Efficacy and efficiency of hepatitis B core antibody in the diagnosis of occult hepatitis B in hemodialysis patients

AL-Sayed M. Rashed[a], Emad A. Mohamed[a], Magdy A. AL-Dahshan[a], Mohamed A. Said[a], Mohamed S. AL-Shorbagy[b]

Background Occult hepatitis B viral infection (OHBI) is defined as hepatitis B virus (HBV) DNA detection in serum by sensitive diagnostic tests in hepatitis B surface antigen (HBsAg) negative patients with or without serological markers of previous viral infection.

Aim This study aimed to evaluate hidden infection of hepatitis B among HBsAg negative chronic kidney disease patients on regular hemodialysis (HD) using hepatitis B core antibody as a marker in the sera of these patients, HBV DNA by PCR, and to evaluate the efficacy and efficiency of hepatitis B core antibodies in the diagnosis of occult hepatitis B in HD patients.

Patients and methods Eighty chronic kidney disease patients on regular HD were included in this study; the mean age of studied patients was 41.8±12.72 years. They were recruited from HD Unit, Internal Medicine Department, Bab Alshearia University Hospital, Al-Azhar University, Cairo, Egypt, after exclusion of HBsAg positive, HBV antibody positive, intravenous drug users, and alcoholic patients. All patients were subjected to a full assessment of history, blood chemistry, HBsAg by ELISA, hepatitis B core immunoglobulin G (anti-HBcIgG), HB DNA by PCR, hepatitis C antibody (HCV Ab) by ELISA, and abdominal ultrasound.

Results Our results showed that HCV Abs were positive in 50% of cases (40 cases); of these patients, 30% (12 cases) were positive for HBcIgG, whereas 50% of the cases (40 cases) were negative for HCV Ab. Of these, 20% (eight cases) were positive for HBcIgG, but the remaining 32 patients were negative for both HCV Abs and HBcIgG. All these results showed negative PCR in all cases (0% of cases).

Conclusion OHBI among Egyptian HD patients is low, with a 0% prevalence by PCR; 6 months of repeated PCR is recommended as liver biopsy is difficult in HD patients and HBc Abs are not sufficient for the diagnosis of OHBI in HD patients.

Sci J Al-Azhar Med Fac, Girls 2018 2:231–236

The Scientific Journal of Al-Azhar Medical Faculty, Girls
2018 2:231–236

Keywords: efficacy, hemodialysis, hepatitis B core, occult hepatitis B viral infection

[a]Department of Internal Medicine and Clinical Pathology, Faculty of Medicine (Boys), Al-Azhar University, [b]Clinical Pathology, Al Azhar University, Cairo, Egypt

Correspondence to Dr. Mohamed S Al Shorbagy, Clinical Pathology, Al Azhar University, Cairo, Egypt. Tel: 002 0100 33 60 94 3; e-mail: drsayedaswad@gmail.com

Received 19 September 2018 **Accepted** 8 October 2018

Introduction

Egypt is considered to be a region of intermediate prevalence of hepatitis B virus (HBV) infection, with a reported figure of 3.3% [1].

Occult hepatitis B viral infection (OHBI) is the major cause of post-transfusion hepatitis B in western countries and in countries such as India and Taiwan, with a higher risk of transmission than for HCV or HIV [2,3].

Aims

The aims of this study were as follows: (a) to screen for the hidden infection of hepatitis B among HBVs Ag negative patients with chronic kidney disease on regular hemodialysis (HD) using the hepatitis B core antibody (HBcAb) as a marker in the sera of these patients in addition to HBV PCR and (b)- to show the efficacy and efficiency of HBc Ab in the diagnosis of OHBI in HD patients.

Patients and methods

A cross-sectional study was carried out of 80 patients with end-stage renal disease on regular HD. There were 44 men and 36 women, with a mean age 41.8 ±12.728 years and a mean HD duration of 52.42 ±39.856 months. Patients were recruited from the HD Unit, Internal Medicine Department, Bab-Alshaeria University Hospital, Cairo, after exclusion of HBsAg –positive patients, intravenous drug abusers, and alcoholic patients.

