Prevalence of Hepatitis B DNA and Serological Markers of Hepatitis B Infection among HBsAg Negative Voluntary Blood Donors in Kashmir Valley

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ABSTRACT

Introduction: Hepatitis B infection has many modes of spread, with blood being the main source in majority of infection. The population at risk for the blood borne infection includes mainly the recipients of blood transfusion and medical professionals. The risk of hepatitis B infection in the recipients of blood and blood products even after screening of donors for HBsAg has been found to be 1 in 60,000 transfusions. This points towards the need to find the reason for this transmission and suggesting preventive measures. Our study focuses on finding the prevalence of HBV DNA among screened blood donors. Objective: to detect HBV DNA and other serological markers in HBs negative voluntary blood donors of Kashmir valley.

Material and Methods: The present study was undertaken in the Postgraduate Department of Medicine, Government Medical College, and Srinagar. This was a hospital based cross sectional study and was conducted on 100 healthy voluntary blood donors of Kashmir region. Blood was tested for HBV DNA through NAAT. Other serological markers were also assessed

Results: All donors were found negative for HBV DNA. Regarding serological markers only 8 patients were positive for HBeAg, 4 were positive for anti HBc antibody.

Conclusion: Our part of the world falls in low endemic zone for hepatitis B infection and voluntary blood donation is also very less. Our study did not find any voluntary donors to be positive for Hepatitis B DNA but further large multicentre funded studies are required to help our blood donation to be more safer.

Keywords: Hepatitis B DNA, Serological Markers, Hepatitis B, HBsAg Negative, Voluntary Blood Donors

INTRODUCTION

An estimated 400 million persons in the world today are carriers of hepatitis B virus which is a small DNA virus that belongs to Hepadnaviridae family.[1] Almost 50% of world's population live in the regions where the prevalence of Hepatitis B surface Antigen (HBsAg), can exceed 8%. Another 40% of the World's population lives in areas with intermediate prevalence, 3%-5%, such as Japan and India. In low-prevalence areas such as United States, Western Europe, and Australia, the rate of HBsAg positivity is 0.1-2%. In endemic areas, most infection occurs in children, whereas in areas of low seroprevalence, most infections occurs in adults.[2] The risk of hepatitis B infection in the recipients of blood and blood products even after screening of donors for HBsAg has been found to be 1 in 60,000 transfusions.[3] However, before transfusion, donor blood is tested for HBsAg, Anti HCV, and HIV status by using sensitive methods as a part of requirement laid by WHO and NACO in this respect. Nevertheless, for reasons that are not clear, occasionally cases of transfusion associated Hepatitis B still occur despite donor blood screening for HBsAg by more sensitive techniques. This means that some blood donor units are infectious for hepatitis B even though they test negative for HBsAg. These blood units may be coming from donors who actually are occult hepatitis B carriers having low levels of circulating HBsAg, i.e. less than 1ng/ml which is too low to be detected even by radio immunoassay.[4]This means that the method used for detection of HBV infection in voluntary blood donors must be highly sensitive and specific.

Keeping in consideration the above stated facts detection of anti-HBc antibodies and HBV DNA in the donors could be used as additional modality for detecting the infectivity of donors. Screening blood for anti-HBc antibodies and HBV DNA will obviously increase the detection rate of number of infective donors who otherwise would be HBsAg negative. However their detection may have limitation because anti-HBc antibodies especially anti-HBc IgG may remain positive lifelong in a donor who was previously infected with HBV even after clinical and biochemical recovery and yet the same donors may be non-infective. Secondly detecting HBV DNA is cumbersome and costly and therefore is not practicable in third world countries.

The above practice is already being used in USA and many European countries. However such a practice will not be feasible in Indian subcontinent due to large patient population as destroying the infective blood would produce shortage of the blood and blood products.[6] Another fact that here deserves a mention is that populations in hyperendemic zones of HB virus infection will have higher rates of anti-HBc antibodies positivity and HBV DNA detection. This means huge

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How to cite this article: Ahmad Wajeed Yousuf, Showkat A Kadla, Najma Saqib, Awhad Mueed Yousuf, Shafat Lone, Bilal A Wani. Prevalence of hepatitis B DNA and serological markers of hepatitis B infection among HBsAg negative voluntary blood donors in Kashmir valley. International Journal of Contemporary Medical Research 2017;4(10):2052-2056.

number of donors will be unsuitable for blood donation which will further aggravate the blood shortage scenario. Therefore currently the practice of screening HBsAg in blood donors is only being performed and screening anti-HBc and HBV DNA is not recommended in Indian subcontinent.[6] Recently studies have shown that blood recipients of HBsAg negative but Anti HBc positive and HBV DNA positive do not develop transfusion associated Hepatitis 'B'. This means that there are other serological markers which are having promoting effect in the development of TAH. These markers could be HBe antigen, Anti HBe antibody and Anti HBs antibody.

