AFRICA UNIVERSITY

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HYPERTRANSAMINASEMIA AMONG HEPATITIS B VIRUS POSITIVE SAMPLES TESTED AT LANCET CLINICAL LABORATORIES FROM 2016 TO 2019

By

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A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE BACHELOR OF MEDICAL LABORATORY SCIENCES HONOURS DEGREE IN THE COLLEGE OF HEALTH, AGRICULTURE AND NATURAL SCIENCES AT AFRICA UNIVERSITY

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ABSTRACT

Serum aminotransferases have always played a key role in the investigation of liver disease, particularly related to HBV. However, recently there has been a discussion on the usefulness of both AST and ALT, due to them being influenced by many other factors and conditions. It is for this reason that this study was conducted to bridge the gap remaining. The study was a Retrospective cross sectional study based on records of patients with HBV positive markers who had serum aminotransferase enzyme levels done at Lancet Clinical Laboratories from 2016 to 2019. Records from January 2016 to December 2019 were retrieved from the Laboratory Information System, Medi-Tech, grouped and analysed using Microsoft excel as a data analysis tool. The study included 214 participants of which 148 were males, 63 females, and 3 not identified. Both AST and ALT elevation had the highest prevalence among females as compared to males and the total ALT prevalence was 55% as compared to 42% for AST. It was also observed that for both AST and ALT, borderline elevation was the most prevalent which corresponds to 1-2 x ULN (upper limit normal). The study revealed a high prevalence of both AST and ALT, with ALT having the highest prevalence among study participants (53,2%) compared to AST (44,3%). These findings were thought to be associated with the fact that early HBV infection causes inflammation and increased hepatocyte death resulting in high aminotransferase levels and terminal illness does the same. It was also discussed that a certain proportion of the population with positive HBV markers remains asymptomatic and has a prolonged illness (Chronic infection) resulting in stable and unaltered serum AST and ALT levels, these patients may however be infectious.

ACKNOWLEDGEMENT

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DEDICATION

I dedicate this Research project to my mother Isabel Damião, my brothers Osvaldo Mariano, Ivan and Valdemiro Damião and nieces Kayla, Nilsa and Patricia. A special dedication to my mother who has always given me the motivation and to Lisa Weaver, my close friend and mentor who has also played a key role in my academic life in the past few years. To these two women I dedicate this research project.

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DEFINITION OF TERMS AND ABREVIATIONS

Aminotransferases- Group of enzymes that catalyse the interconversion of amino acids and oxiacids by transfer of amino groups. Aminotransferases are used in assessing liver diseases, since serum levels are raised in hepatic impairment. Most frequently used aminotransferases are AST and ALT. (Vroom et al., 1990).

AST (Aspartate aminotransferase) - According to medicine net, AST is an enzyme that is normally present in liver and heart cells. It is released into the blood when liver cells are damaged, so blood AST levels are elevated in liver disease such as in viral Hepatitis.

ALT (Alanine aminotransferase)- medicine net defines it as an enzyme present within liver cells, showing increased blood levels when liver function is impaired.

Hypertransaminasemia/ Transaminitis- Refers to high levels of serum transaminases above normal range.

NAFLD (Non-alcoholic fatty liver disease)- defined as deposition of fat in the liver of a non-alcoholic subject, a condition which may progress to end stage liver disease (Dabhi et al, 2008).

Viral Hepatitis- Infection of the liver caused by one of several viruses, most commonly Hepatitis A, B or C. All these viruses may cause similar symptoms but are transmitted in different ways (CDC, 2013).

Alcoholic hepatitis- Acute hepatic inflammation that results from the interaction of factors such as ethanol metabolism effects, inflammation and innate immunity action (Chayanupatkul, el al, 2014).

HCV- Hepatitis C virus infection

HBV- Hepatitis B virus infection

WHO- World health Organisation

BMI- Body mass index, it is a measure of weight adjusted for height, calculated as weight in kilograms divided by the square of the height in meters (CDC, 2015).

HBsAg- Hepatitis B surface antigen

HBcAb- Hepatitis B core antibody

HBsAb- Hepatitis B surface antibody

HCV- Hepatitis C Virus

HAV- Hepatitis A Virus

HBeAg- Hepatitis B Virus e antigen

Anti-HBeAg- Antibodies to HBeAg

AMI- Acute Myocardial Infarction

ULN-Upper limit of normal

Immunity- Defined as the ability of an organism to resist a particular infection by the action of specific antibodies or sensitised white blood cells**.**

Exposure- Defined as the state of having no protection from a harmful agent.

Infection- Defined as the process or state of being infected

Replication – Defined as the process of duplicating or producing an exact copy of a polynucleotide strand such as DNA.

Disease- A condition in which there is impaired normal function within the human body or one of its organs, often manifesting with signs and symptoms.

Virion- An entire virus particle consisting of an outer protein shell called a capsid and an inner core of nucleic acid (either RNA or DNA).

Seroconversion- Defined as the development of detectable antibodies in the blood that are directed against an infectious agent.

Inflammation- Defined as a local response to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat and pain in order to eliminate damaged tissue and infectious agent.

CHAPTER 1 INTRODUCTION

1.1 Introduction

This chapter introduces the study topic, showing the magnitude of the problem in a global and local scale, it brings statistics and previous research that has been done in the area. This chapter also discusses the purpose and objectives of the study, delimitations and possible limitations.

1.2 Background information

Hepatitis is an inflammation of liver cells (Hepatocytes) and damage to the liver. There are different types and causes of Hepatitis including viral, drug toxicity, and immune diseases. The condition can be self-limiting (acute Hepatitis) or progress to scarring of liver tissue (Fibrosis), cirrhosis or liver cancer. Viral hepatitis is caused by infection with any of at least five distinct viruses: Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV) and Hepatitis E virus (HEV). Most severe viral hepatitis infections are due to HBV and HCV, and both are blood borne pathogens transmitted mainly through direct contact with blood but also through other body fluids such as semen and vaginal fluids. HAV and HEV are transmitted through the faecal oral route and are less clinically significant. (WHO, 2018).

Hepatitis B virus (HBV) is transmitted through exposure to infected blood or blood products, semen and other body fluids. The virus can also be transmitted from infected mothers to infants at time of birth or from family members to an infant in early childhood. Unsafe medical procedures, transfusion of contaminated blood or blood products, occupational exposure in Healthcare workers and intravenous drug use have been associated with high numbers of infection (WHO, 2018).

