JOURNAL OF BIOMEDICAL RESEARCH AND ENVIRONMENTAL SCIENCES A MULTI DISCIPLINARY OPEN ACCESS JOURNAL

Medicine Group. 2021 May 27; 2(5):412-417. doi: 10.37871/jbres1251.

LIVER ENZYME PROFILE OF HEPATITIS B POSITIVE BLOOD DONORS IN ENUGU STATE UNIVERSITY TEACHING HOSPITAL.

[1]Department of Microbiology, Faculty of Medical Laboratory Sciences, Enugu State University Teaching Hospital, Parklane

[2]Department of Haematology, Faculty of Medical Laboratory Sciences, Enugu State University Teaching Hospital, Parklane.

ABSTRACT

This study was conducted to Screen for Hepatitis B virus among blood donors and to determine their Liver Enzyme Profiles in ESUTH, Parklane. A cross sectional study was carried out in 170 blood samples and 5mls of blood was collected aseptically into a plain container and were screened using Standard Serological procedures in department of microbiology and Liver enzyme profile of Seropositive donors were determine by standard biochemical procedure in the department of chemical pathology, from July to November 2024. The results revealed that 10% of the blood donors tested positive for HBsAg, a moderate prevalence. Awareness about HBV was found to be high, with (81.4%) of participants being informed, primarily by healthcare practitioners (72.4%). Gender-specific data showed a higher prevalence of blood donation among males (81.4%), The age group 26-35 years showed the highest prevalence of (54.6%), Most participants were single (59.2%), and many had received secondary education (43.3%). The study determined the liver enzyme profiles of HBsAg-positive donors, showing mild elevations in liver enzymes such as Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase, suggesting acute or mild chronic hepatitis.

ABBREVIATIONS

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline phosphatase, GGT: Gamma Glutamyl Transferase, HBV:Hepatitis B Virus, HBSag: Hepatitis B surface Antigen, WHO:World Health Organization, LFT: Liver Function Test, DNPH: 2, 4-dinitrophenylhydrazine, DNA: Deoxyribonucleic acid, HBsAg: Hepatitis B Surface antibody, HCC: Hepatocellular Carcinoma, HIV: Human immuno deficiency virus, EDTA: Ethylene diamine tetraacetic acid, Rpm: revolution per minute, ml: millitre, ESUTH: Enugu State University Teaching Hospital.

INTRODUCTION

Hepatitis B is a potentially life-threatening liver infection caused by Hepatitis B virus and it is a major global health problem (Shepard, *et al.,* 2019). It can cause both acute and chronic disease that affects the liver; it is a type of viral hepatitis. Hepatitis B is a silent killer liver disease for which many carriers are unaware of its clinical status; therefore, they act as a potential source of infection for other sero-negative people (Bosch, 2019). The presence of Hepatitis B surface antigen in the blood indicates that a person has been infected with the virus. Individuals who recover from acute hepatitis B infections clear the blood off HBsAg within approximately four months after the onset of symptoms. Acute HBV infection can be either asymptomatic or present with symptomatic acute hepatitis.

Hepatitis B affects the liver primarily by causing inflammation and damage to liver cells (hepatocytes) and triggers an immune response. This immune response, while targeting the virus, can also cause liver cell damage, potentially leading to fibrosis, cirrhosis, and even liver cancer over time (WHO, 2021). Infection often leaves no visible symptoms because the liver is a non-complaining organ. (Thad, *et al.,* 2019) The disease often gets severe before the symptoms occur Symptoms includes Yellowish skin and eyes (Jaundice), Tiredness, Dark urine and abdominal pain. These individuals develop antibodies to HBsAg (anti-HBs) that provides complete immunity to subsequent hepatitis B viral infection. Similarly, individuals who are successfully vaccinated against hepatitis produce anti-HBs in the blood (World Health Organization, 2012).

