ORIGINAL ARTICLE

Frequency of Anti-Hepatitis B Core Positivity in Patients with Persistently Raised Alanine Aminotransferase

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ABSTRACT

Objective: To find out the positivity for serum hepatitis B core antibody in patients with persistently raised alanine aminotransferase having negative hepatitis B surface antigen and anti-hepatitis C virus antibody status.
Study Design: Retrospective cross-sectional study
Place and Duration of Study: Bilal Medical Trust Hospital, Buner from -1st January 2016 to 31st December 2019.
Methodology: Two hundred and sixty seven cases were included in this study.
Results: One hundred and fifty six (58.4%) were males and 111 (41.6%) were females. One hundred and sixty four (61.4%) cases were positive for anti-hepatitis B core antibody and 103 (38.6%) were negative for anti-hepatitis B core antibody. Anti- hepatitis B core antibody was positive in 57.9% (n=95) males and 42.1% (n=69) females, with a statistical significance, having a p-value of .044. Alanine aminotransferase was raised, (>30 IU/L), in all cases positive for anti-hepatitis B core antibody (61.4%, n=164).
Conclusion: The hepatitis B core alone serological status with raised alanine aminotransferase should warrant clinicians to investigate these patients further by advising polymerase chain reaction for hepatitis B virus deoxyribonucleic acid.
Keywords: Hepatitis B virus, Hepatitis B core antigen (HBcAg), Hepatitis B surface antigen (HBsAg), alanine aminotransferase (ALT)

INTRODUCTION

Hepatitis B is a principal health problem worldwide and a potentially life-threatening infection caused by the hepatitis B virus. It has the paramount importance in causing chronic liver disease, hepatic cirrhosis, and ultimately can leads to hepatocellular carcinoma.[1] In 2015, 257 million people suffered from chronic hepatitis B, which resulted in 887,000 estimated deaths globally.[2] There is marked variation in the worldwide prevalence of hepatitis B virus infection and could be categorized as high, medium, and low endemic areas. Developing countries with a larger population have high endemicity such as Southeastern Asia consisting of China, India, Bangladesh. Sub-Saharan Africa and the Amazon Basin, in these areas, chronic carriers are at least 8% of the total population.[3] Countries grouped under moderate endemic areas are located in Southeast Europe, the Middle East, Japan, and part of South America, evidence of infection in these areas is 10-60% people, and 2-7% population are chronic carriers of Hepatitis B virus.[4] The world's most developed areas have a low endemicity affecting 5-7% of the population, and only 0.5 to 2% are chronic carriers. Names under this are North America, Northern, and Western Europe and Australia.[5]

Pakistan is also one of the highly endemic areas with hepatitis B infection.[6] In Pakistan, the carrier rate of hepatitis B virus is 5% of the total population which approximately make nine million people.[2] The prevalence of hepatitis B infection in Pakistan in 1.98%±1.96.[6] The average incubation period is 75 days, which ranges from 30 to 180 days. The evidence of the virus might even be detected within 30-60 days of infection.[2]

A variety of liver enzymes are measured through liver function tests. Elevated levels of these enzymes in serum are markers of liver or biliary tract diseases.[7] In the acute phase of HBV infection, both aspartate aminotransferase (AST) and ALT (alanine aminotransferase) are elevated. Alanine aminotransferase is known to be a more specific indicator since AST can be elevated in other diseases, such as the diseases of heart, muscles, and kidneys.[7] Normal levels of ALT in the circulation of adult human ranges from 30–50 IU/L. In acute liver disease, ALT levels may reach ten times the normal values. After recovery from acute hepatitis, levels of ALT become normal within 1-4 months.[8]

The serological and viral markers that are used in the diagnosis, tracking of the disease course, and in the follow up tends to be tested at different stages of the disease. In the window phase, neither HBV surface antigen nor HBV DNA can be detected in blood, but the IgM core antibody against HBV core antigen can be detected. After 2-4 weeks of infection, HBV surface antigen and HBV DNA can be detected. Reactive Hepatitis B surface antigen (HBsAg) test in serum manifests current infection, which may be acute or chronic. During recovery from an acute infection, most patients clear the HBsAg within 4-6 months of the infection onset. Persistent HBsAg levels in the patient's blood after six months of infection leads to the diagnosis of chronic hepatitis B infection. The presence of Hepatitis B surface antibodies (anti-HBs) in serum shows the exposure of a person to the hepatitis B virus, but the virus has successfully cleared. It also denotes a successful vaccination against HBV.[9] High levels of HBeAg (Hepatitis B envelop antigen) in blood shows rapid replication of viral DNA and a high risk of infectivity during this phase. With the resolution of the liver disease, HBeAg seroconverts to

HBeAb (hepatitis B envelop antibodies) and manifests a low level of detectable viral DNA.

