Global Journal of Biotechnology & Biochemistry 6 (1): 36-40, 2011
ISSN 2078-466X

Epidemiological Study of Viral Hepatitis B in Different Localities of Faisalabad

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Abstract: All types of Hepatitis viruses causing chronic liver diseases are endemic in Pakistan. The present study indicated lower values of hemoglobin in HBV positive patients while values in HBV negative patients were within normal limits in Faisalabad city. Higher values of ALT, AST and ALP were found with proceeding of age, mostly at the age between 20-32.The maximum values of ALT (80.47 U/L), AST (69.09 U/L), ALP (218.76 U/L), Hemoglobin (11.28 g/dL), Platelets (144.44 10^3/µL) and ESR (47.74 mm/hour) in HBV positive patients were found. As ALT and AST were higher than normal values (10-40 U/L) and had the significance correlation with HBV results. There is no significant correlation of other parameters as for ALT and AST. So, ALT is more sensitive indicator of any liver abnormality and commonly used as the primary indicator of liver function. In conclusion, the prevalence of HBV infection is substantially higher in Faisalabad city and the Microparticle Enzyme Immuno Assay (MEIA) is a useful method for epidemiology of viral Hepatitis B.

Key words: Hepatitis B · ALP · ALT · AST · MEIA

INTRODUCTION

Chronic hepatitis B infection is a major health problem worldwide where 350 million people are affected approximately. The clinical results of chronic HBV infection include inactive carrier state, chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) [1]. Hepatitis B virus exhibits genetic variability with an estimated rate of 1.4-3.2 × 10^{-5} nucleotide substitution per site per year which has resulted in well recognized sub types of the virus. HBV has been classified into 8 genotypes on the basis of an inter-group divergence of 8% or more in the complete genomic sequence, each having distinct geographical distribution [2].

Hepatitis B virus is present in blood, saliva, semen, vaginal secretions and menstrual blood and to a lesser extent, breast milk, tears and urine of infected individuals [3].

In primary infection, HBsAg becomes detectable in the blood after an incubation period of 4-10 weeks, followed shortly by antibodies against HBV core antigen (anti-HBc antibodies), which are mainly of the IgM isotype early infection. Infections occur frequently, 10^9 to 10^{10} virions per milliliter. However, the epidemiology of mixed HBV genotype infections is poorly understood [4]. (HBV) S gene (678dp) encodes small HBV envelope protein of 226 amino acid [5]. The HBsAg contains a high number of cystine, each of which is cross-linked to one another. It may also be glycosylated at Asp 146 and contains a highly antigenic epitope that is used for the subtyping of HBV [6].

The present research work was designed to study the prevalence of Hepatitis B in Faisalabad and find out its co-occurrence with liver enzymes and hematological parameters.

MATERIALS AND METHODS

All the serological and biochemical analyses were performed in Bio-care Lab., Aziz Fatimah Hospital and Enzyme Biotechnology Lab., University of Agriculture, Faisalabad.

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Patients Selection: Sample collection was done from 100 randomly selected individuals of different ages and from different regions of Faisalabad. Patients were considered to have hepatitis B infection if their ELISA was positive and had raised alanine aminotransferase (ALT).

Blood Samples Collection: Six to eight ml of blood was drawn and divided in two tubes (one tube with and the other without EDTA).

Blood samples in the tubes without EDTA were left for more than half an hour at room temperature to allow the blood to clot and finally centrifuged at 4000 rpm for 15 minutes. The separated serum and the EDTA treated blood samples were stored at-20 °C until used.

Biochemical and Hematological Analysis: HBsAg in all samples was tested using an automated immuno-analyzer (Abbott Diagnostics USA), obeying the Microparticle Enzyme Immuno Assay (MEIA) principle [7]. Quantitative determination of total, direct and indirect bilirubin in all samples was carried out using an automated Chemistry analyzer by DPD bilirubin method [8]. ALT, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in all samples were also estimated using Chemistry Analyzer. A kinetic method for the determination of ALT activity according to recommendations of IFCC (International Federation of Clinical Chemistry) without pyridoxal phosphate activation was followed [9]. Hematological parameters including hemoglobin, ESR and platelet count were performed using Sysmex XS800i.

