

Serological Significance of Anti-HBc IgG Marker detected in Sudanese Haemodialysis Patients

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Abstract

Background: Hepatitis is an infectious disease of the liver caused by viruses that cause different types of infections with different symptoms and illness duration. Hepatitis B virus (HBV) leads to a common nosocomial infection that causes a high rate of mortality and morbidity in the population. In addition it causes hepatitis, liver cirrhosis and hepatocellular carcinoma. It can be transmitted through blood or body fluids by both vertical and horizontal routes of transmission. In hemodialysis patients, cross contamination via blood may be responsible for HBV transmission.

Objective: To assess the serological significance of anti-HBc IgG marker detected in Sudanese haemodialysis patients.

Materials and methods: 168 HBsAg negative specimens were collected from haemodialysis patients. All samples were retested for HBsAg and anti-HBc IgG using ELISA. Presence of Hepatitis B virus (HBV) DNA was tested for by polymerase chain reaction (PCR).

Results: From the 168 HBsAg negative haemodialysis patients investigated, 7 (4.2%) specimens were found positive for anti-HBc IgG; while no sample was found positive for HBV-DNA.

Conclusion: Anti-HBc IgG marker was acceptable for detecting HBV colonization among negative HBsAg haemodialysis patients.

Key words: Anti-HBc IgG marker, Haemodialysis patients, HBsAg, HBV DNA.

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Introduction

Hemodialysis patients are at increased risk of parenterally transmitted infections because they are in an immunosuppressed state and exposed to invasive procedures, share the same dialysis machine, and receive more transfusions than the general population. The relatively low acceptance and response rates to the HBV vaccine among dialysis patients also likely contributes to OBI transmission in hemodialysis patients¹.

The prevalence of OBI in hemodialysis patients varies from 0% to 54% according to the diagnostic techniques or HBV endemicity, and several studies suggest that OBI could be a source of viral spread both to other patients and staff within the hemodialysis units.

Therefore, patients and staff need HBV vaccine boosts to maintain levels of protective antibody to HBsAg (anti-HBs). Strict dialysis-specific infection-control programs, including avoidance of dialyzer reuse and use of dedicated dialysis rooms and machines, should be implemented. Staff for infected patients should be educated on preventive method to limit HBV transmission within dialysis units. Furthermore, regular screening for HBV DNA with sensitive PCR-based assays in all dialysis patients should be considered, and more attention should be given to patients who receive immunosuppressant drugs after renal transplantation².

Acute infection with hepatitis B virus is associated with acute viral hepatitis- an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result. The infection may be entirely asymptomatic and may go unrecognized³.

Approximately 300 million individuals are chronically infected with hepatitis B virus in the world. Enzyme linked immune sorbent assay (ELISA) is still a main detection method for HBV infection, but ELISA result can neither efficiently reflect serum viral load or hepatitis activity nor monitor the efficacy of antiviral treatments. For example, with the introduction of new antiviral agents like lamivudine, close monitoring of patients has become increasingly important due to the occurrence of antiviral drug-resistant virus strains or the presence of flares after withdrawal of antiviral therapy⁴.

Hepatitis B virus (HBV) infection can be detected in blood donations by many serologic markers. In this study the routine anti-hepatitis B core antigen (HBc) and HBsAg screening among these population will be repeated and the result of a number of samples will be deferred on the basis of reactivity to anti-HBc IgG, HBsAg test results. Anti-HBc IgG reactive samples will show a variable result.

Investigation of outbreak of hepatitis B infection associated with these population indicate that transmission most likely occurs because of inadequate infection control practice since routine screening of hepatitis B antibody in blood banks is insufficient.

In this study, HBV infection markers were detected to evaluate the potential significance of anti-HBc IgG testing. The aim of the study was to sero-detect anti-hepatitis B core IgG and to assess its impact on the diagnosis of occult HBV infection.

Materials and methods

This was a qualitative, prospective, hospital-based, analytical, descriptive, and cross-sectional study. This study was conducted during the period from June 2013 to December 2016. All blood specimens were collected from haemodialysis patients attending the Dialysis Center; covering males and females at different age groups. All reagents used were pre-tested using control strains; and equipment were calibrated. The software used for the analysis of data was the Statistical Package for Social Sciences (SPSS) program (version 14). For categorical variables, proportions were compared by the Chi-square test as appropriate. The means and medians of the continuous variables were compared by Student's *t* test program depending on the sample distribution. Frequencies, percentages, tables and graphs were used for presentation of the data. Confidentiality of information obtained from participants investigated was maintained. Consent of the participants was taken before being enrolled in the study. Laboratory results of specimens collected were handed to all participants included in the study. Permission to collect the specimens was obtained from the Dialysis Center authorities at Khartoum. Approval to run the study was taken from Sudan University for Science and Technology (Khartoum). Complete information regarding risk factors, if any, was handed to all participants under the study without concealment.

