

# Surface Antigen (HBsAg) Negative Hepatitis B virus in Bangladesh: Warrants the Screening for Blood Donors

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## Abstract

*In every case of blood transfusion, so called proper donor screening is done every day. At the end of beautiful day, after reviewing lots of national and international guidelines and papers of blood transfusion, proved that blood donor screening methods & tests are not adequate & appropriate according to WHO. Lots of multi-transfused thalassaemic & hemophiliac patients getting hepatitis B virus undoubtedly. Implementations of strict donor selection criteria and use of sensitive laboratory screening tests reduce the incidence of HBV transfusion. These courageous heartiest review may awake the decision makers thinking rethinking about HBV free blood transfusion service in Bangladesh.*

**Key words:** Blood transfusion, Blood donor screening, transfusion transmissible infections.

## Introduction

Blood transfusion is the process of transferring blood or blood based products from donor into the circulatory system of recipient. <sup>1</sup> Transfusion of blood & blood component as a specialized modality of patient management saves million of lives each year worldwide. <sup>2</sup> The microbial agents are transmissible by blood transfusion & can cause morbidity & mortality in recipients. The infectious agents may present in the blood for long periods, sometimes in high titers, stability in blood stored at 4°C or lower, long incubation period before the appearance of clinical signs, asymptomatic phase or only mild symptoms in the blood donor, hence not identifiable during the blood donor selection process. <sup>3</sup>

In developing countries, blood safety remains an issue of major concern. Preventing the transmission of HBV through blood transfusion in developing countries are difficult. The priority objective of blood transfusion service is to ensure safety, adequacy, accessibility & efficiency of blood supply at all levels. These strategies have been extremely effective but transmission of HBV still occurs. Inability of the test to detect the disease in the pre-seroconversion or window phase of their infection, immunologically variant viruses, non-seroconverting chronic or immunosilent carriers & laboratory testing errors may be the possible causes. <sup>4</sup>

Bangladesh, a developing country of South-East Asia, has a population of 160 million. HBV is the most common cause of chronic liver diseases including cirrhosis of liver, hepatic failure, & hepatocellular carcinoma. The risk of acquiring new HBV infection has been reduced in Bangladesh with the introduction of hepatitis B surface antigen (HBsAg) screening in blood donors. However, with the advent of 'occult HBV infection', it is now clear that many HBV-infected subjects may not express HBsAg (surface antigen to HBV) but may harbor HBV DNA. <sup>5</sup> From different studies of blood & organ recipients have recently shown HBV transmission from anti-HBc positive (core antibody against the core antigen), HBsAg negative donors, even when HBV DNA was undetectable in the donor. <sup>6</sup> In fact, the blood transfusion system of Bangladesh has not been optimized to tackle these situations. <sup>5</sup>

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## Hepatitis B Virus (HBV)

Hepatitis B virus (HBV) is an enveloped DNA virus that infects the liver and causes hepatocellular necrosis and inflammation. HBV infection can be either acute or chronic, and may range from asymptomatic infection or mild disease to severe or rarely fulminant hepatitis.<sup>7</sup>

## Epidemiology & Burden of HBV

Around 2 billion people have evidence of past or present infection with HBV, & 240 million are chronic carriers of HBV surface antigen (HBsAg). Worldwide, it is estimated that around 650 000 people die each year from the complications of chronic hepatitis B virus infection.<sup>7</sup>

## Hepatitis B Virus (HBV) Infection

New-onset hepatitis B infection that may or may not be icteric or symptomatic.<sup>7</sup> For good understanding of HBV infection should know about some important conditions (Fig 1)<sup>7,8,9,10</sup>