Statistical Analysis: The collected data was statistically evaluated by applying regression and correlation using statistical soft ware SPSS windows version.

RESULTS

Results showed that 70 and 30% of patients of the age group 10-20 years (10% of all individuals) were found negative and positive, respectively for HBsAg. In negative cases; the results revealed that serum ALT, AST and ALP levels were 27.43±5.50, 23.29±3.30 and 167.00±16.92 U/L, respectively, while, positive cases revealed the values of serum ALT, AST and ALP as 69.33±14.50, 57.00±12.52 and 264.33±65.39 U/L, respectively (Figs. 1, 2, 3). The hemoglobin concentration in negative and positive cases was 14.04±1.56 and 11.50±1.18 g/dL, respectively (Fig. 4). The platelet counts of negative cases were 234.71±79.78 and among positive cases, they were 140.00±52.57 $10^3/\mu L$ (Fig. 5). The ESR (erythrocyte sedimentation rate) of negative cases was 9.14±6.04 and it was raised in the positive cases to 40.33±14.57 mm/h (Fig. 6).

The age group of 21-30 years contained 60% individuals, out of which, 68.33% were found negative and 31.67% cases were declared as positive for Hepatitis B. In negative cases, the results revealed that serum ALT, AST and ALP levels were 35.51±18.46, 29.73±8.48 and 173.66±48.11 U/L, respectively, while, positive cases revealed the values of serum ALT, AST and ALP were 85.79±24.04, 74.21±23.13 and 211.21±46.34 U/L, respectively (Fig. 1,2,3). The hemoglobin concentration in negative and positive cases was 14.18±1.41 and 11.43±1.23 g/dL, respectively (Fig. 4). The platelet counts of negative cases were 286.05±80.56 and among positive cases, they were 157.00±69.47 $10^3/\mu L$ (Fig. 5). The ESR of negative cases was 10.78±9.46 and raised in the positive cases to 40.42±20.09 mm/h (Fig. 6).

The age group of 31-40 years contained 11% of the individuals, out of which, 63.64% were found negative and 36.36% cases were declared as positive for Hepatitis B. In negative cases, the results revealed that serum ALT, AST and ALP levels were 33.00±7.00, 30.57±7.52 and 175.43±10.81 U/L, respectively, while positive cases revealed the value of serum ALT, AST and ALP as 61.50±17.14, 52.75±9.36 and 200.00±30.89 U/L, respectively (Fig. 1,2,3). The hemoglobin concentration in negative and positive cases was 13.69±2.12 and 11.48±2.15 g/dL, respectively (Fig. 4). The platelet counts of negative and positive cases were 219.86±94.35 and 151.00±34.37 $10^3/\mu L$, respectively (Fig. 5). The ESR of negative cases was 13.14±8.30 and raised in the positive cases to 26.25±14.55 mm/h (Fig. 6).

The age group of 41-50 years contained 13% of the investigated individuals, out of which, 61.54% were found negative and 38.46% of cases were declared as positive for Hepatitis B. In negative cases, the results revealed that serum ALT, AST and ALP levels were 39.38±15.32, 34.63±14.75, 170.00±14.15 U/L, respectively, while positive cases revealed the value of 57.80±22.94, 53.80±20.14 and 209.00±8.99 U/L, respectively (Fig. 1,2,3). The hemoglobin concentration in negative and positive cases was 15.26±1.11 and 10.36±1.11 g/dL, respectively (Fig. 4). The platelet counts of negative and positive cases were 309.63±52.82 and 120.16±58.56 $10^3/\mu L$, respectively (Fig. 5). The ESR of negative cases was 8.13±6.03 and raised in the positive cases to 83.40±28.61 mm/h (Fig. 6).