The study followed the principles of the ethical committee of AL-Azhar University and an informed consent was obtained from each participant in the study.

Data collected from each patient included assessment of history, focusing on the following: age in years, sex, duration of HD, shifting between dialysis units, number of transfused blood units, number of A-V fistula operation/s, diabetes mellitus, and HBV vaccination.

Blood biochemistry: all patients were investigated for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, albumin, prothrombin time, bilirubin, hemoglobin, calcium, phosphorus, parathyroid hormone, creatinine, and blood urea. Testing for HBV serology included hepatitis B surface antigen (HBsAg) and anti-hepatitis B core immunoglobulin G (Anti-HBcIgG) by ELISA. HBV DNA detection was performed using the PCR technique. Antibody to hepatitis C virus (anti-HCV) was detected by ELISA.

The patients were divided into two groups according to the results of HBVc Abs: group I: negative HBVcIgG and group II: positive HBVcIgG.

Ten mililiteres of venous blood sample was drawn from each patient before dialysis to perform the previous laboratory tests. Abdominal ultrasonographic examination was carried out to assess liver echogenicity and the presence of focal hepatic lesions.

Statistical analysis

Data were analyzed using the SPSS program (M Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.), version 12, and the statistical analysis program, version 100.

Results

Among the patients studied, 44 (55%) patients were men and 36 (45%) patients were women; their mean age was 41.8±12.728 years (Tables 1–3).

Table 1 Demographic data among the patients studied

	Range	Mean±SD
Age (years)	19–67	41.80±12.728
Dialysis duration (months)	9–145	52.40±39.856
Number of blood transfusions	0–20	5.25±4.545
Number of A-V fistulae	1–6	2.22±1.493

Table 2 Detection of hepatitis B virus core immunoglobulin G among patients with chronic kidney disease

Number of patients	Total	Negative HBVcIgG (group I)	Positive HBVcIgG (group II)
All [*n* (%)]	80	60 (75)	20 (25)
Patients with positive HCV Abs	40	28	12
Patients with negative HCV Abs	40	32	8

HBV, hepatitis B virus; HCV Abs, hepatitis C antibody; HBVcIgG, hepatitis B virus core immunoglobulin G.

Fifteen percent (n=12) of our studied patients had a positive history of shifting between dialysis units.

Seventy percent (n=56) of our patients had normal ultrasonic liver parenchyma, whereas 30% (n=24) had coarse liver parenchyma, 0% (n=0) had liver cirrhosis, and 0% (n=0) had hepatic focal lesion. Sixty percent of patients enrolled in the study had coarse liver with positive HCV antibodies.

In terms of the prevalence of hepatitis B infection markers among the patients studied, we found the following. HBV DNA was not detected in any of the patients. However, anti-HBcIgG was positive in 25% (n=20) our studied patients.

In terms of the prevalence of HCV antibodies in the patients studied, we found that 50% (n=40) had positive anti-HCV antibodies and of these, 30% (n=12) were positive for anti-HBcIgG, whereas 50% (n=40) were negative for HCV antibodies and 20% (n=8) were positive for anti-HBcIgG (Tables 4–6).

Also, there was no statistically significant difference between the positive serum anti-HBcIgG group I and the negative serum anti-HBcIgG group II in shifting between dialysis units (Figs 1–3).

Discussion

Undetectable HBsAg in the presence of HBV DNA in the plasma and/or the liver resulted in the introduction of the concept of OHBI [4].

Accordingly, we studied the presence of HBV DNA in the serum of HBsAg-negative patients undergoing chronic HD.

We found that the prevalence of OHBI was 0%; this was in agreement with the rates reported by many investigators. Elrashidy *et al.* [5] reported that in

Table 3 Classification of the patients studied according to hepatitis C virus antibodies

Variables	HCV positive Abs	HCV negative Abs
Total number of patients (80)	40	40
HBV DNA (PCR)	Negative	Negative
HBVcIgG +ve [*n* (%)]	12 (30)	8 (20)
HBVcIgG –ve [*n* (%)]	28 (70)	32 (80)

HBV, hepatitis B virus; HBVcIgG, hepatitis B virus core immunoglobulin G; HCV Ab, hepatitis B virus antibody.