Hepatic injury does not primarily result from the cytopathic effects of the virus in Hepatocytes, but from the body's immune system response to either acute or chronic infection with HBV. Evidence shows that the innate immunity does not play a major role in HBV clearance or liver injury, but the adaptive immunity mediated by cytotoxic T-Lymphocyte cells does have association both with viral clearance and liver injury. Failure by the body mechanisms to clear HBV infected cells can lead to chronic Hepatitis B and development of HBV cirrhosis and hepatocellular carcinoma (Xuanyong, 2011)

The incubation period of HBV infection is 40-150 days and initial symptoms in Acute HBV infection may range from mild disease to a more severe condition (such as fulminant Hepatitis). Following acute episodes about 95% of adults and 5-10% of infected infants develop antibodies to HBsAg with full recovery and about 5% of adults and 90% of infected infants, and 30-50% of children infected at age 1-5 years develop chronic infection (Samji, et al., 2017)

In Africa, the growing HBV incidence and prevalence is attributed to high rates of mother to child transmission, as most of the infections are acquired before the age of 5 years and infected children develop persistent hepatitis and are more likely to develop progressive liver disease and cancer in early adulthood. Unsafe blood transfusions in most African settings, infections with contaminated needles and sharps in healthcare facilities and traditional practices of tattooing have been shown to be important routes of transmission within the continent, as well as unsafe sex, and intravenous drug use. (WHO Africa, 2018)

Aminotransferases are enzymes that are found in different tissues but mainly in Hepatocytes, where they catalyse the reversible transfer of the amino group. They catalyse the interconversion of aminoacids and oxoacids by transfer of the amino group, which is achieved by redistribution of Nitrogen between aminoacids and corresponding oxoacids

participating in both protein metabolism and gluconeogenesis. Elevations of both AST and ALT are highly characteristic of hepatocellular damage in acute Hepatitis and elevation of serum AST above ALT level is seen in chronic hepatitis and liver cirrhosis as a result of hepatic cell necrosis with release of mitochondrial AST (Vroon et al., 1990).

It has been observed that hepatic cell injury is manifested by elevated serum transaminase activity prior to the appearance of clinical symptoms such as jaundice. Mild elevations of aminotransferases have also been associated with toxic or non-ethanol drug induced hepatitis as a result of cellular release of cytoplasmatic enzymes associated with reversible hepatic cell damage, as well as cholestatic lesions either intra-hepatic or post-hepatic may also cause modest transaminase elevations, with AST usually exceeding ALT and also elevations in serum Gamma Glutamyltransferase (GGT) and alkaline phosphatase (ALP) (Israili et al., 1990).

Although aminotransferase elevation may occur with other nonhepatic disorders, elevations exceeding 10 to 20 times the reference are uncommon in the absence of hepatic cell injury and because the concentration of ALT is less than AST in all cells except hepatic cytosol, ALT elevations are less common in nonhepatic disorders such as in Myocardial infarction or skeletal muscle diseases. (Vroon et al., 1990).

Aminotransferases are enzymes that are widely distributed in body tissues and catalyse the reversible transfer of the amino group. The most frequently used aminotransferases in the laboratory are Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST), in the diagnosis of liver function impairment and damage. Usually, serum levels of these enzymes will be above normal range, due to hepatocyte destruction. (Rej,1990).

Liver disease has been ranked as the 5th most common cause of death globally and as the second leading cause of mortality among all digestive diseases in the US alone (Everhart,2009)

It is also estimated that more than fifty million people in the world have chronic liver disease and globally, alcohol and viral hepatitis are the leading causes of chronic liver disease. In 2001, the estimated worldwide mortality from liver cirrhosis was 771,000 people, ranking 14th and 10th as the leading cause of death in the world and in developed countries respectively. It is estimated that by 2020, liver cirrhosis will be the 10th leading cause of death globally (Murray, 1997).

1.3 Problem statement

Despite being of public knowledge that serum aminotransferases are raised in liver injury, different studies conducted on similar topics have shown disagreements among scholars on the usefulness of serum aminotransferases in assessing liver damage such as in viral hepatitis. Some scholars have indicated that serum aminotransferase levels don't always correlate with disease progression or liver injury in patients infected with HBV or in indicating patients at a higher risk for developing liver cirrhosis and liver cancer but others reinforce the fact that serum aminotransferases still remain an effective, less invasive procedure for assessing liver injury, correlating with clinical and other laboratory findings.

The Researcher has anecdotally observed that samples of patients with positive HBV markers at Lancet Clinical Laboratories don't most times present with high serum aminotransferase levels as expected, even in those documented with a chronic infection or thought to be having a progressing liver condition and on the other hand, samples of patients with no documented liver infection or physical hepatic trauma present with abnormally high aminotransferase

levels. In this study, the researcher sought to analyse serum aminotransferase levels in patients found to be positive for HBV, a major cause of liver disease and complications.

Considering that most patients infected with HBV are asymptomatic, serum aminotransferase levels are thought to be a key procedure in assessing liver damage in patients infected with HBV and in doing so, reducing the burden of associated liver cirrhosis, hepatocellular carcinoma, morbidity and mortality in developing countries such as Zimbabwe.

No similar study had been previously done in Zimbabwe to ascertain the burden of Hypertransaminasemia in relation to HBV.

1.4 Justification of the study

The findings of the study may bring a detailed understanding of the usefulness of serum aminotransferases in patients with HBV infection by assessing the prevalence of such elevation in participants with an established infection. The study may help to assess the burden of aminotransferase elevation in those infected with HBV and explore the factors behind normal aminotransferase levels in the same population. The findings from this study may also create ground for further research to be done locally on the same study area and thus give a higher contribution to public health efforts directed at reducing the incidence and prevalence of HBV infections.

1.5 Purpose of the study

The aim of this study was to determine the prevalence of serum Hypertransaminasemia among patients with HBV whose samples were tested at Lancet Clinical Laboratories from 2016 to 2019.

1.6 Research questions

- What was the prevalence of Hypertransaminasemia in relation to HBV infection among patients whose samples were tested at Lancet Clinical laboratories from 2016 to 2019?
- What were the Socio-demographic characteristics of patients with hypertransaminasemia whose samples were tested at Lancet Clinical Laboratories from 2016 to 2019?

1.7 Research Objectives

1.7.1 Broad Objective

- To review Serum aminotransferase levels in Hepatitis B Virus positive patients whose samples were processed at Lancet Clinical Laboratories from 2016 to 2019

1.7.2 Specific Objectives

- To determine the prevalence of Hypertransaminasemia in relation to HBV infection among patients whose samples were processed at Lancet Clinical laboratories from 2016 to 2019.
- To determine the Socio-demographic characteristics of patients with hypertransaminasemia whose samples were processed at Lancet Clinical Laboratories from 2016 to 2019

1.8 Delimitations of the study

The study was conducted at Lancet Clinical Laboratories head office which is located in Harare. The Researcher analysed records from patients with documented HBV infection who had serum aminotransferases tests done. Patients with documentation on positive antibodies

to HCV and HAV (IgM) were excluded from the study. No specific age group or gender was prioritised in this study.