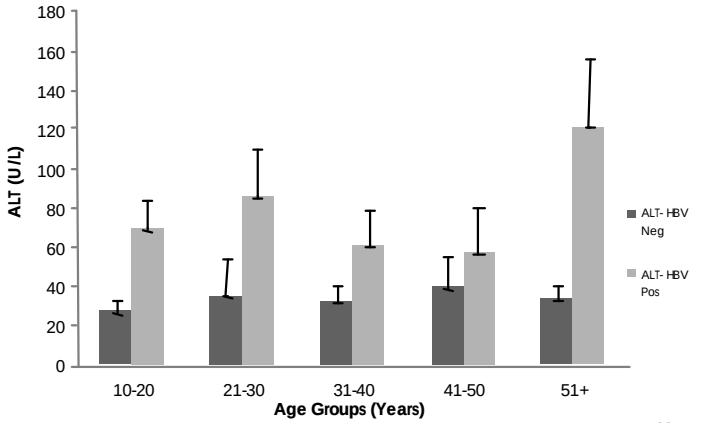

Fig. 1: Serum ALT levels in Hepatitis B among (-ve) and (+ve) cases in different age groups.

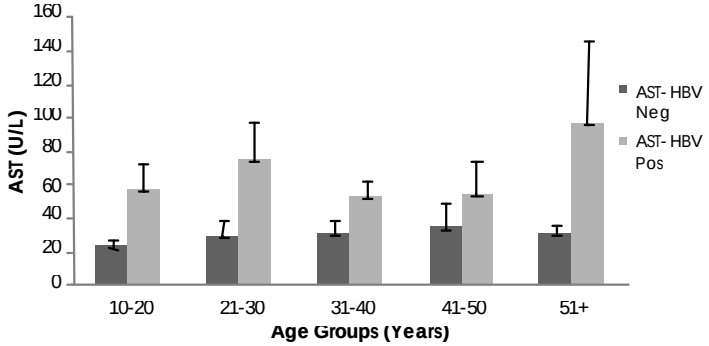

Fig. 2: Serum AST levels in Hepatitis B among (-ve) and (+ve) cases in different age groups.

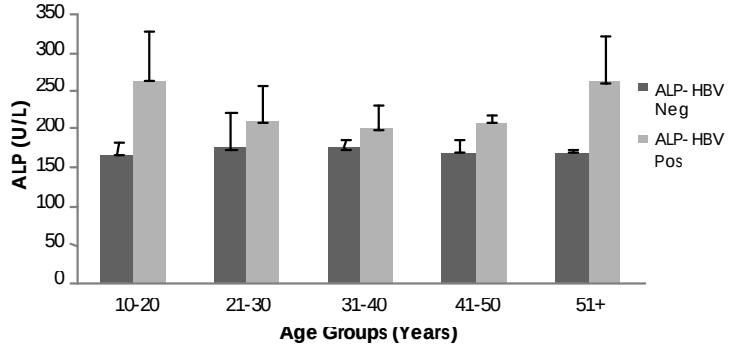

Fig. 3: Serum ALP levels in Hepatitis B among (-ve) and (+ve) cases in different age groups.

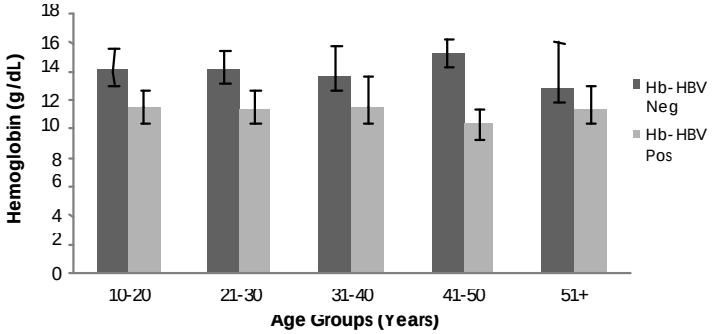

Fig. 4: Serum Hb levels in Hepatitis B among (-ve) and (+ve) cases in different age groups.