Risk factors includes Blood transfusion, Intravenous drug, Persons with current or past sexually transmitted infections (STIs) or multiple sex partners, Persons with HIV infection, Patients receiving dialysis, Living with an infected person, Persons currently or formerly incarcerated in jail, Persons with current or past hepatitis C virus (HCV) infection, Infants born to people who are HBsAg-positive, Persons with elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels of unclear etiology. (Conners, *et al.,* 2023).

The liver plays a crucial role in metabolizing substances, detoxifying the blood, and producing essential proteins. When HBV infects the liver, it disrupts these functions, often resulting in elevated liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver cell injury (Ganem, 2014). Worldwide, it is estimated that 240 million people are chronically infected with HBV (W.H.O.E. Region, 2017). According to the latest reports, 68,600 people worldwide die each year from HBV infection and more than 300,000 from liver cancer as a result of hepatitis B (WHO, 2015). Among voluntary and unpaid blood donors recruited through effective education and screening programs, the prevalence and incidence of HBV infection should be lower than in the general population. Hepatitis B virus is an important transfusion-transmissible infections. The pace of therapeutic advances in the treatment of chronic viral hepatitis, such as hepatitis B and hepatitis C, has accelerated significantly in the past decade. The hepatitis B virus vaccination program also expanded its coverage globally, and limited treatment options in the past have given way to a multitude of

effective drugs for HBV. On the other hand, blood product safety has become increasingly optimized, and improvements in the preparation of existing antiviral medications and better understanding (Helen, *et al.,* 2010). By understanding these profiles, the study can help inform blood donation screening practices and improve transfusion safety, ultimately contributing to better public health outcomes.

It is unethical to screen a Donor as Sero-Positive For Hepatitis B Virus and Just discharge them, Further investigation like the liver Enzyme Profiling which includes the Parameters (ALP (Alkaline Phosphatase), ALT (Alanine Transaminases), AST (Aspartate Transaminases), should be carried out to know if Severity of Liver disease or infection is chronic or Acute. In Nigeria, for example, the seroprevalence of HBV among blood donors ranges from 8% to 15%, with variations depending on geographical location and the donor population's socio-economic status (Ejele *et al.,* 2021).

MATERIALS AND METHODS

Study Design: A descriptive, cross-sectional study design to determine the Liver enzyme profile of Hepatitis B positive prospective blood donors targeting voluntary blood donors who visited Blood bank Centre.

Study Area: The study was carried out in Enugu State University Teaching Hospital (Parklane). G.R.A Enugu Urban, the capital city of Enugu state, Nigeria.

Sample Size determination

The sample size was calculated using the standard formula for calculating the minimum sample size (Charan J, 2013). Sample size (n) is given by Crochan's formula

$$n = \frac{Z^2pq}{d^2}$$

Where;

n = Minimum sample size =?

Z = Standard normal deviation at 95% level of confidence = 1.96

P = (the percentage of target population estimated to have a particular characteristic) = 50% (0.5)

q = 1-p = 1-0.5 = 0.5

d (margin of error) = 10% (0.1)

Therefore, $n = \frac{(1.96^2 * 0.5 * 0.5)}{0.1^2}$

Sample size = 97.

The sample size targeted at 97 subjects.

Inclusion Criteria and Exclusion Criteria

Patients Tested Sero-Positive with Hepatitis B virus, Individuals who provided informed consent to Participate in this Study, All eligible blood donors aged 18-65 years, Hemoglobin level within the normal range for male ≥13.5 mg/dl; for female ≥12 mg/dl were included, Donors with known liver disease or other co-infections (HIV, Hepatitis C), Blood donors who refused to give their consent were excluded.

Study Population

People who Tested Sero-Positive for Hepatitis B virus among Blood donors attending ESUTH, Parklane, during the study period.

Data Collection and Analysis.

