.2-0.3 cm. The posterior of the aneurysm was located at the portal confluence (Figure 1B). Turbulent blood flow was observed inside the aneurysm, and again no signs of thrombosis were observed (Figure 2). The CTA scanning also showed the spleen to be moderately enlarged (oblique diameter: 12 cm), the pancreas to be normal, and presence of gastric varices (Figure 3). No biliary stones were detected. Upper gastrointestinal endoscopy was performed and revealed small gastroesophageal varices.

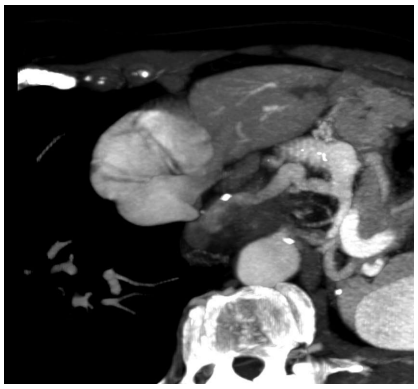

Figure 2 Computed tomography multi-planar reformation oblique and maximum intensity projection of the esophageal varices' venous collector. Observation of slow refilling indicated prehepatic portal hypertension.

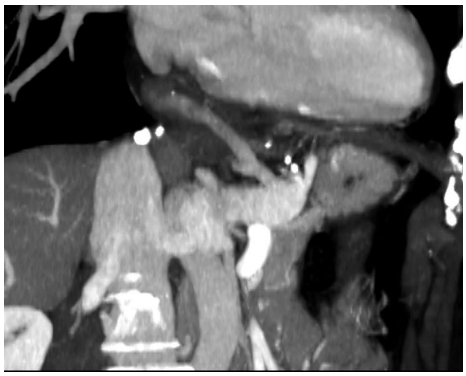

Figure 3 Computed tomography oblique maximum intensity projection on the long axis of the variceal collector showing the gastric varix.

The hepatic vein-portal pressure gradient (HVPG) was normal (6 mmHg), confirming a prehepatic origin of portal hypertension. The presence of esophageal varices and signs of portal hypertension prompted the initial consideration of an intrahepatic portocaval shunt to reduce the portal pressure and maintain blood flow. However, the risk of shunt dysfunction and the patient's refusal for intervention led to the use of a medical therapy approach. The patient was administered carvedilol (50 mg once daily) and monitored by laboratory testing and sonographic imaging every 12 mo. The patient attended routine follow-up appointments, the last being 60 mo after discharge to home. All follow-up examinations indicated asymptomatic status and maintenance of the PVA as stable.

DISCUSSION

Diagnosis of PVA is often overlooked in asymptomatic

patients. Symptomatic clinical presentation is often the result of PVA rupture or secondary thrombosis, portal hypertension, or biliary compression-induced cholestasis[3,7-10]. In the patient described herein, the PVA was diagnosed incidentally by ultrasonographic and CTA examinations, in accordance with its asymptomatic nature. The patient lacked a history of risk factors or indicating signs of PVA, which suggests that the aneurysm may be congenital. The patient's portal hypertension was determined to be secondary to an increased portal blood flow. The prehepatic origin of inflow justified the HVPG, which is typically normal in prehepatic portal hypertension. The asymptomatic nature of the PVA somewhat complicated the choice of therapy and the patient's willingness to accept various treatment options.

Portocaval shunting is a successful treatment approach frequently performed in patients with complicated portal aneurysms accompanied by portal hypertension, and intrahepatic portosystemic shunting is considered an effective option for cirrhotic patients as it may prevent the risk of portal thrombosis[11,12]. Accordingly, we considered that a portosystemic shunt may reduce the portal pressure and turbulent flow within the aneurysm to achieve blood stasis in our patient, thereby reducing the risk of thrombosis and rupture. However, the intrahepatic portosystemic shunt is not a recognized therapy of portal aneurysm, and the risk of dysfunction in a patient without history of variceal bleeding is uncertain. This fact, in conjunction with the patient's preference, led us to apply a conservative management approach. A beta-blocker was selected for the therapy since the patient's gastroesophageal varices could be a contraindication for anticoagulation therapy[13].

In conclusion, the findings from our case highlights the benefit of ultrasonography and CTA scanning to diagnose asymptomatic PVA and suggests its utility for monitoring PVA patients following conservative therapy.