Table 4 Correlation between anti-hepatitis B core immunoglobulin G and anti-hepatitis C antibody

	Anti-HCV antibodies		*P* value	Significance
	Negative	Positive		
Anti-HBcIgG				
Negative number of patients (% within anti-HCV antibodies)	32 (80.0)	28 (70.0)	0.35	NS
Positive number of patients (% within anti-HCV antibodies)	8 (20.0)	12 (30.0)		

HBcIgG, hepatitis B virus core immunoglobulin G; HCV, hepatitis C virus.

Table 5 Correlation between liver parenchyma and anti-hepatitis C virus antibodies

	HCV antibodies		*P* value	Significance
	Negative	Positive		
Liver parenchyma				
Normal number of patients (% within anti-HCV antibodies)	40 (100.0)	16 (40.0)	<0.01	HS
Coarse number of patients (% within anti-HCV antibodies)	0 (0.0)	24 (60.0)		

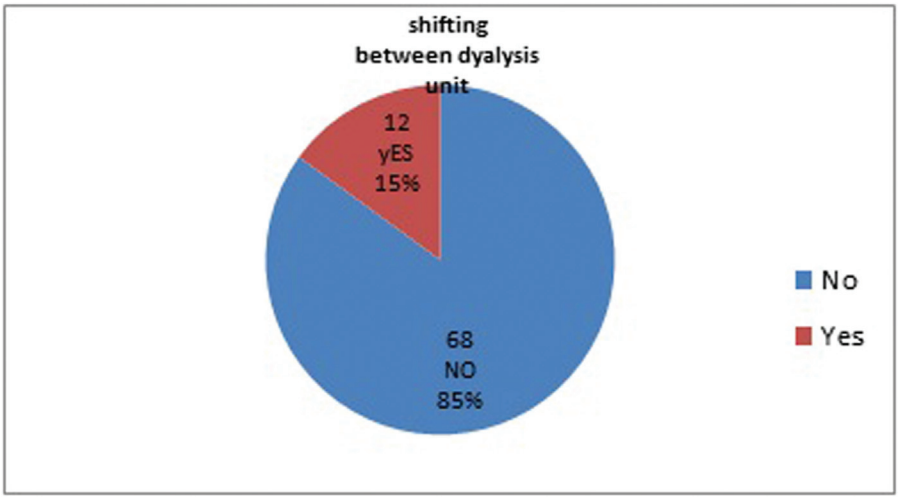
HCV, hepatitis C virus; HS, highly significant.

Table 6 Comparison between group I and group II in terms of liver ultrasonographic findings

	HBcIgG		Total	*P* value	Significance
	Negative	Positive			
Liver parenchyma					
Normal number of patients (% within anti-HBcIgG)	44 (73.3)	12 (60.0)	56 (70.0)	0.33	NS
Coarse number of patients (% within anti-HBcIgG)	16 (26.7)	8 (40.0)	24 (30.0)		

HBcIgG, hepatitis B core immunoglobulin G.

Figure 1



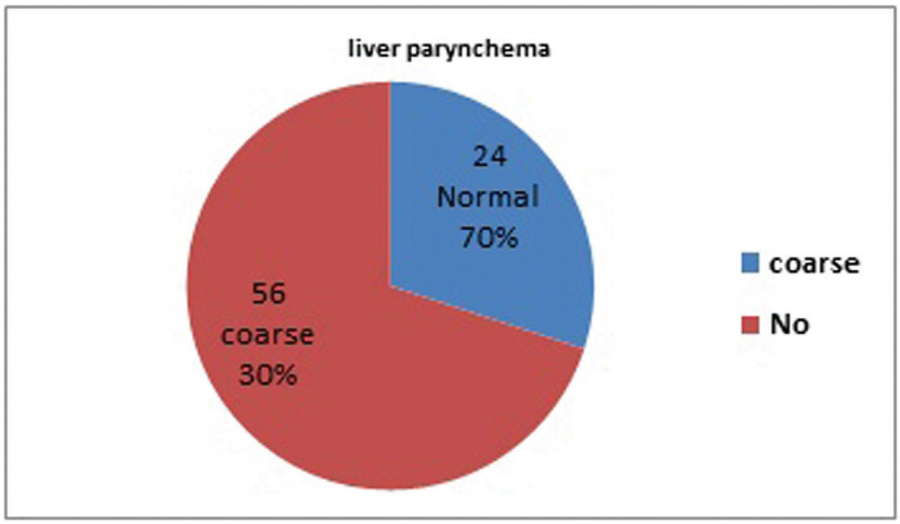
Distribution of patients in terms of shifting between dialysis units.