The socio-demographic data and other relevant information of each participant were obtained using a self-administered questionnaire. Data obtained from this study was analyzed using the statistical package for social sciences (SPSS). Data was presented as mean and standard deviations

Ethical consideration

Ethical clearance for the study was sought and obtained from the ethical committee of Enugu State University Teaching Hospital (ESUTH), Parklane, with the reference number, ESUTH/HREC/2024/10/201.

Sample Collection

The blood sample was taken from each participant aseptically for the serological with a sterile disposable syringe and needle, after disinfection of the selected venipuncture site with 70% alcohol in an expanding circular scrub from the center to the periphery of the needle insertion. About 5ml of blood was collected by venipuncture and was dropped into a plain bottle labeled with corresponding sample number, the plasma was separated and used for serology For Hepatitis b virus screening.

Serology (Hepatitis B Screening)

The HBsAg Rapid Test Strip is a qualitative immunochromatographic assay used to detect the presence of Hepatitis B surface antigen (HBsAg) in serum, plasma, or whole blood. The test strip was removed from the sealed pouch and was placed on a clean and flat surface, The dropper was held vertically and 2 drops of plasma was added to the specimen area of the strip, The recommended number of buffer solution drops was added, if applicable and The specified time (usually 15–20 minutes) was taken and the result was interpreted.

Biochemical Analysis

The serum sample was analyzed at a certified laboratory using standardized methods for each liver enzyme test. Each liver parameter was quantified using appropriate biochemical assays, with results documented.

Alanine Transaminase and Aspartate Amino transferase

Method: Spectrophotometry (Reitman-Frankel)

Principle: Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.

AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD.

It is measured by the reaction with 2,4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution is measured at 540nm.

Materials Buffer/Substrate, 2-4 dinotrophenylhydrazine, water (distilled water), 0.4N NaOH (Sodium Hydroxide solution), Water bath at 37°C, test tube and control serum. The buffer for ALT is called Tris buffer and is contained with substrate which is 2-oxoglutarate. The buffer for AST is called Tris buffer and is contained with substrate which is alpha ketoglutarte and is measured at 540nm.

Control Liver Enzyme for ALT,AST and ALP

	ALP	**ALT**	**AST**
Control	**226.3**	**67**	**81**

Procedure for ALT and AST

	Test	**Control**	**Blank**
Buffer\ Subtrate	0.5ml	0.5ml	0.5ml
Sample	0.1ml	-	-
Control serum	-	0.1ml	-
Water	-	-	0.1ml

- Into test tubes labeled Test and blank were added 0.5 ml each of ALT buffered substrate and AST buffered substrate
- Then 0.1ml of sample was added to the tube labeled Test and 0.1 ml of distilled water into the tube labeled blank
- It was incubated in a water bath at 37°C for 30minutes for ALT, for AST, 40minutes.

2,4 DNPH		0.5ml	0.5ml	0.5ml

- Into each of the tubes were then added 0.5ml of 2, 4-dinitrophenylhydrazine and incubated at room temperature for 20 minutes.
-

0.4N NaOH	5mls	5mls	5mls

- Then 5.0 ml of 0.4N NaOH was added into each of the tubes and allowed to stand for 5 minutes at room temperature.
- The absorbance was then read against reagent blank at 540nm and enzyme activity obtained from a calibration table.

Alanine Phosphatase.

Method: Spectrophotometry (according the standardized method described by DGKC).

Principle: ALP catalyzes the hydrolysis of the colorless organic phosphate ester substrate, p-Nitrophenylphosphate, to the yellow colored product p-Nitrophenol and phosphate. This reaction occurs at an alkaline pH of 10.3.

Materials

Buffer/Substrate, 2-4 dinotrophenylhydrazine, water (distilled water), 0.4N NaOH (Sodium Hydroxide solution), Water bath at 37°C, test tube and control serum. ALP is measured at 405nm.

Procedure

- Into a cuvette was added 0.5 ml of working reagent and 0.01 ml of sample, it was mixed and initial absorbance read at 405 nm and a stop watch started
- The absorbance was further read at 1, 2, and 3 minutes respectively.