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CASE REPORT

Case of acute hepatitis E with concomitant signs of autoimmunity

Catarina Lima Vieira, Cilénia Baldaia, Narcisa Fatela, Fernando Ramalho, Carlos Cardoso

Catarina Lima Vieira, Cilénia Baldaia, Narcisa Fatela, Fernando Ramalho, Carlos Cardoso, Department of Gastroenterology and Hepatology, Hospital Santa Maria, Avenida Professor Egas Moniz, 1649-035 Lisbon, Portugal
Catarina Lima Vieira, Cilénia Baldaia, Narcisa Fatela, Fernando Ramalho, Carlos Cardoso, Department of Virology, Laboratory Dr. Joaquim Chaves, 1500-486 Lisbon, Portugal
Author contributions: All the authors contributed equally to this work.
Correspondence to: Catarina Lima Vieira, MD, Department of Gastroenterology and Hepatology, Hospital Santa Maria, Avenida Professor Egas Moniz, 9, 1649-035 Lisbon, Portugal. rinacilv@gmail.com
Telephone: +351-918839182 Fax: +351-217802775
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Abstract

Sporadic cases of acute viral hepatitis E have been described in developed countries, despite the more common occurrence in endemic areas and developing countries. We present the case of a 58 years old Portuguese female, with no epidemiological relevant factors, admitted with acute hepatitis with positive anti-nuclear antibodies, anti-smooth muscle antibody and high serum gamma globulin (> 1.5 fold increase). Serologies for hepatitis A virus, hepatitis B virus, hepatitis C virus, Epstein-Barr virus, cytomegalovirus, hereditary sensory neuropathy and varicella zoster virus were negative. Liver biopsy histology revealed changes compatible with autoimmune hepatitis. Prednisolone and azathioprine was started. She tested positive for immunoglobulin M anti hepatitis E virus (HEV) with detectable viremia by reverse transcription polymerase chain reaction (RT-PCR) technique. HEV-RNA was confirmed through RT-PCR in a liver specimen, establishing the diagnosis of acute hepatitis E. Immunosuppression was stopped. She clinically improved, with resolution of laboratory abnormalities. Therefore, we confirmed acute hepatitis E as the diagnosis. We review the literature to elucidate about HEV infection and its autoimmune effects.

Key words: Hepatitis; Hepatitis E; Autoimmune; Ribavirin

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INTRODUCTION

Acute hepatitis can result from a variety of etiologies, which include viral [hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus, hepatitis E virus (HEV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus, varicella zoster virus] etiologies, autoimmune hepatitis (AIH), non alcoholic steatohepatitis, toxins and metabolic disorders, like Wilson's disease. Epidemiology is an important factor. HEV, an enterically transmitted virus, occurs mainly in developing countries. Swine seem to function as a reservoir for HEV. The majority of cases have been reported in pregnant women, immunosuppressed patients and in those with contact with swine. However, recent data suggest that the HEV could be more widespread than previously thought, with more sensitive tests increasing detection rates[1]. Cases of sporadic HEV have also been described[1]. To diagnose AIH using the International Autoimmune Hepatitis Group (IAIHG) score for AIH, the exclusion of other etiologies is required before classifying AIH as probable or definitive[2]. When using the recent simplified criteria for AIH[3], the exclusion of viral etiology is one of the 4 criteria, together with liver histology, gamma globulin level and antibody markers [antinuclear antibodies (ANA), anti-smooth muscle antibody

(ASMA), liver kidney microsomal (LKM), soluble liver antigen (SLA)]. More sensitive and accurate tests used for screening for viral etiologies may lead to reclassification of some cases of AIH as being of viral etiology. Two cases of AIH were reclassified as HEV after obtaining the HEV status[4,5]. However, false positive results for anti-HEV have been reported in patients with AIH. In 1997, the Virology Institute of Tour, France, reported that the ABBOT method showed 13% positive results for anti-HEV in chronic AIH which turned out to be negative using the synthetic-peptide based test[6]. Therefore, in a patient presenting as an acute hepatitis and previously considered as being of autoimmune etiology, a positive result of anti-HEV requires the use of more accurate tests, determining viremia or liver immunohistochemistry markers in order to rule out false positive results. We present the case of a patient with acute hepatitis that, according to the IAIHG score, was considered to be a probable AIH, which turned out to be positive for anti-HEV with detectable viremia.