100 HBsAg seronegative chronic dialysis patients, none of them had occult HBV, Franco *et al.* [6] reported that in 198 HBsAg seronegative chronic dialysis patients, none of them had occult HBV. In Japan, among the 82 chronic HBsAg-negative HD patients, the prevalence of occult HBV infection was 0% [7]. In another study carried out in Vietnam, by Pipili *et al.* [8], OHBI was not observed in HD patients. Also, Aghakhani *et al.* [9] identified the epidemiology of OHBI in 285 chronic dialysis patients, and found that OHBI was absent in all patients.

However, some investigators have reported a higher prevalence of occult hepatitis B among their studied patients. In Egypt, among HD patients, Ismail *et al.* [10] reported that occult hepatitis B was detected in six (5.2%) patients from a total of 116 patients on regular HD.

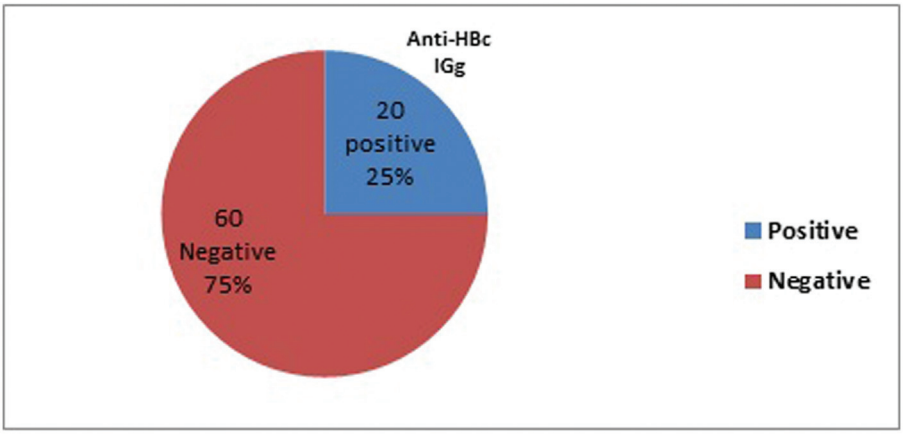
In other countries, Makroo *et al.* [11] reported that in 263 HD patients who were HBsAg negative 4.8% patients were HBV DNA-positive by real-time PCR and Siagris *et al.* [12] reported that HBV DNA was

Figure 2



Distribution of patient in terms of liver parynchemal affection.

Figure 3



Distribution of patient in terms of the presence of anti-HBcIgG. HBcIgG, hepatitis B core immunoglobulin G.

detected in 21.1% HD patients and in 5.9% patients with normal renal function.

IgG anti-HBc is not a neutralizing antibody and remains detectable throughout the patient's life once he or she becomes infected with HBV [13]. The importance of anti-HBc for HBV screening has been reported in numerous studies [11]. In our study, the prevalence of HBcAbs IgG was 25%.

The anti-HBcAbs do not indicate immunity; high titers of anti-HBc, even with the coexistence of anti-HBsAg, are indicative of HBV replication in the liver [14]. Patients who remain anti-HBc positive for years are at risk of transmitting disease on donation of solid organ tissue [15] or reactivation of HBV disease once immunosuppressed [16].

In recent reports, anti-HBc, previously considered a persistent indication of previous HBV infection after all virus has been cleared, has emerged as a reliable marker of occult hepatitis B [17].