- The change in absorbance was then multiplied with factor 2760 to get the activity of the alkaline phosphatase enzyme.

RESULT

Out of 170 blood donors screened, 10% tested positive for Hepatitis B, indicating a moderate level of the infection within the donor population.

For aspartate aminotransferase (AST) showing a narrower range, values range from a minimum of 5.0 to a maximum of 38.0 with a mean of 17.82 and a standard deviation of 10.80. Alkaline phosphatase (ALP) measurements shows a significant range, from a minimum of 22.1 to a maximum of 108.1, with a mean of 62.02 and a standard deviation of 27.56. Alanine aminotransferase (ALT) levels, with a mean of 11.12 and a smaller standard deviation of 6.13, range from 4.0 to 26.0.

Figure 4.1: Prevalence of Hepatitis B Positive Cases Among Donors by Gender

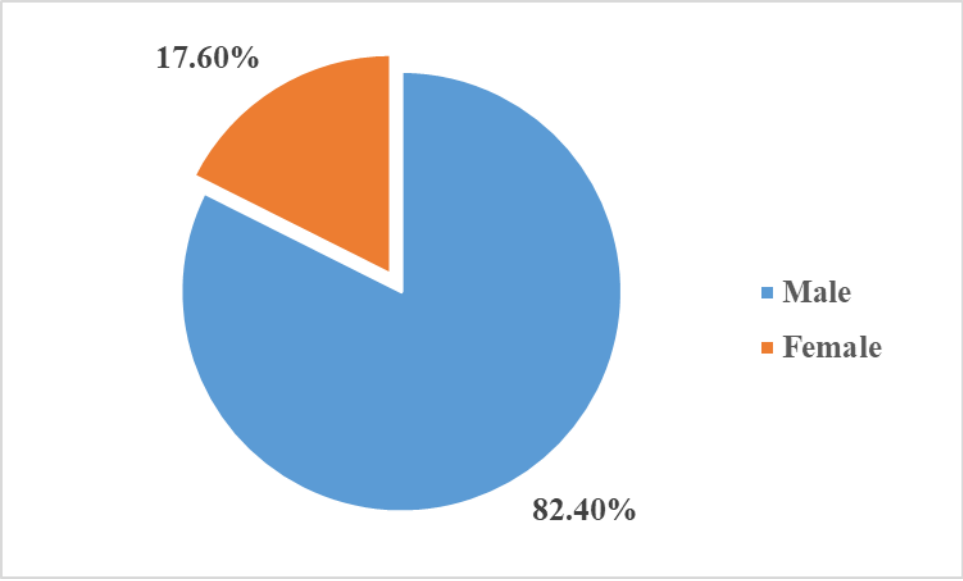
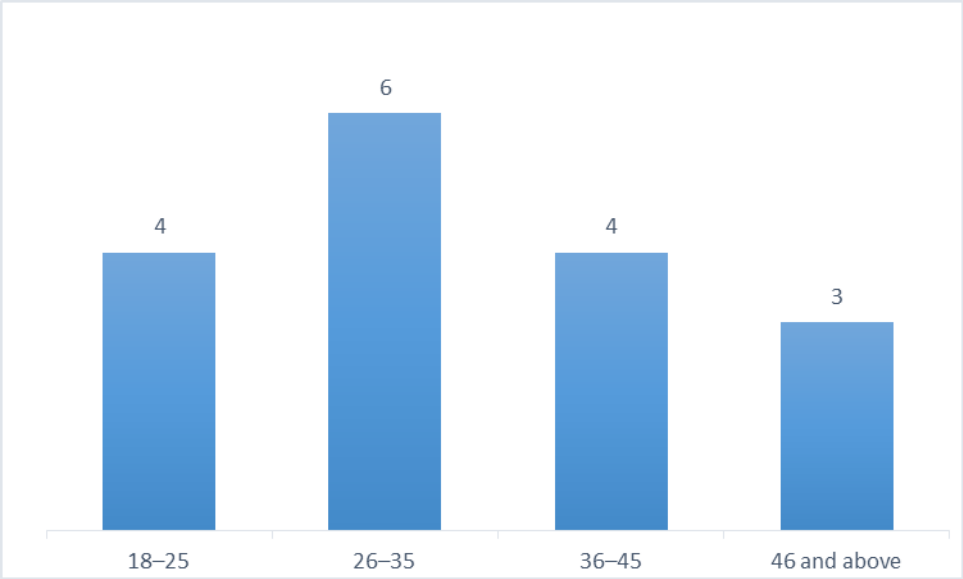


