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Hepatitis B Core Antibody as a Marker of Occult Hepatitis B in Haemodialysis Patients

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Abstract

Background: Occult hepatitis B viral infection (OBI) is defined as hepatitis B virus (HBV) DNA detection in serum by sensitive diagnostic tests in HBSAG-ve patients with or without serological markers of previous viral infection. **Aim of the study:** was to evaluate hidden infection of hepatitis B among hepatitis B surface antigen -vechronic kidney patients (CKD) on regular haemodialysis (HD) by using hepatitis B core antibody (HBcAb) as a marker in the serum of these patients and HBV DNA by polymerase chain reaction (PCR). **Patients and Methods:** Eighty chronic kidney disease (CKD) patients on regular HD were included in this study. They were selected from HD Unit, Internal Medicine Department, Bab Alsharia University Hospital, Al-Azhar University, Cairo, Egypt, after exclusion of HBs Ag+ve, IV drug user and alcoholic patients, all patients were subjected to full history taking, blood chemistry, HBs Ag by ELIZA, hepatitis B core immunoglobulin G (anti HBc IgG), HBDNA by PCR, hepatitis C antibody (HCVAb) by ELIZA and abdominal ultrasound for assessment of hepatic parenchymal function. **Results:** Mean age of studied patients was 41.8 ± 12.72 years, our results showed that HCV Abs were +ve in 50% of cases (40 cases), from these patients 30% (12 cases) were +ve for HBcIgG, while 50% of cases (40 cases) were –ve for HCV Ab and of HCV-ve cases 20% (8 cases) were +ve for HBcIgG, on the other hand the remaining 32 patients were –ve for both HCV Abs and HBcIgG. All these results showed –ve PCR in all cases (0% of cases). **Conclusion:** OBI among group of Egyptian haemodialysis patients is low with 0% prevalence by PCR, 6 months repeated PCR is recommended as liver biopsy is difficult in HD patients and HBc Abs are not sufficient for diagnosis of OBI in HD patients.

Keywords: Hepatitis B core, occult, HD

Introduction

Hepatitis B infections are major health problems in Egypt and the entire continent of Africa. Egypt is considered to be a region of intermediate prevalence for HBV infection with a reported figure of 3.3%[(1)].

OBI is the major cause of post transfusion hepatitis B in western countries and in countries like India and Taiwan, with higher risk of transmission than for HCV or HIV[(2)].

Anti-HBV prophylaxis (with hepatitis B immunoglobulin, lamivudine, or their combination) appears to be very effective in preventing de novo HBV hepatitis in the recipients but not to avoid HBV reinfection [(3)].

Aim of the work

Was to evaluate the hidden infection of hepatitis B among hepatitis B negative CKD patients on regular hemodialysis by using hepatitis B core antibody as a marker in the serum of these patients in addition to HBV PCR.

Patients and Methods

A total of 80 patients with end stage renal disease on regular hemodialysis were included in this study. They were 44 males and 36 females, with a mean age 41.8± 12.728 years with the mean hemodialysis duration 52.42 ± 39.856 months. Dialysis was performed 4 hours three times weekly using bicarbonate buffer and heparin sodium. Patients were selected from Hemodialysis Unit, Internal Medicine Department, Bab-Alshaeria University Hospital, Cairo after exclusion HBs-Antigen positive, intravenous drug abuser and alcoholic patients.

Data collected from each patient included history taking including the following: Age in years, sex, duration of hemodialysis, shifting between dialysis units, number of transfused blood units, number of A-V fistula operation/s, diabetes mellitus and hepatitis B virus vaccination.

Blood biochemistry: All patients were investigated for serum alanine aminotransferase (ALT), aspartate aminotransferase(AST), alkaline phosphatase (ALP), albumin(Alb), prothrombin time (PT), Bilirubin (Bil), hemoglobin (Hb), calcium(Ca), phosphorus(P), parathyroid hormone (PTH),creatinine (Cr) and blood Urea(U).Testing for hepatitis B virus serology included hepatitis B surface antigen (HBsAg), Anti-hepatitis B core immunoglobulin G (Anti-HBc IgG) by ELIZA. Hepatitis B virus DNA (HBV DNA) detection by polymerase chain reaction technique (PCR).Antibody to hepatitis C virus (anti-HCV) by ELIZA.