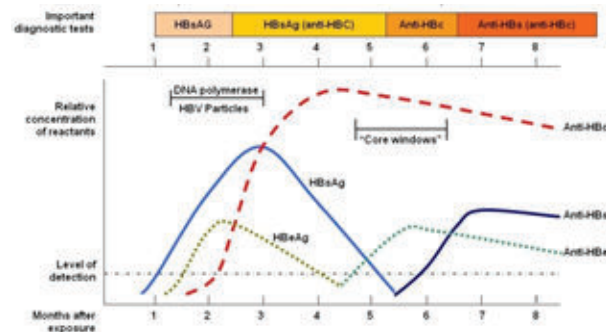


Fig.-1: Profile of HBV infection<sup>11</sup>

## Acute Hepatitis B Virus (HBV) Infection

Diagnosis of acute HBV infection is based on detection of hepatitis B surface antigen (HBsAg) and IgM antibodies to hepatitis B core antigen (anti-HBcIgM).<sup>7,8,9,10</sup>

## Natural Immunity

Recovery is accompanied by clearance of HBsAg with seroconversion to anti-HBs (antibodies to hepatitis B surface antigen) usually within 6 months.<sup>7,8,9,10</sup>

## Chronic Hepatitis B Virus (HBV) Infection

Persistence of hepatitis B surface antigen (HBsAg) for six months or more along with anti-HBcIgG (IgG antibodies to hepatitis B core antigen) after acute infection indicate chronicity. Age is a key factor in

determining the risk of chronic infection (Figure 3.1). Chronicity is common following acute infection in neonates (90% of neonates born to hepatitis B e antigen [HBeAg]-positive mothers) & in young children under the age of 5 years (20–60%), but occurs rarely (<5%) when infection is acquired in adulthood. Worldwide, the majority of persons with CHB were infected at birth or in early childhood.<sup>7,8,9,10</sup>

## Incubation Period:

The time between exposure of HBV and appearance of symptoms.<sup>7,8,9,10</sup>

## Window Period:

The time interval between the disappearance of HBsAg and the appearance of anti-HBs (antibody to surface antigen).<sup>7,8,9,10</sup>

## Occult Hepatitis B Virus (HBV) Infection:

Absence of HBsAg despite presence of anti-HBc (core antibody) and HBV-DNA <1000 copies/ml or <200 IU/ml (Fig 2)<sup>7,8,9,10</sup>

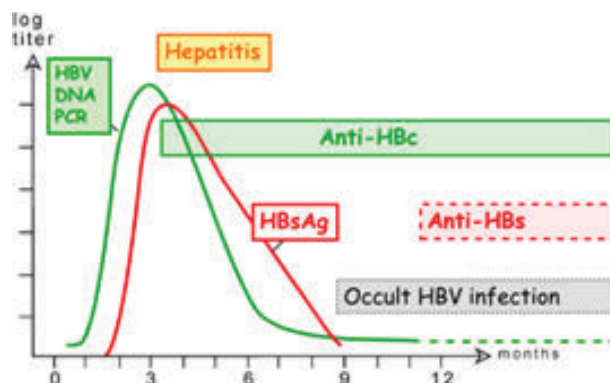


Fig.-2: Occult HBV infection<sup>12</sup>

## Transfusion Transmissible Infections (TTI)

Many viruses [mostly hepatitis B virus, hepatitis C virus, HIV & less commonly cytomegalovirus (CMV) & Epstein Barr Virus (EBV)], bacteria (*Treponema pallidum*) & protozoa (malarial parasite) can be transmitted by transfusion.<sup>13,14</sup> Like other developing countries, blood banking in Bangladesh does not get enough attention for development from authorities. Many blood recipients remained at risk of TTI transmission as a result of poor blood donor recruitment & the use of low-quality testing in TTI screening.<sup>15</sup>

**Transmission of HBV through transfusion**

HBV is spread predominantly by percutaneous or mucosal exposure to infected blood.<sup>7</sup> Several studies from different countries including Bangladesh indicates that, blood transfusion is the main source for transmission of HBV (Table I & II).

