CHANGES IN SERUM LIPIDS AND OTHER BIOCHEMICAL INDICES ASSOCIATED WITH LIVER DAMAGE IN CHRONIC HEPATITIS B INFECTION

By

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil. degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Chronic hepatitis B infection affects nearly 400 million people who are at a risk of death by liver-related complications. The liver plays a vital role in lipid metabolism, hence its consequent degeneration following chronic hepatitis B infection could potentially provoke dyslipidemia. This study therefore, sought to evaluate the effect of the chronic hepatitis B infection on host serum lipid profile. A cross-sectional study was conducted on chronic hepatitis B patients, attending a specialist care at the Oncology Unit at the KATH, during a 9-month period. On the basis of serological and liver enzyme assays, participants were categorized as chronic symptomatic (HBcAb +ve, HBsAg +ve, HBV-DNA $\geq 10^3$ copies/ml and serum AST and ALT $\geq$ 40 IU/l) or chronic asymptomatic (HBcAb +ve, HBsAg +ve, HBV-DNA $\leq 10^3$ copies/ml, serum AST and ALT $\leq$ 40 IU/l) and a control group of apparently healthy individuals (HBcAb -ve, HBsAg -ve, serum AST and ALT $\leq$ 40 IU/l). The relationship between the patients' HBeAg status and pathological stage of infection was evaluated using some indices of lipid metabolism – VLDL, LDL, HDL, triglyceride, and total serum cholesterol, using Chi-square (X^2) analysis. Fifty seven (57) participants fulfilled the inclusion criteria consisting of 41 males (62.9%) and 16 females (37.1%) (p=1.000). Ten (10) of the patients (17.5%) showed active chronic HBV infection (HBeAg +ve) while 47 patients (82.5%) were inactive chronic carriers of the infection (HBeAg-ve) (p=0.1718). Serum triglyceride levels were significantly lower among the chronic HB-infected population, compared to the healthy control (p=0.0051). Serum cholesterol, LDL, HDL, total cholesterol/HDL and HDL/LDL ratios were unaffected by the disease. This work has shown that serum triglyceride is lowered in chronic hepatitis B infection.

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LIST OF ABBREVIATIONS

AST – Aspartate aminotransferase

ALT – alanine aminotransferases

CHB – chronic hepatitis B

HBcAb – Hepatitis B core antibody

HBcAg - Hepatitis B core antigen

HBeAg – Hepatitis B envelope antigen

HBsAg - Hepatitis B surface antigen

HBsAb - Hepatitis B surface antibody

HBI – hepatitis B infection

HBV – hepatitis B virus

HDL – high density lipoprotein

IDL – intermediate density lipoprotein

LDL – low density lipoprotein

TG – triacylglyceride

VLDL – very low density lipoprotein

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DEDICATION

This work is dedicated to my parents, Mr. and Mrs. Kwarteng and my siblings who have contributed in kind to ensure the completion of this work.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Hepatitis B infection (HBI) is a serious global health problem, with 2 billion people infected worldwide, and 350 million suffering from chronic HBI of which nearly 500,000 – 1.2million die annually due to chronic hepatitis, cirrhosis, and hepatocellular carcinoma, and other liver-related complications (WHO, 2008). Mortality due to liver diseases ranks fifth worldwide, even though it ranks higher in developing countries like Ghana, where viral hepatitis infection (hepatitis B and or C) is very endemic with ≈15% prevalence (Sarkodie *et al.*, 2001).

Hepatitis B infection could potentially affect the functional integrity of the liver directly or indirectly in infected hosts. The liver, as a homeostatic organ, plays a pivotal role in modulating intracellular and extracellular cycles of lipid metabolism. It is involved in the sequestration, remodelling (synthesis or recycling), and redistribution of lipid metabolites, including lipoproteins such as very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and their corresponding apolipoproteins - ApoB, and Apo A1, etc., triglycerides (TG), and total plasma cholesterol. Thus, the extracellular circulating levels of these lipids in plasma of hepatitis B-infected patients depend significantly on the functionality of the host liver.

