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Assessment of haematological parameter and Liver enzyme among Hepatitis B Infected Blood Donors

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ABSTRACT

Viral hepatitis poses a substantial global health burden. The most often seen abnormalities after the diagnosis of Hepatitis B Virus (HBV) generally encompass haematological abnormalities and changes in liver parameters. The aim of this study is to evaluate the haematological variables and liver enzymes, specifically aspartate transaminase (AST) and alanine transaminase (ALT), among blood donors who have contracted the hepatitis B virus (HBV) at the Federal Medical Centre (FMC) in Owo. The study encompassed a cohort of 70 individuals, with the experimental group comprising 50 individuals who exhibited positive results for the hepatitis B virus (HBV). The control group consisted of a sample of twenty (20) apparently healthy adults. The mean levels of red blood cells (RBC), hematocrit (HCT), haemoglobin (HGB), and platelets (PLT) demonstrated a statistically significant reduction in persons relative to the control group, as evidenced by the p-values of 0.002, 0.001, 0.035, and 0.002, respectively. No statistically significant decrease in the average concentration of white blood cells (WBC) was reported among the participants in comparison to the control group (p=0.236). The study revealed a significantly increased mean concentration of alanine aminotransferase (ALT) in the participants as compared to the control group (p=0.016). The study findings revealed that there was no statistically significant difference in the levels of AST observed between the participants and the control group (p = 0.285). The present study has provided evidence to support the use of haematological parameters and liver enzymes as a reliable and accurate approach for assessing the severity of hepatitis B virus (HBV) infection. Therefore, it is crucial to incorporate the surveillance of patients even after their recovery from hepatitis B virus infection as an essential element of their healthcare.

Keywords: Hepatitis B, Liver, Haematological Parameter, Liver Enzyme

INTRODUCTION

The prevalence of Hepatitis B (HB) infection is a matter of great concern on a global scale, as it has the potential to greatly impact public health [1-4]. The hepatitis B virus (HBV) has exhibited a substantial global prevalence, impacting a population over two billion individuals [5-8]. Moreover, a significant proportion of this affected population, estimated to be between 360 and 400 million individuals, is known to suffer from chronic HBV infection [10-16]. However, it has been determined that the anticipated annual mortality attributable to HB infection spans a range of 0.61 million to one million [17-20]. Furthermore, the annual global incidence of HB infection amounts to around 4.5 million new cases. Notably high prevalence of HBV infection in the Western Pacific and African Region. Specifically, the infection rates among the adult population in both regions were found to be 6.2% and 6.1% respectively [21-25]. Numerous studies have reported the prevalence rates in sub-Saharan Africa, revealing a considerable variation ranging from 3% to 50%. It is worth noting that Nigeria has documented a prevalence rate of 9.5% for hepatitis B infection [26-30].

The liver is accountable for the synthesis of approximately 90% of all proteins, encompassing the entirety of albumin [31-38]. As a result, a notable decrease in blood protein concentrations is found in cases of extensive liver tissue destruction. Liver function tests are diagnostic procedures used to identify, diagnose, and evaluate hepatic diseases. The enzymes aspartate amino-transferase (AST) and alanine aminotransferase (ALT) are intracellular enzymes that are released into the circulation as a result of liver cell membrane disruption, causing the cytoplasmic contents of the cell to be expelled [39-43]. The enzymes alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) are predominantly localised on the canalicular membrane of hepatocytes. In the event of cholestasis, these enzymes are released into the plasma. The correlation between an elevated alkaline phosphatase (ALP) level and the existence of a cholestatic disease can be verified through an augmentation in gamma-glutamyl transferase (GGT) levels [43-47]. The usage of haematological parameters is a widely employed method for evaluating the overall health status of individuals, since each component plays a vital role in diagnosing particular illnesses such as hypertension and hemostatic disorders [30-40]. The aforementioned criteria are acknowledged to demonstrate variety with respect to aspects such as age, gender, race, geographic location, as well as special circumstances such as pregnancy and certain medical disorders. The measurements listed above demonstrate intra-individual variability, as observed by [9]. Therefore, the main aim of this research is to evaluate the haematological parameters and liver enzymes, specifically aspartate transaminase (AST) and alanine transaminase (ALT), in individuals who have contracted the hepatitis B virus (HBV) and have made blood donations at the Federal Medical Centre (FMC) in Owo.

