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Original Article

Prevalence of occult hepatitis B infection in patients visiting tertiary care hospital

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ABSTRACT

Background: To study the prevalence of occult hepatitis B virus infection (OBI) in a tertiary care hospital.

Methods: 50 HBsAg negative individuals, each amongst blood donors, alcohol dependence syndrome (ADS), alcoholic cirrhotics, hepatitis C virus (HCV)/cryptogenic cirrhotics, end-stage renal disease (ESRD) on maintenance haemodialysis for one year, all malignancies prior to chemotherapy and HIV positive patients were evaluated for anti-HBc total antibody, and blood hepatitis B virus (HBV) DNA amplification in those tested positive.

Results: A total of 60/369 (16.2%) individuals were anti-HBc total positive, 13/50 (26%) of HCV/cryptogenic cirrhotics, 13/52 (25%) of HIV positive, 10/50 (20%) of patients with malignancy, 10/51 (19.6%) and 7/59 (11.9%) of alcoholic cirrhotics and ADS respectively had intermediate prevalence, while, blood donors 5/55 (9.1%), ESRD patients 2/52 (3.8%) had low prevalence. 12 patients (20% of all anti-HBc total positive cases) were HBV DNA positive, 5 HCV cirrhotics (10% of total HCV/cryptogenic), 4 HIV positive (7.69%), 1 each of ADS (1.69%), alcoholic cirrhotics (1.96%) and malignancy group (2%). Blood donors and ESRD patients were negative for HBV DNA.

Conclusion: HBV DNA amplification may under diagnose OBI and anti-HBc total positivity may be a better surrogate marker. Nucleic acid testing of blood donors, however is preferred, especially in high endemic areas. OBI must be looked for in cirrhotics, HIV infection, and patients with cancers prior to chemotherapy, as they may contribute to morbidity in them.

Introduction

Hepatitis B virus (HBV) is a major public health problem worldwide. Blood transfusion is one of the most common routes for spread of infection. In order to reduce the transmission of HBV, pretransfusion screening of blood donors by serum HBsAg was being carried out. However it was observed that HBV transmission can still occur from HBsAg negative blood donors despite above screening measures. Thus, the term occult

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hepatitis B virus infection (OBI) was introduced.[1] OBI is defined as condition when HBsAg is undetectable in serum, despite the presence of HBV DNA in liver or blood.[2]

There are several mechanisms, which have been hypothesized for OBI are studied and are due to interplay of host and viral factors. These includes: (a) residual low viremia following overt HBV infection due suppression of replication following strong immune response; (b) APOBEC-3 proteins deamination-dependent and deamination-independent actions reduce replication of HBV DNA[3]; (c) genomic integration into host's chromosomes as ccc HBV DNA leading to decrease replication and reduced expression of HBsAg[4]; (d) epigenetic mechanisms[3]; (e) HBV-containing immune complexes are formed, which may cause non detection of HBsAg due to its masking with anti-HBs antibodies; (f) co-infection of HBV with other viruses (hepatitis C virus, HCV), which causes inhibition of replication of HBV; (f) extra hepatic replication of HBV in peripheral blood mononuclear cells.

The clinical significance of OBI includes; (a) can cause fulminant hepatitis due to reactivation of frank infection in immune-compromised hosts like in HIV, patients on Chemotherapeutic drugs[5]; (b) potential risk of transmission of infection through blood donors, transplant donors, and haemodialysis[6,7]; (c) association with development of hepatocellular carcinoma[8]; (d) effects the progression of disease and treatment response in chronic HCV patients; (e) may be associated with cryptogenic liver disease.[7]

Hence, there is a requirement for evaluation of Occult HBV infection (OBI). HBV DNA screening, therefore, carries lot of significance in certain clinical contexts. As HBV DNA is expensive and laboratory intensive; anti-HBc total, as a marker of previous HBV infection, in this situation, becomes an appropriate screening tool.