CASE REPORT

A 58 years old female Caucasian patient was admitted to our hospital with a history of progressive fatigue and anorexia over 6 wk and markedly increased liver enzymes. She had a past medical history of rheumatic fever, with normal cardiac function, nephrolithiasis, arterial hypertension, diabetes mellitus, major depression and recent traumatic fracture of the wrist (3 mo before), medicated with venlafaxine, mirtazapine, lisinopril, nebivolol, bromazepam and diclofenac (last taken 3 mo before admission and maximum 1 per day). She had no history of contact with animals or previous stays in regions endemic for hepatotropic viruses, and mentioned regular swine meat ingestion. There was no history of immunosuppressive therapy. On examination, she was apyretic and subicteric. There was no evidence of clinical encephalopathy, pruritus, dermatological lesions, hemorrhagic dyscrasia or abdominal pain. Laboratory analysis showed altered liver enzymes with aspartate aminotransferase (AST) 2088 IU/L (0-34); alanine aminotransferase (ALT) 2638 IU/L (10-49); alkaline phosphatase 233 IU/L (45-129); gamma-glutamyl transpeptidase 197 IU/L (< 38); total bilirubin 3.17 mg/dL (< 1.0); prothrombin time 13.7/11.6 s; V factor 122% (50-100); Ⅶ factor 40% (70-130); gamma globulin 3.15 g/dL (0.68-1.60); and immunoglobulin G (IgG) 3850 mg/dL (700-1600). The serological tests for autoimmune hepatitis revealed positive ANA (titer 1:320 with cytoplasmic pattern); anti-DsDNA (824 IU/mL; < 200); ASMA positive, thyroid auto-antibodies, type anti-thyroid peroxidase antibody (243 IU/mL; < 50) with negative tests for anti-liver kidney microsomal, anti-myocardial antibody, SSA, single-strand binding protein, ribonucleoprotein, HS70 and JO1. Viral serology was negative for HAV [IgM (-); IgG (-)], EBV [IgM (-); IgG (-)], CMV [IgM (-); IgG (-)], HBV (AgHBs, AgHbe, AcHBc, antiHBs), negative anti-HCV with undetectable RNA of HCV and HIV antigens types 1 and 2 both negative. Serological tests for Coxiella burnetti and leptospirosis were negative. Tests for iron and copper metabolic disorders were negative. Laboratory tests showed normal alpha1 antitrypsin complement and IgG4 levels. Thyroid function tests were also normal. The abdominal Doppler ultrasound was normal. A percutaneous liver biopsy was performed and compatible with autoimmune hepatitis, revealing intense interface hepatitis, with lymphoplasmacytic portal, septal and acinar infiltrate with focal hepatocellular necrosis and areas of periportal fibrosis. The patient did not have an epidemiological history favoring HEV and, prior to knowing its results, the IAIHG pre-treatment score was 14 points considering a negative viral study [female sex (+2); ALP/AST < 1.5 (+2); IgG > 2 × normal upper limit (+3); ANA > 1:80 (+3); negative AMA; alcohol intake < 25 g/d (+3); hepatotoxic drugs present (-4); interface hepatitis (+3)]. As the patient met the absolute criteria with AST, ALT > 10 × the upper limit of normal, a diagnosis of type 1 AIH was considered and therapy was started on day 7[7]. Retrospectively, we realized that both the AST and ALT levels (Figure 1) were already slowly decreasing 24 h before the immunosuppressive therapy was started. After one week on prednisolone 30 mg/d and azathioprine 50 mg/d, there was a significant reduction in the laboratory values: AST 245 U/L; ALT 835 U/L; ALP 148 U/L; GGT 214 U/L; total bilirubin 1.06 mg/dL; prothrombin time 12.5/11.6 (Figure 1, day 12). She was discharged with the combined immunosuppressive treatment for AIH. There was a progressive improvement of symptoms. One month after discharge, she was asymptomatic with normal total bilirubin (0.67 mg/dL) and gamma globulin (1.25 g/dL) but AST and ALT were still > 2 × the upper limit (AST 94 U/L; ALT 158 U/L). The results of serological tests for HEV were obtained after the patient was discharged. The patient had positive IgM serology for HEV and negative serology for IgG. The tests were performed using the enzyme-linked immunosorbent assay method by MP Biomedicals (IgM determination: sensitivity 95.8%; specificity 97.6%). Positive viremia was detected by real time polymerase chain reaction (PCR) but not quantified. This technique has

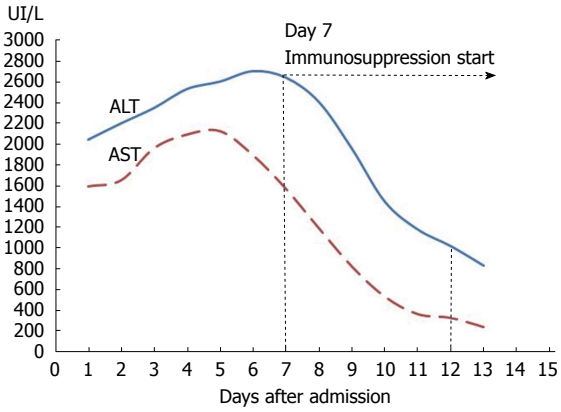

Figure 1 Evolution of aspartate aminotransferase and alanine aminotransferase during the hospitalization period. Introduction of immunosuppression was as shown in the figure at day 7, when aspartate aminotransferase (AST) levels were already lowering. ALT: Alanine aminotransferase.