All patients were divided into two groups according to the results of HCV Abs.

10 ml venous blood sample was drawn from each subject before initiation of dialysis and divided in tubes as follows:

1) Tubes in which blood samples were centrifuged and serum aliquoted and stored at - 20 ^{o}C until processed for HBV and HCV serology and PCR.

2) Tubes in which blood samples were centrifuged and serum aliqouted where routine investigations were done.

3) Tubes containing EDTA for hemoglobin measurement.

Serological tests for hepatitis B markers (HbsAg and Anti-HBcIgG), serum Anti-HCV antibodies were estimated by enzyme linked immuno-sorbant assay**(ELISA)** technique [(4)]using kits from **Dia Sorin Italy** using a fully automated ELISA apparatus **Dia Sorin Italy**.

HBV DNA was estimated by polymerase chain reaction **(PCR)** technique **(Piratvisuth et al., 2013)** [(5)]using commercial kits from **AJ Roboscreen, Germany** by real time PCR ABI 7000 apparatus. The sensitivity level was 20IU/ml while the specificity for HBV DNA was 100%.

Abdominal ultrasonographic examination:

To asses liver echogenicity and presence of focal hepatic lesions.

Statistical analysis of data

Data were analyzed using SPSS program version 12 and statistical analysis program version 100. The tests used were:

1. $\overline{X}$ Mean, SD standard deviation: to measure the central tendency of data and the distribution of data around their mean.
2. Student's t test: for testing statistical significant difference between means of two samples.
3. Median: is a measure of central tendency when extremes of values are found in data.
4. X^2 test (Chi square test) to test statistical significant relation between different variable or grades (qualitative data) or percentages.

Results were reported by probability values (p-value): if > 0.05 Non significant (NS), < 0.05: Significant (S), < 0.001 Highly significant (HS)

Results

Among the studied patients, 44 patients were males (55%) and 36 patients (45%), were females, with the mean age was 41.8±12.728 years.

Table (1): Distribution of history data among the studied patients.

	Range	Minimum	Maximum	Mean	Std. Deviation
Age (years)	48	19	67	41.80	12.728
Dialysis duration (months)	136	9	145	52.40	39.856
Number of blood transfusions	20	0	20	5.25	4.545
Number of A-V fistulae	5	1	6	2.22	1.493

Table (2): Group (1) and group(2))as regard hepatitis C.

			Anti-HCV antibodies		p-value	Significance
			Negative	Positive		
Anti-HBc IgG	Negative	Count	32	28	0.35	N.S
		% within Anti-HCV antibodies	80.0%	70.0%		
	Positive	Count	8	12		
		% within Anti-HCV antibodies	20.0%	30.0%		

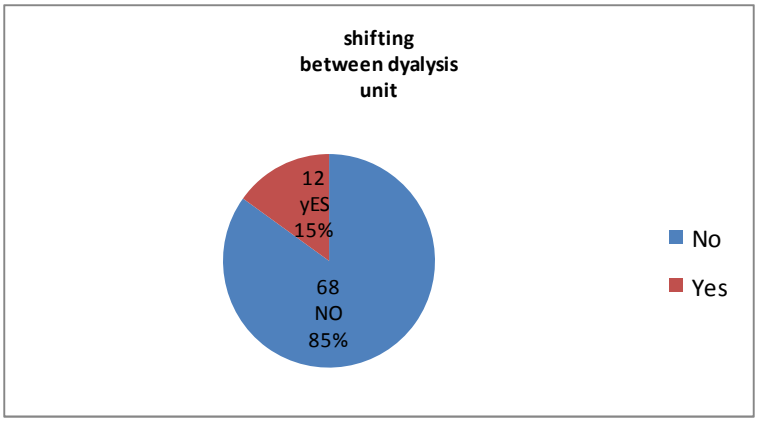

Figure (1): Distribution of patient as regard shifting between dialysis units 15% (n =12) of our studied patients have positive history of shifting between dialysis units.

