Significance of Screening HBc-Ab among HBs-Ag Negative Blood Donors in Syria

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Abstract

Transfusion transmitted hepatitis B has always been a dreaded disease, with incidence of increased transmission through donated blood. A total of 180 negative HBsAg blood samples were collected from Blood Bank in Lattakia, Syria. Blood units were selected randomly regardless of age, gender and blood group. The samples were drawn on anticoagulant tubes and then the appropriate dissolution for each sample was performed and a blood plasma was obtained. The competitive enzymatic immunoassay method (ELISA) was used with the appropriate kit to detect the presence of total HBc antibodies, as antibodies to HBc antigen may be the only marker for the recent infection by the virus (Window Period) and also for some chronic patients or due to presence of mutations in the structure of the virus. The results showed 17 positive samples for anti-HBc total antibodies to HBc antigen. which refers to a ratio of 9.44 % of all tested samples. This is of critical diagnostic and medical importance to detect these antibodies in blood donors along with the HBs antigen, in order to increase the level of safety in blood transfusion and to reduce the risk of hepatitis B infection through blood, because Blood Transfusion Centers in Syria investigate only the HBs antigen in blood donors to eliminate the transmission of HBV infection.

Keywords —*ELISA, Hepatitis B, Anti-HBc, HBs Ag, Blood units.*

I. INTRODUCTION

Hepatitis B Virus is classified as the type species of the Orthohepadnavirus, which contains eight other species. The genus is classified as a part of the Hepadnaviridae family[1]. The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. It composed of an outer lipid envelope containing hepatitis B surface antigen (HBsAg) and an inner nucleocapsid consisting of hepatitis B envelope antigen (HBeAg) and hepatitis B core antigen (HBcAg). HBc antigen is the main structure protein of HBV icosahedral nucleocapsid and it has function in replication of the virus[2]. Corresponding antibodies to each of these antigens are Hepatitis B surface antibody (anti-HBs or HBsAb), Hepatitis B envelope antibody (anti-HBe or HBeAb) and hepatitis B core IgM and IgG antibody (anti-HBc or HBcAb) [3]. The viral core also contains double stranded DNA genome and DNA polymerase (Figure 1 shows the structure of the virus).

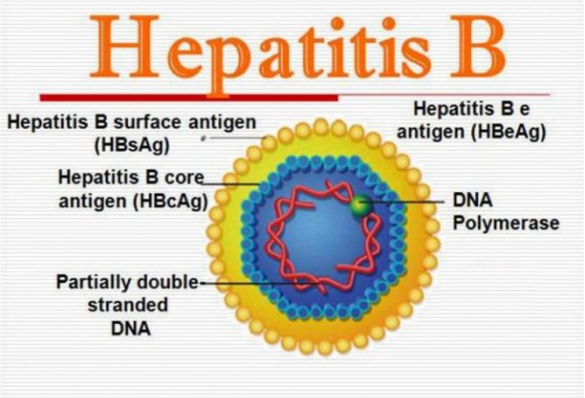

Fig 1: Structure of Hepatitis B Virus

The serological markers of Hepatitis B virus are HBsAg, anti-HBs, HBcAg, anti-HBc (IgM and IgG), HBeAg, anti-HBe, and HBV DNA; these are important as they can be used in the diagnosis of the infection and to determine the severity of the infection [4]. Hepatitis B virus (HBV) infection with its associated sequel is a disease of major public health importance, being the 10th leading cause of death globally[5], [6]. HBV infection accounts for 500,000 to1.2 million deaths each year[7]. Of the approximately 2 billion people infected worldwide, more than 350 million are chronic carriers of HBV[8]. Approximately 15-40% of infected patients will develop cirrhosis, liver failure or hepatocellular carcinoma (HCC)[9], [10]. Hepatitis B surface antigen (HBsAg) is the main diagnostic marker for hepatitis B infection and for screening of donated blood in Syria and many other countries[11]. Antibody to hepatitis B core antigen (anti-HBc) is the most sensitive marker of previous hepatitis B contact. It appears in acute phase of HBV (hepatitis B virus) infection and usually persists after virus clearance[12]. Diagnostic problems may arise when anti-HBc is found without HBsAg or anti-HBs seropositivity[13]. Isolated anti-HBc serological profile may be associated with: (a) a chronic carrier state in which HBsAg is not detectable; (b) remote infection with loss of measurable anti-HBs; (c) passive transfer of anti-HBc; (d) nonspecific, cross-reacting antibody; and (e) the period when HBsAg

