

Inclusion of Seromarkers to Exclude Hepatitis B Virus Infection *via* Transfusion in Voluntary Blood Donors

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Abstract

Introduction: Hepatitis B Virus (HBV) infection is a serious viral infection causes acute and chronic hepatitis. Transfusion of blood and blood products is the predominant route of transmission of HBV. As per NACO guidelines, in India, donor blood is screened for HBsAg, anti-HCV antibodies, anti-HIV antibodies; slide/card rapid screening for malarial parasites and VDRL for Syphilis.

Blood banks in India are only equipped to carry out rapid card test or ELISA for routine screening of HBV, HCV and HIV infections. In developed countries use Nucleic Acid Testing (NAT) were in place for screening and diagnosis of viral infections during window period. NAT assays are highly sensitive, specific and robust but require skilled manpower and specialized infrastructure and thus may not be cost-effective.

Objectives: To explore the current sero-prevalence of HBsAg, Anti-HCV antibodies and anti-HIV antibodies (and other relevant seromarkers) by routine screening tests in a cohort of voluntary blood donors and to determine the efficiency of NAT over the routinely used rapid card and ELISA tests. To include any additional seromarker of HBV infection to detect the antigen in the window period.

Methods: In this cross-sectional observational study, 3160 voluntary blood donors were screened for HBsAg. Samples were also subjected to screening of additional seromarkers such as HBeAg, anti-HBs and anti-HBc antibodies. Thirty samples were randomly selected from each HBsAg positive and HBsAg negative groups and were subjected to HBV DNA PCR for viral load estimation.

Results: ELISA testing is more specific than rapid card test in detecting HBV infection, whereas NAT testing was beneficial in identification of added HBV infection. Both ELISA and rapid card test methods were found to be equally sensitive and specific in diagnosing HCV and HIV infections. NAT testing did not yield any significant increase in positive results compared to serological/rapid methods. HBeAg was found to be positive in 2 samples which are tested negative for HBsAg. HBV DNA was also found to be positive in one sample which is negative for HBsAg (1 out of 30), which might be cryptic HBV infection.

Conclusion: Though NAT would be the choice of screening for Hepatitis B, it not cost-effective. Therefore, inclusion of one or more seromarkers for screening HBV, such as HBeAg, might be helpful in preventing transfusion transmitted infection

Keywords: Hepatitis; Seromarkers; Blood donors; Human immunodeficiency virus

Abbreviations: NACO: National Aids Control Organization; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; TTI: Transfusion-transmitted Infections; ELISA: Enzyme-linked Immunosorbent Assay; NAT: Nucleic acid Amplification Test

Introduction

Hepatitis B is one of the most common infectious diseases globally, which spreads through blood and other infected fluids such as semen. Individuals can be infected with the virus through sexual contact, by sharing needles or by accidental needle-stick, by getting a body

piercing or tattooing, and by vertical transmission from mother to foetus.

Viral and immune markers are detectable in blood, and characteristic antigen-antibody patterns evolve over time. Hepatitis B Surface Antigen (HBsAg) in serum is the first sero-marker to indicate active HBV infection, either acute or chronic. The first detectable viral marker is HBsAg, followed by HBeAg and HBV DNA. Serological tests, such as HBsAg, anti-HBs and anti-HBc are used to distinguish acute, self-limited infections from chronic HBV infections and to monitor vaccine-induced immunity [1-4].

Hepatitis B Core Antigen (HBcAg) appears in hepatocytes within 2 weeks after HBV infection. Anti-HBcIgG forms during the recovery phase of infection and is persistent for life, thus, the presence of this antibody in blood indicates past HBV infection. Anti-HBc testing was introduced in the mid-1980s for screening of blood donors in HBV

non-endemic countries, such as the United States. However, HBV endemic countries were unable to implement anti-HBc screening because many blood products would be discarded due to positive screening tests even though most of the blood would be safe for transfusion. In 1989, Japan introduced anti-HBc testing with a modified algorithm in which anti-HBc-reactive blood with titers <1:32 or 1:32 with anti-HBs 200 IU/mL were used for transfusion.

In India, a total of 30 million blood components are transfused each year and most of the blood banks are equipped only to carry out card test or the ELISA method for the routine screening for HBsAg, anti-HCV antibodies and anti-HIV antibodies. Blood safety thus becomes a top priority; especially with a population of around 1.23 billion and a high prevalence rate of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in general population. Hence any diagnostic assays used in the blood banks should be sensitive, specific, user-friendly, rapid, robust and affordable. Thus increasing the sensitivity of the assays especially those that are used in blood banks becomes more important and valuable [2].

