Efficacy of Double Dose Recombinant Hepatitis B Vaccination in Chronic Hepatitis C Patients, Compared to Standard Dose Vaccination

Mohammad Minakari, Afshin Tahmasebi[1], Mahyar Hosseini Motlagh[2], Behrooz Ataei[2], Majid Yaran[3], Hamid Kalantari[4], Hamid Tavakkoli[4]

Department of Gastroenterology, Infectious Disease and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Alzahra University Hospital, Isfahan, Iran, [1]Department of Internal Medicine, Isfahan University of Medical Sciences, Alzahra University Hospital, Isfahan, Iran, [2]Department of Infectious Disease, Infectious Disease and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Alzahra University Hospital, Isfahan, Iran, [3]Infectious Disease and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Department of Gastroenterology, Isfahan University of Medical Sciences, Alzahra University Hospital, Isfahan, Iran

Correspondence to:
Dr. Hamid Tavakkoli,
Department of Gastroenterology,
Isfahan University of Medical Sciences,
Alzahra University Hospital, Isfahan, Iran.
E-mail: h_tavakoli@med.mui.ac.ir

Date of Submission: Oct 26, 2012

Date of Acceptance: Oct 06, 2013

How to cite this article: Minakari M, Tahmasebi A, Motlagh MH, Ataei B, Yaran M, Kalantari H, *et al*. Efficacy of double dose recombinant hepatitis B vaccination in chronic hepatitis C patients, compared to standard dose vaccination. Int J Prev Med 2014;5:145-51.

ABSTRACT

Background: Hepatitis B virus (HBV) vaccination is a well-known, safe and effective way for protection against HBV infection; however, non-responders remain susceptible to infection with HBV. This is so important in patients with any kind of chronic liver disease, especially chronic hepatitis C virus (HCV) patients in whom acute HBV infection may lead to decompensation of liver disease. Some of the studies have shown that immunogenicity of HBV vaccination is decreased in these patients. The aim of this study was to evaluate the efficacy and safety of double dose vaccination of HBV in these patients, compared with standard dose vaccination in similar patients and healthy adults.

Methods: A total of 64 patients with chronic HCV infection were randomized into 2 groups of 32. Group A received standard dose HBV vaccine, at 0, 1, 6 months, whereas group B received double dose HBV vaccine. Group C consisted of 32 healthy adults who also received standard dose vaccination. At 1 month after the end of vaccination, Hepatitis B surface antibody (HBsAb) titer was checked in all participants and the results were compared.

Results: There was no significant difference in age or sex among three groups. The response rate in groups B and C was 100% (all had HBsAb titer >10 mIU/mL), while in group A, 4 patients (12.5%) were non-responders (HBsAb titer < 10 mIU/mL). The difference in response rate was statistically significant between Group A and the other two groups ($P < 0.05$).

Conclusions: The efficacy of standard dose HBV vaccination in patients with chronic HCV infection was suboptimal. Using double dose vaccination in these patients was an effective way to increase the antibody response.

Keywords: Chronic hepatitis C, Hepatitis B vaccination, Double dose vaccination

Original Article

INTRODUCTION

Despite declining incidence of acute viral hepatitis in recent years, it remained the most common cause of chronic liver disease (CLD) world-wide.[1]