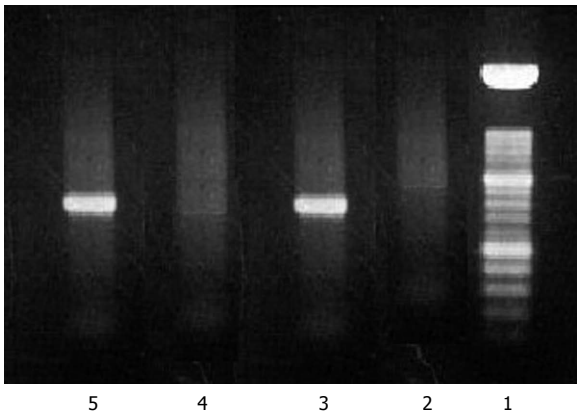

Figure 2 Electrophoresis with agarose gel 2%. Reverse transcription polymerase chain reaction (RT-PCR) for hepatitis E virus (HEV) RNA from the liver specimen was performed using a First Strand cDNA synthesis kit for RT-PCR from Roche Diagnostics (Indianapolis, United States) according to the manufacturer's instructions. This figure demonstrates the positive detection of HEV RNA with compatible strands 5 and 3. 1: Marker 100 bp (Promega); 2: White amplification; 3: Positive control for HEV RNA; 4: Negative control for HEV RNA; 5: Liver biopsy sample (HT370).

Table 1 The evolution of antibodies

Day after admission	ANA; anti DsDNA titers (UI/mL)
0	1/320; 840
30	1/160; 520
90	Negative; < 200
180	Negative; < 200

ANA: Anti-nuclear antibodies.

an overall sensitivity of 98% with 100% specificity and uses a primer targeting the ORF2 protein. The sample was processed with the MagnaPure system (Roche) and amplified with StepOnePlus (Applied Biosystems). To complete the study, HEV RNA was extracted from liver tissue using Roche MagnaPure kit (Indianapolis, United States). Two sets of primers for reverse transcriptase (RT)-PCR and nested PCR were synthesized by TIBMol, based on the highly conserved region of HEV ORF 2 of the United States (GenBank accession No. AF060668 e AF060669), Japanese (AB089824), Burmese (M73218 and D10330) and Korean swine (AF516178, AF516179, and AF527942) strains. RT-PCR was performed using a First Strand cDNA Synthesis kit for RT-PCR (AMV) from Roche Diagnostics (Indianapolis, United States) according to the manufacturer's instructions. Figure 2 demonstrates the positive detection of HEV-RNA. Serotype determination was not available at the laboratory. Therefore, the detectable viremia and the positive molecular testing in liver biopsy allowed the diagnosis of acute hepatitis E in our patient. The patient was reassessed at the outpatient clinic nearly two months after hospitalization. She was asymptomatic and because of the positive tests for HEV, immunosuppressive therapy was stopped. HLA study revealed a DRB1*03 subtype. Nearly 6 mo after hospitalization, AST, ALT, gamma globulin, ANA, anti DsDNA

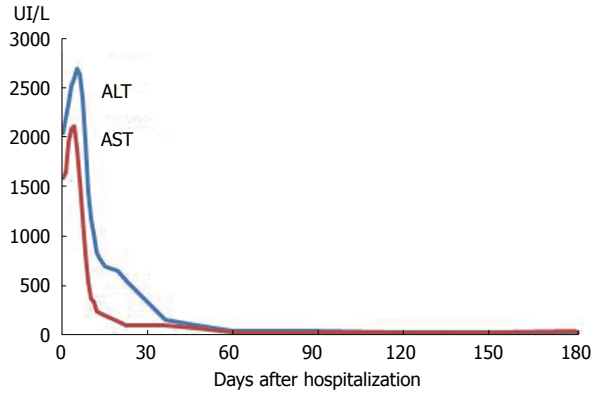

Figure 3 Evolution of aspartate aminotransferase and alanine aminotransferase until the 6th month after the hospitalization, with normalization soon after day 30, under no immunosuppression. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

and ASMA were within normal range, after more than 4 mo with no immunosuppression treatment (Figure 3 and Table 1). First day, the serum gamma globulin was 3.15 g/dL; 30 d, the serum gamma globulin was 3.0 g/dL; 90 d, 1.5 g/dL; 180 d, 1.4 g/dL. The serum gamma globulin levels evolved over time, with normal values 6 mo after the admission under no immunosuppression. The patient remained asymptomatic during the 6 mo follow up period.