Hepatitis B core antigen cannot be detected in serum as it is an intracellular antigen while HBcAb IgM (HBV core antibody) can be detected in serum and indicates previous infection by HBV. HBcAb IgG is the indicator of exposure to HBV, and its isolated raised levels lead to the diagnosis of occult HBV infection.[8] Occult hepatitis B is defined as the presence of HBV DNA in the liver or serum of person without detectable HBsAg, irrespective of the status of HBV antibodies.[10] Multiple studies suggest that occult hepatitis B infection might play a role in favoring the progression of liver cirrhosis and the development of hepatocellular carcinoma in patients that might be having some other chronic liver disease.[11] It is suggested that due to a very low viral DNA load (<200 IU/mL), without detectable HBsAg, the diagnosis of occult hepatitis is quite challenging.[12] In populations highly exposed to HBV, there is a higher prevalence of HBV DNA in HBcAb positive patients in the absence of HBsAg, and these individuals are on the higher side of infectivity.[13] This study aims to find out the prevalence of HBcAb in patients presented with persistently raised serum ALT levels that are negative for HBsAg and anti HCV antibody, and having no other cause of raised ALT.

MATERIALS AND METHODS

This research was conducted as a retrospective cross-sectional study and data were collected from the patient's record register from January 2016 till December 2019. A total of 267 cases were included in this study. The research reported here was conducted at the Bilal Medical Trust Hospital, a private hospital in District Buner. Buner is a district in the Malakand division of Khyber Pakhtunkhwa province in Pakistan. This hospital is a trust hospital and provides healthcare to different social classes of people all over Buner. The inclusion criteria for this study were all the patients with persistently raised ALT, who were negative for both HBsAg and anti-HCV antibody. All other causes of raised ALT, such as cirrhosis, active hepatitis, hemochromatosis, and rhabdomyolysis, were excluded from this study. Patients who were HBsAg or anti-HCV positive were also excluded from this study. Venous blood was collected from patients and tested for hepatitis B core antibody using 3rd generation enzyme-linked immune-sorbent assay (ELISA) by Stat Fax® 4200 microplate reader. Serological markers for hepatitis B surface antigen and HCV were tested using ElectroChemiLuminescence (ECL) technology for immunoassay analysis by Cobas e411 analyzer system. ALT levels were measured using SelectraProM clinical chemistry analyzer.

The normal reference range of ALT taken until now is 30-50 IU/L[14], but this was established in the 1980s a fairly obsolete data. According to new data and new research in the field, recently dictates to lower the normal upper limit of ALT.[15] For this reason, the normal upper limit for men was 30 IU/L, and women were 23 IU/L in this study. For our research, we categorized ALT into four i.e. ALT <30 IU/L, ALT between 31-60 IU/L, ALT between 61-90 IU/L and ALT >90 IU/L. The data were entered and analyzed in IBM SPSS software package version 23. Pearson's Chi square test was used to compare gender and anti-HBc antibody.

RESULTS

Out of a total of 267 cases that were included in this study, 156 (58.4%) were males, and 111 (41.6%) were females. 164 (61.4%) cases were positive for anti-HBc antibody, and 103 (38.6%) were negative for anti-HBc antibody. Gender wise comparison of anti-HBc antibody is given in Table 1. There was statistical significance between male and female positivity of anti-HBc antibody, with a p-value of .044. The relationship of ALT with anti-HBc antibody is given in Table 2; no statistical significance was seen.

Table 1: Gender and anti-HBc antibody

Gender	Anti-HBc antibody		Total
	Positive	Negative	
Male	95 (57.9%)	61 (59.2%)	156 (58.4%)
Female	69 (42.1%)	42 (40.8%)	111 (41.6%)
Total	164 (100%)	103 (100%)	267 (100%)

Table 2: ALT levels and Anti-HBc antibody

Anti-HBc antibody	ALT levels				Total
	<30	31-60	61-90	>90	
Positive	- -	11 (6.7%)	101 (61.6%)	52 (31.7%)	164 (100%)
Negative	1 (1%)	33 (32%)	66 (64.1%)	3 (2.9%)	103 (100%)
Total	1 (0.4%)	44 (16.5%)	167 (62.5%)	55 (20.6%)	267 (100%)

