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Serological profile and interpretation of different hepatitis B virus marker

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Abstract

Hepatitis B virus (HBV) infections are a global public health problem. Chronic hepatitis B (CHB) infection is associated with an increased risk of cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC). In virus-infected liver cells, HBsAg is produced in excess and secreted into the blood, where it serves as a marker for active infection and infectivity. Currently, recombinant HBsAg is used for HBV vaccination, and the development of antibody to HBsAg is typically associated with protective immunity. The likelihood of developing CHB is related to the age at which infection is acquired; the risk being lowest in adults and >90% in neonates whose mothers are hepatitis B e antigen positive. During hepatitis B virus (HBV) infection, Different markers of hepatitis B virus are detectable in blood. Presence of this markers in serum carry importance in diagnosing the stage of illness. Most common marker of clinical significance is: HBsAg, HBeAg, anti-HBs, anti-HBe, Anti-HBcIgM, Anti HBc (total). This marker can be easily detected by different serological methods eg, ICT, ELISA, CMIA, MEIA. We have studied the records of this serological parameters, advised earlier by the physicians of BIHS General Hospital. Last year, about **1,364** patients were tested for hepatitis B serological markers: hepatitis B surface antigen and its antibody (anti-HBs); hepatitis B e-antigen and its antibody (anti-HBe); and antibody to hepatitis B core antigen (anti-HBc) in BIHS General Hospital.

Keywords: HBV; CHB; HBsAg; BIHS; HCC

1. Introduction

Hepatitis B virus (HBV) infections are a global public health problem. It is caused by HBV which is a DNA virus belonging to a family called *Hepadnaviridae* which can cause acute or chronic infection [1]. It is currently estimated that 2 billion individuals have been in contact with the virus. Of those, about 350 million are chronically infected. Each year there are between 600,000 and 1.2 million deaths due to complications of acute or chronic hepatitis. About 25% of chronic cases develop cirrhosis and hepatocellular carcinoma (HCC) of which HBV is considered to be the main agent [2]. The natural course of hepatitis B virus (HBV) infection is determined by the inter- relationship between viral replication via HBV protein production and the host's immune response, and, therefore, clinical practice diagnosis of HBV infection is established by the serological detection of HBV protein products (antigens) as well as host produced antibodies [3]. During HBV infection, four structural antigens–antibody systems are observed: hepatitis B surface antigen (HBsAg) and its antibody (anti- HBs); the preS antigens associated with HBsAg particles and their antibodies; the particulate

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nucleocapsid antigen (HBcAg) and anti-HBc; and an antigen structurally related to HBcAg, namely, hepatitis B e antigen (HBeAg) and its antibody (anti-HBe). Through the examination of these antigen–antibody systems, hepatitis B infection is diagnosed and the course of the disorder may be observed [4]. HBsAg is the serologic hallmark of HBV infection. It is detected in the serum by radioimmunoassays (RIA) or enzyme immunoassays (EIA). It appears in serum 1-10 weeks after an acute exposure to HBV and approximately 2-6 weeks before the onset of symptoms [5].