The nature and extent of association of chronic hepatitis B disease and serum transaminases and lipid dysregulation has become the subject of interest for most biomedical researchers over the past decade. Dyslipidemia in liver diseases, as reported by most researchers, has been largely attributed to the decrease in the biosynthetic and bioprocessing capacity of the

liver in the diseased state, as with chronic hepatitis B infection. Reports on the profile of lipids in cases of liver diseases have been very diverse, showing slight to marked variations in plasma lipoprotein and apolipoprotein patterns. In one study of the dyslipidemia in chronic hepatitis, liver cirrhosis and hepato-cellular carcinoma (HCC), the TG and cholesterol levels decreased while LDL – cholesterol fraction increased with HDL-fraction remaining fairly unchanged (Altiparmak, *et al.,* 2005). Altogether, the increased LDL-Cholesterol and the normal plasma HDL-Cholesterol may pose increased cardiovascular risk to patients with liver disease.

Pathophysiologically, there are two main stages of chronic hepatitis B infection – chronic symptomatic and chronic asymptomatic stages with their attendant pathological manifestations. However, most studies of chronic hepatitis B infection have not investigated the relationship of these stages on serum transaminases and lipid profile of infected hosts.

1.2 PROBLEM STATEMENT

Dyslipidemia and other biochemical changes are common findings in liver-related diseases as with chronic hepatitis B-infection. Particularly, dyslipidemia poses a threat to the patients' prognosis. For instance, perturbed lipid profile has been implicated in various dreadful clinical and physiological states, such as coronary artery diseases, depression, non-insulin dependent diabetes mellitus, *et cetera*. (Shepherd *et al.,* 2000). Worldwide, dyslipidemia has been well documented not only in liver-related illness but also in other non-liver related illnesses such as renal failure (Amin *et al.,* 2006), prostate and breast cancers (Shalini *et al.,* 2011), HIV-1 and HIV-2 infections (Aberg, 2009), etc. much as hepatitis B infections has

been studied, there is still paucity of data relating the HBI and dyslipidemia among the chronic hepatitis B – infected patients in Ghana.

1.3 MAIN OBJECTIVE:

This study was therefore, aimed at finding the probable link between the pathophysiological stages of the hepatitis B infection and changes in some serum biochemical indices among chronic HBV-infected patients in Kumasi.

1.4 SPECIFIC OBJECTIVES

To achieve the above-stated objective, this work set out to;

1. Categorize some chronic HBV – infected patients for the study into either of the two general stages – chronic symptomatic or chronic asymptomatic – using a combination of serum HBV- related serology and serum ALT levels.
2. To find out the relative distribution of plasma lipids and aminotransferases among chronic HBV – infected patients at the different stages of the disease.
3. Use the pattern of the biochemical parameters to explain the pathophysiological features of the patients under study.

1.5 JUSTIFICATION

Hepatitis B infection is one of the major diseases of humankind and is a serious global public health problem. This has necessitated the WHO to rank it among its top 10 priorities of diseases requiring urgent attention (WHO, 2008). Also, several advocacy groups have been established, both locally and worldwide to create awareness and offer subsidized diagnostic screening and vaccinations. It is at the moment one of the major infections screened for in every donated blood, prior to transfusion. Ghana, a West African country, is also endemic for

HBV infection with the hepatitis B surface antigen (HBsAg) carrier rate approximately 15.8% among children aged 6–18 years (Martinson *et al*., 1996). In Kumasi, Ghana, the prevalence of the hepatitis B (HBsAg) was found to be approximately 15% among young volunteer blood donors at the Komfo Anokye Teaching Hospital (Sarkodie *et al.,* 2001), while the WHO reports that 8-10% of the people in the general population in the developing countries become chronically infected (WHO, 2008).