Over the last decade, nucleic acid amplification testing (NAT) has become a routine part of donor blood screening in developed countries, as well as also being introduced in some developing countries.[7] As a screening tool, individual donor NAT detects infection before serological tests; 25-36 days earlier for HBV 10-16 days earlier for HIV-1, 49 – 65 days for HCV.[8,9] These undetected window period infections are responsible for most of the transfusion transmission of these viruses.[10,11] In addition, NAT is also useful for determining the incidence of active infection by these viruses in blood donor populations.

The infection with HBV in our community is very less (Intermediate zone for HBV infection). But studies regarding this issue are very limited in our part of the world. Currently till date no study has been performed in which the HBV DNA and other serological profile of HBsAg negative voluntary blood donors has been studied.

MATERIAL AND METHODS

The present study was undertaken in the Postgraduate Department of Medicine, Government Medical College, and Srinagar between December 2013 to November 2014. This was a hospital based cross sectional study and was conducted on healthy voluntary blood donors of Kashmir region. One hundred healthy voluntary blood donors hailing from the valley of Kashmir (age range 18-60 yrs.) were taken by consecutive sampling. Each voluntary blood donor was asked detailed history. A detailed clinical examination was performed in each donor. We excluded those donors who had Past history of jaundice / hepatitis like illness,Previous history of surgical procedure, invasive procedure like UGIE / LGIE / Coronary angiography,Previous dialysis, Previous transfusion of blood or blood products or ant haemophilic factors, Tattooing / body piercing, clinical examination revealing stigmata of chronic liver disease, history of high risk behaviour, all subjects who had received hepatitis "B" vaccine, family history of hepatitis B and C infection.

Blood Sampling

7mls of blood from each voluntary blood donor was put in a EDTA containing vacutainer tube and 3 mls of blood was put in gel tubes. They were stored at a temperature of 2-8. Each tube was labelled with numerical number as a code. The blood was investigated for following parameters:-

1) HBsAg / anti HCV/HIV (I, II) /VDRL screening by rapid test and ELISA. Procedure was performed as per guidelines laid down by the kit manufacturer.

The details of the kits used are as follows

▲ Rapid screening kits- with specifications to be used:

- HBsAg - Noo2076
- HCV - 019133
- Biostandard (India)
- HIV(I, II, III) - 023421
- VRDL/Syphilis - SYP 0120025 {Acon Biotec (China)}

2)ELISA screening kits -with specifications to be used:

- HBsAg - Benesphera - IHF - 15001007 (Kit Manufactured in USA)
- HCV - Benesphera - IHF-1500997 (Kit Manufactured in USA)
- HIV - Benesphera - IHF - 1500992 (Kit Manufactured in USA)

3) Anti HBs antibodies, Anti-HBc IgM, Anti-HBc Total antibodies, HBe Antigen and Anti HBe Antibody screening was performed by ELISA method.

The details of the kits used are as follows:

▲ ELISA screening kits specifications as per manual of the kits.

- Anti HBs Antibody - DS-EIA-B-551 (Kit Manufactured in USA)
- HBe Antigen - DSI-EIA-B-951 (Kit Manufactured in USA)
- Anti HBe Antibody - DSI-EIA-B-851 (Kit Manufactured in USA)
- Anti HBc IgM - DS-EIA-B753 (Kit Manufactured in USA)
- Anti HBc Total - DRG-EIA-3894 (Kit Manufactured in USA)

4) HBV DNA was done using NucleicAcid Amplification

Technology (NAAT---- using machine ROCHE COBAS S201).

The blood was processed in mini pools of six samples for HBV DNA, HCV RNA and HIV RNA. The procedure was performed as per the steps described in the machine manual.

RESULTS

Out of total number of 100 voluntary blood donors, maximum (44%) were between 26-35 years of age and 59% were from rural areas. All the voluntary blood donors studied were negative for HBsAg, Anti HCV and HIV I/II by rapid card test and ELISA. Seventeen percent of the voluntary blood donors had bilirubin between 1 and 1.5. Four percent of the voluntary blood donors had bilirubin between 1.6 and 2 mg/dl. None of the voluntary blood donors had bilirubin >2.6mg/dl. Six percent of voluntary blood donors had serum protein between 5 and 5.5 g/dL, one percent of voluntary blood donors had serum albumin between 2.8 and