Material and methods

The study was done at a tertiary care center from June 2012 till Dec 2013. At least 50 patients in each subset with negative HBsAg test (by standard kits) were included. The inclusion subsets of patients were (i) blood donors, (ii) alcohol dependence syndrome (ADS) fulfilling CAGE criteria, (iii) alcoholics with cirrhosis of liver, (iv) patients of HCV or cryptogenic cirrhosis (diagnosis of cirrhosis of liver was considered on the basis of history, clinical signs and symptoms, USG abdomen for liver echotexture, portal vein size, ascites, and or UGI endoscopy for esophageal varices), (v) patients of malignancy prior to chemotherapy, (vi) end-stage renal disease (ESRD) patients on at least one year of haemodialysis (diagnosis of ESRD was considered on the basis of uremic symptoms), GFR <15, which was calculated as creatinine clearance by Cockcroft gault formula and deranged RFT for 3 months or more duration, (vii) HIV positive individuals. The following patient groups were excluded; (i) HBsAg positive individuals in above categories, (ii) age <18 and >75 year, (iii) pregnancy, (iv) patients having overlap between two categories.

All patients were subjected to detailed history, complete physical examination, hematological and biochemical investigations as per protocol, an ultrasonography abdomen for features of cirrhosis was done in all patients for fulfilling inclusion and exclusion criteria; HBsAg and anti-HCV, HIV TEST was done by ELISA in all patients, anti-HBc total was measured in all the patients. HBV DNA analysis was done by real time PCR in patients, who were found positive for anti-HBc total.

Table 1 – Total number of patients and their mean age in each group.

Category	N	Mean age
Blood donors	55	32.67
ADS	59	36.25
Alcoholic cirrhotics	51	47.94
HCV/Cryptogenic cirrhotics	50	56.10
ESRD on MHD for atleast one year	52	50.02
Malignancy patients prior to chemotherapy	50	58.18
HIV positive patients	52	35.81
Total	369	44.87

Results

This study included 369 patients of various categories. The mean age of the patients was ranging from 32 to 58 years (Table 1). A majority of males were noted in the study population more so in the subsets of ADS patients and alcoholic cirrhotics (Table 2). There were 55 blood donors, 59 ADS patients, 51 patients of alcoholic cirrhosis, 50 patients of HCV/cryptogenic cirrhosis and 52 patients of ESRD on maintenance haemodialysis, 50 patients of malignancy before starting chemotherapy and 52 HIV positive patients. A total of 60/369 (16.2%) individuals were found to be anti-HBc total positive, who were negative for HBsAg (Table 3). Maximum prevalence of anti-HBc total was found in HCV or cryptogenic cirrhosis group 13/50 (26%), followed by HIV positive patients

Table 2 – Sex wise distribution of population in each group.

		Group							Total
		Blood donor	ADS	Alcoholic cirrhotics	HCV/Cryptogenic cirrhotics	ESRD on MHD for atleast one year	Malignancy patients prior to chemotherapy	HIV positive patients	
Sex	F	1	1	0	22	16	13	0	53
	M	54	58	51	28	36	37	52	316
Total		55	59	51	50	52	50	52	369

Table 3 – Number of anti-HBc total positive/negative patients found in each group.

		Anti-HBc total		Total
		Negative	Positive	
Group	Blood donors	50	5	55
	ADS	52	7	59
	Alcoholic cirrhotics	41	10	51
	HCV/Cryptogenic cirrhotics	37	13	50
	ESRD on MHD for atleast one year	50	2	52
	Malignancy patients prior to chemotherapy	40	10	50
	HIV positive patients	39	13	52
Total		309	60	369

Table 4 – Number of patients found to have HBV DNA positive in each group.

		No of patients	HBV DNA positive
Group	Category-blood donor	55	0
	ADS	59	1
	Alcoholic cirrhotic	51	1
	HCV + Cryptogenic cirrhotics	46 + 4 = 50	5 (all HCV)
	ESRD on MHD for atleast one year	52	0
	Malignancy patients prior to chemotherapy	50	1
	HIV positive patient	52	4
Total		369	12

13/52 (25%), malignancy patients prior to chemotherapy 10/50 (20%), alcoholic cirrhosis 10/51 (19.6%), ADS 7/59 (11.9%), blood donors 5/55 (9.1%) and ESRD patients on haemodialysis for at least one year 2/52 (3.8%) (Table 3).