The Researcher used three HBV markers (HBsAg, HBsAb and Total HBcAb) wherever possible, to rule out confounders such as recent vaccination that may give a false positive HBsAg, since a positive Total HBcAb indicates a true infection, either in the past or currently, being it a combination of both IgG and IgM antibodies to the core antigen of the virus found within infected hepatocytes.

1.9 Limitations of the study

A major limitation to my study was the fact that Lancet Laboratories is a private laboratory, whose charges are very expensive, so the data collected could be biased towards people of higher economic status.

The researcher also conducted a retrospective data analysis. Data had been originally collected by subjects other than the researcher and for purposes other than the study that was conducted, so the information could have some discrepancies or incomplete variables, which may have an impact on the findings of the study.

Other limitations include scientific evidence which shows that serum aminotransferase levels may suffer a mild elevation depending on a patient's diet, BMI, and non viral hepatic diseases such as alcoholic liver disease and fat storage disorders. The researcher may not have been able to trace all the listed confounding variables.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter brings scientific research done on areas of concern to the study topic as well as areas of scientific discussion between different scholars and implications to the study topic.

2.2 Literature review in relation to research objectives

Aminotransferases are analysed in serum to asses and monitor liver damage, such as in viral infections. ALT is mainly found in the liver, but also in small amounts in the kidneys, heart, muscles and pancreas, whilst AST is found in the liver, but also in considerable amounts in other tissues.

In recent years there has been some controversy on the usefulness of serum aminotransferases in assessing liver disease, due to many other non viral factors which may influence their serum levels such as diet, alcohol consumption, obesity and the presence of these enzymes in other tissues. Still, serum aminotransferase levels remain a key procedure in assessing liver damage such as in viral hepatitis, for being less invasive and more effective even in resource limited settings.

A study conducted in Germany, with the aim of scrutinising the evidence for using ALT as a primary screening parameter for liver diseases concluded that elevated serum ALT levels indicate a high specificity and a reasonable sensitivity to liver injury. The study also concluded that ALT levels are increased in majority, but not all patients with acute or chronic liver disease and that elevated ALT levels are also a risk factor for liver specific mortality (Wedemeyer et al., 2010).

An article on mild elevation of liver transaminase levels, its causes and evaluation published in 2017 indicated that based on a large prospective study performed in the UK on patients

with abnormal transaminase levels or liver function, only 1.35% of 1,300 patients with abnormal transaminases levels had serious liver disease that required immediate treatment. About 1% of the cases had viral hepatitis and 0.3% hereditary hemochomatosis and even after repeating liver enzyme testing, 84% of the results remained abnormal after one month and 75% after two years (Robert et al., 2017).

A study done in 1987 to investigate the relationship between ALT levels and the prevalence of serologic markers of viral hepatitis concluded that patients with viral Hepatitis had the highest elevations in ALT serum levels. Serologic evidence of acute or chronic HBV infection was detected in 6.1% of specimens with elevated ALT levels compared with 1.3% with normal ALT levels (Donald et al., 1987).

A similar study conducted on the relationship between serum ALT levels and liver histology in chronic HCV infected patients concluded that patients with elevated ALT levels had higher histological activity than those with normal ALT levels. Among patients with chronic HCV infection, liver lesions were milder in those with normal serum ALT than those with abnormal ALT levels. The researchers also noted that some patients with normal ALT levels may have advanced liver disease (Mohammadi et al., 2005).

An article published by the American Association for the study of liver diseases (AASLD) journal on serum activity of ALT as an indicator of health and disease indicates that ALT is a sensitive test to detect individuals with liver disease, due to proved association between ALT levels and prediction of mortality. The researchers also highlighted that abnormal ALT levels must trigger an appropriate clinical evaluation (Kim et al., 2008).

A study done in 2009 on the correlations of serum HCV RNA and ALT with liver histopathological changes in patients with chronic HCV concluded that serum HCV RNA titter had no correlation with the ALT level and it could not reflect the degree of liver

histological damage. Researchers also found out that the ALT level had no correlations with the stages of liver fibrosis, but it did reflect the grades of liver necroinflammatory activity to some extent (Liu et al., 2009)

A study done in Italy on the prevalence of significant liver disease in people with asymptomatic HCV infection showed that ALT levels normal in 46% of the viremic patients and elevated in 54% of the cases. Abnormalities were detected in 19% of the people who had normal ALT levels and in 61% of the viremic persons who had elevated ALT levels. Researchers also concluded that the severity of liver disease correlates with abnormal ALT levels and increases with age (Alberti et al., 2002).

A similar study done in 2003, to determine the validity and clinical utility of the AST/ALT ratio in assessing liver disease severity indicated that there was significant correlation between abnormal serum levels of AST and ALT, and severity of liver disease. The AST and ALT ratio had a sensitivity of 81.3% and specificity of 55.3%. The researchers concluded that the AST/ALT ratio is correlated with both histological stage and clinical evaluation of liver disease and that progressive liver impairment is marked by an increase in the AST/ALT ratio (Risso et al., 2003).

A study done in 2001 in order to assess the clinical utility of serum ALT and AST in chronic HCV infection, showed that both ALT and AST are useful markers of chronic hepatic disease , however it is important to note that the AST may elevate alone, suggesting that measuring AST may be useful when the ALT is consistently normal (Anderson et al., 2000).

Mzingwane M, et al. (2014) in a cross sectional study conducted on the seroprevalence of HBV and its serology patterns in Zimbabwe indicate a total HBsAg prevalence of 17.1% (males 19% and females 15.8%). The researchers also tested the participants for other HBV markers such as HBsAb, HBcAb, HBeAg and Anti-HBeAg, with participants being grouped

as having a resolved infection, active HBV carriers and inactive carriers with a resolving infection.

CHAPTER 3 RESEARCH METHODOLOGY

3.1 Introduction

This chapter makes a detailed description of the methodology to be used in order to conduct the study, including the research design, data collection instruments and ethical considerations.

3.2 Research Design

The researcher used a retrospective cross sectional design. A retrospective cross sectional study design measures parameters at a single point of time through analysis of past records or secondary data on the desired variables. The researcher reviewed laboratory records originally collected from January 2016 to December 2019.

3.3 Study setting

This study was carried out at Lancet Clinical Laboratories in Harare due to the organisation's testing capacity of HBV markers and biochemical parameters of interest to the study.

3.4 Data collection procedure

The researcher conducted a retrospective data analysis, through collection of available laboratory records on patients from the organisation's data base. Individual records on positive HBV markers and serum aminotransferases were grouped for presentation and analysis.

3.5 Study population

In this study, the population was comprised of samples processed at Lancet Clinical Laboratories in the period starting from 2016 to 2019 that had tested positive for HBV markers (HBsAg, HBsAb, and HBcAb). Patients with documented confounders to the study were excluded.