MATERIALS AND METHODS

Research Area

This research was carried out at FMC Owo, situated in Ondo State, Nigeria. Owo is located in the southwestern region of Nigeria, positioned at the southern edge of the Yoruba Hills, and serves as a crossroads for routes from Akure, Kabba, Benin City, and Siluko. Owo is situated equidistantly between the towns of Ile Ife and Benin City.

Research Design

The study utilized a cross-sectional approach to examine HBV-infected blood donors at the Federal Medical Centre (FMC) Owo in Ondo State. This investigation took place from March to June 2022.

Determination of Research Sample Size

The sample size (N) was calculated using the formula for sample size determination commonly employed in health studies, the prevalence of HBV in Ondo State is reported at 3.1% based on a study by Owolabi et al. in 2014.

$$\mathbf{N} = \frac{\mathbf{Z^2 p\,(1-P)}}{\mathbf{D^2}}$$

N = Minimum sample size
Z = standard normal deviation (1.96)
P = estimated prevalence of HBV in Ondo state is 3.1% (0.031)
D = margin of error at 5% (standard value = 0.05)

$$N = \frac{1.96^2 \text{ X } 0.031\ (1\text{-}0.031)}{0.05^2}$$

$$N = \frac{3.8416 \text{ X } 0.031\ (0.969)}{0.0025}$$

N =46 minimum sample sizes

Therefore, a total of 50 subjects and 25 controls were recruited for this study.

Research Data Collection

Data were collected using a well-structured self-administrated preceded questionnaire which was specifically designed to obtain information that helped in either including or excluding certain individuals from this study respectively.

Inclusion and exclusion criteria

In this study, the inclusion criteria encompassed individuals aged ≥18 years who were present at the Federal Medical Centre in Owo, Ondo State during the study period were enlightened on the nature of this study and they provided their consent. However, individuals with pre-existing medical conditions and those who did not grant consent were excluded from participation.

Ethical Approval

The approval of the Ethical Review Committee of the Federal Medical Centre (FMC) Owo, was obtained. All experiment was performed in accordance with Good Laboratory Practice (GLP) regulations.

Specimen Collection and Processing

Aseptically and with minimal stasis, 5 millilitres (5mls) of venous blood were drawn from each subject using a sterile syringe and needle from the ante-cubital vein. Of the collected blood sample, 2mls were placed into an EDTA bottle for haematological analysis, while the remaining 3mls were transferred into a lithium heparin anticoagulated sample bottle for biochemical analysis and stored at 5°C until required for further analyses. Blood collected in the lithium heparin bottles underwent centrifugation at 3000RPM for 5 minutes, after which the plasma was extracted into a sterile container for analysis within 4 hours

Methodology

Haematological parameters were manually analysed while Liver function biomarkers (AST and ALT) were spectrophotometrically estimated.

Red Blood Cell Count

1:200 dilution of blood was prepared in the diluting fluid and mixed. The improved Neubauer counting chamber was carefully charged with the well-mixed diluted blood. The cells were allowed to settle in a moist chamber for 3-5 minutes. The ruled area of the counting chamber was microscopically located using a x10 objective lens. The total number of red blood cells in five groups of 16 small squares in the central ruled area was counted using a x40 objective lens.

Haematocrit estimation

The capillary tube was filled to 75% of its length with thoroughly mixed blood sample. The dry end of the capillary tube was sealed using plasticine. The capillary tube was positioned in a microhematocrit centrifuge, with the sealed end facing the periphery, and centrifuged at 10,000 RPM for 5 minutes. The haematocrit level was ascertained using a microhematocrit reader.

Haemoglobin estimation

Test tubes were labelled as blank, standard and test. In test tubes labelled as test and standard, 0.02ml of blood and cyanomethaemoglobin standard were added respectively. 5ml of Drabkin's solution was added to all test tubes including blank. The solutions were thoroughly mixed and allowed to stand for 10minutes. Absorbance reading was taken at 540nm.