DISCUSSION

Liver function tests, which include AST, ALT, and bilirubin, are the most common laboratory tests used to detect liver pathologies. Of this, serum alanine aminotransferase (ALT) is most specific for the liver. ALT is widely distributed in the population and is influenced not only by hepatic conditions but also in various other conditions unrelated to the liver, such as cardiac, skeletal, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes.[7] ALT is also dependent on demographics such as age, gender, and body mass index.[16] From the above argument, the cut-off value for serum ALT that differentiates healthy from the diseased liver has not been defined clearly. The normal range of ALT levels in the blood is 30-50 IU/L with an upper normal limit (UNL) set at 40 IU/L. However, many researchers have argued to lower the UNL of serum ALT for multiple reasons, the most important one being that the current normal value of ALT was introduced in the 1980s, which was almost four decades ago.[14]

In the present study, all cases that were positive for anti-HBc antibody had raised ALT levels (>30 IU/L) i-e 61.4% (n=164 out of the total 267 cases). Majority of the positive cases had ALT in 61-90 IU/L category (n=101, 61.6%) while having very high ALT (>90 IU/L) were 31.7% (n=52). Patients that were negativity for anti-HBc antibody, yet having raised ALT were 38.57% (n=103). The cases that were positive and negative for raised ALT showed no statistical significance. A study that was done by Zhang et al[17] about ALT as an indicator of health and disease argued about many aspects of ALT and its relation to various disease processes. They argued that in the case of hepatic diseases, ALT levels warrant the extent and urgency of further investigations. Patients having raised ALT should have a repeat ALT and be subjected to further investigations if ALT is persistently high. Another author concluded from their study that prolonged low-level viremia is reflected as raised ALT level at least 1-2 times above the upper normal limit of ALT and thus a having a risk of

development of complications associated with chronic hepatitis B and occult hepatitis B infection.[18] New emerging data suggest ALT being a potential indicator of the overall health and survival of human beings. ALT activity and mortality have strong correspondence, even when the main focus of raised ALT is not from the liver.[17]

In our study, males were 156 (58.4%), and females were 111 (41.6%). Out of the total 267 cases, 164 (61.4%) cases were positive for anti-HBc antibody, and 103 (38.6%) were negative for anti-HBc antibody. We compared gender with anti-HBc antibody, and males turn out to be more positive than females. Out of the positive cases (n=164), males were 96 (57.9%), and females were 69 (42.1%). There was statistical significance between anti-HBc antibody positivity among males and females. A cohort study done in China showed that males (71.42%) were more positive for anti-HBc antibodies than females (28.57%).[19] In comparison to this, a study done in Iran on occult hepatitis B infection showed that females (53.12%) were more positive for anti-HBc antibody than males (46.87%) with no statistical significance.[20]

Males are more prone to hepatitis B infection than females. Many factors influence the transmission of the hepatitis B virus. Specific to our region, there lie many notorious means of viral transmissions. A study done by Asad et al[21] shows various ways of viral transmission common in Pakistan. These include high-risk groups such as; unsterile use of syringes and needle pricks, blood transfusion and transfusion products, injectable drug users (heroin addicts), occupational risk such as healthcare workers and sex workers, dental practice by quacks, shaving by barbers and spousal transmissions.

A study done in China showed that the patients with high ALT levels had a higher level of anti-HBc antibodies ($p<0.001$) without any other viral markers.[19] Much similar work is done worldwide, in Iran[20], the prevalence of anti-HBc positive markers, who have HBsAg-negative status, is 8%. A similar study was conducted on Italian blood donors, where 6313 consecutive blood donors were analyzed for viral screening; the anti-HBc antibody was positive in 4.85% cases.[22] All of these studies further analyzed the anti-HBc antibody cases for viral DNA by performing PCR and found viral DNA being positive in a handful of cases confirming occult hepatitis B infection. From the studies mentioned above as a reference, due to high positive rates of anti-HBc antibody in HBsAg negative patients, this test should also be done with other routinely done viral screening. Many authors have argued by far to include this test as part of routine investigations so that these can be managed on time to prevent the consequences of occult hepatitis that is cirrhosis and hepatocellular carcinoma.[11]

Wu et al[23] have argued that anti-HBc antibody alone can be used to detect occult hepatitis B without doing PCR for HBV DNA, especially in the general population due to its cost-effectiveness in routine screening tests. Patients that are in the high-risk category and in those who clinically presents with raised ALT without obvious etiology, with/without positive viral serological markers should be screened by performing PCR for HBV DNA only, in an attempt as cost-effectiveness to lessen the economic burden on patients in developing countries.

CONCLUSION

As positivity of Anti-HBc antibody is an entity that represents resolved infection, occult hepatitis B infection or chronic infection. Other possibilities are not easily distinguished by routine serological testing, but they can have clinical implications and important consequences. In addition to this, raised ALT levels in subjects with no obvious cause indicate ongoing liver damage. Patients presenting to clinicians with raised ALT levels in HBV endemic areas should warrant clinicians to investigate them further.

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