Sampling was a non- probability purposive sampling type, and sample strategy was convenience where participants were chosen on the basis of accessibility. The sample frame was blood donor participants. Sample size was 168 specimens collected from HBsAg negative haemodialysis patients. Demographic and clinical data were collected from all patients using a structured questionnaire with a written informed consent. Information collected from each patient included: medical history, physical examination, past history of HBV vaccination, past HBV infection, and past blood transfusion or organ transplantation.

All HBsAg negative and anti-HBc reactive samples were tested for the presence of HBV DNA. Molecular biology investigations were conducted at the Central Laboratory of the Ministry of Science and Technology and laboratories of Al-Neelain University (Khartoum). The DNA product was estimated and verified at the National Laboratory of Ministry of Health (Sudan). Hepatitis B surface antigen (HBsAg) was detected by enzyme-linked immuno-sorbent assay (ELISA). Negative HBsAg results were confirmed by a further HBsAg immunoassay. All technical procedures were performed according to the manufacturers' instructions. HBsAg was initially determined in all blood samples collected by ELISA technique (Fortress Diagnostic). Positive HBsAg samples were excluded from the study; and only negative samples were included and subjected to PCR testing using a single set of primers. Blood samples collected were assayed for serological markers of HBV infection, i.e. HBsAg and anti-HBc IgG; using ELISA (Biokit, Barcelona, Spain). A further repetition for at least ten samples (using the same ELISA technique) were carried out as a control. The test results were considered valid if the quality control criteria were verified. Appropriate quality control system was established with quality control material similar to or identical with the patient sample being analyzed.

Detection of anti-HBc IgG was performed as per Foretress manufacturer's instructions, following the same procedure adopted for detection of HBsAg as detailed above. Also anti-HBc IgG test methods for interpretation of results, quality control, and calculation of cut-off values were similar to the methods performed for detection of HBsAg.

HBV-DNA was extracted by the help of the standard phenol/chloroform extraction method (Vivantis GF-1 Nucleic acid extraction kit). Vivantis GF-1 Nucleic acid extraction kit provides a rapid and efficient method for purification of nucleic acid from various samples. Reconstitution of solutions, wash buffers, and complete procedures were performed as per manufacturer's instructions.

The extracted DNA was tested for HBV DNA using PCR techniques using the Maxime PCR PreMix Kit (Invitex, Germany). HBV DNA was amplified using a thermocycler (Perkin Elmer 9700). One set of primer pairs was used: HBV-F 5-TCGGAAATACACCTCCTTTCCATGG-3 HBV-R 5 GCCTCAAGGTCGGTCGTTGACA-3). The diagnosis of occult HBV infection was made when PCR amplifiable HBV DNA in sera was noticed at least in two different regions (surface, pre-core and core) during absence of detectable HBsAg. The round of PCR was performed by setting the primer for 35 cycles. PCR products were subjected to electrophoresis on a 3% agarose gel stained with ethidium bromide, and DNA was observed under ultraviolet light. PCR protocol procedure was conducted according to the manufacturer's instructions. Negative controls that contain everything except the template DNA was used for detecting contamination or non-specific amplification reaction. Positive controls were used for the verification of negative amplification results and the positive control reaction contains the same components as the sample except it includes a template that amplifies the reaction.

Results

168 blood specimens were collected from HBsAg negative haemodialysis patients. Out of these blood specimens, 7 (4.2%) were anti-HBc IgG positive. The PCR test was carried out for 168 HBsAg negative blood donors to detect occult HBV DNA. All samples showed no positive HBV DNA. All DNA bands were accumulating in align with the length of 100 base pair in the ladder column.

Chi-square analysis (p value) was calculated to determine any significant difference between HBsAg and anti-HBc IgG results among haemodialysis patients. HBsAg test outcome revealed few positive cases (3/1.8 %) in haemodialysis patients. This pattern of association was found to be statistically significant (p-value =0.000).

Anti-HBc IgG test outcome also revealed few positive cases (7/4.2%) among haemodialysis patients. This pattern of association was found to be statistically significant (p-value = 0.000). This also means that the mean frequency rate of anti-HBc IgG differed according to the group of population investigated. Also Chi-square analysis was calculated to compare the statistical difference of positive specimens investigated by PCR to exclude occult HBV infection among the patients investigated. This indicates that the relation between negative HBsAg, positive anti-HBc IgG, and HBV-DNA was insignificant. Frequency rates of positive anti-HBc IgG and HBsAg according to age incidence were higher among patients aged 20-40 years and lower above 60 years of age. Positive HBsAg was found more prevalent among vaccinated patients studied, while most positive anti-HBc IgG were more detected among non-vaccinated patients. The specificity of HBsAg, anti-HBc IgG, and HBV DNA was calculated by using PCR results as a gold standard. All samples were found to have true negative and false positive values.

Determination:

Specificity = No. True Negatives / No. True Negatives + No. False Positives
 Specificity measures the proportion of population that are correctly identified as negatives.

True negative: Healthy people correctly identified as healthy

False positive: Healthy people incorrectly identified as sick

The negative predictive values (NPV) are the proportions of negative results in statistics and diagnostic tests that are true negative results.