In patients who subsequently recover, HBsAg usually becomes undetectable after 4-6 months. Persistence of HBsAg for more than 6 months implies chronic infection. The disappearance of HBsAg is followed by the appearance of anti-HBs. The appearance of anti- HBs marks the recovery from hepatitis B. Anti-HBs is the only protective antibody induced by most of the currently available vaccines that consist of recombinant HBsAg only. [6] Coexistence of HBsAg and anti-HBs has been reported in about 10-25% of HBsAg positive individuals. This occurs more commonly in those with chronic hepatitis B. [7] HBcAg is an intracellular antigen that is expressed in infected hepatocytes and is not detectable in serum. Its antibody, anti-HBc,can be detected throughout the course of HBV infection. During acute HBV infection, anti-HBc is predominantly immunoglobulin M (IgM) class. IgM anti-HBc is the first antibody to be detected. It usually appears within 1 month after the appearance of HBsAg, approximately 1-2 weeks before the elevation of aminotransferases. [5]. Thus, the detection of IgM anti- HBc is usually taken as an indication of acute HBV infection. IgG anti-HBc persists along with anti-HBs in patients who recover from acute hepatitis B and in association with HBsAg in those who progress to chronic hepatitis B. HBeAg is generally considered to a marker of HBV replication and infectivity, and is usually associated with the detection of HBV DNA. [9] Transmission rates of HBV infection are much higher when the source is HBeAg positive. [10,11] During acute infection, HBeAg appears shortly after the appearance of HBsAg. In patients who recover, HBeAg to anti-HBe seroconversion precedes HBsAg to anti- HBs seroconversion. [4] Anti-HBe may persist for many years after resolution of acute hepatitis B. In patients with chronic infection, HBeAg may persist for years to decades. During the HBeAg positive phase, most patients have detectable HBV DNA and active liver disease [12]. Seroconversion from HBeAg to anti-HBe is usually associated with the disappearance of HBV DNA in serum and remission of liver disease. However, a small proportion of anti-HBe positive patients continue to have active liver disease and detectable HBV DNA in serum [13].

Hepatitis B virus (HBV) infection is a major cause of morbidity and mortality worldwide. It is estimated that approximately 65 million die from liver disease due to their HBV related infection [2]. HBV diagnosis is accomplished by testing for a series of serological markers of HBV. Moreover, serological tests are used to distinguish acute, self-limited infections from chronic HBV infections and to monitor vaccine- induced immunity. This study provides an overview of the natural history of HBV infection, diagnosis, serological Marker and its Interpretation.

2. Material and methods

It was observational cross-sectional study. The study population was both male & female attending at BIHS General Hospital. The study was conducted from July 2017 to July 2018. Sampling technique was generally convenience. The general objective of this study to determine the prevalence of Hepatitis B virus attending at BIHS General Hospital. The General objectives of this study is to determine the prevalence of Hepatitis B virus attending at BIHS General Hospital and those who have develop jaundice are coming to the hospital and we were testing different serological markers of HBsAg.

2.1. Data collection procedure

This was a cross-sectional study conducted at the BIHS General Hospital between 1st July,2017 to 31st July 2018. Data including age, gender, which test have been performed like HBsAg, HBeAg, Anti-HBe, Anti-HBc, Anti-HBs, were collected from previous record book.

2.2. Laboratory procedure

2.2.1. Detection of HBV serological markers by Enzyme Immunoassay

It is a direct immunoenzymatic method of the «sandwich» type in which guinea pig anti-HE antibodies coated to microplate wells act as the capture antibody and goat anti-HBs antibodies marked with peroxidase serve as conjugate antibodies. The sample to be analyzed is incubated in one of the antibody-coated wells. If the sample contains HBsAg, the antigen will bind to the antibody on the plate. After washing to eliminate, any unbound material, goat anti-HBs conjugate to peroxidase is added to the well and allowed to react with the antigen-antibody complex formed in the first incubation. After a second incubation and subsequent washing, an enzyme substrate containing a chromogen is added, the substrate e will develop a blue colour if the sample is positive for HBsAg. The blue color changes to yellow after

blocking the reaction with sulphuric acid. The intensity of the color is proportional to the amount of HBsAg in the test specimens [18].

- Interpretation of result

Positive result for HBsAg is indicative of a Hepatitis B virus infection. To determine whether it is acute or chronic, other serological markers of hepatitis B should be analysed taking into account the clinical picture of the patient. All samples were analyzed in BIHS Immunology Laboratory by —Enzyme-Linked Immunosorbent Assay (ELISA) for detection of the following HBV infection markers: HBsAg, anti-HBs, HBcAg, HBeAg, anti-HBe and total antibodies against HBV core protein (total anti-HBc). Bioelisa R brand kits were used (Biokit, SA - 08,186 Llica d'Amunt - Barcelona, Spain). Moreover, Nucleic acid testing for HBV-DNA is increasingly being used to quantify HBV viral load and measure the effectiveness of therapeutic agents [14].