Even though evidences of dyslipidemia in chronic liver diseases are real, data regarding the relationship between the pathological stages of the chronic HBV infection and the serum lipid distribution in infected hosts is still lacking especially in this country. For this reason, the study aimed at examining the correlation between hepatitis B disease stages (chronic symptomatic and chronic asymptomatic stages) and the associated distribution of serum lipid in infected hosts. To do this would require a longitudinal study to monitor at which point the infected host switches from a mild stage to a worse stage and the peculiar lipid indices involved. However, this approach was faced with several challenges including:

a) Late report for treatment by most of the infected patients. By which time they may have switched over from one stage to the other.
b) Inconsistent attendance for OPD care even for those who do report at the hospital hence making follow-up difficult.
c) Death of chronic hepatitis B patients a few days after reporting and admission at the hospital.

Per the advice of the specialist Clinicians, in the face of these challenges, this study rather used a cross-sectional approach to evaluate the serum lipid and aminotransferase distribution.

CHAPTER TWO

LITERATURE REVIEW

2.1 GLOBAL EPIDEMIOLOGY OF HEPATITIS B INFECTION

Hepatitis B infection (HBI) is a disease caused by the hepatitis B virus (HBV) which infects the liver of *hominoidae*, including humans, and causes an inflammation (AASLD, 2007). Originally known as "serum hepatitis" (Barker, 1996), the disease has caused epidemics in parts of Asia and most tropical African countries (WHO, 2008). About a third of the world's population, *i.e.* more than 2 billion people have been infected with the hepatitis B virus *(ibid)*. This includes 350 million chronic carriers of the virus distributed throughout the world (Williams, 2006). Transmission of hepatitis B virus results mainly from exposure to infectious blood or body fluids containing the infective form of the virus.

The prevalence of HBV infection varies depending on the geographical area (*ibid*). In the Far East, the Middle East, Africa, and parts of South America, the prevalence is high, with hepatitis B surface antigens (HBsAgs) detection ranging from 8% to 15% (Andrė, 2000). Areas of intermediate prevalence (2 – 7%) include Japan, parts of South America, Eastern and Southern Europe, and parts of central Asia (WHO, 2008). Areas with low HBV endemicity (prevalence of chronic infection <2%) include Northwestern Europe, North America, and Australia as shown in the fig. 2.1 below (Lavanchy, 2005). The source of infection in these areas (North America, Northwestern Europe, and Australia) is mainly through sexual contacts and needle sharing among injection drug users, with a peak incidence in the 15 – 25-year-old age group (Martinson *et al*., 1996). Africa, as a highly endemic region for HBV infection, has an HBV carrier rate of 10.4% among Black Africans (Kew, 1996) and 15% in young volunteer blood donors in Ghana (Sarkodie *et al.*, 2001). Ghana, a West

African country, has HBsAg carrier rate approximately 15.8% in children aged 6–18 years (Martinson *et al.*, 1996). In regions of high HBsAg endemicity, serologic evidence of prior HBV infection (anti-HBc and/or anti-HBsAg) is almost universal in subjects without active infection (WHO, 2008). As a general rule, in these areas with high HBV endemicity, the source of infection is mainly through perinatal transmission from the chronically – infected mother or through infection during early childhood *(ibid).*

Fig.2.1: Geographical distribution of HBV infection worldwide (WHO, 2008).

2.2 STRUCTURE OF THE HBV PARTICLE

Hepatitis B virus (HBV) is a member of the *Hepadnavirus* family (Zuckerman, 1996). The virus particle or virion consists of an outer lipid envelope and an icosahedral nucleocapsid core, composed of protein (Locarnini, 2004). The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (*ibid*). The outer envelope contains embedded proteins or the ligand which are involved in viral binding to, and entry into, susceptible hepatocytes. The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical

bodies, lacking a core (Howard 1986). These pleomorphic forms are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen (HBsAg), and is produced in excess during the life cycle of the virus as shown in the fig. 2.2 below *(ibid):*