Nucleic acid Amplification Testing (NAT) in blood donor screening has been implemented in many developed countries to reduce the risk of Transfusion Transmitted Infections (TTIs). NAT takes care of the dynamics of window period of viruses and offers the safest blood pack for donation. Automated triplex NAT assays are highly sensitive, specific and robust. But these assays require highly skilled manpower which is scanty in India. Also, the cost of equipment and reagents is very high. In resource-limited settings, it will remain out of reach to a large population. In the present study, our objective is to find out the efficiency of NAT tests over the regularly used card test and ELISA tests [3].

Aims and Objectives

The clinical outcome of occult HBV transmission primarily depends on recipient immune status and the number of HBV DNA copies present in the blood products. The presence of donor anti-HBs reduces the risk of HBV infection by approximately five-fold. Furthermore, Occult Hepatitis B Infection (OBI) is transmissible by blood transfusion [4].

The amount of viral DNA in the serum is typically very low in cases of true OBI. Because testing liver tissue is not always practical or possible, OBI is often diagnosed through serum HBV DNA and viral marker tests. A positive OBI test may be found in blood donors as a result of various clinical conditions, including: (1) the incubation period of acute infections; (2) the tail-end stage of chronic hepatitis B; (3) low-level viral replication after recovery from hepatitis; and (4) escape mutants not detected by current HBsAg tests [5-8].

Therefore, our study aims to:

- Explore the current sero-prevalence of HBsAg, anti-HCV and anti-HIV antibodies and their co-infection, among voluntary blood donors by the routine rapid assays (card test) and by ELISA methods.
- Explore the real efficiency of NAT tests in terms of increased sensitivity in identifying the potential pathogens in voluntary blood donors who are found negative by the regular serological assays used by the blood banks for the routine screening and compare these results with those of the routine tests.
- Study the role of HBV sero-markers in identifying the HBV disease status in HBsAg positive cases-to study the pattern of HBV

serological profile for identifying the stage of HBV stage in chronic HBV infected subjects among voluntary blood donors who are asymptomatic, and also identify the cryptic cases and non-responders.

- Assess the relevance of the sero-markers and HBV viral load in randomly selected HBsAg positive and negative cases to establish the fact that inclusion of one or more sero-markers in routine blood screening could be more effective in the prevention of transfusion induced Hepatitis B infection.

Materials and Methods

The study protocol was reviewed and approved by the Institutional Review Board (IRB) [9-12].

Design: This is a cross sectional observation study which includes 3160 individuals enrolled between 2010 and 2014.

Inclusion criteria: The study population was participants who came to the blood bank centers to donate their blood. All these 3160 samples were screened.

Exclusion criteria: Volunteers with acute febrile illness ongoing or in the previous two weeks, ongoing chronic illness, recent donation of blood in the previous 3 months, long-term medication for a serious chronic disorder such as epilepsy, history suggestive of a bleeding disorder and substance abuse.

Method: Hepatitis B was screened using rapid card test, ELISA and NAT. HBV viral load-done by RT PCR (AB 7500) to measure number of copies of the virus in the infected normal individual.

Discussion and Results

The major objective of this study was to estimate the sero-prevalence of TTI markers and to compare the efficiency of rapid card test and ELISA in detecting the TTIs among the voluntary blood donors. The HBV prevalence by rapid test was 3.9% whereas by ELISA the prevalence was 4.1%. The HCV and HIV prevalence was 0.06% by the rapid and ELISA method, and there was no difference in the sensitivity between the rapid test and ELISA (Tables 1 and 2).

The next objective of this study is to explore the efficiency of NAT tests in comparison with the serological assays. Though NAT screening did not improve the positivity significantly, it was able to detect additional positives for HBV samples which were HBsAg negative. This would help reduce the rate of TTIs further. ProcleixUltra qualitative in vitro nucleic acid assay was found to be more sensitive (100%) than other serological assays for the diagnosis of HBV infection [13].

Among HBV vaccinated group, 23% had >10 mIU anti-HBs compared to non-vaccinated group 1.9% whereas HBeAg was found to be significantly higher in non-vaccinated (91.7%) group showing the rate of non-responders (Table 3).