White blood cell count

0.38ml of diluting fluid (blood) was dispensed into a clean test tube and mixed with 0.02ml of EDTA anticoagulated blood. The diluted blood sample was re-mixed using a Pasteur pipette held at an angle 45^0. One grid of the counting chamber was charged with well mixed diluted blood sample making sure it does not overflow the area. The chamber was left undisturbed for about 2 minutes to allow time for the white blood cell to settle. Fluid was placed in petri-dishes to prevent them from drying up. The underside of the chamber was dried and placed on a microscope stage. The rulings of the chamber and white cell was focused until they appear as small black dots. Cells that appear in the four large corner squares of the chamber (total area of 4mm2) including the cells lying on the lines of the two side of each large square on the lines of the two side of the chamber were counted.

Platelet count

In a clean test tube 380µl of 1% ammonium oxalate solution was pipetted. 20µl of whole blood collected into EDTA bottle was added to the test tube. The improved Neubauer counting chamber was charged by moistening the grid area and placing cover slip on it firmly. The sample was remixed gently using the pipette. One of the grid areas of the chamber was filled with the mixture. The chamber was placed in a moist environment to allow the platelets to settle undisturbed for 15min. The counting chamber was then focused under the microscope using x10 to focus and x40 to count the platelets.

Estimation of Alanine Aminotransferase (ALT)

Tubes were designated as "blank" and "sample." In both the blank and sample tubes, 500µl of reagent 1 (R1) was carefully pipetted. 100µl of distilled water was pipetted into blank tube. 100µl of the sample was pipetted into the sample tube. Thorough mixing was ensured, followed by incubation for 30 minutes at 37°C. 500µl of reagent 2 (R2) was added into each tube. Mixing followed, and the tubes were incubated at room temperature for 20 minutes. In each tube, 5ml of sodium hydroxide (NaOH) was added. Further mixing and incubation at room temperature were performed for 5 minutes. Absorbance readings were

taken at 546nm. The obtained absorbance values were compared to a reference graph provided in the manual.

Estimation of aspartate aminotransferase (AST)

Tubes were designated as "blank" and "sample." In both the blank and sample tubes, 500µl of reagent 1 (R1) was carefully pipetted. 100µl of distilled water was pipetted into blank tube. 100µl of the sample was pipetted into the sample tube. Thorough mixing was ensured, followed by incubation for 30 minutes at 37°C. 500µl of reagent 2 (R2) was added into each tube. Mixing followed, and the tubes were incubated at room temperature for 20 minutes. In each tube, 5ml of sodium hydroxide (NaOH) was added. Further mixing and incubation at room temperature were performed for 5 minutes. Absorbance readings were taken at 546nm. The obtained absorbance values were compared to a reference graph provided in the manual.

Statistical analysis

The result obtained was organized and subjected to appropriate statistical analysis. Statistical Package for Social Science (SPSS) version 26.0 was used in all the statistical analysis. Data from 2 groups were compared using students 2 tailed t-test for paired samples.

RESULTS

A total of 75 participants, with a mean age of 35.76±7.23 years, were enrolled in this study. The participants were divided into two groups: a subject group comprising 50 individuals who tested positive for HBV, and a control group consisting of 25 apparently healthy individuals. Table 1 presents a comparison of the mean concentrations of the measured parameters between the subjects and the controls. Among the findings, it was observed that the mean concentrations of RBC, HCT, HGB, and PLT were significantly lower in the subject group compared to the control group, with p-values of 0.002, 0.001, 0.035, and 0.002, respectively. However, the mean concentration of WBC did not exhibit a statistically significant difference between the subjects and controls (p=0.236). Additionally, the mean concentration of ALT was significantly higher among the subjects compared to the control group (p=0.016), while the mean concentration of AST did not show a significant increase among the subjects compared to the controls (p=0.285). Table 2 provides a comparison of the mean concentrations of the measured parameters among the subjects, considering their gender. The results revealed that the mean concentrations of RBC and WBC were not significantly lower among male subjects compared to female subjects, with p-values of 0.051 and 0.070, respectively. However, the mean concentrations of HCT, HGB, and PLT were significantly lower among male subjects compared to female subjects, with p-values of 0.032, 0.045, and 0.025, respectively. Furthermore, the mean concentrations of ALT and AST were significantly higher among male subjects compared to female subjects, with p-values of 0.002 and 0.005, respectively. Table 3 displays a comparison of the mean concentrations of the measured parameters among the subjects based on their age groups. The results indicated that the differences in the mean concentrations of RBC, HCT, HGB, WBC, PLT, ALT, and AST in relation to different age groups were not statistically significant, with p-values of 0.071, 0.060, 0.120, 0.061, 0.200, 0.102, and 0.082, respectively.