Interpretation of Results		
I.	HBsAg / anti HCV / HIV (I, II) Rapid test)	A positive test meant appearance of coloured line as compared to the "control line" (given on Test Strip).
II.	Anti-HBc IgM antibody	a. Titre of → ≥ 1.00 was labelled as reactive and detected.
III.	Anti-HBc Total antibody	a. combination of anti HBc IgM and anti HBc IgG.
IV.	If anti-HBc IgM antibody was negative, anti-HBc total was positive, it meant that blood was positive for anti-HBc IgG	
V.	If anti-HBc IgM was positive and anti-HBc total was negative, it meant that blood was positive for IgM anti HBc antibody.	
VI.	If blood was positive for both (anti-HBc IgM and anti-HBc Total), it meant that blood was positive for anti-HBc IgG with persistent positivity of anti-HBc IgM antibody which is seen in 20% of patients. It would also mean, a chronic HBV infected patient with current acute exacerbation.	
VII.	HBe Antigen a. Titer of → ≥ 1.00 was labelled as reactive and detected.	
VIII.	Anti HBe Antibody: Titer of → ≥ 1.00 was labelled as reactive and detected.	
IX.	HBV DNA was a qualitative estimation. The results interpreted were as positive or negative.	

Sex	Number	Percentage
Male	100	100
Age group (Years)		
18-25	24	24%
26-35	44	44%
36-45	19	19%
46-55	12	12%
56-60	1	1%
Demography	**No. of Voluntary Blood Donors**	**Percentage**
Rural	59	59
Urban	41	41
Total	100	100

Table-1: Socio demography of study participants

Serological markers for Hepatitis B infection	Positive	Negative	P value
HBeAg	8	92	0.5794
Anti HBeantibody	6	94	
ANTI HBc IgM	4	96	0.7004
ANTI HBc Total	3	97	

Table-2: Association between serological markers of hepatitis B in the study population

3.50. More than half of voluntary blood donors had ALP <70 IU/L, 47% had ALP between 70.01 and 140 IU/L. Nearly half of voluntary blood donors had ALP <2times the upper normal limit of ALP mean ALP was 71.59IU/.Majority of the voluntary blood donors had AST <40 U/L, 13% had AST between 41-70U/L and 3% had AST between 71-100 U/L. Sixteen donors had AST <2 times the upper limit mean AST was 29U/.Majority of the voluntary blood donors had ALT <40 U/L, 4% had ALT between 71 and 100 U/L, 1% had ALT between 101 and 130U/L and 2% had ALT between 131 and 160U/L. Twenty eight percent of the voluntary blood donors had ALT <2 times the upper normal limit of ALT while as seven (7%) had ALT >2 times the upper normal limit. Minimum and maximum ALT was 5.3 U/L and 134 U/L respectively. Mean ALT was 33.72 U/L. All the voluntary blood donors were negative for HCV RNA, HBV DNA, and HIV RNA by NAAT. 7 voluntary blood donors had 1 serological marker positive for Hepatitis B, 7 were having 2 serological markers positive and 1 was with 3 serological markers positive for Hepatitis B. Liver function tests including amino-transferases were normal in all of them (table 1,2).

DISCUSSION

There is very limited research done on prevalence of hepatitis B infection among normal Kashmiri population. A study conducted by Makroo et al in 1974 has revealed a prevalence rate of 1.1%[12] as per which Kashmir falls in low prevalence area (HBV carrier rate < 2%).In our study, 100 voluntary blood donors were studied. In this study the blood from these voluntary donors was routinely tested for HBV, HCV, HIV I/II, syphilis and Malaria as per the guidelines laid down by the Drugs and Cosmetics Rules of the Ministry of Health and Family Welfare (Regulatory Authority for Indian Blood Banks).[13] Blood from RCT and ELISA negative donors was studied by NAAT. The results of our study did not detect even a single HBV DNA positive donor. These results are slightly different from the studies which have been performed in this regard in the various parts of the globe.[13-23] In these studies the prevalence of HBV DNA has been found to range from 0.00007% to 0.038%.

In India, one study regarding NAAT based screening of blood have been performed in 2014[24] and another study in this regard has undertaken in AIIMS, New Delhi in October 2009 to July 2010.[13] In former study 28,134 samples were studied. It was only in 25 (0.088%) out of 28,134 that HBV DNA was detected, while as in AIIMS study (2010), 18354 donors were studied. The result of this study was 07(0.038%) donor samples detected to have HBV DNA despite negative serology.[23] There are other studies from Japan, Switzerland, Bangkok, Italy where more than 5-10 lac samples have been studied and these studies have shown NAAT HBV DNA detection rate of 0.001-0.005% with Japan having a percentage of 0.001% consistently in two studied sample. These studies showed HBV DNA by NAAT in India is about 30 times more than that of Western countries. A large chunk of total blood donors in western countries are voluntry, but in India, nearly half of the blood donors are replacement donors (who in turn are high prevalence donors) and only 15% (2008 data) of all donors are voluntary who in turn have less prevalence of HCV.[13] Futhermore the majority of Indian voluntary blood donors are first time donors this could

explain the high NAAT yield in Indian studies.