3.6 Study Sample

The researcher used a total sample size of 214 laboratory records with documented positive HBV markers and serum aminotransferases.

To come up with the sample size, the researcher used a formula for sample size calculation in Cross sectional medical studies by Pourhoseingholi MA, et al, 2015.

$$n = \frac{z^2 P(1\text{-}p)}{d2}$$

Where:

n is the sample size

Z is the statistic corresponding to level of confidence

P the expected prevalence (obtained from previous studies or pilot study conducted by the researcher) and d is the precision.

Pourhoseingholi MA, et al, also states that the level of confidence usually aimed for is 95% and that if the prevalence is between 10% and 90% a precision of 5% should be used.

For this research, Z for a 95% CI= (1.96)95%, P=(0.17)17%, According to Mzingwane M et al, in a study the total prevalence of HBV in Zimbabwean adults is 17% (Mzingwane M et al, 2014) and d= 0.05(3%).

$$n = \frac{(1.96)2*0.17(1\text{-}0.17)}{(0.05)2}$$

$$n = \frac{3.92 * 0.17 * 0.83}{0.0025}$$

$$n = \frac{0.5531}{0.0025}$$

$n=221$

3.7 Sampling procedure

The researcher used a non-probability convenience sampling technique to select the desired sample. Records with documented positive HBV markers with requested aminotransferases were selected until the sample size was reached.

3.8 Data collection instruments

The formula below was used as a data collection instrument to calculate the prevalence of hypertransaminasemia in HBV positive patients.

Prevalence is the number of HBV positive participants with elevated aminotransferases (AST/ALT) amongst all HBV positive participants with aminotransferases.

$$\%\text{Prevalence of}\uparrow\text{AST/ALT}=\frac{\text{Total number of HBV positive participants with elevated AST/ALT}}{\text{Total number of HBV positive participants with AST or ALT done (normal+elevated)}}$$

x100

3.9 Ethical considerations

The study was conducted after receiving approval from the Africa University Research Ethics Committee (AUREC and securing approval from Lancet Clinical Laboratories management to carry out the study.

The researcher did not deal with individual patients, but with records, and these were coded according to a sample number in order to ensure patient privacy and confidentiality. Due to the nature of the study (Retrospective data analysis), the researcher did not use any consent forms prior to data collection.

CHAPTER 4 DATA PRESENTATION AND ANALYSIS

4.1 Introduction

In this chapter, the researcher presents the study findings in respect to research objectives, making a detailed description of the results obtained from the study population.

4.2 Baseline characteristics of the study participants

Table 1: Characteristics of study participants

Characteristic	**N = 214**
Age (Median, IQR) yrs	(41, 20-79)
Gender	
Females	148 (69, 1)
Males	63 (29, 4)
Not stated	3 (1,4)
HBsAg Concentration	
<1000 IU/L	22(10.3)
>1000 IU/L	192(89.7)

NB. Data is given as n(%) unless defined

A total of 214 patient results were analysed using Microsoft Excel, of which the majority were from males (69.1%). There were 3 records in which the sex was not indicated by the clinician. The sampled data comprised of patient records from 2016 to 2019 whose samples had been processed at Lancet clinical laboratories. The median age was 41 years. A total of 22 study participants had HBsAg levels below 1000 IU/l as compared to 192 participants with HBsAg levels above a 1000 IU/l, which may point out that a true infection was present, for HBsAg levels below 1000 IU/L may not necessarily be an indication of an infection with HBV. It is also an indicator of the severity of viral HBsAg concentration in the patient's serum and possibly higher infectivity. The researcher used a 1000 IU/L as a cut off point for the HBsAg value following how the results are reported by the Architect i1000 analyser which does the quantitative HBsAg testing at the site where the data was collected.

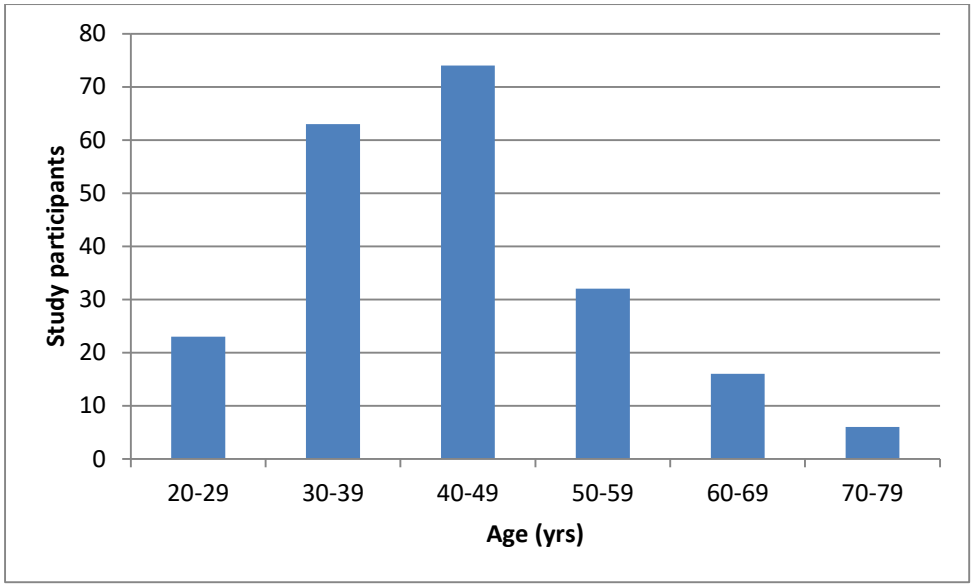

Figure 1: Age distribution for the Study participants

The age distribution for the study participants is shown in Fig 1. The results show that most study participants where aged between 40-49 years, followed by those aged between 30-39 years. A lower number of participants is seen in the 70-79 age group, as well as those aged between 60-69 years.

4.3 Aminotransferase results of the study participants

In the present study the researcher analysed two aminotransferases, the AST and ALT. The AST reference rage used in this study for classification is 15-40 IU/L and for ALT 10-40 IU/l. The standard classification of aminotransferase elevation is shown in Table 2.The classification is based on the extent of elevation observed, allowing an estimation of the severity of the hypertransaminasemia.

Table 2: Standard hypertransaminasemia classification guide

Hypertransaminasemia Classification	**Reference Value**
Borderline elevation	1-2 x ULN
Mild elevation	2-5 x ULN
Moderate elevation	5-15 x ULN
Severe elevation	>15 x ULN
Massive elevation	>10,000 IU/L

The ULN (Upper Limit of Normal) represented in the table above is equivalent to 40 IU/L which is the upper value in the reference range. It is the element used to find the type of elevation present.

The unit used for quantifying the aminotransferase value is IU/L (International units per litter). Massive elevation is defined as an AST/ALT level that is above 10,000 IU/L.