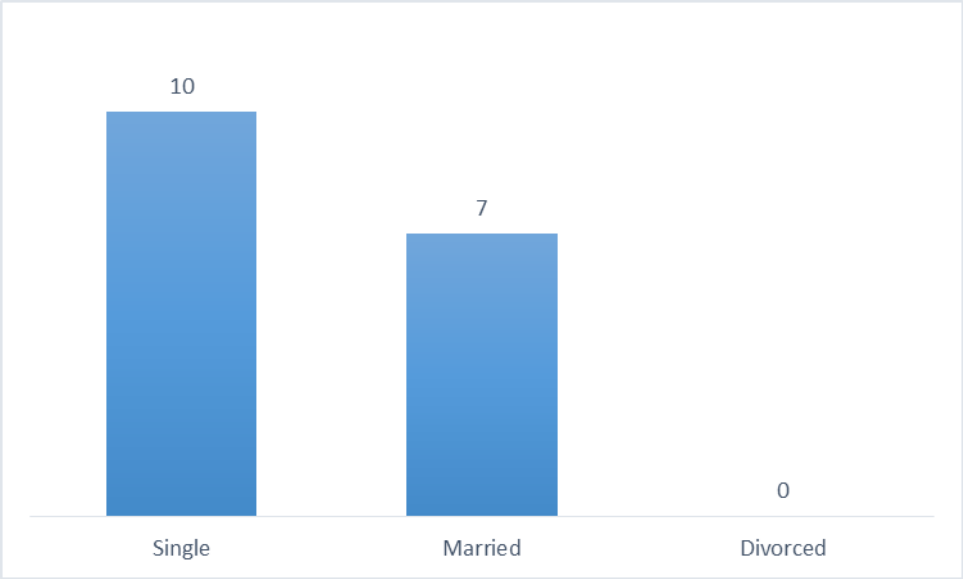
Figure 4.1 illustrates the prevalence of Hepatitis B among male and female blood donors. The prevalence was higher in male donors (82.4%) than in female donors (17.6%).

Figure 4.2: Prevalence of Hepatitis B Positive Cases Among Donors by Age



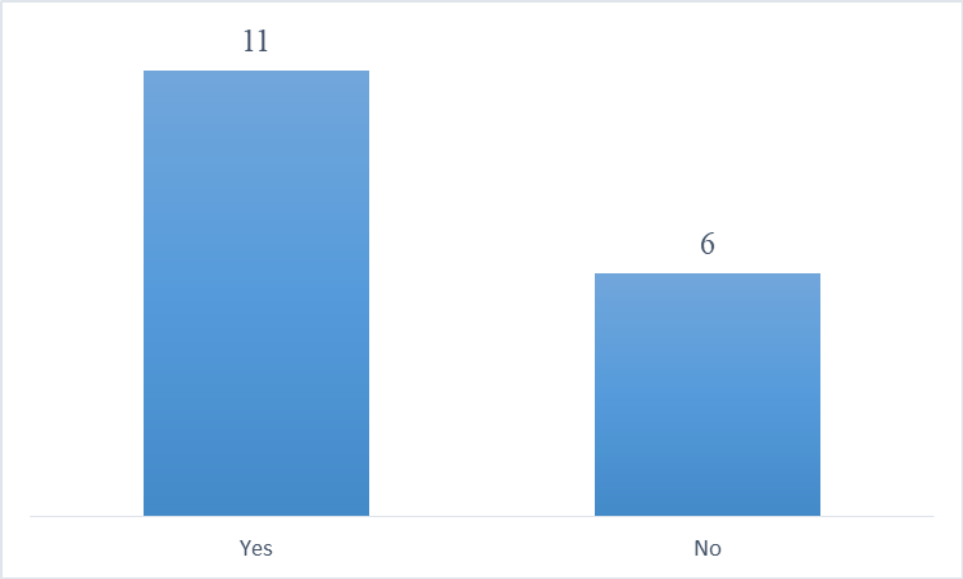
This chart illustrates the prevalence of Hepatitis B positive cases among different age groups of blood donors. The 26–35 age group had the highest frequency, with 6 positive cases. The 36–45 age group followed with 4 positive cases. The 18–25 and 46 and above age groups each had 4 and 3 positive cases, respectively.