has disappeared and anti-HBs has not yet been detected[14], [15]. Therefore, a reliable serological tests are required for the diagnosis of occult hepatitis B infection. The aim of this study is to detect occult hepatitis B among hepatitis B surface antigen negative blood donors using anti-HBc as an only marker to evaluate its importance in blood transfusion.

II. MATERIALS AND METHODS

Between August and November 2019, A total of 180 selected hepatitis B surface antigen-negative sera from blood donors were collected from Blood Bank in Lattakia, Syria. Blood samples were randomly selected regardless of age, gender and blood group. 5 milliliters of blood were collected from each donor in a sterile, capped tube. Then, the appropriate centrifugation was done for each sample and the plasma was obtained and stored at -50 C0 until it was needed for the test. We used the competitive enzymatic immunoassay method (ELISA) with the appropriate kit (DIA Source, Belgium) for screening the total hepatitis B core antibody (anti HBc IgM + IgG). The presence or absence of anti-HBc antibodies was determined by comparing the recorded absorbance with the calculated cut-off values. All the steps were followed according to the manufacturer's instructions. All donors were checked for HBs Ag, HCV Ab, and HIV. In addition, donors with medical histories that included symptoms suggestive of acquired immunodeficiency syndrome, a blood transfusion or a history of jaundice were not accepted for the study. All samples that were found to be 'anti –HBc alone' positive were retested by another commercially available ELISA with a sensitivity of 100% and specificity of 99.6%.

III. RESULTS

Out of the 180 samples studied, 17 samples were found to be positive for HBc Ab. The prevalence rate of HBc Ab was {(17/180) x 100% = 9.44%} (Fig 2).From the 180 blood donors in this study, 155 (86.11%) were male and 25 (13.89%) were female. The mean age was 32.4 years, ranging from 18 to 45. The relationship between age and HBc Ab results was not significant (P value > 0.05). Also, the relationship between gender and HBc Ab results was not significant.