Table 3: AST results of the study participants

[AST] elevation	**N = 214**	
	[HBsAg] <1000 IU/L	**[HBsAg] >1000 IU/L**
Normal	16 (7,4)	106 (49, 5%)
Borderline	4(1, 8)	26 (12, 1)
Mild	1 (0, 4)	21 (9, 8)
Moderate	0	25 (11, 6)
Severe	1 (0, 4)	14 (6, 5)
Massive	0	0

NB. Data is given as n(%)

Represented above is the criteria used in classifying transaminase elevation in respect to AST levels observed in study participants. Study participants with a normal AST level are defined as those with a serum AST within the normal reference range (15-40 IU/L). A total of 16 (7,4%) participants with [HBsAg] <1000 IU/L had normal AST levels, followed by 4 (1,8%) participants with borderline elevation. Mild elevation was observed in 1 (0,4%) participant, as well as severe elevation which had 1 study participant, both with an [HBsAg] <1000 IU/L.

A total of 106 participants with [HBsAg] >1000 IU/L had normal AST levels, followed by 26 (12,1%) participants with borderline elevation. Mild elevation was seen in 21 (9,8%) study participants and moderate elevation in 25 (11,6%) study participants. The last 14 (6,5%) participants showed severe elevation and massive elevation was not present in any of the participants.

Table 4: ALT results of the Study participants

[ALT] elevation	**N = 214**	
	[HBsAg] <1000 IU/L	**[HBsAg] >1000 IU/L**
Normal	12 (5,6)	84 (39,2)
Borderline	5(2,3)	50 (23,3)
Mild	3 (1,4)	26 (12,1)
Moderate	0	14 (6,5)
Severe	3 (1,4)	17 (7,9)
Massive	0	0

NB. Data is given as n(%)

Represented above is the criteria used in classifying transaminase elevation in respect to ALT levels in study participants. A total of 12 (5,6%) study participants with [HBsAg] <1000 IU/L had normal ALT levels, followed by 5 (2,35) study participants with borderline

elevation and 3 (1,4%) participants with mild elevation respectively. No participants showed moderate elevation, in contrast to 3 (1,4%) participants with severe elevation.

A total of 84 (39,2%) study participants with [HBsAg] >1000 IU/L had normal ALT levels, followed by 50 (23,3%) participants with borderline elevation and 26 (12,1%) participants with mild elevation respectively. Moderate elevation was seen in 14 (6,5%) study participants and severe elevation in 17 (7,9%) study participants.

4.4 Prevalence of Hypertransaminasemia in Study Participants

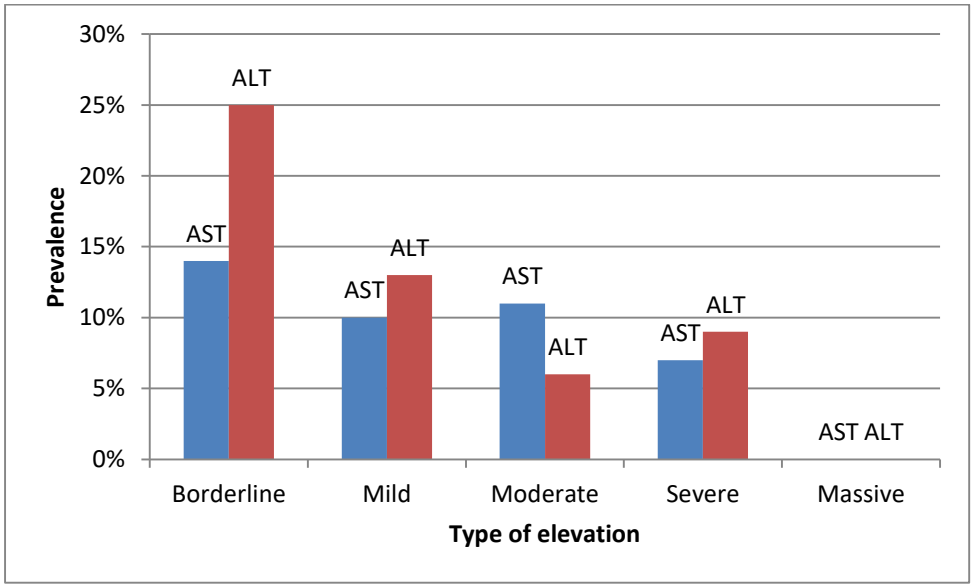

Figure 2: Prevalence of hypertransaminasemia in study participants

Depicted in Fig 2 is the prevalence of hypertransaminasemia by each classified form. Fig 2 also depicts a comparative analysis of both AST and ALT elevation by each specific type. A higher proportion of participants with normal AST level is seen compared to ALT. The same can be observed with borderline elevation, where ALT elevation is more prevalent as compared to AST and mild elevation where ALT elevation is more prevalent as compared to AST mild elevation. AST moderate elevation is also seen to be more prevalent as compared ALT elevation, and ALT severe elevation is more prevalent compared to AST elevation.

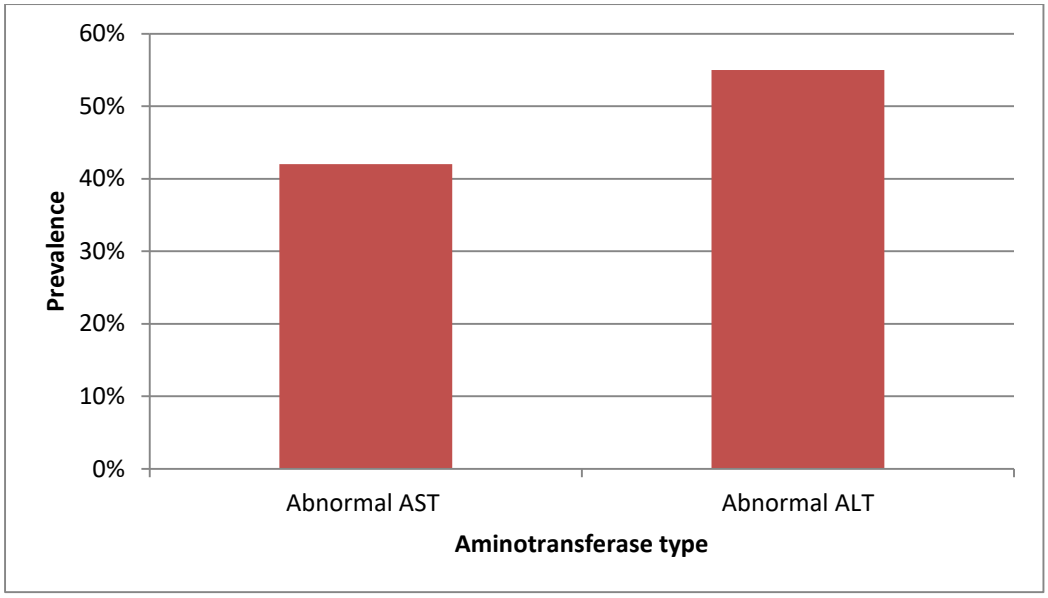

Figure 3: Cumulative prevalence of AST and ALT elevation

The bar graph shows the cumulative prevalence for both AST and ALT with ALT having the highest prevalence in aminotransferase elevation (55%) as compared to the prevalence of AST elevation (42%).