2.2.2. Detection of HBV by ICT

When the liquid sample is dropped on the sample pad, th*e antigen in the sample form an immunocomplex with the antibody labeled with colloidal gold. It is complex moved along with the liquid sample and make a contract with the antibody immobilized on the membrane antibody resulting in generating a colored red purple line. Appearance of red purple on the membrane indicate the presence of antigen of interest in the sample. Since the liquid of the sample migrates through the membrane very fast, it makes it possible to detect the presence or absence of antigen within 15 minutes. [21]

- Interpretation
  - **Positive:** Two distinct pink-colored bands appear, on in the patient test region (T) and on the control region (C).
  - **Negative:** Only one pink colored band appears in the control region (C). No apparent pink band appears in the patient test region (T).
  - **Invalid:** A total absent of pink colored bands in both region is an indication of procedural error or that test reagent deterioration has occurred.

2.3. Data analysis:

Data were analyzed with the help of the software SPSS (Statistical Package for Social Sciences) version 23 and Microsoft Excel 2016. The results were expressed as mean ±SD (standard deviation).

3. Results

This study investigated the serological markers in 1,364 patients from BIHS general hospital during the period of July,2017 to july,2018. Demographic variables such as gender and age were analyzed. Diagnosis can be made serologically by different methods eg. ICT, ELISA, CMIA, MIEA. For this dissertation all the test was perform by ELISA method. Interpretation of the result can be done by following Table 1.

Table 1 Interpretation of the different serological profile

HBs Ag	Anti-HBs	Anti-HBc IgM	Anti-HBc Tot	HBe Ag	Anti-HBe	HBV/ DNA	Interpretation
+	-	+	+	+	-	+	Viral activity (mainly by wild virus)
+	-	+	+	-	+/-	+	Viral activity (mainly pre-core minus variant)
+	-	+	+	-	+/-	-	Resolving acute hepatitis
+	-	-	+	-	+/-	-	Healthy carrier
-	-	+	+	-	-	-	Core window
-	+	-	+	-	+/-	-	Immunization post-natural infection
-	+	-	-	-	-	-	Immunization post-vaccination

(+) Positive Result, (-) Negative Result

The female: male ratios were 1.40, In total 58 % were Female and 42 % were Male. Of these, 66 male had HBV positive and 501 had negative. on the other hand, 27 female had HBV positive and 770 had negative (Figure 1).

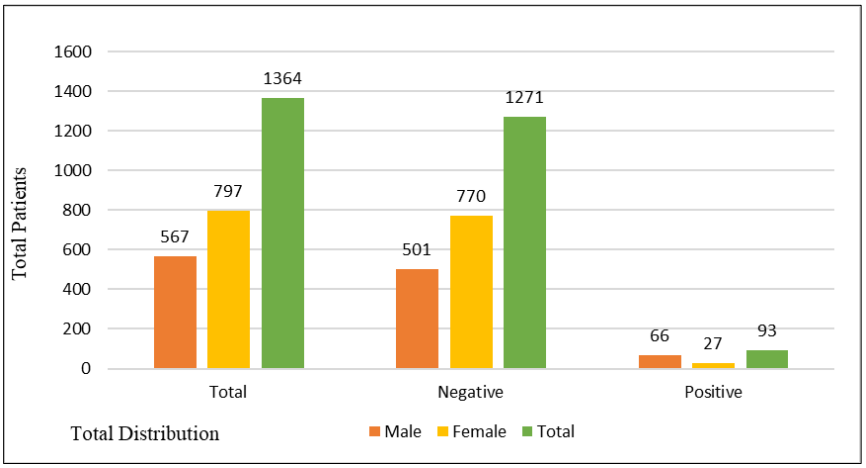

Figure 1 Overview of HBV infection

Participants ages ranged from 18 to 89 years old. According to the test results from the record book, active HBV infections were present in 1% of the population, resolved infections in 3% Acute infection 6%. Moreover 89% had no serological markers identifiable with the serological tests employed and were defined as susceptible.