Globally, more than 350 million people are chronic carriers of hepatitis B virus (HBV) and up to 40% of these individuals may progress to cirrhosis, liver failure or hepatocellular carcinoma.[2] HBV infection causes 600,000-1,200,000 deaths every year around the world. Many of the estimated 130-170 million people with chronic hepatitis C virus (HCV) infection may also progress to cirrhosis and hepatocellular carcinoma.[3] Due to the gradual course of disease, its morbidity and mortality is often underestimated. CLD is the tenth cause of mortality in the United States and is responsible for 25,000 deaths annually.[4] It is estimated about 40% of all CLDs in the United States is due to HCV infection. HCV-associated cirrhosis is the most common indication for orthotopic liver transplantation in adults.[5] The Centers for Disease Control (CDC) estimates that 8,000-13,000 deaths occur each year in the United States due to chronic HCV infection. A number of preventive measures for patients with CLD have been recommended to improve quality-of-life, prevent the development of complications and improve overall survival. One of them is the prevention of HBV super-infection by HBV vaccination and is recommended for people at high risk of either exposure to or severe morbidity of HBV infection, such as patients with other CLD,[6] particularly chronic hepatitis C. In co-infection with chronic HBV and HCV, replicating HBV lowers the replication of HCV,[7] but both independently enhance the severity of hepatitis[7] and the risks of liver cirrhosis and hepatocellular carcinoma.[7-9] Therefore, many organizations endorse HBV vaccination for patients with CLD. These include the World Health Organization, the CDC and prevention, the National Institutes of Health, the Veteran's Health Administration, the American Liver Foundation and the Advisory Committee on Immunization Practices.[10-12] The first HBV vaccine was produced by the inactivation and purification of hepatitis B surface antigen (HBsAg) obtained from the plasma of chronic HBV carriers.[13-15] This was replaced by the production of HBsAg using recombinant deoxyribonucleic acid (DNA) technology.[16] All the studies have shown that available ribosomal deoxyribonucleic acid (rDNA) HBV vaccines of 5-10% or more do not create an antibody response to the HBsAg component present in these preparations (non-responders) or they respond poorly (hypo-responders) in individuals with healthy immune system.[17-20] The definition of non-responsiveness and hypo-responsiveness, generally, is anti-HBs level less than 10 mIU/mL and 100 mIU/mL, respectively.[14,18,21] Non-responders remain susceptible to infection with HBV. Several factors affect the antibody response to HBsAg, including the site of injection, sex, advancing age, being overweight and immunodeficiency. Available information suggests that the immunogenicity of the HBV vaccine is reduced in patients with CLDs and in liver transplant recipients, but is unimpaired in patients with fatty liver.[22]

Testing for post-immunization antibody titers is not recommended; exceptions include patients at increased risk for recurrent exposure. This includes health-care workers, patients on chronic hemodialysis, gay or bisexual men and sexual partners of carriers. Some experts also test for post-immunization antibody titers in patients with CLDs, due to the high risk of severe infection in this subgroup.

METHODS

Precipitants selection and study design

The study was designed as an open prospective trial to compare the immunogenicity of the recombinant HBV vaccine of 40 and 20 μg in patients with chronic HCV infection. The study was approved by the Ethical Committee of Isfahan University of medical sciences.

Trial was performed in two out-patient clinics "Infectious Department of Research Center of Sedighe Tahere and Imam Reza Hepatitis Clinic" of Isfahan city, Iran, from May 2010 to February 2011. Blood sample was obtained to assess the anti-HBs concentration 1 month after the basic vaccine schedule was completed to determine the rate of non-responsiveness (anti-HBs < 10 mIU/mL), seroconversion (anti-HBs between 10 and 99 mIU/mL) and seroprotection (anti-HBs ≥ 100 mIU/mL).[14,18,21]

Criteria for inclusion were subjects between 18 and 65 years old with chronic HCV infection and negative serum markers for HBV infection. Chronic HCV infection was defined based on positive HCV-Ab and HCV-ribonucleic acid (RNA), in a patient without clinical or laboratory findings, indicating acute

hepatitis (jaundice, very high aminotransferases). The exclusion criteria were transplanted patients or those on waiting lists, dialysis therapy and chronic renal patients with GFR < 60 ml/min/1.73 m^3, HIV-positive patients, patients with neoplasias, immunosuppressed patients, pregnant women and alcoholic hepatitis or cirrhosis. Individuals already immunized against HBV (presence of anti-HBs) were excluded too. HIV-Ab was tested in all patients and also the control group and those with positive results were not recruited. Alcoholic patient was considered in the study, if he/she did drink at least 40 g of alcohol/day (males) and 20 g/day (females) for at least 5 years. Presence of chronic hepatitis or cirrhosis in proven HCV infected patients (by HCV RNA or Recombinant Immuno Blot Assay (RIBA), was detected based on clinical, laboratory, ultrasonographic, endoscopic and histopathological findings.

Cirrhotic patients were sorted using the original classification proposed by Child-Turcotte and modified by Pugh.

HBsAg and quantitative anti-HBs were detected by Enzyme-linked immunosorbent assay.