Squadrito *et al.* [18] recommended that if we use serum samples in the diagnosis of OHBI, a highly sensitive and specific test should be used, such as HBV nucleic acid amplification testing, a PCR technique with detection limits of less than 20 copies HBV DNA per reaction. Only if this highly sensitive HBV DNA testing is not possible should anti-HBc be used to identify potential seropositive OHBI cases.

From all previous reports, we can consider our HBcAb-positive patients as potential seropositive OHBI cases with low or nonreplicative phase and we can expect

some of these patients to have the viral DNA in their liver cells.

This could be attributed to the quantitative differences in the levels of HBV viremia during the course of the disease when positive patients may be identified to be negative because of no or low replicative period of the virus; this suggestion is based on the data reported by Tseng *et al.* [19], who examined repeated sera from the same patients for the presence of HBV DNA, and reported inconsistent results, with previously negative samples being positive for HBV DNA, and vice versa, which suggests a fluctuating level of viremia during the course of the disease.

Also, it should be stated that the detection of HBV DNA in serum samples rather underestimates the actual prevalence of OHBI. Indeed, the most correct and precise methodological approach for the determination of the prevalence of OHBI is the analysis of liver DNA extracts. However, the availability of liver tissues is often limited by restrictions on the performance of liver biopsies, which, in the setting of HD, is often very difficult and usually relatively contraindicated [20].

Raimondo and Pollicino [21] carried out a study to evaluate the long-term effect of chronic HBV infection after seroconversion (about one decade after resolution of the acute infection). The investigators had reported persistence of cccDNA in all liver biopsies (nine out of nine samples), whereas serum HBV DNA by quantitative PCR technique was detected only in two patients.

In our study, both the duration of HD and the number of transfused blood units were significantly higher in IgG anti-HBc-positive patients; this result is in agreement with that of Ismail *et al.* [10], who reported a significant relationship between HBcAbs and longer HD duration, as well as a history of number blood transfusion. Again, Elrashidy *et al.* [5] reported a significant relationship between HBcAbs and longer HD duration, as well as a history of number blood transfusion. The significant relation between acquiring hepatitis B infection in our study and the higher dialysis duration led us to suggest that some anti-HBc-positive patients may have had positive viremia and was the source of the transmission of infection in our unit. Our results are in agreement with those of Tseng *et al.* [19], who reported that viremia in hepatitis B infection is intermittent and occult HBV has the potential to spread silently by nosocomial transmission within the HD unit as concluded by Motta *et al.* [22].

The significant relation between acquiring hepatitis B infection in our study and the number of blood transfusions highlight the significance of routine screening for HBsAg in the transfused blood units and the need to use erythropoietin-stimulating agents in treating anemia in our patients instead of blood transfusion.

In the current study, there was no correlation between positivity or negativity of HBcAbs and serum levels of ALT, AST, albumin, alkaline phosphatase, prothrombin time, and bilirubin. Also, our results were in agreement with those of Aghakhani *et al.* [9], who reported similar results.

A possible explanation for these results may be the lack of association between liver injury and levels of liver enzyme in HD patients. The same conclusion was reported by Ray *et al.* [23], who reported that identification of liver damage by estimation of serum transaminase may be affected by a reduction in aminotransferase values in these patients. Although the exact cause is unknown, possible underlying reasons may be related to pyridoxine deficiency (pyridoxal phosphate is a necessary coenzyme for ALT and AST) and/or the presence of an inhibitory substance in the uremic milieu [23].

Another explanation could be o an intermittent increase in the quality and magnitude of host immune responses against HBV infection that led to an intermittent increase in liver enzyme; this mechanism was defined by Ramaty *et al.* [24]. They found a very low or nonreplicative state of HBV within liver cells, which could explain this nonsignificant impact of positivity of HBcAb on liver enzymes.