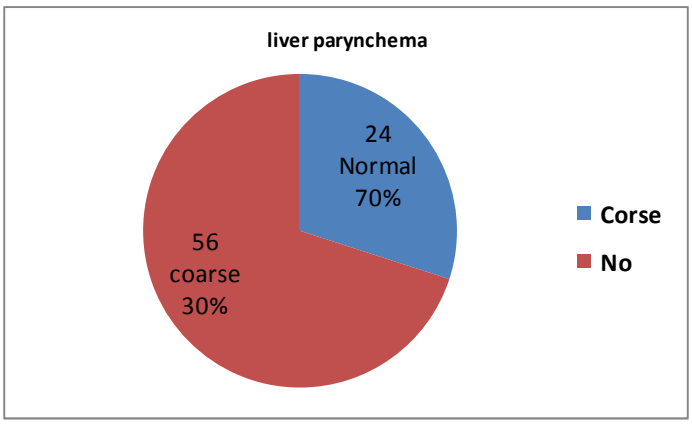

Figure (2): Distribution of Patient As Regard Liver Parynchemal Affection 70% (n=56) of our population had normal ultrasonic liver parenchyma, while30% (n=24) had coarse liver parenchyma, 0% (n = 0) had liver cirrhosis, 0%(n =0) had hepatic focal lesion. All coarse liver paryncheme are associated with HCV antibodies Positive and consider about 60% (n=48).

The prevalence of hepatitis B infection markers among studied patients we found the following. Hepatitis B virus DNA was not detected in the studied patients. On the other hand anti-hepatitis B core immunoglobulin G was positive in 25% (n =20) in our studied patients.

The prevalence of HCV antibodies in the studied patients we found that 50% (n=40) had positive Anti-HCV antibodies from them30% (n=12) patients were positive for anti-hepatitis B core immunoglobulin G, while 50% (n=40) were negative for HCV antibodies and 20%(n=8) were positive for anti-hepatitis B core immunoglobulin G. On the other hand the remaining 30 patients were negative to both HCV and anti-hepatitis B core immunoglobulin G.

Table (3): Relationship between Anti-HBcIgG and Anti-HCV Ab

			Anti-HCV antibodies		p-value	Significance
			Negative	Positive		
Anti-HBc IgG	Negative	Count	32	28	0.35	N.S
		% within Anti-HCV antibodies	80.0%	70.0%		
	Positive	Count	8	12		
		% within Anti-HCV antibodies	20.0%	30.0%		

Table (4): Relationship between Liver parenchyma and Anti-HCV antibodies

			Anti-HCV antibodies		p-value	Significance
			Negative	Positive		
Liver parenchyma	Normal	Count	40	16	<0.01	H.S
		% within Anti-HCV antibodies	100.0%	40.0%		
	Coarse	Count	0	24		
		% within Anti-HCV antibodies	.0%	60.0%		

Table (5): Comparison between group (1) and patients group(2)) as regard liver ultrasonographic findings.

			Anti-HBc IgG		Total	p-value	Significance
			Negative	Positive			
Liver parenchyma	Normal	Count	44	12	56	0.33	N.S.
		% within Anti-HBc IgG	73.3%	60.0%	70.0%		
	Coarse	Count	16	8	24		
		% within Anti-HBc IgG	26.7%	40.0%	30.0%		

Also there was no significant difference between Positive serum Anti-HBcIgG group (1) and Negative serum Anti-HBc IgG group (2) as regard Shifting between dialysis units.

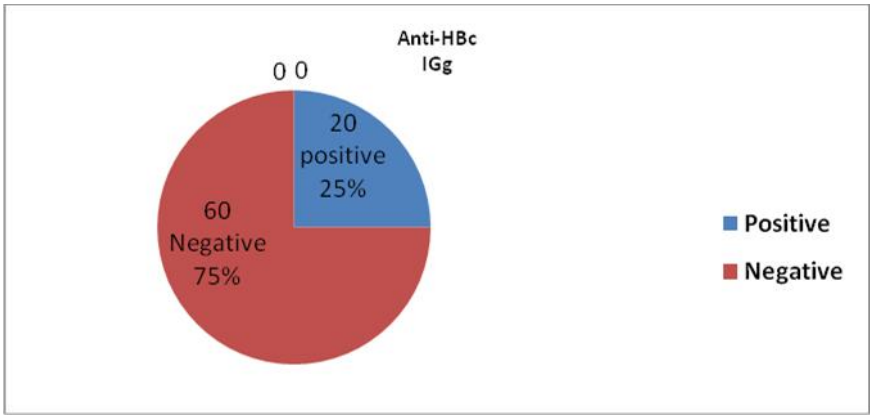

Figure (3): Distribution of patient as regard presence of anti-HBc IgG

Discussion

The presence of HBV infection with undetectable HBs Ag in the presence of HBV-DNA in plasma and or liver resulted in the introduction of the concept of occult HBV infection **(Kitab et al., 2014)**[6].