All precipitants who did not match the exclusion criteria and had given written, informed consent were recruited. A total of 64 patients with chronic infection of HCV and 32 healthy volunteers, as a control group, were included in the study. Healthy volunteers were chosen from newly arrived hospital staff who were not vaccinated against HBV and needed to be vaccinated before starting their job as medical staff. The control group matched to the patient groups by age. We didn't match the patients and the controls by sex, because sex is not an important factor in response to HBV vaccination. Chronic HCV infected patient were randomized to two groups (standard dose and a double dose groups), using a computerized randomization software. Each group consisted of 32 patients. For all participants, body mass index (BMI) was calculated at the beginning of the study and the quantitative anti-HBs titer was checked. Patients were also inquired about the high-risk behaviors such as drinking, smoking, IV abuse and history of tattooing and transfusion.

Sample size

The sample size was calculated by the formula:

$N = (z_{1-\alpha 2} + z^{\beta})^2 [p_1 (1 - p_1) + p_2 (1 - p_2)]/(p_1 - p_2)^2$

$z_{1-\alpha 2} = 1.96$

$z_{\beta} = 1.64$

$p1 = 0.9$, $p2 = 0.5$

$n = 27.5$.

Vaccine

HBV vaccine (Hepavax-Gene®) that used in this study was a liquid containing highly purified, non-infectious particles of HBsAg, produced by DNA recombinant technology in *Hansenula polymorpha* cells. The vaccine was adsorbed on to aluminum hydroxide gel. Thiomersal was used as a preservative (0.01 v/w%). The quantity of HBsAg in the vaccine was 20 μg/mL.

Vaccination protocol

Recombinant DNA HBV vaccine (Hepavax-Gene®) with a standard dosage, 1 mL (20 μg) was administered to all healthy volunteers in the control group and 32 patients who were selected randomly from HCV infected group whereas the double dosage, 2 mL (40 μg), was administered to 32 remaining HCV infected patients, intramuscularly in the deltoid muscle at three different times (months 0, 1 and 6). Local and systemic side-effects related to the vaccine were documented following the injection of each dose of vaccine. For data missing prevention, we called every participant 1 day before the scheduled time for vaccination or blood sampling.

Statistical analyses

The SPSS statistical analysis package (version 18) was used for all calculations. Patient and control groups were compared in relation to continuous variables with an independent *t*-test for independent variables and Chi-square test for categorical variables. Furthermore, one-way ANOVA and Pearson's correlation test were used. For all these comparisons, level of significance was considered to be less than 5%.

RESULTS

Precipitants characteristics

A total of 64 patients were eligible according to the inclusion criteria and divided into two equal groups, randomly; 32 ones received standard dose vaccine and 32 patients received a double dose vaccine. About 32 healthy individuals

from hospital staff were set as a control group. Baseline characteristics and relative frequencies of risk factors of all participants are showed in Table 1. The mean age of the entire group was 34.4 ± 9.0 years (range, 21-65 years). The mean age of patients in the standard dose and a double dose vaccine group was 33.8 ± 9.6 and 33.7 ± 9.1 years, respectively and 32.1 ± 7.6 years in the healthy group. The variance analysis showed that the age of the control group wasn't significantly different from the age of patients groups ($P = 0.45$, $P = 0.18$ for comparison to standard dose and a double dose groups, respectively). The majority of patients were male (90.6%) and had high-risk behaviors such as smoking, Intraravenous drug users (IVDU) and tattooing. It was not surprising that injection drug abuse was the most common identifiable risk factor of HCV infection in our study (75%). A total of 56 patients were HCV RNA positive proven by PCR and viral genotype was obtained in 49 patients. Prevalence of genotype 1 and genotype 3 was 51% and 49%, respectively. Sex, BMI and genotype had similar distribution in all groups ($P > 0.05$). On physical examination of patients, there was no significant abnormality such as icterus, hepatosplenomegaly, ascites and edema. None of them had features of cirrhosis or end stage liver disease. Serum ALT (mean 49.8 ± 36 IU/L) and AST (mean 36.5 ± 18) were mildly elevated in most patients. Alkaline phosphatase, prothrombin time and bloodcell count were normal and the platelet count was more than 100,000/μL in all subjects.