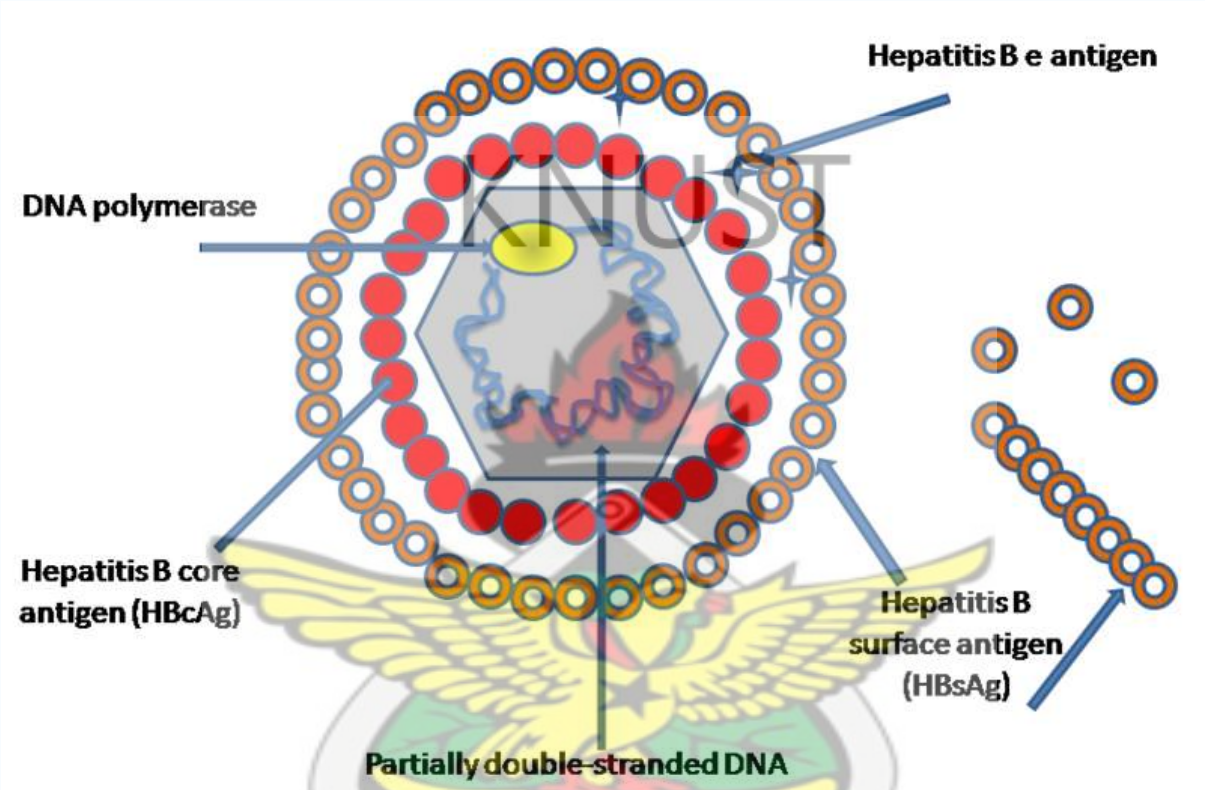


Fig. 2.2 : *The structure of the Hepatitis B virus as first described by Dane et al.(1969) and Jokelainen-* (Krohn *et al., 1970)*

2.3 TRANSMISSION

The HBI can be transmitted by the same modes as with the human immunodeficiency virus (HIV), even though the HBV is hardier and 50-100 times more infectious than the HIV (WHO, 2008). Unlike HIV, the virus can survive outside the body for at least 7 days. During that time, the virus can still cause infection if it enters into the body of a person who is not infected *(ibid)*. Transmission of hepatitis B virus results from exposure to infectious blood or body fluids. Possible modes of transmission include but are not limited to unprotected sexual

contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission from mother to child during childbirth. Without intervention, a mother who is positive for HBsAg confers a 20% risk of passing the infection to her offspring at the time of birth (WHO, 2008). This risk is as high as 90% if the mother is also positive for HBeAg. The HBV infection can be transmitted between family members within households, possibly by contact of non-intact skin or mucous membrane with secretions or saliva containing HBV (Petersen *et al.,* 1976). However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor (Shapiro, 1993). In many developed countries (e.g. those in Western Europe and North America), patterns of transmission are different from those mentioned above. Today, the majority of infections in these countries are transmitted during young adulthood by sexual activity and injecting drug use. HBV is a major infectious occupational hazard of health workers (WHO, 2008).