Table 1: Comparison of the mean concentration of measured parameters between subjects and controls (mean ± standard deviation).

Parameters	Subjects (n=50)	Controls (n=20)	t-value	p-value
RBC (x 10^{12}/L)	4.28 ± 1.20	5.86 ± 1.81	2.280	0.002
HCT (%)	35.61 ± 3.82	40.37 ± 4.50	3.123	0.001
HGB (g/dl)	11.24 ± 1.23	13.62 ± 1.79	-2.014	0.035
WBC (x 10^{9}/L)	5.85 ± 3.67	6.40 ± 1.82	1.460	0.236
PLT (x 10^{9}/L)	165.49 ± 4.16	225.5 ± 5.18	2.460	0.002
ALT (U/L)	25.43 ± 26.35	15.50 ± 12.05	1.820	0.016
AST (IU/L)	28.65 ± 22.59	23.39 ± 17.55	0.847	0.285

Table 2: Comparison of the mean concentration of measured parameters among subjects in relation to their gender

Parameters	Male (n=35)	Female (n=15)	t-value	p-value
RBC (x 10^{12}/L)	4.10 ± 2.10	4.37 ± 1.21	-1.004	0.051
HCT (%)	34.61 ± 2.90	37.42 ± 3.71	2.503	0.032
HGB (g/dl)	11.16 ± 1.32	12.68 ± 1.11	-1.014	0.045
WBC (x 10^{9}/L)	5.72 ± 5.55	6.21 ± 2.46	0.981	0.070
PLT (x 10^{9}/L)	152.91 ± 3.25	182.00 ± 5.12	1.900	0.025
ALT (U/L)	28.25 ± 7.94	20.43 ± 16.10	2.019	0.002
AST (U/L)	32.35 ± 21.71	23.76 ± 16.81	1.678	0.005

Table 3: Comparison of the mean concentration of measured parameters among subjects in relation to their age group

Parameters	18-30years (n=19)	31-40years (n=24)	Above 40years (n=7)	p-value
RBC (x 10^{12}/L)	4.51 ± 1.89	4.30 ± 1.52	3.93 ± 0.99	0.071
HCT (%)	37.91 ± 4.21	35.76 ± 2.39	32.83 ± 3.54	0.060
HGB (g/dl)	12.61 ± 1.62	11.29 ± 1.67	10.34 ± 73	0.120
WBC (x 10^{9}/L)	6.12 ± 3.21	5.89 ± 1.27	4.50 ± 1.45	0.061
PLT (x 10^{9}/L)	167.21 ± 4.31	159.48 ± 4.86	150.32 ± 3.61	0.200
ALT (U/L)	24.11 ± 23.19	24.32 ± 23.91	27.21 ± 28.33	0.102
AST (U/L)	26.92 ± 25.29	28.10 ± 25.09	31.48 ± 27.32	0.082