DISCUSSION

In the clinical setting of an acute hepatitis, according to the IAIHG score, the positive serologies for ANA, ASMA, high immunoglobulin and a compatible liver histology added up to a score of 14 points, which suggests a probable diagnosis for AIH. Even without considering the 3 points given for the viral negativity, the score would still be suggestive of probable AIH. Our patient improved, both clinically and biochemically on immunosuppressive treatment. The positivity for thyroid autoantibodies favored a diagnosis of autoimmune disease. However, the improvement could be coincident with a self-limited viral hepatitis with a gradual recovery course, considering that both AST and ALT values started to decrease before the therapy was started. Sporadic cases of HEV in non-endemic areas are rare[1]. The patient's history and previous health status were not suspicious for viral hepatitis E. There have been case reports on the effect of HEV in cirrhotic patients and false positive results of anti-HEV antibodies in patients with chronic liver disease of autoimmune etiology[6]. However, our patient had no evidence of liver cirrhosis. The presence of detectable viremia and positive liver molecular biology confirmed the diagnosis of hepatitis E infection. There are several relevant issues which arise because of this patient. Firstly, even in non-endemic areas, HEV must be considered in the differential diagnosis when approaching a patient with an acute hepatitis. Secondly, if a patient tests positive for IgM, it can be a false positive result and viremia and/or molecular liver histology

and immunohistochemical testing should be performed. IgM and IgG titers and their evolution in serial analysis could also be used to determine the hepatitis time frame. Viremia is detectable for 4 mo and is better determined with real-time PCR techniques. Compared to single and nested gel-based RT-PCR, the various real-time PCR assays have a higher sensitivity, are less laborious, save time and are less prone to cross contamination. Thirdly, it is known that a minority of patients (3%-5%) with chronic AIH can present with low HEV viremias[6], highlighting the possibility of HEV also being a trigger for the development of AIH. Also, one must consider the possibility of HEV inducing serological abnormalities such as hyperimmunoglobulinemia, possibly through polyclonal stimulation mimicking AIH[5]. The anti-DSDNA positivity in our patient, with no other criteria for systemic lupus erythematosis[8], could be interpreted as a non-specific crossed reaction finding. Although we gave the diagnosis of a viral hepatitis E infection in our patient, follow up will be necessary to determine if the serological and histological findings were due to mechanisms of non-specific reactions to a viral infection or even a specific reaction to a specific HEV strain that could trigger AIH in the future. Also, reassessing serology and serum HEV RNA detection through RT-PCR could be of value to characterize this case as an acute episode with spontaneous virological clearance or evolution to chronic hepatitis E, as it is now well known that this can occur, although mainly in transplanted livers. Acute hepatitis E can evolve to hepatic failure and ribavirin in monotherapy use has been reported in some patients to avoid the need for liver transplantation. In the setting of chronic hepatitis E in liver transplanted patients, after failure of immunosuppression reduction, ribavirin monotherapy for a minimum period of 3 mo is advised in order to clear the virus, through mechanisms yet not fully understood[9]. Given the normalization of symptoms and laboratory parameters and considering the high cost of the serological and PCR techniques used at our institution, and also considering the high diagnostic accuracy of the tests used during work up, we did not reassess the HEV RNA determination at 6 mo. In 1991, hepatitis A was identified as a trigger for autoimmune chronic hepatitis type 1 in individuals with a familial history of AIH. Our patient was a case of HEV and had no familial history of autoimmune diseases. However, the authors elucidated the mechanism through which virus could trigger AIH, besides molecular mimicry. In this case, HEV induced a defect in suppressor-inducer T lymphocytes specific for the asialoglycoprotein receptor, a surface receptor of the hepatocyte, which increases in HAI and induces cell inflammation[10].

In conclusion, HEV should be considered in the differential diagnosis of an acute hepatitis, even in patients with a possible diagnosis of AIH. Further studies are needed to understand the interaction between AIH presenting as acute hepatitis and viral hepatitis E infection.

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CASE REPORT

Genetically confirmed Wilson disease in a 9-month old boy with elevations of aminotransferases

Joo Whee Kim, Jong Hyun Kim, Jeong Kee Seo, Jae Sung Ko, Ju Young Chang, Hye Ran Yang, Kyung Hoon Kang

Joo Whee Kim, Jeong Kee Seo, Jae Sung Ko, Ju Young Chang, Hye Ran Yang, Department of Pediatrics, Seoul National University Children's Hospital, Jongro-gu, Seoul 110-744, South Korea
Jong Hyun Kim, Department of Pediatrics, St. Vincent's Hospital, The Catholic University of Korea, Paldal-gu, Suwon, Kyounggi-do 423-067, South Korea
Kyung Hoon Kang, Department of Pathology, Seoul National University Children's Hospital, Jongro-gu, Seoul 110-744, South Korea
Author contributions: Kim JW, Kim JH, Seo JK, Ko JS, Chang JY and Yang HR wrote the case report; Kang KH provided the slides from the patient.
Correspondence to: Jeong Kee Seo, MD, PhD, Professor, Department of Pediatrics, Seoul National University Children's Hospital, 28 Yongon-dong, Jongro-gu, Seoul 110-744, South Korea. jkseo@snu.ac.kr
Telephone: +82-2-20723627 Fax: +82-2-7433455
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Abstract