Further HBV DNA analysis among anti-HBc total patients revealed 12 patients (20% of all anti-HBc total positive cases) were found to have HBV DNA in their blood. Among those 5 were HCV cirrhotics (10% of total HCV/cryptogenic), 4 were HIV positive (7.69%), 1 each in ADS (1.69%), alcoholic cirrhosis (1.96%) and malignancy prior to chemotherapy group (2%). Blood donors and ESRD patients did not show any HBV DNA in their blood (Table 4).

Discussion

By using routine serological markers (HBsAg) for screening of HBV infection, though the incidence of post transfusion hepatitis B infection has come down but number of cases have been reported in past, where the HBV infection has been transmitted by donors having OBI raising concerns about the safety of blood transfusions and organ donation.[1] OBI is defined as the presence of HBVDNA in liver with undetectable hepatitis B surface antigen (HBsAg) in blood. Though it is not practical to perform liver biopsy in all cases, serum HBV level is taken into account for estimating low grade viral replication with cut off of <200 copies/ml. Anti-HBc total has been used as surrogate marker for HBV infection in some of studies for detecting OBI and has been made mandatory by some of the countries with low prevalence of HBV infection. Anti-HBc total may not be used as surrogate marker for OBI in countries with high prevalence rates of anti-HBc total such as India (10.82–58.8%) and Pakistan (17.28%), as it can lead to high rejection rates of blood.[9–12] Accurate diagnosis of OBI requires sensitive HBV-DNA PCR assay, which may be ideal in such countries but is not cost effective. A study in Egypt on 3167 patients revealed 14.2% positivity for anti-HBc total, while 17.2% among those were found to have HBV DNA positive.[13] In our study 5 out of 55 blood donors (9.1%) were found to be positive for anti-HBc; however, prevalence of OBI was found to be 0% in healthy blood donors, which further makes a case for not making anti-HBc total mandatory for screening for OBI in blood donors. In our study, we found 7/59 patients (11.9%) of ADS to be anti-HBc total positive. Among these anti-HBc total patients, only 1 patient was detected to have HBV DNA in the blood. Prevalence of OBI in this group is 1.6%. In fact, literature search did not reveal any study in the above study population.

In our study, we found 10/51 patients (19.6%) of Alcoholic cirrhotics to be anti-HBc total positive. 10% of such anti-HBc total positive patients were detected to have HBV DNA in their blood. Hence, prevalence of OBI in this group overall was found to be 1.9%, though limited work has been done to find out prevalence in above subject. Nevertheless, the difference in the prevalence between ADS and alcoholic cirrhotics is insignificant, suggesting a non-significant role of OBI in the progression of disease and cirrhosis in ADS or contribution of OBI in the pathogenesis of alcoholic cirrhosis.

OBI has highest prevalence among HCV patients.[14–16] A study by Cacciola et al.[15] revealed OBI in 33% of HCV infected cirrhotic patients, which was higher in anti-HBc positive group and can also possibly enhance the progression to cirrhosis. In patients with cryptogenic liver disease, there is less available information than in HCV patients, but the prevalence is thought to range from 19% to 31%.[17,18] Work done to find prevalence of OBI in cryptogenic cirrhosis has been limited. In our study, we had 46 patients of HCV cirrhosis and 4 patients of cryptogenic cirrhosis. A total of 13/50 patients (26%) were found to be anti-HBc total positive in this combined group. None of the cryptogenic patient was found to be anti-HBc total positive. 28.2% HCV cirrhosis patients were anti-HBc total positive and HBV DNA was detected in 5/13 of them. Prevalence of OBI is 10.46% in HCV cirrhosis and 0% in cryptogenic cirrhosis. Over all prevalence of OBI in this group was 10%. The clinical significance of OBI in this group of patients lies in the accelerated progression to cirrhosis and HCC with OBI and also the altered response to interferon therapy.