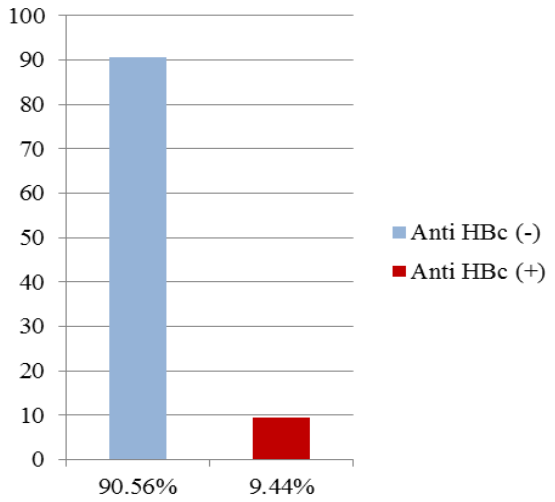

Fig 2: Prevalence of anti-HBc positive

IV. DISCUSSION

The risk of transmitting occult hepatitis B virus continues to be a major problem despite mandatory screening for Hepatitis B surface Antigen (HBsAg)[16]. The results of this limited study show that a relatively high prevalence of 'anti-HBc alone' (9.44%) was found in the 180 blood donors investigated. Anti-HBc has been found to be an excellent indicator of occult HBV infection during the window period[17], [18], [19]. Other markers for detecting occult HBV infection in an HBsAg negative blood donor, such as detection of HBV DNA by polymerase chain reaction (PCR), may not be cost effective[20]. Detection of anti-HBc has contributed significantly in reducing the incidence of post transfusion hepatitis B amongst patients.'Anti-HBc alone' could also be due to false-negative HBsAg tests. Very low concentrations of HBsAg (below detection limits) or mutations in the major antigenic determinant of HBsAg may lead to false negative HBsAg results[21], [22]. Mutation in codon 144 was also reported to give false-negative HBsAg results but these mutants were not investigated in Syrian studies. The world can be divided into three areas based on the prevalence of chronic HBV infection ; high (> 8%), intermediate (2-8%) and low (< 2%) as shown in Figure 3. Most countries in the world are still considered intermediate to high endemicity for HBV infection [23], [24]. In Syria the prevalence of hepatitis B varies from 2.2 to 6.4%, depending on the different areas of the country[25]. As shown in various studies the prevalence of HBc antibody among blood donors varies from 0.11 to 22% (Table I).

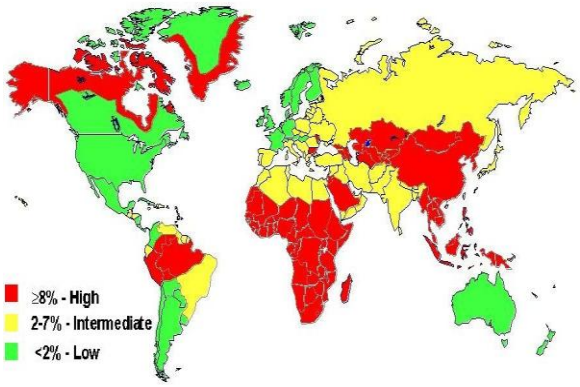

Fig 3: Geographic Distribution of Chronic HBV Infection

TABLE I
Comparison of Seroprevalence of Anti HBc in Negative HBs Ag Blood Donors from Various Studies

Place of the study	Seroprevalence of Anti HBc	Year of the study [Reference]
Egypt (Tanta)	18.9%	2015 [26]
Egypt (different areas)	7.8%	2010 [27]
Germany	1.52%	2002 [28]
India (New Delhi)	18.9%	2006 [29]
India (Chandigarh)	8.4%	2006 [30]
Bangladesh	20.6%	2013 [31]
Iran (Bandar Abbas)	2.3%	2012 [32]
Iran (Shiraz)	6.55%	2002 [33]
Iraq (Basra)	2.1%	2013 [34]
Italy	4.85%	2005 [35]
Japan	3%	2011 [36]
KSA	21.47%	1999 to 2000 [37]
UK	0.07 %	2008 to 2009 [38]
Korea	13.5%	2008 [39]
Lebanon	2.2%	2002 to 2003 [40]
Libya (Tripoli)	10%	2014 [41]
Malaysia (Kuala Lumpur)	5.5%	2015 [42]
Nigeria (South East)	5.4%	2009 [43]
Pakistan	19.15%	2005 [44]
Switzerland (Berne)	1.7%	2005 [45]
Taiwan	0.11%	2007 [46]
Turkey	15%	2006]

V. CONCLUSIONS

The result of this study revealed a 9.44% prevalence of anti-HBc among Syrian healthy blood donors. The study shows the need to include anti-HBc in routine screening of blood dnors in Syria and confirms the fact that testing blood donors for HBs Ag alone is not sufficient to eliminate the risk of HBV infection through blood transmission. Although the possibility to reduce the risk of transfusion associated HBV infection by DNA testing for all the collected blood units is difficult to be done due to the cost in many developing countries including Syria. Our study also emphasizes that a national study including a larger number of blood donors from different blood donation centres in the country should done to increase the safety of the blood transfusion in Syria.

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