Therefore, we have studied the presence of HBV DNA in the serum of HBs Ag-negative patients undergoing chronic hemodialysis.

In the current study, the prevalence of occult hepatitis B virus infection was 0%, this prevalence was in agreement with the rates reported by many investigators. This result go in agreement with **Elrashidy et al.**[7],who reported that in 100 HBsAg seronegative chronic dialysis patients, none of them had occult HBV. This result also go in agreement with **Franco et al.**[8]**,** who reported that in 198 HBsAg seronegative chronic dialysis patients, none of them had occult HBV. In Japan among the 82 chronic HBsAg-negative hemodialysis patients, the prevalence of occult HBV infection was 0% [9]**.** In a another study conducted in Vitnam, **by Pipili et al.,**[10]found that occult HBV infection(OHBI) wasnot observed in hemodialysis patients. Also **Aghakhani et al.**[11] addressed the epidemiology of occult Hepatitis B infection in 285 chronic dialysis patients, and found that occult hepatitis B virus infection was absent in all patients.

On the other hand, other investigators reported a higher prevalence of occult hepatitis B among their studied patients. In Egypt among hemodialysis patients**, Ismail et al.,** [12] reported that occult hepatitis B was detected in 6 patients (5.2%) from total of 116 patients on regular hemodialysis.

In other countries, **Makroo et al.,**[13]included 263 hemodialysis patients who were hepatitis B surface antigen-negative, (4.8%) patients were HBV DNA-positive by real-time PCR. In another study, by **Siagris et al.**[14] reported that HBV DNA was detected in (21.1%) hemodialysis patients, and in (5.9%) patients with normal renal function.

In our study, the prevalence of hepatitis B core antibodies IgG was 25%. IgG anti-HBc is not a neutralizing antibody and remains detectable throughout the patient's life once he get infected with HBV[15]. The importance of anti-HBc for HBV screening has been demonstrated in numerous studies [13].

The anti-HBc antibodies does not indicate immunity, high titer of anti-HBc, even with the coexistence of anti-HBsAg, are indicative of HBV replication in the liver[16]**.**Even patients who remain anti-HBc positive for years are at risk of transmitting disease on donation of solid organ tissue[17] or reactivation of HBV disease once immunosuppressed[18]**.**

In recent reports anti-HBc, previously considered a persisting indication of previous HBV infection after all virus has been cleared, has emerged as a convincing marker of occult hepatitis B [19]**.**

Squadrito et al.[20]reported that if we use serum sample in diagnosis of occult hepatitis B virus infection to use a highly sensitive and specific test, like HBV nucleic acid amplification testing (NAT), a PCR technique with detection limits of < 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 case.

From all these reports we can consider our HBcAb positive patients as a potential seropositive OHBI cases with low or non replicative phase andwe can expect some of these patient have the viral DNA in their liver cells.

One could bethe quantitative differences in the levels of HBV viremia during the course of the disease when positive patients may appear negative due to non or low replicative period of the virus, this suggestion is based on data by**Tseng et al.**[21] who examined repeated sera from the same patients for the presence of HBV DNA, and demonstrated inconsistent results with previously negative samples being positive for HBV DNA, and vice versa, which suggests a fluctuating level of viremia in the course of the disease.

Also it should be stated herein that, the detection of HBV-DNA in serum samples rather underestimates the true prevalence of occult HBV infection. Indeed, the most correct and precise methodological approach for the determination of the prevalence of occult HBV infection 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[22]**.**

Raimondo et al.[23]conducted 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), while serum HBV-DNA by quantitative PCR technique was detected only in two patients.