**Table I**  
*Seroprevalence of HBV among multi-transfused Thalassaemic children*

Country	Year	Total Children	HBsAg Positive
India <sup>16</sup>	2017	211	10 (2.3%)
Egypt <sup>17</sup>	2016	97	4 (4.1%)
Iran <sup>18</sup>	2016	90	3 (3.3%)
Pakistan <sup>19</sup>	2015	145	5 (3.5%)
Malaysia <sup>20</sup>	2015	100	4 (4.0%)
Bangladesh <sup>21</sup>	2014	200	13 (6.5%)
Bangladesh <sup>22</sup>	2013	100	3 (3.0%)
India <sup>23</sup>	2013	218	8 (3.6%)
Bangladesh <sup>24</sup>	2012	95	21 (22.1%)
Bangladesh <sup>25</sup>	2007	42	8 (19%)
Bangladesh <sup>26</sup>	2003	152	21 (13.8%)

**Table II**  
*Seroprevalence of HBV among multi-transfused Hemophiliac children*

Country	Year	Total Children	HBsAg Positive
Pakistan <sup>27</sup>	2017	396	12 (3.0%)
Iran <sup>28</sup>	2015	146	2 (1.4%)
USA <sup>29</sup>	2012	400	32 (8.0%)
Iran <sup>30</sup>	2007	81	8 (9.8%)
Iran <sup>31</sup>	2002	101	27 (26.7%)

Donor screening tests for HBV in Bangladesh and Abroad

Nowadays in Bangladesh, routine screening of blood donors for HBV is only HBsAg (surface antigen to HBV).<sup>32</sup> Along this test, International organizations added some recent tests for prevention of HBV

transmission. With HBsAg (surface antigen), American red cross,<sup>33</sup> Australian red cross,<sup>34</sup> U.S. food and drug administration (FDA),<sup>35</sup> United Kingdom (UK)<sup>36</sup> & Singapore health science authority<sup>37</sup> recommends for blood donor screening anti-HBc [core antibody against the core antigen (HBcAg) (these antigen usually present in liver not in serum)] and Nuclieic acid testing (NAT)<sup>38</sup> (It is a molecular technique for screening of HBV which providing an additional layer of blood safety). But Centers for disease control and prevention (CDC)<sup>39</sup> and World Health Organization (WHO)<sup>13</sup> recommends cost effective approach for developing countries like Bangladesh are serum HBsAg and Anti-HBc. When both are negative or HBsAg negative but Anti-HBc positive then they advised should do anti-HBs titer & if it is greater than 100 mIU/ml then they allow the donor for transfusion.<sup>13</sup>

**Limitations of Hepatitis B Virus Surface Antigen (HBsAg)**

Surface antigen (HBsAg) usually appear after 3-4 weeks of exposure & disappear before window period if not develop chronicity. Core antibody usually appear just after appearance of HBsAg & persist throughout the life. HBV-DNA appear within a week of exposure & diagnose even <75 copies/ml.<sup>7,8,9,10</sup> So, Only HBV surface antigen (HBsAg) marker does not always reflect the presence or absence of HBV infection. HBsAg may absent in incubation period,<sup>40,41</sup> window period,<sup>40,41,42</sup> occult HBV infection,<sup>5,38</sup> mutant to surface antigen (HBsAg)<sup>41,42</sup> & even after reactivation of resolved HBV infection.<sup>40,42</sup> Blood that is free of HBsAg but has high-titer antibodies against hepatitis B core antigen (anti-HBc) in the absence of antibodies against hepatitis B surface antigen (anti-HBs) can also transmit HBV infection. However, a small proportion of donors with anti-HBc in the absence of HBsAg have circulating HBV DNA & may have a risk of infectivity.<sup>43</sup>

**Core antibody Situation in Bangladesh and Abroad**

Transmission of hepatitis B virus (HBV) infection through donated blood is a common occurrence. In blood bank, screening for HBsAg is carried out routinely to detect HBV infection. Despite testing for HBsAg in blood donors, transfusion-associated HBV infection continue to be a major problems in Bangladesh. Antibodies to hepatitis B core (HBc) antigen are marker of acute, chronic, or resolved HBV infection & remain detectable for life. These can be present in the absence of both HBsAg & anti-HBs

antibodies. Anti-HBc is therefore detected in anyone who has been infected with hepatitis B virus.<sup>41</sup>