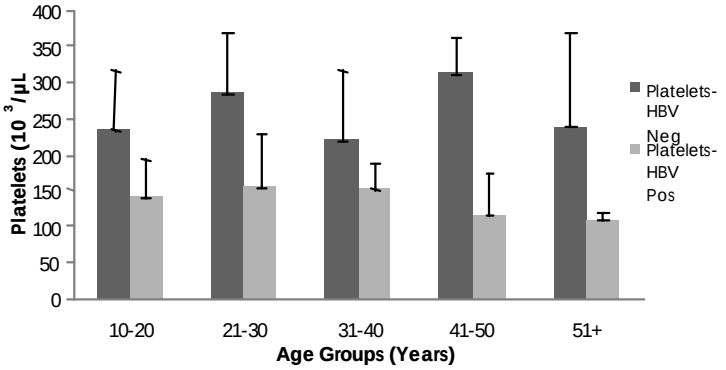

Fig. 5: Platelets levels in Hepatitis B among (-ve) and (+ve) cases in different age groups.

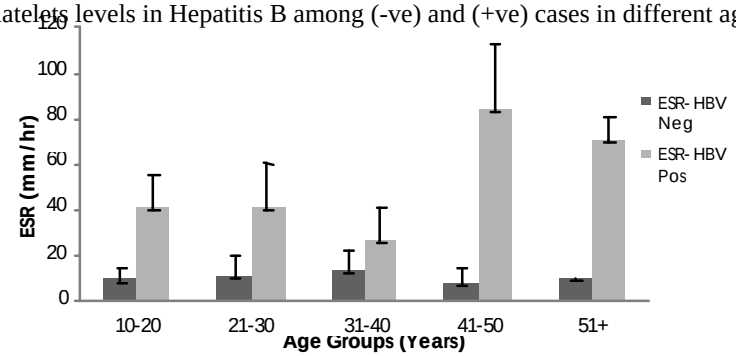

Fig. 6: ESR levels in Hepatitis B among (-ve) and (+ve) cases in different age groups.

The age group of 51^{+} years contained 6% of the tested individuals, out of which 50% were found negative and 50% cases were declared as positive for Hepatitis B. In negative cases, the results revealed that serum ALT, AST and ALP levels were 33.67±511.6, 30.67±5.51, 170.67±3.06 U/L, respectively, while positive cases revealed the value of 121.00±34.66, 96.00±49.39, 261.67±62.42 U/L, respectively (Fig. 1,2,3). The hemoglobin concentration in negative and positive cases was 12.87±3.15 and 11.33±1.78 g/dL, respectively (Fig. 4). The platelet counts of negative and positive cases were 239.00±128.83 and 107.67±11.93 10^3/µL, respectively (Fig. 5). The ESR of negative cases was 10.00±5.51 and it raised in the positive cases to 70.67±9.81 mm/h (Fig. 6).

DISCUSSION

The present study indicated higher values of ALT in HBV positive patients as compared to HBV negative patients that were in normal range, hence significance correlation was found between ALT and HBV. Increased levels of ALT however are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis. Normally ALT remains within the hepatocytes, but on damaging of hepatocyte, the enzyme escape out from hepatocytes and raised levels are found in blood [10].

In this present study, the increased levels of AST was found in HBV positive patients, while normal values are found in HBV negative individuals with significant correlation of AST with HBV [11]. ALT level found to be increased with age [11]. Increase in both transaminases (ALT and AST) with ALT much higher than AST was found in liver diseases. Serum ALT remains the most accessible test for monitoring the chronic Hepatitis B viral infection [11]. AST/ALT ratio is highly specific and predictive (100%) of cirrhosis in patients with chronic HBV infection [12].

In the present study, results revealed higher values of ALP in HBV positive patients and lower values in HBV negative individuals. Serum ALP level increases with increasing age, body mass index, C-reactive protein, liver diseases, lesion of liver and cardiovascular disease. The

old-aged and very old-aged subjects had higher levels of serum ALP [13].

The present work indicated lower values of hemoglobin in HBV positive patients as compared to HBV negative patients (within normal limits). The main causes for decreased hemoglobin include impaired hepatic function as in hepatitis and cirrhosis, increased albumin turnover and liver disease [14].

CONCLUSION

We concluded that blood screening must be most important tool to minimize the incidence and epidemiology of HBV. In rural areas there must be some awareness programs to educate people about HBV transmission.

ACKNOWLEDGEMENT

The present work is from MSc thesis of Muhammad Amjad which was carried out under the supervision of Dr. Muhammad Anjum Zia in Enzyme Biotechnology Lab.

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