

Occult Hepatitis-B Infection in Volunteer Blood Donors in Different Hospitals of Peshawar, Khyber Pakhtunkhwa

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ABSTRACT

Background: Although screening tests are performed in hospitals of Khyber Pakhtunkhwa, but still some tests are being ignored due to that potential infection is being transmitted.

Objective: This study was designed to find out potential HBV infection transmitted through blood transfusion.

Methodology: In this regard, 200 blood samples, negative for both (anti-HCV and HBsAg) were screened for HB core infection through ELISA technique after screening of routine procedures in the blood banks. Anti-HB core Positive samples were then processed for active HBV infection using Real time PCR.

Results: Out of 200 samples, 17 (8.5%) were positive for anti-HB core and about 4 (23.5%) of the subjects had active HBV infection.

Conclusion: It was concluded that HB core infection was present in apparently healthy individuals. Hence this study suggests that anti-HB core test should also be included in routine screening procedures in different hospitals of KPK.

Keywords: HBV (Hepatitis B Virus), KPK (Khyber Pakhtunkhwa), PCR (Polymerase Chain Reaction).

Introduction

Hepatitis means inflammation of the liver cells.¹ It is also called blood born disease. Viral hepatitis is caused by three common viruses, hepatitis A, B and C. Other types causing hepatitis are hepatitis D, E and the recently identified hepatitis G virus.² A serious global infectious disease caused by Hepatitis B is a major cause of mortality and morbidity. Hepatitis B virus (HBV) is one of the major cause of death, about two billion people are infected with HBV and an estimated 400 million are suffering from chronic hepatitis B infection, worldwide.³ In Asian and Western countries, chronic HBV infection rate is over 10%, while in United States and northern European countries it is under 0.5%.⁴

Hepatitis B core Antigen (or HBcAg of HBV) is the major component of the core particles. In spite of availability of sensitive screening assays for hepatitis B surface antigen (HBsAg), occasional cases of post-transfusion HBV infections (PTH) are common.⁵ Generally, detection of HBV-DNA in the serum of blood donors who have negative test for HBs-Ag is called occult HBV infection.⁶ The frequency of HBc infection varies considerably, like in Europe and North America, HBV prevalence is low and hence 5% of the blood donors are characterized as having occult hepatitis B. The areas where HBV prevalence is high such as Ghana, occult HBV is the major cause of transfusion transmitted HBV infection.⁷

Presently, for the detection of HBV infection in the blood bank of Pakistan, HBsAg is the only routinely screening test. HBsAg screening of blood does not totally reduce risk of transmission of hepatitis B. Sometime the person is infected with HBV, but the sign and symptoms do not appear that is called core window period phase. In core window period, HBsAg cannot be detected in the blood, although hepatitis B infection is present.

Evaluating usefulness of anti-HBc screening is critical, particularly for Pakistan and other countries that have intermediate and high hepatitis B endemicity.⁸ Anti-

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HBc was initially introduced in the blood banking as a surrogate marker for hepatitis C virus (HCV) and to a certain extent for human immunodeficiency virus (HIV) as well. It could be an excellent indicator for occult HBV infection during the "core window" period. For these reasons some countries like in India there are a few blood banks that have incorporated anti-HBc testing in their donor screening protocols. Determination of anti HBcAg IgM antibodies are very important for the fast classification of the virus, for the phase of the illness and for the monitoring of patients under treatment with interferon.

In Pakistan and especially in Khyber Pakhtunkhwa, this sort of study has not been done and there is no trend of screening HBV core infection in blood banks of hospitals. Hence, it was the need of the hour to conduct such study to create awareness in general population as well as in concern authorities to screen themselves and implement such screening procedures in hospitals. In this way transfusion associated transmission of HBV infection can be prevented.

Methodology

Blood samples from volunteer blood donors in the blood banks of three major hospitals, Lady Reading, Khyber Teaching and Hayatabad Complex were collected. Serum was isolated by centrifugation and then kept at -4 °C. After serum isolation, the following procedure was adopted to find out HB core infection.

Screening tests: All samples were subjected to routine screening procedures for anti-HCV, HBsAg and anti-HIV, using Immunochromato Graphic Technique (ICT) (Standard, USA).

Anti-HB Core Test: Samples negative for the above-mentioned screening were processed for HB core antibodies. A total of 200 samples were processed for detection of anti-HB core antibodies. This was done using Enzyme linked immunosorbant Assay (ELISAs) (Biotech, USA).