In a study of Bangladesh, Rahman et al.<sup>41</sup> stated that among 52 core antibody (Anti-HBc) positive donor who are HBsAg negative, HBV-DNA positive in 16 (30.7%) cases. Again those who are HBV-DNA positive their anti-HBs (antibody to surface antigen) titer were higher (>10 mIU) in only 6 (33.33%) cases. They concluded that, core antibody should be routinely included on blood donor screening & if found positive regardless of anti-HBs titer blood should be discarded.<sup>41</sup> Jahan et al.<sup>5</sup> also stated the same phenomena. Among 398 HBsAg negative donors, core antibody (anti-HBc) positive 82 (20.6%). Again who are anti-HBc positive, HBV-DNA present in 7 (8.5%) donors. They concluded with surface antigen negative blood is not capable of blocking HBV transmission. Developing country like Bangladesh may apply core antibody (anti-HBc) for donor screening.<sup>5</sup>

A study from West Bengal, India, a neighboring Indian province of Bangladesh has reported that 21.3% HBsAg negative & anti-HBc-positive blood donors were harboring HBV DNA in their blood.<sup>44</sup> Another study from India also showed that 7.5% of HBsAg-negative, anti-HBc positive blood was expressing HBV DNA. Surprisingly, 4.6% people expressing both anti-HBc & anti-HBs were also expressing HBV DNA in their blood.<sup>45</sup>

Presence of HBV DNA in HBsAg-negative blood donors has also been reported from other Asian countries, such as Iran that showed HBV DNA among 12.2% HBsAg-negative, anti-HBc positive donors.<sup>46</sup> Studies from Lebanon & Pakistan have revealed HBV DNA among 2.8% & 2.9% HBsAg negative, anti-HBc-positive blood donors, respectively.<sup>47,48</sup>

Despite all these beneficial & informative study, No blood transfusion centers of Bangladesh do core antibody except two corporate hospitals. One is Square Hospital Limited<sup>49</sup> & another is Apollo Hospital, Dhaka.<sup>50</sup> They routinely done anti-HBc for donor screening & if positive then deferred them from blood transfusion. Based on these observations, policy makers & professional organizations of these countries have been trying to optimize their blood transfusion system.<sup>5</sup> However, these scientific developments have not influenced the ongoing blood transfusion program of Bangladesh, even though high prevalence of anti-HBc (22.6%) has been reported among healthy subjects of Bangladesh. Additionally, anti-HBc-

positivity is extremely high even among children below the age of 5 years (16.3%) & increased to 30% among family members.<sup>51</sup>

In this dangerous situation, almost nothing is known about the real extent of problem with anti-HBc positivity in this country. Recently, nucleic acid testing (NAT) has been adopted by many countries to avoid transfusion-induced hepatitis.<sup>52</sup> Also, we would not advocate for immediate starting of NAT for donor screening in Bangladesh, as this is neither feasible nor acceptable in the context of present socio-economic condition of this country. It is usually discussed that NAT establishment is a costly endeavor in Bangladesh & other developing countries.<sup>5</sup>

### Tests methods

According to WHO & National guideline, the main types of assay used for blood screening of HBV are:<sup>32</sup>

- Immunoassays (IAs):
- Enzyme immunoassays (EIAs)
- Chemiluminescent immunoassays (CLIAs)
- Rapid/simple single use assays (rapid tests)
- Nucleic acid amplification technology (NAT) assays.