DISCUSSION

Hepatitis B infection has been recognized as one of the major extra-hepatic manifestations such as polyarteritis, polyarthritis nodosa, and glomerulonephritis, as well as hematological disorders that affect about 20% of individuals around the world [10]. Haematological parameters and liver enzymes like aspartate transaminase (AST) and alanine transaminase (ALT) have been identified as vital laboratory indicators to ascertain the overall health status of the liver[11], which was the reason for assessing the haematological parameters and some liver enzymes among HBV-infected blood donors in the Federal Medical Centre (FMC) Owo. This study had 75 participants with a mean age of 35.76±7.23 years, with an age distribution seen as (48%) within the age group 31–40 years, (38%) within the age group 18–30 years, and the remaining 14% being above 40 years. Also, the majority (70%) of the subjects were male, while 30% were female, as seen in figures 4.2 and 4.1, respectively. The high proportion of male participants was similar to the study conducted by [11-12] indicating the need to educate the female gender more about blood donation due to the assertion that that male blood donors far dominate the female donors [13]. The study observed a decrease in the haematocrit level, haemoglobin level, total erythrocyte count, total leucocyte count and platelet count of the HBV seropositive subjects when compared with the healthy individuals as seen in table 1. The findings of this study were in consistent with the study conducted by [14-15], in which the decrease in those haematological parameters may be inversely proportional to the degree of hepatic damage that determines the involvement of the liver. Marked anaemia has been observed as well-known hematological complication of viral infections, including hepatitis viruses [10,16-17], but then the hemocrit level in most patients with acute viral hepatitis decreases gradually during the first three weeks of illness as a result of temporary bone marrow suppression and autoimmune hemolytic anaemia, which may accompany viral hepatitis as a result of shared epitopes of viral and human antigens presented by antigen-presenting cells to trigger autoimmune reactions [18]. Another reason for the decreased value was attributed to an increased haemolysis in patients with acute hepatitis due to extravascular defect in the red cells which leads to shortened red cell life span [10].

The findings of this report also observed pancytopenia among donors who are infected with hepatitis B in comparison with the control as seen in Table 4.1 with a reduction in WBC and PLT level respectively. The findings from this study were in consistent with that of [19] The typical course of pancytopenia is two to three months after severe hepatitis, while some cases like the one presented in the study by [19] had present in as little as one week. Low level of WBC and PLT was consistent with the study of [20] this reduction could be negatively correlated with the level of hepatic damage, which

establishes the liver's involvement [15]. HBV can directly infect bone marrow, suppress the generation of new platelets, and hasten the death of existing ones by triggering the immune system and monocyte-macrophage system [21].

With respect to the liver enzymes, ALT and AST in this study, there was an observed significant increase in the mean concentration of ALT of Hepatitis B infected individuals when compared to the control ($p=0.016$) while a non-significantly increased value was seen in the AST value ($p= 0.285$). As the illness progressed and reached its lowest points, the concentration of the liver enzymes began to reduce, and when liver cirrhosis developed, the liver enzymes began to rise again, though still less than when the infection was new and acute [11]. Aspartate aminotransferase is among the enzymes that the HBV uses to detect damage to the liver tissue. However, as a marker of liver injury, AST is less precise. Additionally, its concentration rises with the breakdown of other body tissues, including the kidney, heart, striated muscles, and brain. Additionally, since hepatocytes alone create the increase in ALT, it is a more accurate and specific indicator of liver injury than AST [16]. Hepatitis virus infection causes abrupt and severe necrosis of the hepatocytes, which allows the cytoplasm of the cell to release the enzymes ALT and AST into the bloodstream [3].

The results also show that the difference in the mean level of HCT, HGB, PLT, ALT, and AST between males and females was significant. This might be a result of lifestyle factors such as alcohol consumption, smoking, and eating habits, which can directly or indirectly affect the liver, resulting in an alteration in the haematological parameters and liver enzymes. However, there was no significant difference in the parameters evaluated with respect to age group. The study has some limitations owing to its inability to group the hepatitis B virus among the donors and ascertain the progress of infection, giving room for future studies to advance in that area. However, the strength of the study lies in its ability to provide an understanding of medication responses, optimising treatments, and potential drug development specific to this population. The novelty lies in addressing a critical knowledge gap, potentially leading to tailored therapeutic approaches for hepatitis B patients in general so as to minimise liver damage [22-25].

CONCLUSION

The association between HBV, haematological alterations, and liver function disruption is established in this study. The concentration of haematological parameters in HBV-infected individuals differs from that of healthy individuals, with a significant difference in the levels of HCT, HGB, RBC, and PLT, as well as liver parameters such as ALT and AST. The present study has established that assessment of haematological parameters and liver enzymes is an effective, reliable, and competent way of diagnosing the severity of HBV infection.

RECOMMENDATION

In the present study, variation in the haematological parameters has been observed in patients with the seropositive Hepatitis B virus. This determines the need for routine haematological investigations in acute viral hepatitis patients. The follow-up of patients even after recovery from hepatitis B virus infection should be an essential part of these patients' management.

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