Among HBsAg negative groups (Controls), anti-HBs positivity was found to be much higher (61%) than other HBV serological marker suggestive of naturally resolved HBV infection (Tables 4 and 5).

Randomly selected n=30 (financial constraint) from each group of HBsAg positive (vaccinated and unvaccinated) and negative were subjected to HBV DNA PCR, 26.7% found to viremic among HBsAg positive group compared to 3.3% of occult HBV in HBsAg negative group (Table 6). This forms the occult group of patients.

In recent years, Occult HBV Infection (OBI) has been widely investigated. OBI is identified as the persistence of HBV-DNA in HBsAg negative patient's liver with or without other serological markers of previous HBV infection. To explain this phenomenon three mechanisms have been proposed. For two of these, the common factor is the change in the steric configuration in HBsAg molecule, determined by mutations located within the "a" determinant. These modified HBsAg are actually very weakly exposed in the surface of hepatocytes due to a poor recognition by the immune system. Finally, several authors suggest that host immune surveillance and epigenetic mechanisms are probably involved [14].

| Marker | Rapid Test # of positive (%) | ELISA # of positive (%) | Prevalence (%) |
|--------|---------------------------------|----------------------------|----------------|
| HBV | 126 (3.9) | 132 (4.1) | 4.1 |
| HCV | 2 (0.06) | 2 (0.06) | 0.06 |
| HIV | 2 (0.06) | 2 (0.06) | 0.06 |

Table 1: Prevalence of TTI markers and comparison of rapid card versus ELISA.

| Markers | Rapid card Test # of positive (%) | ELISA # of positive (%) | NAT #of positives (%) |
|---------|--------------------------------------|----------------------------|--------------------------|
| HBV | 126 (3.9) | 132 (4.1) | 134 (4.2) |
| HCV | 2 (0.06) | 2 (0.06) | 2 (0.06) |
| HIV | 2 (0.06) | 2 (0.06) | 2 (0.06) |

Table 2: Overall prevalence of TTI markers among blood donors.

| HBV Vaccination | Number n=134 | % |
|-----------------|--------------|------|
| Vaccinated | 26 | 19.4 |
| Non Vaccinated | 108 | 80.6 |

Table 3: Group showing the rate of non-responders.

| Donor category | HBsAg Positive | HBeAg | | Anti-HBc | | Anti-HBs | |
|----------------|----------------|-------|------|----------|------|----------|------|
| | | No. | % | No. | % | No. | % |
| HBV vaccinated | 26 | 15 | 57.7 | 2 | 7.7 | 6 | 23.1 |
| Non vaccinated | 108 | 99 | 91.7 | 12 | 11.1 | 2 | 1.9 |

Table 4: Prevalence of HBV markers among HBV Positive blood donors.

| Donor category (Control s) | HBsAg Negative | Anti-HBc | | | Anti-HBs | |
|-------------------------------|----------------|------------|------------|------------|------------|------------|
| | | Positive % | Positive % | Negative % | Positive % | Negative % |
| | | | | | | |

| | | | | | | |
|----------------|-----|---|---|----|----|----|
| HBsAg Negative | 100 | 2 | 4 | 96 | 61 | 39 |
|----------------|-----|---|---|----|----|----|

Table 5: Prevalence of HBV markers among HBV Negative blood donors.

| Donor category | Number screened | HBeAg | | | | HBV Viral load | | | |
|----------------|-----------------|----------|-----|----------|------|----------------|------|-------|------|
| | | Positive | | Negative | | >1000 | | <1000 | |
| | | No | % | No | % | No | % | No | % |
| HBsAg positive | 30 | 12 | 40 | 18 | 60 | 8 | 26.7 | 22 | 73.3 |
| HBsAg negative | 30 | 2 | 6.7 | 28 | 93.3 | 1 | 3.3 | 29 | 96.7 |

Table 6: HBV viral load among HBV positive and negative blood donors.

Conclusions

Based on our findings, we suggest:

A. Inclusion of additional seromarkers in the following 3 groups:

Vaccinated HBsAg positive individuals- HBeAg

Unvaccinated HBsAg positive individuals-HBeAg /Anti-HBc

Control group (HBsAg negative)-Anti-HBs

B. Molecular characterisation for characterisation of protective genes and those that increase and risk of susceptibility to Hepatitis B infection and its complications are recommended. This will enable further understanding of susceptibility risks.

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