The prevalence of HBcAbs in HCV-positive patients was 30%, which was higher than that in HCV-negative patients, 20%. In previous studies, Elrashidy *et al.* [5] reported similar results. This could be explained by the fact that both HBV and HCV have common routes of transmission.In our study, there was no significant correlation between anti-HBcAbs and HCV antibodies. Our result was in agreement with that of Alavian [25], who concluded that HCV positivity is not a contributing factor to HBV infection in HD patients. Also, the same result was reported with Sav *et al.* [26]. However, studies by Bivigou-Mboumba *et al.* [27] found a high correlation between anti-HCV and HBcAbs. In the current study, there was no significant impact of HBcAbs on liver parenchyma or presence of hepatic focal lesions. Also, there was no significant impact of positivity of HBcAbs among

the HCV-negative or HCV-positive patients in terms of parenchymal ultrasonic changes. Our results were in agreement with those of Myers *et al.* [28] and Sagnelli *et al.* [29], who studied the impact of positive anti-HBc on liver histology and they concluded that previous HBV infection does not affect liver histology. Similarly, Emara *et al.* [30] reported nonsignificant differences in histological activity and fibrosis between anti-HBc-positive and anti-HBc-negative patients as well as between anti-HBc-positive/DNA-positive and anti-HBc-positive/DNA-negative patients.

Conclusion

OHBI (serum hepatitis B viral DNA in the absence of HBsAg) among Egyptian HD patients is low, with a detected prevalence of 0%, and it is recommended that the test be repeated regularly because of difficulty of carrying out a liver biopsy. HBcAb is a good screening test for the diagnosis of OHBI in HD patients, but not sufficient or as effective as HBV DNA.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Zeid WMA, Ramadan DI, Shemis MA. Prevalence of mutations within major hydrophilic region of hepatitis B virus and their correlation with genotypes among chronically infected patients in Egypt. *Arab J Gastroenterol* 2016; **13**:750–757.

2 Kennedy B. Occult hepatitis B virus infection: implications in transfusion. *Hepat Mon* 2012; **11**:714–718.

3 Cholongitas E, Papatheodoridis G, Burroughs A. Liver grafts from anti-hepatitis B core positive donors. *A systematic review J Hepatol* 2010; **52**:272–279.

4 Kitab B, Ezzikouri S, Alaoui R, Nadir S, Badre W, Trepo C, Benjelloun S. Occult HBV infection in Morocco: from chronic hepatitis to hepatocellular carcinoma. *Liver Int* 2014; **34**:e144–e150.

5 Elrashidy H, El-Didamony A, Elbahrawy G, Mostafa M, George NM, Elwassief A, Abdelbasseer MA. Antibody levels against hepatitis B virus after hepatitis B vaccination in Egyptian diabetic children and adolescents. *Hum Vaccin Immunother* 2013; **9**:2002–2006.

6 Franco E, Bagnato B, Marino M, Meleleo C, Serino L, Zaratti L. Hepatitis B: epidemiology and prevention in developing countries. *World J Hepatol* 2012; **4**:74–80.

7 Nagakawa O, Miyatomi Y, Shigeta Y, Inayama E, Murakami K, Sakai T, Yoshida T. Occult hepatitis B virus infection in Japanese chronic hemodialysis patients. *Ther Apher Dial* 2013; **17**:289–292.

8 Pipili CL, Papatheodoridis GV, Cholongitas EC. Occult hepatitis B virus infection in immuno-compromised patients. *Kidney Int* 2013; **84**:880–885.

9 Aghakhani A, Banifazl M, Velayati AA, Eslamifar A, Ramezani A. Occult hepatitis B virus infection in hemodialysis patients: a concept for consideration. *Ther Apher Dial* 2012; **16**:328–333.

10 Ismail H, Soliman M, Ismail N. Occult hepatitis B virus infection in Egyptian hemodialysis patients with or without hepatitis c virus infection. *Pathol Lab Med Int* 2010; **2**:113–120.

11 Makroo RN, Chowdhry M, Bhatia A, Arora B, Rosamma NL. Hepatitis B core antibody testing in US blood donors: a double-edged sword!. *Asian J Transfus Sci* 2012; **43**:696–704.

12 Siagris D, Christofidou M, Triga K, Pagoni N, Theocharis GJ, Goumenos D, *et al.* Occult hepatitis B virus in hemodialysis patients with chronic HCV infection. *J Nephrol* 2011; **19**:327–333.