CHAPTER 5 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

An appropriate laboratory diagnosis of HBV infection is mostly dependent upon HBV markers, of which HBsAg is the most frequently used, due to its cost effectiveness and availability even in a resource limited environment. Bonino F, et al. outlines the specific roles of each of the HBV serological markers used in clinical practice as listed in the table below (Bonino et al. 2010)

Table 5: Diagnostic importance of HBV serological markers

HBV Serological Marker	Diagnostic Category
HBsAb	Immunity
HBcAb	Exposure
HBsAg	Infection
HBeAg	Replication
IgM HBsAb	Disease

All of the samples analysed in the present study had a positive HBsAg test, of which 89.7% of the study participants had an HBsAg value greater than 1000 IU/L and only 10.3% had an HBsAg value of less than 1000 IU/L. The HBsAg is a serum marker for the external envelope of the virion, released from infected hepatocytes in high quantities in an infected person. Persons with a positive HBsAg have overt HBV infection but may not have an active infection, because most inactive carriers have a normal liver (Bonino et al. 2010).

After infection, HBsAg levels increase in the blood within the next few weeks following exposure, this is called the acute phase of the infection which may result in development of HBsAb and clearance of HBsAg (Recovery and immunity) or development of a Chronic infection (Persistent HBsAg for more than 6 months, with or without HBsAb), so HBsAg is a good indicator of infection, be it acute or chronic. (CDC, 2020).

All samples analysed where also positive for total HBcAb. A total HBcAb does not screen for either IgM HBcAb or IgG HBcAb but gives the total, which is a combination of the two. HBcAb appears at onset of symptoms in acute HBV infection and persists for life. It is an indication of an ongoing infection (IgM type) or a previous infection (IgG type) (CDC, 2020).

The HBcAb helps determine whether the infection is acute or chronic in conjunction with a positive HBsAg, but in this study only the Total HBcAb was being assayed, establishing only the existence of infection at some point in time, but considering the HBsAg which is positive there is a higher probability of it having been a chronic infection in most if not all cases

The study findings also indicated that more than half of the study participants had a negative HBsAb test. This indicates the presence of infection with no identifiable immunity or seroconversion occurring. The Presence of HBsAb indicates recovery and immunity from HBsAg either from infection or vaccination and the absence of HBsAb in a patient with a positive HBsAg may result from a recent infection or inability from the host to clear the HBsAg, the late is observed in Chronic infection. (CDC, 2020).

A few participants had been screened for HBeAg and HBeAb, and for these, most patients presented with a negative HBeAg and Positive HBeAb. The Presence of HBeAg indicates ongoing viral replication and high levels of HBV in the patient serum. HBeAg is found in the serum of both Acute or chronic individuals who have failed to develop HBeAb and is associated with poor disease prognosis (CDC, 2020).

The absence of HBeAg and Presence of HBeAb in most patients indicates that most of them were less infectious and had a lower HBV viral replication rate and had no signs or symptoms of the infection, due to inactive infection. When HBV markers indicate a possible infection, serum aminotransferases are used to assess liver damage or impairment. Serum aminotransferases (AST and ALT) are useful measures of hepatocyte injury, although AST is

less specific than ALT. Diseases that affect hepatocytes, such as in HBV infection, show an elevation of the AST and ALT levels (Murali et al. 2017).

Findings of the present study regarding AST results in study participants with [HBsAg] <1000 IU/L showed that approximately 7.4% of the subjects had normal AST levels and only 2.6% had elevated AST levels of which 1.8% borderline elevation, 0.4% mild elevation and the remaining 0.4 severe elevation. On the other hand, 49.5% of the participants with a HBsAg value >1000 IU/L had normal AST levels and 40% had elevated AST levels, of which 12.1% borderline elevation, 9.8% mild elevation, 11.6% moderate elevation and 6.5% severe elevation.

Ahadi M et al (2017) showed that serum HBV DNA levels did not correlate with AST levels, which can be applicable to the HBsAg value, which tends to be higher in patients with a high HBV DNA viral load as indicated by Yakovlev A et al. Based on that it is safe to assume that a high or normal AST value does not always correlate with the HBsAg value as seen with the current study which had more than half of the participants with HBsAg value >1000 IU/L having a normal AST level.

Another possible explanation of the AST value could be drawn from Shao J et al who indicates that in HBeAg patients there is no correlation between serum levels of HBV DNA (hence HBsAg) and AST levels. As indicated above, the findings of the current study show that most study participants had a negative HBeAg value. It is also important to note that AST is less specific in Viral Hepatitis, since it is elevated in non hepatic conditions such as AMI, or general muscle injury (Carey et al. 2014).

Regarding the ALT results, the current study showed that 5.6% of the study participants with [HBsAg] <1000 IU/L had normal ALT levels in comparison to 39.2% of participants with HBsAg value >1000 IU/L that had normal ALT levels. Among those with [HBsAg] <1000

IU/L another 5.1% had elevated ALT levels of which 2.3% borderline elevation, 1.4% mild elevation and another 1.4% severe elevation. It is important to note that only 10.7% of the total study participants had [HBsAg] <1000 IU/L in comparison to 89.2% of participants that had an HBsAg value >1000 IU/L.

Shao J et al (2007) indicates the existence of correlation between serum ALT levels and HBV DNA viral load mostly in HBeAg negative individuals. This would then indicate that more than half (56%) of the total of those with HBsAg value >1000 IU/L in this study had elevated levels of ALT in comparison to 43.9% participants with normal ALT levels and of these, the majority (26.1%) had borderline elevation.

A normal AST and ALT value in patients with a positive HBsAg value can be explained by a lower level of infection characterised by reduced viral replication, less or no hepatic inflammation and reduced AST and ALT levels. This mostly occurs in inactive chronic HBV infection often with detectable HBeAb and negative HBeAg (Carey et al. 2014).

Based on findings from the present study, it was established that the prevalence of AST elevation in study participants was less marked as compared to the ALT elevation. All types of elevation, except for moderate elevation show a higher prevalence of ALT elevation in relation to AST. A higher proportion of patients with a positive HBsAg value and yet normal aminotransferase level is seen with the AST value which could support findings by Carey W et al that point out that AST is less specific in viral hepatitis. The prevalence of hypertransaminasemia seemed to drop as the type of elevation got to the top, with borderline elevation showing the highest prevalence (14% for AST and 25% for ALT) and massive elevation with a prevalence of 0% for both AST and ALT. The cumulative prevalence of hypertransaminasemia in the present study also shows a higher prevalence of ALT elevation (55%) in comparison to AST elevation (42%).