Figure 4.3: Prevalence of Hepatitis B Positive Cases Among Donors based on Marital Status



This chart shows the Prevalence of Hepatitis B positive cases based on the marital status of blood donors. The majority of positive cases were found among single donors, with a frequency of 10 positive cases. Married donors had a lower frequency, with 7 positive cases. There were no positive cases reported among divorced donors.

Figure 4.4: Prevalence of Hepatitis B Positive Cases Among Donors based on Awareness of Hepatitis B



This chart displays the prevalence of Hepatitis B positive cases based on whether blood donors have heard of Hepatitis B Virus (HBV). Among donors who have heard of HBV, 11 individuals tested positive for Hepatitis B. In contrast, among those who have not heard of HBV, 6 individuals tested positive.

DISCUSSION

The result of this study showed that 17(10%) blood donors out of 170 had HBsag as the serological marker. A prevalence of 10% observed for HBSag in this study is moderate compared to those obtained in 18.1% in Usmanu Danfodiyo University Teaching Hospital, Sokoto State (Hussaini, 2021). This may due to proper awareness from Table 4.4 by health Practioners 79(81.4%), media 11(12.6%), friends and relatives 5(5.6%) while about 18(18.6%) were not aware of this silent virus.

The gender-specific difference shown that male blood donors were higher 87(89.7%) than the Female counterpart 10(10.3%) This contradicts the report of Uneke, 2005, who stated that females donates more than the male. The reason for this difference might be due to a large

number of male blood donors in this study than the females. However, his observation contradicts what had been previously reported by some authors, Mehmet, 2005 in his study reported a higher prevalence rate of blood donation in male than females in both rural and urban, A similar study also reported a higher prevalence in males than females among patients attending dental Clinic, University College Hospital, Ibadan, Nigeria (Lukhwareni, 2009).

Blood donors within the age group 26-35years, 53(54.6%) had the highest prevalence of blood donation, followed by 18-25 years, 22(22.7%), 36-45years 17(17.5%) and 45-65 years as shown in Table 4.3. This study contradicts the earlier report with a high prevalence of HBV in older subjects 40 years and above than in younger people Lawal (2009) and Luka (2008), in their study, reported higher HBV prevalence among the older age group 30-34 years. The finding in this study supports the work of Buseri (2009), who noted that HBV infection is more prevalent in younger subjects within ages 20-29 years Buseri (2009), the possible reason for this high prevalence rate in younger people than older people may be attributed to their active sexual activities and druRegarding marital status, the majority of respondents were single 58 (59.2%), while 36 (36.7%) were married, and a small percentage were divorced 4(4.1%). None of the respondents were widowed. In terms of education, most respondents had completed secondary school 42(43.3%), followed by tertiary education 28 (28.9%). Additionally, 17 (17.5%) had completed primary school, while 10 (10.3%) had no formal education. The largest group of respondents were self-employed 35(36.1%), followed by students 26(26.8%), unemployed 19(19.6%), and employed 17(17.5%). Lastly, regarding blood donation history, the majority of respondents 80(82.5%) reported having donated blood before, while 17 (17.5%)