13 Marijt-van der Kreek T, Molenaar-de Backer MW, Hogema BM, Zaaijer HL. The yield of universal antibody to hepatitis B core antigen donor screening in the Netherlands, a hepatitis B virus low-endemic country. *Transfusion* 2015; **55**:1206–1213.

14 Whang DH, Seo DH, Song EY, Han KS. Hepatitis B virus DNA in blood donors with anti-HBc as a possible indicator of active hepatitis B virus infection. *World J Hepatol* 2015; **7**:600.

15 Dindoost P, Chimeh N, Hollinger BF, Saberfar E, Norouzi M, Jazayeri SM. The pigeonhole of occult hepatitis B. *Acta Med Iran* 2014; **52**:582–590.

16 Gerlich H, Bremer C, Saniewski M. Occult hepatitis B virus infection: detection and significance. *Dig Dis* 2010; **28**:116–125.

17 Behzad-Behbahani A, Mafi-Nejad A, Tabei SZ, Lankarani KB, Rashidi M, Rasouli M, Salah AR. Indication of anti-HBc antibody screening and HBV-DNA detection in diagnosing latent hepatitis B virus infection. *Iran J Med Sci* 2015; **30**:28–33.

18 Squadrito G, Spinella R, Raimondo G. The clinical significance of occult HBV infection. *Ann Gastroenterol* 2013; **27**:15.

19 Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Chen DS. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. *Hepatology* 2012; **57**:441–450.

20 Ching LK, Gounder PP, Bulkow L, Spradling PR, Bruce MG, Negus S, McMahon BJ. Incidence of hepatocellular carcinoma according to hepatitis B virus genotype in Alaska Native people. *Liver Int* 2016; **21**:11931.

21 Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Chen DS. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. *Hepatology* 2012; **57**:441–450.

22 Motta JS, Mello FC, Lago BV, Perez RM, Gomes SA, Figueiredo FF. Occult hepatitis B virus infection and lamivudine-resistant mutations in isolates from renal patients undergoing hemodialysis. *Gastroenterol Hepatol* 2010; **25**:101–106.

23 Ray L, Nanda SK, Chatterjee A, Sarangi R, Ganguly S. A comparative study of serum aminotransferases in chronic kidney disease with and without end-stage renal disease: need for new reference ranges. *Int J Appl Basic Med Res* 2015; **5**:31.

24 Ramaty E, Maor E, Peltz-Sinvani N, Brom A, Grinfeld A, Kivity S, Segal G. Low ALT blood levels predict long-term all-cause mortality among adults. *A historical prospective cohort study. Eur J Intern Medicine* 2014; **25**:919–921.

25 Alavian SM. Is hepatitis C virus positivity a contributing factor to occult hepatitis B virus infection in hemodialysis patients? *Hepat Mon* 2012; **12**:242–243.

26 Sav T, Gursoy S, Torun E, Sav NM, Unal A, Oymak O, Utas C. Occult HBV infection in continuous ambulatory peritoneal dialysis and hemodialysis patients. *Ren Fail* 2010; **32**:74–77.

27 Bivigou-Mboumba B, François-Souquière S, Deleplancque L, Sica J, Mouinga-Ondémé A, Amougou-Atsama M, Rouet F. Isolated antibody to hepatitis B core antigen in patients with chronic hepatitis C virus infection. *World J Gastroenterol* 2016; **12**:4406–4410.

28 Myers RP, Thibault V, Poynard T. The impact of prior hepatitis B virus infection on liver histology and the response to interferon therapy in chronic hepatitis C. *J Viral Hepat* 2010; **10**:103–110.

29 Sagnelli E, Pisaturo M, Martini S, Sagnelli C, Filippini P, Coppola N. Advances in the treatment of hepatitis B virus/hepatitis C virus coinfection. *Expert Opin Pharmacother* 2014; **15**:1337–1349.

30 Emara M, El-Gammal N, Mohamed L, Bahgat MM. Occult hepatitis B infection in Egyptian chronic hepatitis C patients: prevalence, impact on pegylated interferon/ribavirin therapy. *Virol J* 2010; **7**:324.