As per NACO statistics, in India there are 43 million with HBV.[13] Keeping in view, the higher prevalence in India and a prevalence of 1.1% (Makroo et al) in Kashmir, it is important to do NAAT for HBV DNA as a screening for HBV.

The reason for the different result in our study could be because of low prevalence of HBV infection in our valley as compared to the rest parts of India. In our study none of the donors had detectable Anti HBs antibodies. In our study we performed HBe Antigen by ELISA. Eight patients had this antigen positive. The relevance of this is that possibly these donors are infected but do not have HBsAg antigen, HBV DNA titers in the range of detection. The implication of a positive HBe Antigen would mean replication, however negative HBe antigen would mean either non replicating phase or infection with pre core mutant. Anti HBe was positive in 5 donors (5%) and results were equivocal in 4 donors (4%). Those 5 samples which were positive for anti HBe were all positive for HBe antigen, however, 4 donors who had equivocal anti HBe antibody only one was positive for HBe antigen.While studying the Anti HBc status in our study, we have seen that IgM Anti HBc was positive in 4 (4%) donors and equivocal in 1 (1%) donor. IgM Anti HBc is a marker of acute HBV related hepatitis. Anti HBc Total was positive in 3 (3%) donors. The significance of Anti HBc Total is that it is a marker of chronic HBV related status i,e either indication of resolved HBV infection in the remote past, low grade chronic infection or occult HBV infection with a mutant strain of HBV.[6] In our study one sample which was positive for Anti HBc Total was also positive for IgM Anti HBc, but was negative for HBe antigen and anti HBe. Another Anti HBc Total positive donor had equivocal IgM Anti HBc but negative HBe antigen and Anti HBe. The third sample positive for anti HBc Total was negative for all HBV related parameters. Overall in our study there were 7 (7%) samples positive for Anti HBc, out of this 4 (4%) were positive for IgM Anti HBc and rest that is 3(3%) were positive for Anti HBc Total. A study by O'Brien Et al was undertaken in Canada in 2007. In this study 4, 93,344 were studied for HBV by NAAT. In their study they detected HBV DNA positivity in 29(0.52%) samples. All of them were HBsAg negative and Anti HBc detectability rate was 1.13%. A study has been undertaken in India in 2007 by Brig AC Anand et al. In this study they detected 522 samples out of 5619 samples positive for Anti HBc IgG giving a positive rate of 10%.[6] The results of this study are not in accordance with the results of our study. The main reason for this is that India is falling in the intermediate zone prevalence zone.[2]

Bernvil et al from Saudi Arabia studied prevalence of anti HBc in voluntary blood donors.[25] They found IgG anti HBc positive in 16.4% However in their study, it is not mentioned what percentage of donated blood was positive for Anti HCV antibody, since anti-HCV positivity itself has a suppressive role in detection of HBsAg antibody thereby bias due to HCV positivity cannot be ruled out. Lok ASF et al 1998 screened 1801 Chinese subjects aged 1-90 years,[26] and found 11.90% subjects had positive anti-HBc which is close to the results of our study. Regarding the detectability of Anti HBc Antibody in NAAT positive HBV DNA but HBsAg negative donors, it has been found in an India study that only in 48% Anti HBc was detected despite positive HBV DNA. Thus, it implies that Anti HBc Antibody is not a complimentary marker for HBV DNA but it is a marker with huge consequences as its positivity will add to the quantum of donors who were rendered useless by positive DNA by NAAT thereby resulting in wastage of the donated blood. HBV DNA detection has been found to be highest in Anti HBc positive and Anti HBsAg negative individuals. Further, it has been found that risk of transmitting HBV infection from IgG Anti HBc positive donors is 2.1-8.6%.[27]

At an HBV prevalence rate of 4% in India[6], the most of the studies available in India have detected HBV by NAAT in about 0.03 -0.08% of donors. When we take a prevalence rate of around 1.1% in Kashmiri population (Makroo et al)[12], there is a chance that out of 15,000 donations(which take place in our blood banks per year) we will get a HBV positive rate of 2-4(0.013-0.026%) donors.

As screening of donor blood for Anti HBc Total is not done in our blood bank, a positive percentage of 3 donors would mean that 450 out of 15000 blood donors will be infected with HBV. Thus as per literature 2.1% to 8.86%[48] of these 450 donors may transmit the infection to the recipients.

The main drawbacks of our study were; a low sample size and the inclusion of only voluntary blood donors, which in turn resulted in low sample size.

CONCLUSION

The recommendation from our study is that the NAAT for HBV DNA detection should be employed as pre transfusion screening requisite in our blood banks and secondly the application of Anti HBc Total and Anti HBs titer screening should be done on donor's blood as a screening protocol also. By doing this we will be actually preventing transfusion of 450 donor units with HBV positivity per year.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 24-09-2017; **Accepted:** 20-10-2017; **Published:** 03-11-2017