Wilson disease (WD) is an autosomal recessive disorder of copper transport caused by alteration of the adenosine triphosphatase 7B gene. It is rare to diagnose WD below the age of three years. Molecular genetic testing is one of the most important diagnostic methods and may confirm the diagnosis in equivocal cases. We report a case of a 9-mo old boy with WD who presented as chronic hepatitis. Genetic analysis showed compound heterozygotes of p.G1186S and c.4006delA.

Key words: Hepatolenticular degeneration; Wilson disease; Early diagnosis; Molecular genetics; Mutation

Kim JW, Kim JH, Seo JK, Ko JS, Chang JY, Yang HR, Kang KH. Genetically confirmed Wilson disease in a 9-month old boy with elevations of aminotransferases. *World J Hepatol* 2013; 5(3): 156-159 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i3/156.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i3.156

INTRODUCTION

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism that results in an accumulation of copper in the liver, brain and other tissues. The prevalence of WD is between 1 in 30 000 and 1 in 100 000 persons worldwide and in a nationwide survey, the prevalence of WD in South Korea is 1:37 000[1,2]. It has been demonstrated that children with WD usually have no symptoms below the age of 3 years. However, cases of WD presenting as a liver disease at 13 mo[3] and 2 years[4] have been reported, as well as diagnosis by asymptomatic sibling screening. Recently, molecular genetic testing has shown to be one of the most important diagnostic methods and may confirm the diagnosis in equivocal cases. We report a case of a 9-mo old infant with WD diagnosed by direct molecular genetic testing, whose hypertransaminasemia was normalized with penicillamine therapy.

CASE REPORT

A 9-mo old male infant was found to have hypertransaminasemia on routine laboratory investigation when he visited for acute diarrhea. Although clinical symptoms of enteritis improved, aminotransferase levels remained persistently high. He had no other symptoms and his development was normal for his age. All the etiological investigations of hypertransaminasemia (hepatitis A virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, rubella virus, herpes virus, toxoplasmosis, autoimmune hepatitis, myopathies) were negative. However, the serum ceruloplasmin level was below normal (< 3 mg/dL) and serum copper level was 37.4 μg/dL. He had no abnormal neurological findings and a Kayser-Fleischer

Table 1 Clinical and biochemical features of the patient

Age (mo)	9	22	23	24	26	27	30
AST/ALT (IU/L)	102/122	113/144	90/105	171/194	185/285	262/401	111/162
Cu in urine (μg/24 h)			14.9	15.2	62.2		421.4
Ceruloplasmin (mg/dL)		< 9	< 9		< 8		< 8
Mutation			p.G1186S/c.4006delA				
K-F ring		No					
Cu in the liver (μg/g dry weight)						748	

AST: Aspartate aminotransferase; ALT: Alanine aminotransaminase.

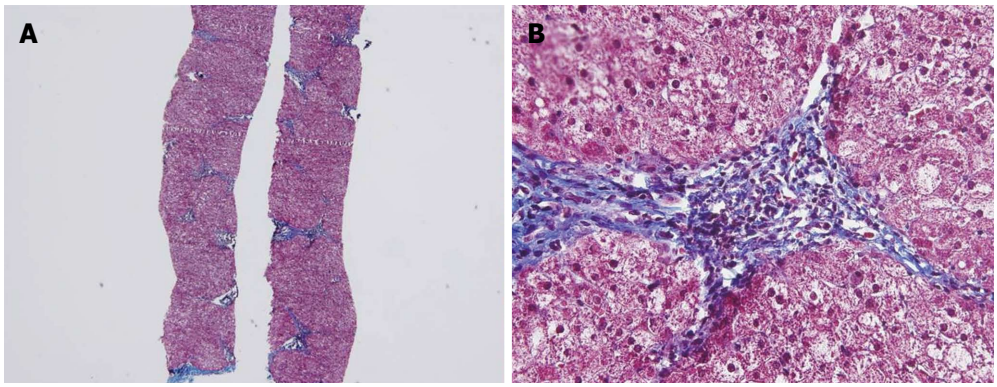

Figure 1 Histological findings. Biopsy specimen showing mild lobular activity, mild portoperiportal activity and periportal fibrosis with frequent portal to portal bridging fibrosis. A: Masson's Trichrome stain, 40×; B: Masson's Trichrome stain, 400×.