In our study both duration of hemodialysis and number of transfused blood units were significantly higher in IgG anti-HBc positive patients. This result go in agreement with **Ismail et al.**[12]who reporteda significant relationship between hepatitis B core antibodies and longer hemodialysis duration, as well as history of number blood transfusion**.** Also this result go in agreement with **Elrashidy et al.**[7]who reported a significant relationship between hepatitis B core antibodies and longer hemodialysis duration, as well as history ofnumber blood transfusion**.** The significant relation between acquiring hepatitis B infection in our study and the higher dialysis duration gave us the rational to suggested that someanti-HBc positive patients had positive viremia at some time and was the source of the transmission of infection in our unit. In concordance with us **Tseng et al.**[21]reported that viremia in hepatitis B infection is intermittent and occult HBV has the potential to spread silently by nosocomial transmission within the hemodialysis unit as concluded by **Motta et al.**[24].

On the other hand, the significant relation between acquiring hepatitis B infection in our study and number of blood transfusion highlight the significance of the routine screening for HBS Ag 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 hepatitis Bcore antibodies and serum levels of alanine aminotransferase,aspartate aminotransferase, albumin, alkaline phosphatase, Prothrombin time, and bilirubin. Also**,** our results go in agreement with **Aghakhani et al.**[11]who reported that no correlation was found between increased biochemical values of hepatic functions and anti-HBc positivity.

The possible explanation of these results may be due to lake of association between liver injury and level of liver enzyme in hemodialysis patients. The same conclusion was reported by **Ray et al.**[25] who reported that recognition of liver damage by estimation of serum transaminase may be affected by 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[25]

Another explanation, could be dueto an intermittent increase in quality and magnitudeof host immune responses against hepatitis B virus infection that lead to intermittent increase in liver enzyme this mechanism was explained by **Ramaty et al.**[26]. Also we had demonstrated a very low or non replicative state of hepatitis B virus inside liver cell which could explain this nonsignificant impact of positivity for HBcAb on liver enzymes.

The prevalence of hepatitis B core antibodies in HCV positive patients was 30% which was higher than in HCV negative patients 20%. In previous studies **Elrashidy et al.** [7] reported thatthe prevalence of hepatitis B core antibodies in HCV positive patients was 24.39% which was higher than in HCV negative patients 15.25%.Among Egyptian chronic hepatitis C patients the prevalence of anti-HBc was 57% as reported by **Sabry et al.**[27] reported that anti-HBc antibodies were positive in 9.2% of HCV positive hemodialysis patients. Among Egyptian chronic hepatitis C patients the prevalence of anti-HBc was 57% as reported by **Mohamoud et al.**[28]and 59% as

reported by **Kishket al.** [29]. In another study the prevalence of anti-HBc in Chronic Hepatitis C Patients was 12.6% [30]**.** This could be explained by that both HBV and HCV share common routes of transmission.

Also, there was no significant correlation between anti-HBc antibodies and HCV antibodies. Our result go in agreement with**Alavian**[31] who concluded that HCV positivity is not a contributing factor to HBV infection in hemodialysis patients**.** Also the same result was addressed with **Sav et al.**[32] On the other hand, studies by **Bivigou-Mboumba et al.**[33] found a high correlation between anti-HCV and hepatitis B core antibodies.

In the current study there was no significant impact of hepatitis B core antibodies on liver parenchyma or presence of hepatic focal lesion. Also there was no significant impact of positivity of hepatitis B core antibodies among the HCV negative or positive patients as regard parenchymal ultrasonic changes.

Our results go in agreement with **Myers et al.** [34] and **Sagnelli et al.** [35] who studied the impact of positive anti-HBc on liver histology and they concluded that previous HBV infection does not affect liver histology. In the same way, **Emara et al.** [30] showed non significant differences in histological activity and fibrosis between anti-HBc positive and negative patients as well as between anti-HBc positive/ DNA positive and anti-HBc positive/DNA negative patients.

Conclusion

Occult hepatitis B virus infection (serum hepatitis B viral DNA in absence of hepatitis B surface antigen) among Egyptian hemodialysis patient is low with the detected prevalence was 0% and it is better to repeat the test regularly because of difficulty of doing a liver biopsy.

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