The CKD patients on hemodialysis are at high risk of acquiring transmitted infections, which may be due to increase in the number of invasive procedures, blood transfusions and immunosuppressed state. In a prospective study of 96 patients undergoing hemodialysis done in Mansaura University Hospital, OBI was detected in 18.8% patients.[19] In a study conducted in eastern Japan, Nagakawa et al. showed that the prevalence of occult HBV infection in chronic hemodialysis patients was 0.3%.[20] A study conducted in Sudan by Nafisa et al. on patients undergoing hemodialysis

did not show any OBI.[21] Makarem et al.[22] showed OBI in 4.1% patients and anti-HBc positivity of 20% in a study of 145 patients in Egypt.

In our study, anti-HBc was detected in 2 out of 52 patients (3.8%). HBV DNA was not detected in any anti-HBc positive patient. Prevalence of OBI was found to be 0% in CKD patients on hemodialysis for at least one year, which is commensurate with some of the above studies.

Patients having malignancy with OBI may be at risk of reactivation on starting chemotherapy.[23] A study conducted by Laurenti et al. in Italy on 397 patients of CLL prior to chemotherapy revealed 34 (8.4%) patients to have OBI.[24] A study conducted in India by Sodhi et al. in Kashmir for the presence of overt HBV and occult HVB infection among 690 cancer patients prior to starting chemotherapy revealed occult HBV infection in 1.9% (13/690) patients and overt HBV in 14% (98/690) patients.[25] In our study, we found 10/50 patients (20%) of all malignancy, freshly diagnosed, prior to start of chemotherapy to be anti-HBc total positive. 1/10 anti-HBc total positive was detected to be HBV DNA positive and prevalence of OBI was found to be 2%. Thus, reactivation of HBV following chemotherapy can be anticipated in a significant number of patients.

The various studies on prevalence of OBI in HIV patients have shown variable results with prevalence ranging from 0% to 89%.[26–30] In our study, we found 13/52 HIV positive patients (25%) to be anti-HBc total positive. 4/13 anti-HBc total positive patients were found to have HBV DNA in their blood. Prevalence of OBI was found to be 7.69% in this group. The prevalence of OBI in HIV positive patients in our study was similar to few of the above-mentioned studies.

The importance of such a high prevalence of anti-HBc total positivity in this subset of patients needs to be defined. Immune reactivation of HIV patients after ART could possibly lead to reactivation of HBV infection and hence the need to consider dual anti-HBV antiviral in such cases. This needs further evaluation by prospective studies.

The limitations of our study were that actual prevalence of OBI in various groups of patients is likely to be more than as concluded in our study as patients who were anti-HBc total negative were not included for DNA analysis (to make study cost effective), and liver biopsy for HBV DNA was not done, which has high yield for HBV DNA as per various studies. Also, exact comparison of prevalence of OBI with previous studies could not be authenticated, due to lack of standardization of diagnostic tools for OBI and difference in material and methods used.

Conclusion

It is concluded from our study that using only DNA amplification as a marker for OBI among blood donors may underdiagnose OBI and using anti-HBc total for detection may be a better surrogate marker. However in countries, where HBV infection is endemic, anti-HBc donor screening cannot be used because of its high prevalence, nucleic acid testing screening of pooled blood donors is presumed to be necessary and very beneficial. Furthermore, patient with cryptogenic or HCV cirrhosis, HIV infection, cancers prior to chemotherapy need to be evaluated for OBI, as they may contribute to morbidity and possible risk of reactivation of HBV in such a group of patients.

Future directions

The importance of the presence of OBI leading to worsening of pre-existing cirrhosis needs consideration and further study. In addition, patients with HIV infection and OBI requiring HAART should receive dual drugs which are active against HBV also (Tenofovir based). Further prospective studies are required to ascertain the role of OBI.

Conflicts of interest

The authors have none to declare.

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