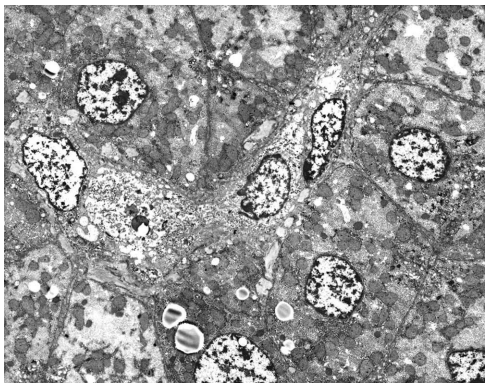

Figure 2 Electron microscopic examination. There was no evidence of abnormal mitochondria. Some fat vacuoles are found. In the portal area, a few inflammatory cells and collagen depositions are observed (original magnification 2000×).

corneal ring was not found on a slit-lamp examination. Laboratory investigations revealed a hemoglobin level of 12.8 g/dL, with normal white blood cell and platelet counts. The serum total bilirubin was 0.3 mg/dL, albumin level was measured at 4.1 g/dL. Prothrombin time was 12.7 s and the international normalized ratio was 6.54. However, a 24-h urinary copper excretion was normal in a repeated test (Table 1). Percutaneous liver biopsy was performed. Histological evaluation revealed mild lobular activity, mild portoperiportal activity and periportal fibrosis with frequent portal to portal bridging fibrosis (Figure 1). Quantitative determination of hepatic copper level was elevated (748 μg/g dry weight of liver tissue). On electron microscopic examination, there was no evidence of abnormal mitochondria. Some fat vacuoles were found. In the portal area, a few inflammatory cells and collagen depositions were observed (Figure 2). The diagnosis of WD was confirmed by molecular analysis of the adenosine triphosphatase 7B (*ATP7B*) gene, which showed that the child had a compound heterozygote of p.G1186S and c.4006delA. The mutation p.G1186S has previously been described in a Japanese patient[5]. The mutation c.4006delA was a novel frameshift mutation, resulting in a stop codon (Figures 3 and 4).

Therapy with zinc 24 mg twice a day was immediately started, as well as a low copper diet. However, after commencing zinc treatment, aminotransferase level increased; aspartate transminase 262 IU/L; alanine transminase 401 IU/L (Table 1). The therapeutic regimen was switched to D-penicillamine 125 mg twice a day (22 mg/kg per day), pyridoxine 12.5 mg once a day and zinc. At regular follow-ups under medical therapy, hypertransaminasemia was normalized and currently he continues to do well.

DISCUSSION

WD is an autosomal recessive disorder of copper transport that results in an accumulation of copper, primarily in the liver, brain and the cornea, and was first described as a syndrome by Wilson[6] in 1912. WD is the most common inherited liver disease, with a prevalence of 1:37 000 in the pediatric population in South Korea[2,7].

In recent years, molecular testing for *ATP7B* muta-

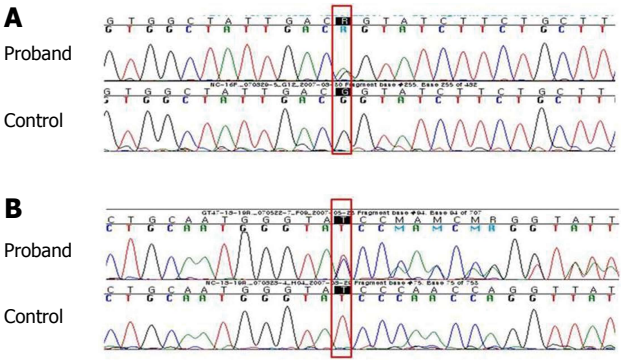

Figure 3 Adenosine triphosphatase 7B sequencing. A: c.3556G>A, p.G1186S, heterozygote; B: c.4006delA, p.Ile1336TyrfsX57 (reverse complementary sequence, STOP 1392), heterozygote.