ELISA HBsAg Specificity = $165 / (3+165) = 98.214\%$

False positive rate (α) = Type I error = $1 - \text{Specificity} = 1 - \text{TN} / (\text{FP} + \text{TN})$
 $= 1 - 165 / (3+165) = 0.018 \%$

Therefore, HBsAg test with 98.214% specificity correctly reports 98.214% of patients without the disease as test negative (true negatives) but 1.786% patients without the disease are incorrectly identified as test positive (false positives).

ELISA anti-HBc IgG Specificity = $161 / (161+7) = 95.83\%$

False positive rate (α) = Type I error = $1 - \text{Specificity} = 1 - \text{TN} / (\text{FP} + \text{TN})$
 $= 1 - 161 / (161+7) = 0.042 \%$

Therefore, anti-HBc IgG test with 95.83% specificity correctly reports 95.83% of patients without the disease as test negative (true negatives) but 4.17 % patients without the disease are incorrectly identified as test positive (False positives).

PCR- HBV DNA Specificity = $168 (\text{True negative}) / (168+0) = 100\%$

False positive rate (α) = Type I error = $1 - \text{Specificity} = 1 - \text{TN} / (\text{FP} + \text{TN})$
 $= 1 - 168 / (168+0) = 0\%$

A DNA test show 100% specificity correctly identifies all patients without disease. The analysis concluded that the test applied to detect HBsAg in this study was more specific than anti-HBc IgG test. DNA test was the most specific test to identify HBV (Table 1)

Discussion

This study dealt with determination of the serological and molecular markers of HBV using ELISA and PCR techniques. It correlated the findings obtained with HBV vaccination, previous blood transfusion, previous organ transplantation, and past history of bilharziasis. Sudan had been classified among the African countries with high HBsAg endemicity. This endemicity rate ranged from as low as 6.8% in Central Sudan to as high as 26% in other parts of Sudan. Our study found a high seropositivity of HBsAg and anti-HBc IgG tests among patients aged less than 45 years.

In this study no HBV DNA was detected by PCR in all sera with positive or negative HBsAg and anti-HBc IgG among blood donors. This proved that the presence of positive anti-HBc IgG does not rule out the endemicity of HBV DNA or HBV transmission. The molecular characterization of occult HBV was not confirmed in our study.

Table (1): True negative and false positive values in samples investigated

Rates	HBsAg	HBcAb	PCR
True positive	0	0	0
False positive	3	7	0
True negative	165	161	168
False negative	0	0	0
Specificity	98.21%	95.83%	100%
False positive rate	0.018 %	0.042 %	0%

This study showed that the frequency rate of HBsAg among haemodialysis patients was not statistically significant in relation with gender ($p = 0.294$). This finding was similar to that reported by other authors who found a non-significant correlation between anti-HBsAg and gender ($p > 0.05$)⁵.

Also our study found a significant frequency rate of HBcAb ($p = 0.007$) when comparing haemodialysis male patients (62.3%) with females patients (37.7%). This result agreed with previous reports⁶ which documented a high frequency rate of positive HBV markers. Among hemodialysis patients, the present study revealed that the HBsAg carrier rate was less frequent (3/1.8%) than that of HBcAb (7/4.2%). Similar findings were documented by a large Italian study on chronic hemodialysis patients, where the frequency rate of HBcAb was more (36%) than HBsAg (1.9%) and HBV DNA (0%). Similar findings were reported in studies⁷. Gasim and his coworkers in Sudan reported similar frequency rates for HBsAg (4.5%) and for HBcAb (39.4%).

In the present context, the frequency rate of positive HBsAg was found significantly higher (15.3%) among males than among females (1.9%) investigated ($p = 0.000$). This finding was similar to what reported elsewhere where males were found to be six times more likely to become carriers of HBsAg than females⁸.

Furthermore, the frequency rate of positive HBcAb was found insignificantly higher (15.7%) among males than among females (12.4%) investigated ($p = 0.332$). This finding was different from that reported by other workers⁹, who found a frequency rate of 93.2% among males and 85.7% among females ($p > 0.05$).

Pre-seroconversion window period (WP) infections are most likely transmit HBV but transmission from occult HBV infection remains debated¹⁰. In immunocompetent recipients, there is no evidence that anti-HBs-containing components (even at low titer) are infectious. Anti-HBc IgG only, with HBV DNA, can be associated with infectivity, as can rare cases of HBV DNA without any serological HBV marker¹¹. Addition of anti-HBc IgG testing for donor screening (although will lead to rejection of a large number of donor units), shall definitely eliminate HBV infected donations and help in reducing

HBV transmission with its potential consequences, especially among the immunocompromised population¹².

In this study, in spite of past history of HBV vaccination, three patients were found negative for HBsAg and 28 HBsAg positive samples were detected among non-vaccinated patients. None of these patients were found co-infected with anti-HBc IgG and HBV DNA. Conclusion: Positive anti-HBc IgG and HBsAg markers did not exclude OBI. The diagnosis of OBI requires the use of highly sensitive Real Time or Nested PCR techniques.

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