Immunogenicity of rDNA HBV vaccine in chronic HCV infected patients

Administration of HBV vaccine with three-doses of 20 and 40 μg protocols in two chronic HCV groups and 20 μg in all healthy controls created 65.6%, 62.5% and 53.1% seroprotection rate, respectively. Non-responsiveness was observed in 12.5% of patients with standard dose (20 μg) protocol, whereas there was no non-responder in patients with a double dose (40 μg) protocol and healthy control group. Hypo-responsiveness (seroconversion) was detected in 30.0%, 37.5% and 29.5% of standard dose protocol, double dose protocol patients and healthy group, respectively. Responsiveness rates and anti-hepatitis B surface antibody titers of all participants are shown in Table 2.

Surprisingly, our results showed significant lower rate of seroprotection in the healthy group compared with HCV positive patients with the same dose protocol vaccination. Conversely, non-responsiveness was lower in healthy groups ($P = 0.026$). However, anti-HBs mean level had no significant difference among three groups ($P = 0.052$). However, age distribution was differed among all groups ($P = 0.047$). Pearson correlation test showed no correlation between age and anti-HBs mean level ($r = –0.07$, $P = 0.52$). There was no correlation between BMI and anti-HBs mean Level ($r = –0.03$, $P = 0.77$). However, anti-HBs mean level was differed with sex. It was 165.3±150 mIU/mL in males and 92.5±56 mIU/mL in females ($P = 0.04$). Mean anti-HBs level in genotype 1 and 3 was 174.0 ± 125 mIU/mL and 151.3 ± 120 mIU/mL, respectively ($P = 0.52$). From 96 participants who completed the vaccination protocol, nine individuals (8.3%) reported local adverse reactions (pain at the site of injection and erythema) after vaccination. Neither severe systemic adverse reactions nor neurologic symptoms occurred in the follow-up period.

Table 1: Demographic characteristics and risk factors of participants

HBV vaccination groups	Standard dose HCV+	Double dose HCV+	Healthy control	Total
Age (mean±SD)	33.7±9.6	37.5±9.0	32.0±7.6	34.4±9.0
BMI (mean±SD)	22.3±3.31	23.5±3.8	23.7±3.9	23.2±3.7
Sex				
Female (%)	3 (9.4)	4 (12.5)	9 (28.1)	16
Male (%)	29 (90.65)	28 (87.5)	23 (71.9)	80
IVDU (%)	25 (78)	23 (72)	0	75
Tattooing (%)	11 (35)	12 (37)	0	36
Transfusion (%)	8 (25)	5 (16)	0	20
No. identified risk factor (%)	4 (12)	6 (18)	100	15

HBV=Hepatitis B virus, HCV=Hepatitis C virus, SD=Standard deviation, BMI=Body mass index, IVDU=Intraravenous drug users

Table 2: Responsiveness rates and anti-HBsAb titers

HBV vaccination groups response rate	Standard dose HCV+	Double dose HCV+	Healthy control
Non-response rate			
No.	4	0	0
Percent	12.5	0	0
Hypo-response rate			
No.	7	12	15
Percent	21.9	37.5	46.9
Seroprotection rate			
No.	21	20	17
Percent	65.6	62.5	53.1
HBsAb titer			
Mean	163.5	158.7	111.0
SD	127.5	123.5	74.6
Minimum	2	13	12
Maximum	447	454	368

SD=Standard deviation, HBsAb=Hepatitis B surface antibody, HCV=Hepatitis C virus

DISCUSSION

Recombinant HBV vaccine is one the safest vaccines. Mild local reaction that occurred in 8.3% of precipitants in this study was similar to another study (7% of vaccines).[23] Shaw *et al*. reported extremely rare neurologic adverse events that did not exceed the incidence of that in the general population except the Guillain-Barre' syndrome that occurred more frequently than expected (9 cases vs. 3.6, respectively) in follow-up of more than 850,000 vaccines in the United States.[24] There is no doubt that the development of HBV vaccines is a major accomplishment in modern medicine. It is a cost-effective manner in preventing HBV infection and its squeals. Dual-infection with chronic HBV and HCV enhances the severity of hepatitis[7] and the risks of liver cirrhosis and hepatocellular carcinoma[7-9] and response to treatment is lower. Literatures suggest that the immunogenicity of the HBV vaccine is reduced in patients with CLDs[22]