HBV is not spread by contaminated food or water, and cannot be spread casually in the workplace. The virus incubation period is 90 days on average, but can vary from about 30 to 180 days (AASLD, 2007). HBV may be detected 30 to 60 days after infection and persist for widely variable periods of time *(ibid).*

2.4 STAGES OF HBV INFECTION

Remarkable progress has been made in the understanding of the three (3) main natural stages of the HBV infection in hosts: acute infection, chronic asymptomatic and chronic symptomatic stages (AASLD, 2007). However, not all HBV-infected patients go through all the three stages. The risk to develop liver–related complications, such as cirrhosis and hepatocellular carcinomas increases as patient progresses from acute to chronic stage of the infection. Indeed, most HBV infections end up at the acute stage (~ 90%) with a few progressing on to the chronic stage *(ibid).*

2.4.1 Acute HBV infection

This is the initial stage of the infection and every HBV- infected patient goes through this, even though not all patients transit beyond this stage. Early phases of this stage of the infection are characterized serologically by the presence of HBsAg, high serum HBV DNA, HBeAg, and normal level of serum aminotransferase level (ALT), and minimal or insignificant inflammation on liver biopsy (Altiparmak *et al.*, 2005). A later phase, also called immunity phase, is marked by increased serum titres of anti-HBsAg IgG (HBsAb), anti-HBcAg IgG, lowered or disappearance of HBsAg and HBV DNA, normal liver histology *(ibid).* This is true for those who recover fully from the infection after attaining full and permanent immunity through exposure. The duration of either phase differs among patients but generally lasts between 5 - 8 months (AASLD, 2007). However, those patients who fail to mobilize adequate immune response factors to combat the infection end up with the fate of living with the disease their entire lifetime. In this case, it is said the disease has become chronic. The physical signs and symptoms, such as jaundice, fever, dark-urine formation, nausea, among others, would occur, even though they will last shortly after which they get resolved following recovery. Generally, transition from the acute stage to the chronic stage depends on several factors including: age, gender, viral genotype, and host immune competence *(ibid).*

2.4.2 Chronic HBV infection

This occurs as a progression of the early phase of the acute HBV infection due to the host's failure to mount the necessary immune stimulus to ensure total viral clearance and consequent resolution of the disease. It is serologically marked by relative rise in serum anti-HBcAg IgG, disappearance or lower titres of anti-HBsAg IgG, and either normal or

significant liver damage as shown by ultrasonography (WHO, 2008). Also, this stage of the disease may be characterized by normal or elevated serum aminotransferase levels (aspartate amino transferase (AST) and alanine amino transferase (ALT)) (AASL, 2007). Chronic HBV – infected patients fall into one of the two pathologically progressive forms, namely:

a) chronic asymptomatic, marked by :

- presence of HBsAg in serum
- anti-HBsAg IgG (HBsAb) positive
- normal liver histology indicated by apparently normal ALT levels
- relatively lower viral load ($<10^3$ copies/ml) (AASLD, 2007)

b) chronic symptomatic, marked by;

- presence of HBsAg in serum
- anti-HBsAg IgG (HBsAb) positive
- relatively higher viral load ($>10^3$ copies/ml),
- significant damage on liver histology, showed by elevated ALT levels (AASLD, 2007).

Patients at the chronic symptomatic stage may show mild to severe liver cirrhosis. Terminal stage of liver damage is accompanied by complications such as hepatomegaly, lower abdominal bleeding, elevated serum endogenous mercaptans, hepatic encephalopathy, hepatic coma, membranous glomerulonephritis, etc. (Lok and Mahon, 2007).