The participants, 79 (81.4%) had heard of HBV, with the majority 63(72.4%) learning about it from health workers, followed by media 11 (12.6%) and friends/relatives 5 (5.6%). When asked if HBV is a serious liver infection, 37 (38.1%) acknowledged it as serious, while 60 (61.9%) did not. Regarding transmission, the most recognized routes were blood transfusion 82 (23.2%), unprotected sexual intercourse 73(20.7%), sharing needles/syringes 73(20.7%), and contact with contaminated items 70(19.8%). Additionally, 76 (78.2%) of respondents were aware that HBV could be prevented through vaccination, and all respondents 97(100%) believed screening for HBV before blood donation is important.

The Liver Enzyme Profile of Hepatitis B Positive Blood donors, For aspartate aminotransferase (AST) showing a narrower range, values range from a minimum of 5.0 to a maximum of 38.0 with a mean of 17.82 and a standard deviation of 10.80, this slight elevation could be attributed to other factors such as intense exercise, are unrelated to liver function and can markedly increase AST and ALT. as AST is not a specific marker for liver damage. Showing not much elevation the samples could be termed as they are in their “acute phase” of viral infection as AST has a shorter half-life of 17hours circulating in the body, and is specific for acute damage infection compared to ALT having a longer half-life.

Alkaline phosphatase (ALP) measurements shows a significant range, from a minimum of 22.1 to a maximum of 108.1, with a mean of 62.02 and a standard deviation of 27.56. ALP is next indicator of hepatocellular damage. Elevated levels of alkaline phosphatase activity usually reflect impaired biliary tract function (Burtis and Bruns 2007). In this case, it may be due to recent attack of hepatitis B.

Alanine aminotransferase (ALT) levels, with a mean of 11.12 and a smaller standard deviation of 6.13, range from 4.0 to 26.0. Showing not much elevation, as ALT being a specific marker for liver damage. Elevations in ALT is specific for chronic damages in the liver as ALT has a longer short life of 48hours being circulated, compared to AST.

The samples showing the elevations in ALT and AST are lesser elevation than ALP. For the fact there is a significant increase or elevation in both ALT and AST this suggests viral hepatitis. Lesser elevations are encountered in mild acute viral hepatitis as well as in both diffuse and focal chronic liver diseases e.g. chronic active hepatitis, cirrhosis, and hepatic metastases (Burtis and Bruns 2007). Hence, the cases in the study with lesser elevations may be of mild acute hepatitis due to Hepatitis B virus infection. Tsai et al. (1997) Although results of liver function tests can be normal in HBsAg carriers, Cirrhosis of the liver or fulminant liver failure secondary to hepatitis commonly reach values for both ALT and AST in the >1000 U/L range; however, many people with liver disease have normal transaminases.

CONCLUSION

The study confirms that Hepatitis B infection significantly affects liver enzyme profile. Leading to liver damage. Early detection and intervention are crucial to mitigating the long term impact of HBV on liver health.

RECOMMENDATION

1. HBV screening: It is recommended that all blood donors be routinely screened for HBV profile as part of the routine blood donation process, particularly during their first visit.

2. Routine Liver Function Test: HBV seropositive individuals should undergo regular liver functions tests to monitor enzyme levels and detect liver damage early. There should be public awareness campaign for Hepatitis B infection and public vaccination/ immunization. Hospitals should prioritize the accessible and affordable liver function facilities.

3. Awareness Campaign: Increased public health campaigns are needed to educate individuals about the Causes of HBV, their transmission route, prevention and treatment.

REFERENCES

Charan J, Biswas T. (2013). How to calculate sample size for different study designs in medical research? *Indian Journal of Psychology Medicine* ;35(2):121-6.