Available information from previous studies indicates that aminotransferase elevations exceeding 10 to 20 times the reference rage are uncommon in the absence of hepatic cell injury, considering that the concentration of ALT is less than AST in all cells except hepatic cytosol, ALT elevations are less common in nonhepatic disorders such as in Myocardial infarction or skeletal muscle diseases. (Vroon et al, 1990).

Wedemeyer et al (2010) also indicates that ALT levels are increased in majority, but not all patients with acute or chronic liver disease and that elevated ALT levels are also a risk factor for liver specific mortality. This statement strengthens the point that not all patients with an acute or chronic viral HBV infection has abnormal aminotransferase levels, thus not all study participants had a significantly raised AST/ALT value.

5.2 Implications of findings to public health

The results from the study revealed that there is a high prevalence of hypertransaminasemia among patients with positive HBV markers. Study findings have also indicated that high serum AST and ALT levels may be indication of early hepatic disease or the terminal stage of the disease when there is inflammation and hepatocyte cell death. It is also important to note that a certain proportion of the study population that had positive HBV markers had normal serum AST and ALT levels, indicating a possible inactive chronic infection with no visible signs of infection and yet the patient may be infectious and spreading the infection,

Based on these findings, there is need to put more effort in screening for HBV infection in the general population due to the risk it poses, specially through the asymptomatic and inactive carriers that could still be spreading the infection.

5.3 Limitations to the study

The study was carried out by analysing electronic records from an organisation's data base. Information on confounders could not always be traced or retrieved, thus this may have resulted in some Bias to the study.

The Researcher was limited to working with the total HBcAb and not specific parameters such as the IgM type HBcAb and IgG type HBcAb in order to trace the infection if it is Chronic or acute.

5.4 Study conclusion

The study revealed a high prevalence of both AST and ALT enzyme levels among patients with positive HBV markers. ALT had the highest prevalence (55%) as compared to AST (42%).

5.5 Recommendations

From the findings of this study, the Researcher recommends that a prospective study be conducted on serum aminotransferases in relation to HBV infection in the Zimbabwean population, focusing clearly on all HBV markers including the IgM and IgG HBcAb and the role of HBeAg and HBeAb in Chronic infection.

5.6 Suggestions for further study

The researcher suggests that a prospective study be conducted on the usefulness of serum aminotransferases in patients chronically infected with HBV.

5.7 Dissemination of results

The dissemination of the study findings involves raising the awareness of clinicians in local hospital settings on the usefulness of aminotransferases in the initial assessment of HBV infection, especially when used in combination with other HBV infection markers and the

need for care and expertise when interpreting apparently normal aminotransferase levels in patients chronically infected. The researcher will share the study findings with the organisation where the study was conducted.

REFERENCES

Walker, H., Hall, W., & Hurst, J. (1990). The History, Physical, and Laboratory Examinations. Boston : Butterworth publishers

Giannini, E., Risso, D., Chiarbonello, B., Fasoli, A., Malfatti, F., Romagnoli,P., Testa, E., Ceppa, P., Testa, R. (2003). Validity and Clinical Utility of the Aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. Journal of internal medicine, 163(2), 218-224. doi: 10.1001/archinte.163.2.218

Alberti, A., Noventa, F., Benvegnu, L., Bocatto, S., & Gatta, A. (2002) Prevalence of liver disease in a population of asymptomatic persons with hepatitis c virus infection. Journal of internal medicine, 137(12), 961-964. doi:10.7326/0003-4819-137-12-200212170-00009

Tswana, S., Chetsanga, C., Nystrom,L., Moyo,S., Nzara,M., &Chieza, L. (1996). A sero-epidemiological cross-sectional study of hepatitis B virus in Zimbabwe. South African medical journal, 86(1), 72-75

Kim,W., Steven, F., Bisceglie, A., & Bodenheimer, H. (2008). Serum activity of Alanine aminotransferase (ALT) as an indicator of health and disease. Journal of hepatology, 47(4), 1363-1370. doi:10.1002/hep.22109

Lau, G. (2017). Liver disease in Chinese perspective from a practicing hepatologist, 27(1), 12-28

Wang, X., Lin, S., Tao, J., Wei, X., Liu, Y., Chen, Y., & Wu, B. (2014). Study of liver cirrhosis oven ten years in southern China, World journal of Gastroenterology, 20(37), 13546-13555. doi:10.3748/wjg.v20.i37.13546

Rej, R. (1990). Aminotransferases in disease. Clinics in laboratory medicine, 9(4), 667-687.

Clark, J., Brancati, F., & Diehl, A. (2003). The prevalence and etiology of elevated aminotransferase levels in the United States. The American journal of gastroenterology, 98 (5), 960-967. doi: 10.1111/j.1572-0241.2003.07486.x

Chisari, F., Isogawa, M., & Wieland, S. (2010). Pathogenesis of Hepatitis B virus infection. Pathol Biol, 58(4), 258-266. doi: 10.1016/j.patbio.2009.11.001

Esmaeelzadeh, A., Saadatnia, H., Memar, B., Mokhtari, E., Ganji, A., Vosoughinia, H., & Ahadi, M. (2017). Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative hepatitis B patients. Gastroenterology Hepatology journal, 10(1): 39-43. doi: 28331563

Belopolskaya, M., Avrutin, V., Yakovlev, A. (2015). HBsAg level and hepatitis B viral load correlation with focus on pregnancy. Annals of gastroenterology, 28(3); 379-384.

Shao, J., Wei, L., Wang, H., Sun, Y., Zhang, L., Li, J., & Dong, J., (2007). Relationship between hepatitis B virus DNA levels and liver histology in patients with chronic hepatitis B.