The serological presence of HBeAg is real in all stages of the disease. The presence of this antigen together with elevated viral load (HBV DNA > 10^3 copies/ml) and higher ALT (> 60 IU/l) is a strong indication of viral activity, replication, and infectivity (WHO, 2008). Patients

with such manifestations are put on retrovirals. A key event in the natural history of HBeAg – positive CHB patients is HBeAg seroconversion (Sharma *et al.,* 2005). It is believed that seroconversion of HBeAg to HBeAb is accompanied with cessation of HBV replication and remission of liver disease. Several studies have shown that seroconversion with a marked reduction in HBV replication is associated with biochemical and histological remission of inflammatory activity in the majority of patients (Sharma *et al.,* 2005 ; Elghouhari *et al.,* 2008; McMahon 2005). Some studies showed that the mean annual rate of spontaneous HBeAg seroconversion ranges from 8% - 15% in children or adults with an elevated ALT level (Sharma *et al.,* 2005; McMahon, 2005). Although the ALT level is normal in most Asian children, their spontaneous HBeAg seroconversion rate is less than 2% during the first 3 years of age and then increases to 4%-5%. In some cases, spontaneous recurrence of hepatitis is not frequently recognized because it is usually asymptomatic. Since subsequent HBeAg seroconversion would not occur in such situations of hepatitis, it can thus be viewed as an abortive attempt at seroconversion *(ibid).* However, regression of fibrosis occurs several months or years after HBeAg seroconversion (McMahon *et al.,* 1990). The recurrence of hepatitis may precede the disappearance of HBeAg and development of HBeAg antibody, culminating in the remission of hepatitis activity (Furusyo *et al.,* 2002; Chan *et al.,* 1999).

Absence of HBeAg could rather be indicative of viral dormancy/hibernation (Tsai *et al.,* 1992). Although a high serum DNA level in patients with liver disease with minimal or no inflammation is considered as a sequela to immune tolerance to HBeAg, it has been shown that HBeAg may promote HBV chronicity by functioning as an immunoregulatory protein (Milich and Liang, 2003). Such a mechanism may be responsible for the high chronic HBV infection rate (~ 90%) observed in babies infected by their HBeAg – positive mothers, accounting for the inability of infants to clear perinatal HBV infection. HBeAg can also enter thymus. It has been

reported that HBeAg specific Th_2-like cells can preferentially survive tolerance production to a greater extent than HBeAg – specific Th_1-like cells (Huang *et al.,* 2006). Therefore, chronicity resulting from vertical transmission of HBV, characterized by the predominance of HBeAg - specific Th_2-like cells and secretion of anti-inflammatory cytokines, such as IL-4, IL-5, and IL-10, can enhance antibody production, and viral persistence would characterize the HBeAg-specific T-cell response. Presence of HBeAg and absence of the HBeAb increases a patient's risk of developing liver – related complications, such as cirrhosis, fibrosis, carcinoma, etc *(ibid).*

2.4.3 Occult HBV infection

Recent investigations into the serologic and pathological distinctions among the various stages of HBV infections have led to the discovery of a new form, though rare, called occult HBV infection (HBI). Occult HBI is defined as the existence of HBV DNA in serum marked with absence of HBsAg (Torbenson and Thomas, 2002; Conjeevaram and Lok, 2001). In addition to a symptomatic and serologically evident infection, occult persistent HBV carriage has been identified since nucleic acid amplification assay enhances its sensitivity to hepadnaviral genomes and their replicative intermediates. There is evidence that occult HBV infection is a common and long-term consequence of acute hepatitis B resolution. This form of residual infection is termed as secondary occult infection (SOI) (Conjeevaram and Lok, 2001).

2.5 SIGNS AND SYMPTOMS

Acute infection with hepatitis B virus is associated with acute viral hepatitis – an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. These signs may last for

a few weeks and then gradually improve in most affected people. A few patients may have more severe liver disease (fulminant hepatic failure), and may die as a result of it. The infection may be entirely asymptomatic and may go unrecognized (Lai *et al.*, 1991).