In 2004, Mattos *et al*. in a prospective study of 85 patients with chronic HCV infection and 46 healthy adults, reported non-response rate of 45 and 2% to three-dose regimen of the recombinant HBV vaccine in patients and control group, respectively.[25] Earlier studies[25-27] showed the response rates in HCV infected patients varying between 69% and 100%. Impaired immunogenicity has been described in patients with alcoholic liver disease,[28-30] liver transplant recipients and in those awaiting liver transplantation.[31-33]

A randomized, double-blind trial in 110 alcoholic patients was conducted to assess whether the use of high-dose versus standard-dose HBV vaccine would be more effective.[30] Patients were randomly assigned to 20 μg at baseline, 1 and 6 months versus 40 μg at baseline, 1, 2 and 6 months. Seroconversion rates were improved in the high-dose arm (46 vs. 75%, respectively). In our study, non-responsiveness to three-dose (20 μg) regimen of the recombinant HBV vaccine, observed in 12.5% of 32 patients with chronic HCV infection versus 0.0% of 32 healthy control group. This was slightly more than expected response in the normal population (5-10%) reported in other studies. Although with a three-doubling dose (40 μg) regimen in other 32 chronic HCV patients, non-response rate was the same as that of healthy control group (0.0%).

Several factors may affect the antibody response to HBsAg components in HBV vaccine. This trial showed age, BMI and viral genotype were not influential risk factors. But, female sex was accompanied with lower anti-HBs response.

CONCLUSIONS

Overall, according to the findings in this study and given the fact that the antibody response simply was divided into three groups of non-response, hypo-response and seroprotection, we may fail to appropriately decide about the borderline responses. There were four non-responders (12.5%) in chronic HCV infected patients with standard dose vaccination compared with 100% response rate in double dose vaccination group. We conclude that using double dose vaccination in these patients is an effective way to increase the response rate.

REFERENCES

1. Schiff ER. Viral hepatitis. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Diseases of the Liver. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2003. p. 741-4.
2. Lok AS. Chronic hepatitis B. N Engl J Med 2002;346:1682-3.
3. World Health Organization. Fact Sheet No. 164: Hepatitis C. Available from: http://www.who.int. [Last accessed on 2003 Nov 14].