World journal of gastroenterology, 13(14), 2104–2107. doi:10.3748/wjg.v13.i14.2104

Jamali, R., Khonsari, M., Merat S., Koshnia, M., Jafari, E., Bahram, A., Abolghasemi, H., Amini, S., & Pourshms, A. (2008). Persistent alanine aminotransferase elevation among the

general Iranian population: Prevalence and causes. World journal of gastroenterology. 14(18), 2867-2871. doi:10.3748/wjg.14.2867

Mzingwane, M., & Tafadzwa, M. (2014). Hepatitis B Virus Seroprevalence and Serology Patterns in a Cohort of HIV Positive Individuals from Harare, Zimbabwe. The journal of viruses. doi: 10.1155/2014/691953

Ioannou, G., Boyko, E., & Lee, S. (2006). The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. The American journal of gastroenterology, 101(1), 76-82. doi: 10.1111/j.1572-0241.2005.00341.x

Ruhl, C., & Everhart, J. (2003). Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology, 124 (1), 71-79. doi: 10.1053/gast.2003.50004

Anderson, F., Zeng, L., Rock, N., &Yoshida, E. (2000), An assessment of the clinical utility of serum AST and ALT in chronic hepatitis C. Hepatology research, 18(1), 63-71. doi: 10.1016/s1386-6346(99)00085-6

Sarin, S., & Maiwall, R.. (2020). Global burden of liver disease: A true burden on health sciences and economies. Retrieved from the World gastroenterology website https://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-point-of-view-articles-collection/global-burden-of-liver-disease-a-true-burden-on-health-sciences-and-economies

Everhart, J., Ruhl, C. (2009) Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. Journal of gastroenterology,136(4),1134–1144. doi: 10.1053/j.gastro.2009.02.038

Lu, X. (2011). Pathogenesis of Hepatitis B virus mediated liver injury. doi:10.7156/v4i1p001

Murray, C., & Lopez. A. (1997). Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet; 349,1498–1504. doi: 10.1016/S0140-6736(96)07492-2

Asrani, S., Devarbhavi, H., Eaton, J., Kamath, P. (2018). Burden of liver diseases in the world. Journal of Hepatology, 70(1),151-171. doi:10.1016/j.jhep.2018.09.014

Spearman, W., Afihene, M., Ally, R., Apica, B., Awuku, Y., Cunha, L., Dusheiko, G., Gogela, N., Kassianides, C., …Kew, M. (2017). Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. The Lancet, 2(12), 900-909. doi: 10.1016/S2468-1253(17)30295-9

Kramvis, A., & Knew, M. (2007). Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. Hepatology research,37(s1),S9-S19. doi:10.1111/j.1872-034X.2007.00098x

Chen, C., Huang, M., Yang, J., Nien, C., Yang, C., Yeh, Y., & Yueh, S. (2007). Prevalence and etiology of elevated serum alanine aminotransferase level in an adult population in Taiwan. Journal of gastroenterology and hepatology,22(9),1482–1489. doi:10.1111/j.1440-1746.2006.04615.x

Wedemeyer, H., Hofmann, W., Lueth, S., Malinski, P., Thimme, R., Tacke, F., & Wiegand, J. (2010). ALT screening for chronic liver diseases: scrutinizing the evidence. Journay of gastroenterology, 48(1), 46–55. doi:10.1055/s-0028-1109980

Mohammadi, T., Daryani, N., Bashashati, M., Hashtrudi, A., Haghpanah, B., &Shakiba, M. (2005). Relationship between serum alanine aminotransferase levels and liver histology in chronic hepatitis C-infected patients. Indian Journal of Gastroenterology, 24(2):49-51

Robert, C., Hustead, T., Ali, S., & Pantsari, M. (2017). Mildly elevated liver transaminase levels: Causes and Evaluation. American Family Physician, 96(11),709-715.

Jensen, D., Dickerson, D., Linderman, M., & Kessler, H. (1987). Serum Alanine aminotransferase levels and prevalence of hepatitis A,B and Delta in outpatients. Journal of internal medicine, 147(10),1734-1737.

Centres for Disease Control and prevention. (2020) . Viral Hepatitis: Overview and statistics. Retrieved from https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm

WHO regional office for Europe. (2019). Data on HBV statistics. Retrieved from the WHO website http://www.euro.who.int/en/health-topics/communicable-diseases/hepatitis/data-and-statistics

Rodriguez, E., Rogers, K., (Ed). (2017). Virion:Viral structure. Britannica encyclopedia, Retrieved March 2020 from https://www.britannica.com/science/virion

Samji, N (2017). What is the pathophysiology of hepatitis B virus infection? Retrieved March 2020 from the website https://www.medscape.com/answers/775507-38235/what-is-the-pathophysiology-of-hepatitis-b-virus-hbv-infection#qna

Merriam Webster dictionary (2020) Disease: Definition of disease. Retrieved from https://www.merriam-webster.com/dictionary/disease

AUREC Approval letter

AFRICA UNIVERSITY
(A United Methodist-Related Institution)
INVESTING IN AFRICA'S FUTURE

AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE (AUREC)

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Ref: AU1120/19

4 November, 2019

Edipo Ilton Isabel Damiao
C/O CHANS
Africa University
Box 1320
Mutare

RE: **HYPERTRANSAMINASEMIA AMONG HEPATITIS B VIRUS POSITIVE SAMPLES PROCESSED AT LANCET CLINICAL LABORATORIES IN 2019**

Thank you for the above titled proposal that you submitted to the Africa University Research Ethics Committee for review. Please be advised that AUREC has reviewed and approved your application to conduct the above research.

AFRICA UNIVERSITY
RESEARCH ETHICS COMMITTEE (AUREC)
04 NOV 2019
APPROVED
P.O. BOX 1320, MUTARE, ZIMBABWE

The approval is based on the following.

a) Research proposal
b) Questionnaires
c) Informed consent form
- **APPROVAL NUMBER** AUREC1120/19

This number should be used on all correspondences, consent forms, and appropriate documents.

- **AUREC MEETING DATE** NA
- **APPROVAL DATE** November 4, 2019
- **EXPIRATION DATE** November 4, 2020
- **TYPE OF MEETING** Expedited

After the expiration date this research may only continue upon renewal. For purposes of renewal, a progress report on a standard AUREC form should be submitted a month before expiration date.

- **SERIOUS ADVERSE EVENTS** All serious problems having to do with subject safety must be reported to AUREC within 3 working days on standard AUREC form.
- **MODIFICATIONS** Prior AUREC approval is required before implementing any changes in the proposal (including changes in the consent documents)
- **TERMINATION OF STUDY** Upon termination of the study a report has to be submitted to AUREC.

Yours Faithfully

MARY CHINZOU – A/AUREC ADMINISTRATOR
FOR CHAIRPERSON, AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE

Lancet Clinical Laboratories Approval letter

Lancet Clinical Laboratories

22 five Avenue cnr Blakiston Street

Harare

Zimbabwe

08 October 2019

Africa University

P.O BOX 1320

Mutare

Zimbabwe

Dear Édipo Damião

RE: CLEARANCE TO CARRY OUT ACADEMIC RESEARCH

I refer to your request for the above subject matter and hereby clear you to carry out your academic research on HYPERTRANSAMINASEMIA AMONG HEPATITIS B VIRUS POSITIVE SAMPLS PROCESSED AT LANCET CLINICAL LABORATORIES IN 2019.

Please note that the ethical principles that are relevant when conducting any research should be observed.

Yours sincerely

Shingai Chikutye

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Laboratory manager