### Universal Choice

The blood transfusion department of medical colleges, institutes & specialized hospitals should perform EIAs & CLIAs for blood screening in addition to the current system of rapid testing.<sup>32</sup>

### In Special Situation

Only rapid tests may be considered in case emergency screening (when blood is needed urgently) or in remote areas with low workloads or limited number of tests are performed daily & limited facilities, when equipment is lacking or where there may be no electricity e.g. district blood centers & Upazila health complexes.<sup>32</sup>

### Why EIAs & CLIAs Rather than Strip

In case of rapid test, lots of false positive/negative results may occur. It is most commonly used, only 30 taka/strip, less sensitive & specific, manual entry of test results & not recommended by WHO as a universal screening of blood donors in Bangladesh. When false positive results occur, prospective blood donors are unnecessarily excluded from blood donation. On the other h&, when false negatives occur, this poses a great challenge to the quality & reliability



of blood screening & to patient safety. Errors may also be produced if samples for re-testing are improperly stored &/or transported. On the other side, quite unlikely to occur when screening is done by EIA, Chemiluminoassay, or PCR technology.<sup>32</sup>

### Present Situation in Bangladesh

In Bangladesh, Transfusion Transmissible Infections (TTI) screening is done mainly by rapid assay. Only at private sector centers (Square Hospital Limited<sup>49</sup> & another is Apollo Hospital, Dhaka<sup>50</sup>) are screening done by EIA/ CLIA (Chemiluminescent Assay). The blood donation system in Bangladesh is decentralized; all centers collect, & process & distribute blood. A beautiful study was done in 2013, which was organized by WHO, DGHS (Director General Health Services) & IEDCR (Institute of Epidemiology, Disease Control & Research) to assess the donor screening quality of different blood transfusion centers of Bangladesh. A total of 12 centers from Dhaka city (Apollo hospital, Square hospital, United hospital, LabAid hospital, Armed forces institutes of pathology Bangladesh medical college hospital, Cancer & rehabilitation hospital, Chest diseases hospital, Kidney & urology hospital, Institutes of child & maternal health etc) & 15 centers from outside the Dhaka (Rajshahi medical college hospital, Khulna medical college hospital, Faridpur medical college hospital, Comilla medical college hospital & lots of district hospitals) were included in this study. A total 915 blood samples were received from testing centers & all samples were accepted for re testing in IEDCR. Out of 27 testing centers, results from 18 centers were correct (66.67%) & those from 9 centers were incorrect (33.33%) when compared with the results obtained at IEDCR. Among incorrect results, HBV (53.3%) is the dominant one followed by HCV (40%) & malaria (6.7%). No results of disparity observed among HIV & syphilis cases.<sup>53</sup>

### Conclusions

Although so called proper donor screening prior to blood transfusion & vaccination present, actually they are unable to prevent transmission of HBV. The physicians, hepatologists, academicians & policy makers are equally unaware of the seriousness, loop holes & magnitudes of ongoing blood transfusion program of this country. The hazards of blood transfusion, specially the risk of transfusion transmissible infections specially HBV are now the burning issue. The risk of disease transmission increases many fold if blood donor selection is

inappropriate & method of testing is inadequate. Percentage of core antibody (anti-HBc) positivity with surface antigen (HBsAg) negativity of healthy donors warrants the decision makers for rethinking about occult/silent HBV infection. Transfusions should be planned judiciously & side by side, efforts should be made to minimize the risk of hepatitis B transmission through adopting the guidelines from WHO for safe blood transfusion. Awareness & knowledge would be the key to prevent the transfusion of transmissible diseases.

### Recommendations

- Donor screening should be on Enzyme immunoassays (EIAs) & Chemiluminescent immunoassays (CLIAs) in place of strip method.
- Regarding HBV, Core antibody (anti-HBc) should include in national donor screening program.
- In future, nucleic acid testing (NAT) for HBV should be included for safe blood transfusion in Bangladesh.

### Future Prospect

Establishment of a nationally coordinated blood transfusion service, collection of blood only from voluntary donors, testing of all blood for compatibility & transfusion transmissible infections (TTIs) with appropriate method of testing & reduction of unnecessary transfusion will be the key factors for excellent blood transfusion service (BTS) in future. It is widely expected that BTS will be able to supply HBV free blood from voluntary blood donors to all parts of the country in timely manner with sufficient quantity.

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