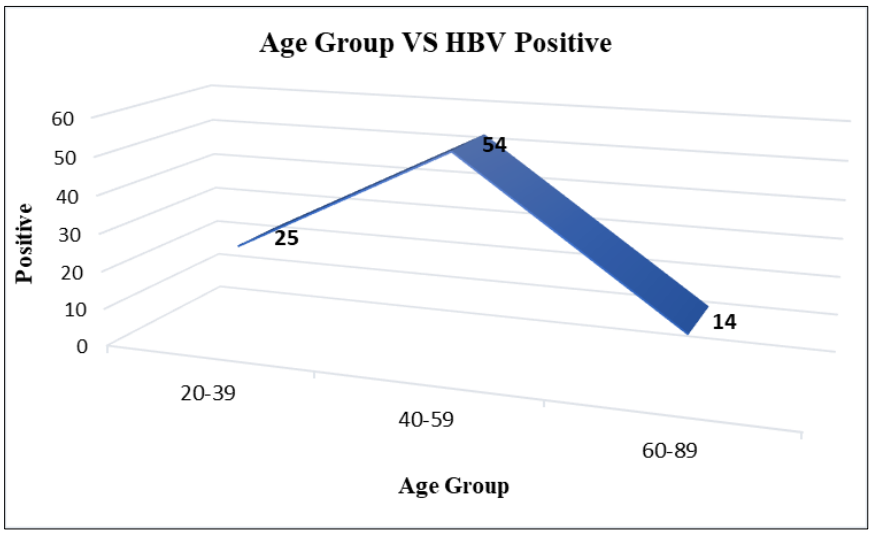

Figure 2 Prevalence of Hepatitis B virus according to the Age group

In this study, there are high prevalence of Hepatitis B virus are found Between the age of 40 to 59. Moreover, the low prevalence of hepatitis B at the age of 60-90.

4. Discussion

Immunity to HBV is acquired from a resolved infection or from vaccination. The HBV vaccine has been shown to induce protective immunity in 90% to 95% of vaccines. Most vaccines will have protective levels of anti-HBs for five to 10 years after vaccination, although the exact duration of immunity remains undefined. When anti- HBs levels have waned below the protective threshold of 10 mIU/mL, a booster dose of HBV vaccine has been shown to induce a strong anamnestic immune response in such individuals. It is therefore probable that protection from chronic HBV infection may last for decades and may well be lifelong [13]. Hepatitis B virus (HBV) infection are a significant public health problem in the developing countries including Bangladesh due to the lack of health education, poverty, illiteracy and lack of universal hepatitis B vaccination programs. Although not significant, the present study shows that the prevalence of HBV infection

among BIHS General hospital is 6.8. Another retrospective study among the blood donors in Dhaka has also shown the decreasing trend of HBV infection during the year 2006 to 2008.

5. Conclusion

However, ignorance, illiteracy, poverty, urbanization, industrialization, socio cultural changes, poor infection control measures in health-care settings and unscreened blood transfusion practices are possible causes of HBV infection spread. Both the government and non-government sectors must take some major steps in educating the masses, including the youth, in schools, colleges, universitiesand working areas for better knowledge of such problems. Health screening in these areas along with the awareness programs and the government and non-government organizations must strictly monitor the compulsory vaccination programs in the country in order to prevent further spread of HBV infection. In conclusion, observed a low prevalence of HBV infection and poor vaccination coverage.

Compliance with ethical standards

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Disclosure of conflict of interest

The author hereby declares that there are no conflicts of interest concerning this paper.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

Statement of informed consent

Informed consent was obtained from all individuals included in this study

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