Polymerase Chain Reaction (PCR): Samples positive for HB core antibodies were processed further for detection of active HBV infection (HBV DNA). Simply DNA was extracted from anti-HB core positive samples according to manufacturer’s instructions (Roboscreen, German). Extracted DNA, 5ul of each positive subject was mixed with 20ul PCR master mix in a reaction tube, according to manual of manufacturer’s (Roboscreen, German). Reaction tubes were placed in the heating block of Real time

PCR (Mini-Opticon USA), and in 2 hours results were obtained in the form of graphs.

Results

In the present study, occult and active HBV infection was determined in the blood of volunteer blood donors, collected from three hospitals of Peshawar. The results were summarized as,

Out of 200 samples, only 17 (8.5%) were positive for anti-HB core and all of them were male. Among 17 anti-HB core positive samples, only 4 (23.5%) were positive for HBV DNA. Moreover ALT level of all PCR positive samples was high and the age of the positive subjects was in the range of 20-40 years (Table 1).

Table 1: Correlation of anti-HB core, ALT and HBV DNA

| S.NO | AGE | SEX | Anti-HBc | ALT | PCR |
|------|-----|------|-----------|-----|-----|
| 1 | 30 | Male | + (0.043) | 10 | - |
| 2 | 25 | Male | + (0.224) | 99 | + |
| 3 | 21 | Male | + (0.230) | 12 | - |
| 4 | 29 | Male | + (0.234) | 50 | - |
| 5 | 22 | Male | + (0.172) | 11 | - |
| 6 | 32 | Male | + (0.206) | 137 | + |
| 7 | 23 | Male | + (0.188) | 50 | - |
| 8 | 22 | Male | + (0.193) | 81 | - |
| 9 | 32 | Male | + (0.65) | 11 | - |
| 10 | 26 | Male | + (0.173) | 10 | - |
| 11 | 48 | Male | + (0.26) | 89 | + |
| 12 | 39 | Male | + (0.491) | 45 | - |
| 13 | 36 | Male | + (0.299) | 31 | - |
| 14 | 24 | Male | + (0.577) | 28 | - |
| 15 | 35 | Male | + (0.326) | 59 | + |
| 16 | 20 | Male | + (0.05) | 22 | - |
| 17 | 28 | Male | + (0.67) | 35 | - |

Cut of value for positivity is < 1

Discussion

Blood transfusion associated transmission of HBV infection is common in some part of the world, mostly in developing countries like in India and Pakistan.⁹ Carrier can transmit infection, as routine examination does not exclude the infection and that’s why viral hepatitis is becoming a major public health problem in all parts of the world. Pakistan is in the intermediate HBV prevalence zone with a carrier rate of 3–4%.¹⁰ Chronic hepatitis B is a severe problem in Pakistan.^{11, 12} In the present study anti-HB core positivity is 8.5% [Table.1]. This is an almost an agreement with the

previous studies conducted in Pakistan, that has shown that prevalence of anti-HB core was in the range of 9.19% to 31 %.^{13, 14} In India, prevalence rate of HB core infection also varies as it has been shown by different studies that its prevalence ranges from 8 to 18%.¹⁵ Similar prevalence rate might be due to close geographical boundaries and almost similar traditions and habitats.

Active HBV infection in the present study is 23.5%, in anti-HB core positive subjects [Table 1]. Results of the current study regarding active HBV infection as compared to other parts of the world varies. Like proportion of HBV DNA positivity in blood donors is higher than in Europe and USA but relatively similar or lower to other regions in the Middle East, like a study from Japan and India has shown HBV DNA positivity in 38 and 30% of persons respectively.^{16- 18} while in North America it was 3.7%.⁴ Moreover it is evident that persons having active HBV infection had high ALT level [Table.1]. This is another strong indicator of having HBV infection as it has been observed that ALT is the strongest indicator of liver problem.¹⁹ There is increasing evidence that occult HBV infection is associated with severe liver disease and hepatocellular carcinoma HCC, in addition to transmission of HBV infection.²⁰ Therefore, high rate of occult HBV infection is an alarm for KPK province, as these persons will transmit potential HBV in spite of routine screening tests. Thus, our study suggests that there should be proper implementation of anti-HB core test in routine screening tests. This will reduce transfusion associated transmission of HBV infection although this will increase cost of examinations and will also cause in rejection of donations of blood, but in return will get rid of HBV infection. As According to Laperche.²¹, HBV DNA screening would be more effective in countries with high or medium endemicity, and where anti-HBc testing is not routinely done.

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Competing Interest: The authors declare that they have no competing interest.

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Authors' contributions:

1. Bashir Ahmed conceived the idea
2. Mehwish Iqbal did the experimentation
3. Nourin Mehmood did analysis
4. Sajjad Ali wrote the manuscript
5. Shumila Bashir facilitated analysis