Chronic infection with Hepatitis B virus may be either asymptomatic or may be associated with cirrhosis of the liver over several years leading to accumulation of waste materials and fluids in the liver. This results in enlargement of the liver (hepatomegaly). This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer *(ibid).*

2.6 HBV DIAGNOSIS

The tests, or assays, for detection of hepatitis B virus infection involve serum or blood tests that detect either viral antigens (proteins) or antibodies produced by the host. Interpretation of these assays is complex (Bonino *et al.*, 1987). The first step in identifying patients with chronic HBV infection is to screen those with risk factors. Screening is focused on patients in high-risk groups, such as persons born in endemic areas, patients engaged in high-risk sexual behaviours, injection drug users, dialysis patients, HIV-infected and other immunosuppressed patients, pregnant women, and persons with occupational exposure, as well as family/household members and sexual contacts of HBV-infected persons. The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. Testing for antibody to hepatitis B core (anti-HBc), and antibody to hepatitis B surface antigen (anti-HBs) indicate whether an individual has been previously exposed to HBV. The HBV DNA levels are not required for preliminary screening for the HBV-infection. The HBsAg is the first detectable viral antigen to appear during infection. However, the length of time within which detectable amount of HBsAg may persist in host depends on efficiency of

host immune function at clearing the virus-infected hepatocytes and establishing enduring immunity (AASLD, 2007). This antigen may persist when infection has become chronic stage of the infection. The molecular mechanism underlying this adaptation remains yet unknown. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or HBcAg. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (anti-HBc IgM) may be the only serological evidence of disease *(ibid)*.

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Lok and McMahon, 2007). Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase levels and inflammation of the liver, as revealed by biopsy. Carriers who have seroconverted to HBeAg negative status, particularly those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others (Chu and Liaw, 2007). Additionally, polymerase chain reaction (PCR) tests have been developed to detect and measure the amount of viral nucleic acid in clinical specimens. These tests measure viral loads and are used to assess a person's infection status and to monitor treatment (Zoulim, 2006).

2.7 PATHOGENESIS – VIRUS / HOST INTERACTION

Viral hepatitis, characterized by diffused inflammatory reaction, is associated with cell damage and death. It has been recently reported that HBV replication is associated with cell death, which is different from the widely accepted non-cytopathic characteristics of HBV (Philip *et al.*, 2006). The mechanism of cell damage is generally defined as the result of cytotoxic T-lymphocyte (CTL)-mediated immune responses to viral infection. Indeed, whatever the undesirable

pathological effect that may result from the infection, it is only the consequence of the interaction of the virus with host factors *(ibid)*. Generally, the hepatitis B virus interacts with the host hepatocytes at two levels:

a) Virus protein interacting with host proteins (mainly transcriptional / signalling factors).
b) Virus genome interacting with host proteins (mainly transcriptional factors).