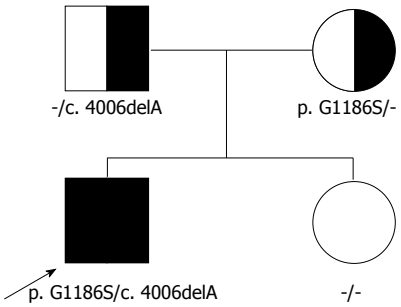

Figure 4 Pedigree. The patient has a compound heterozygote of p.G1186S and c.4006delA.

tions has been used to aid in diagnosis. More than 500 mutations in the *ATP7B* gene are now recognized (www.medicalgenetics.med.ualberta.ca/wilson/index.php). The p.R778L (an allele frequency of 37%), p.A874V (13%), p.L1083F (8%) and p.N1270S (6%) are the common major mutations in South Korea[2,7]. The identification of one mutation may be adequate to confirm the diagnosis if characteristic clinical symptoms and at least some of the biochemical features are present and if the one mutation detected is clearly established as a disease-causing mutation[8]. Direct DNA sequencing did confirm WD in 98% of the Korean patients. Two mutations were detected in 70% and one mutation in 28% of the patients who showed characteristic biochemical and clinical findings of WD[2]. Iorio *et al*[3] reported a case of a 13-mo old child with WD who was diagnosed by biochemical family screening. However, the molecular analysis had not revealed known mutations in either the patient or her 8-year old brother. The 9-mo old infant in the present study represents the youngest patient confirmed by two *ATP7B* mutations, even in the absence of a family history of WD.

The diagnosis of WD is based upon the clinical setting combined with compatible biochemical, histological and physical findings. None of the laboratory parameters alone allows for a definite diagnosis of WD, particularly in infants and young children. At a consensus meeting, a scoring system was devised by Ferenci *et al*[9] in 2003, based on a composite of biochemical, histological and genetic features. In 2008, the American Association for Study of Liver Diseases guidelines provided diagnostic approaches and specific recommendations on the basis of published data and the researcher's experience in caring for patients with WD[10]. The biochemical diagnosis relies upon demonstration of abnormal copper parameters, such as low serum ceruloplasmin concentration, high urinary copper and increased hepatic copper concentration. A combination of any two of these three findings is strong support for a diagnosis of WD[8]. In South Korea, in a nation-wide survey of WD, low serum ceruloplasmin (< 20 mg/dL), high 24-h urine copper excretion (> 100 μg), high hepatic copper contents (> 250 μg/g of dry liver) and Kayser-Fleischer (KF) rings were found in 96%, 86%, 88% and 73% of the patients with WD respectively[2,7].

There are numerous pitfalls in the diagnosis of WD because of the lack of any single sensitive and specific diagnostic test. Serum ceruloplasmin concentrations are elevated by acute inflammation and may be low in conditions associated with a hypoproteinemic state. Particularly in early infancy to the age of 6 mo, serum ceruloplasmin concentrations are physiologically very low. In one series of WD in children, 2 of 57 cases had a normal ceruloplasmin level and the diagnosis was confirmed by genetic analysis[11].

A 24-h urinary copper excretion is useful for the diagnosis of WD and for monitoring treatment. However the interpretation of 24-h urinary copper excretion can be difficult because similar results may be seen in chronic liver diseases, including autoimmune hepatitis. Furthermore, heterozygote carriers could also have intermediate levels. Twenty-four hours urinary copper excretion may show false negative results at presentation in patients diagnosed with WD and it may also show false negative results in asymptomatic children.

Hepatic copper content greater than 250 μg/g dry weight is usual in patients with WD but the concentration can be falsely low due to an inhomogeneous distribution of copper. In a pediatric study, sampling error was common in rendering this test unreliable in patients with cirrhosis and clinically evident WD[12] and hepatic copper concentrations can be highly increased in long-term cholestasis. KF rings also are present in of 44%-62% of patients with mainly hepatic disease at the time of diagnosis[9]. Especially in children presenting with liver disease, KF rings are usually absent. In the genetically confirmed present case, the serum ceruloplasmin level was below normal and the amount of hepatic copper was elevated, but 24-h urinary copper excretion was normal at presentation and a KF ring was absent. Specific ultrastructural changes may be visible in WD. Typical findings include variability in size and shape, increased density of material, inclusions of lipid and granular material, and increased intracristal space with dilatation of the tips of cristae[13] but ultrastructural analysis of liver specimens revealed no evidence of ab-

normal mitochondria in our case.

The treatment of first choice among chelators and zinc in specific clinical situations of WD is still debated. Not only the agent, but the age at which presymptomatic patients with WD should be treated is still a matter of debate. Brewer *et al*[14] recommended an early treatment of presymptomatic patients during pediatric years with zinc. Iorio *et al*[3] had treated the 13-mo old child with zinc. In this case, although therapy with zinc was continued, his transaminase levels increased but after changing the regimen to penicillamine, the liver enzyme levels normalized. Further research on management of very young children with WD is needed.

In conclusion, even in infancy, elevation of transaminase should lead to exclusion of WD if other etiologies of hypertransaminasemia are ruled out. Genetic testing is the only reliable tool to identify very young patients with WD. We report a case of a 9-mo old boy with WD presenting as chronic hepatitis with compound heterozygotes of p.G1186S and c.4006delA.

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