4. Murphy SL. Deaths: Final data for 1998. Natl Vital Stat Rep 2000;48:1-105.
5. Verna EC, Brown RS Jr. Hepatitis C virus and liver transplantation. Clin Liver Dis 2006;10:919-40.
6. Lemon SM, Thomas DL. Vaccines to prevent viral hepatitis. N Engl J Med 1997;336:196-204.
7. Zarski JP, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, *et al*. Characteristics of patients with dual infection by hepatitis B and C viruses. J Hepatol 1998;28:27-33.
8. Kaklamani E, Trichopoulos D, Tzonou A, Zavitsanos X, Koumantaki Y, Hatzakis A, *et al*. Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. JAMA 1991;265:1974-6.
9. Simonetti RG, Cammà C, Fiorello F, Cottone M, Rapicetta M, Marino L, *et al*. Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case-control study. Ann Intern Med 1992;116:97-102.
10. Kane M. Global programme for control of hepatitis B infection. Vaccine 1995;13 Suppl 1:S47-9.
11. Centers for Disease Control and Prevention. Recommendations and Reports: Hepatitis A and B vaccines. Morb Mortal Wkly Rep 2003;52:34-6.
12. National Institutes of Health. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002 - June 10-12, 2002. Hepatology 2002;36:S3-20.
13. Szmuness W, Stevens CE, Zang EA, Harley EJ, Kellner A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): A final report. Hepatology 1981;1:377-85.
14. Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF, *et al*. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. N Engl J Med 1986;315:209-14.
15. Jilg W, Schmidt M, Deinhardt F. Immune response to hepatitis B revaccination. J Med Virol 1988;24:377-84.
16. Jilg W, Lorbeer B, Schmidt M, Wilske B, Zoulek G, Deinhardt F. Clinical evaluation of a recombinant hepatitis B vaccine. Lancet 1984;2:1174-5.
17. Westmoreland D, Player V, Heap DC, Hammond A. Immunization against hepatitis B – What can we expect? Results of a survey of antibody response to immunization in persons 'at risk' of occupational exposure to hepatitis B. Epidemiol Infect 1990;104:499-509.
18. Dienstag JL, Werner BG, Polk BF, Snydman DR, Craven DE, Platt R, *et al*. Hepatitis B vaccine in health care personnel: Safety, immunogenicity, and indicators of efficacy. Ann Intern Med 1984;101:34-40.
19. Craven DE, Awdeh ZL, Kunches LM, Yunis EJ, Dienstag JL, Werner BG, *et al*. Nonresponsiveness to hepatitis B vaccine in health care workers. Results of revaccination and genetic typings. Ann Intern Med 1986;105:356-60.
20. Wood RC, MacDonald KL, White KE, Hedberg CW, Hanson M, Osterholm MT. Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. JAMA 1993;270:2935-9.
21. Hadler SC, Margolis HS. Hepatitis B immunization: Vaccine types, efficacy, and indications for immunization. In: Remington JS, Swartz MN, editors. Current Clinical Topics in Infectious Diseases. Boston: Blackwell Scientific; 1992. p. 282-308.
22. Kośliñska-Berkan E, Kuydowicz J. The comparison of the humoral response among the patients with liver cirrhosis and steatosis of the liver after HBV vaccination. Przegl Epidemiol 2006;60:199-203.
23. Wiedmann M, Liebert UG, Oesen U, Porst H, Wiese M, Schroeder S, *et al*. Decreased immunogenicity of recombinant hepatitis B vaccine in chronic hepatitis C. Hepatology 2000;31:230-4.
24. Shaw FE Jr, Graham DJ, Guess HA, Milstien JB, Johnson JM, Schatz GC, *et al*. Postmarketing surveillance for neurologic adverse events reported after hepatitis B vaccination. Experience of the first three years. Am J Epidemiol 1988;127:337-52.
25. Mattos AA, Gomes EB, Tovo CV, Alexandre CO, Remião JO. Hepatitis B vaccine efficacy in patients with chronic liver disease by hepatitis C virus. Arq Gastroenterol 2004;41:180-4.
26. Keeffe EB, Krause DS. Hepatitis B vaccination of patients with chronic liver disease. Liver Transpl Surg 1998;4:437-9.
27. Lee SD, Chan CY, Yu MI, Lu RH, Chang FY, Lo KJ. Hepatitis B vaccination in patients with chronic hepatitis C. J Med Virol 1999;59:463-8.
28. Mendenhall C, Roselle GA, Lybecker LA, Marshall LE, Grossman CJ, Myre SA, *et al*. Hepatitis B vaccination. Response of alcoholic with and without liver injury. Dig Dis Sci 1988;33:263-9.
29. Bronowicki JP, Weber-Larivaille F, Gut JP, Doffoël M, Vetter D. Comparison of immunogenicity of vaccination and serovaccination against hepatitis B virus in patients with alcoholic cirrhosis. Gastroenterol Clin Biol 1997;21:848-53.
30. Rosman AS, Basu P, Galvin K, Lieber CS. Efficacy of a high and accelerated dose of hepatitis B vaccine in alcoholic patients: A randomized clinical trial. Am J Med 1997;103:217-22.
31. Villeneuve E, Vincelette J, Villeneuve JP. Ineffectiveness of hepatitis B vaccination in cirrhotic patients waiting for liver transplantation. Can J Gastroenterol 2000;14 Suppl B: 59B-62.

32. Arslan M, Wiesner RH, Sievers C, Egan K, Zein NN. Double-dose accelerated hepatitis B vaccine in patients with end-stage liver disease. Liver Transpl 2001;7:314-20.
33. Domínguez M, Bárcena R, García M, López-Sanroman A, Nuño J. Vaccination against hepatitis B virus in cirrhotic patients on liver transplant waiting list. Liver Transpl 2000;6:440-2.

Source of Support: This study was supported by Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.