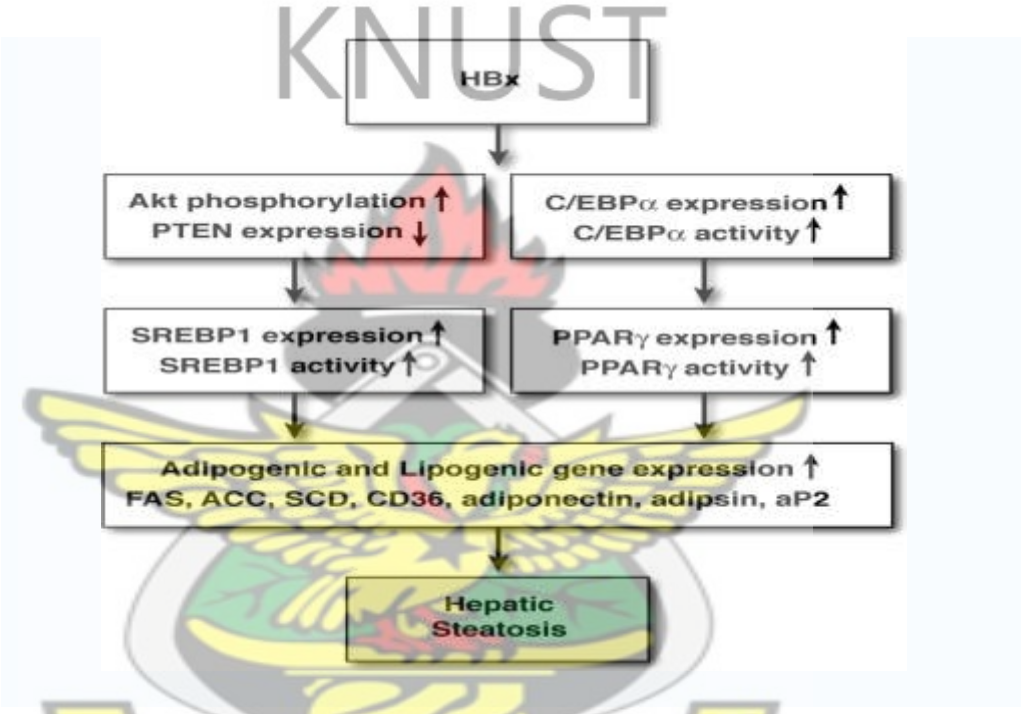


Figure 2.6.1: HBx protein of HBV interacting with host metabolic genes (Kim *et al.*, 2007)

2.8 THE LIVER AS A HOMEOSTATIC ORGAN

The liver is located in the upper right-hand portion of the abdominal cavity, beneath, and on top of the stomach and right kidney. Shaped like a cone, the liver is a dark reddish-brown organ that weighs about 2.5 - 3 pounds (1.1 – 1.3kg) depending on age and size of individual (Williams, 2006). There are two distinct sources that supply blood to the liver, they are

- oxygenated blood flowing in from the hepatic artery

- nutrient-rich blood flowing in from the hepatic portal vein

The liver holds about 0.5 litres (13 percent) of the body's blood supply at any given moment *(ibid)*. It consists of two main lobes, both of which are made up of thousands of lobules. These lobules are connected to small ducts that connect with larger ducts to ultimately form the hepatic portal system. These portal systems transport repackaged materials/nutrients and recycled wastes produced by the liver cells to the main circulatory stream of blood. Thus, the liver regulates the amount of substrates present in the blood at a time. Dietary triacylglycerides (TAGs) are broken down to produce fatty acids and 2–monoglycerides as it travels through the alimentary canal. The products (fatty acids and 2-monglycerides) diffuse into the absorptive enterocytes of the small intestines where ther are reconstituted back to TAGs which are more usable to the body. Again, in the endoplasmic recticulum of the enterocytes, the TAGs are packaged with cholesterol esters, apoproteins, and other lipids into complex particles called chylomicrons. The chylomicrons are drained from the enterocytes into the lymphatic vessels that be-lie the villi called lacteals. The lacteals merge to form larger lymphatic vessels that drain their content into the thoracic duct which finally empties their content into the main bloodstream at the subclavian vein. The lipds in the blood are then transported to adipocytes for storage. In the fasting state, the lipid mass in the adipocytes are broken down and re-packaged into very low density lipoproteins (VLDLs) and transported into the bloodstream for circulation. In the liver, the VLDL is processed and repackaged with apoprotein into low density lipoproteins (LDLs) for transport to the peripheral tissues. The lipoproteins - VLDL, high density lipoprotein (HDL), and low density lipoproteins (LDL) contain cell receptors binding elements (apoproteins) that enable them to identify and release lipids to only specified/target cells. This helps to prevent the potential of lipid poisoning in the blood and infiltration of lipids to privileged sites/cells in the body. It is therefore, by

function, more adapted to handling larger doses of fats and lipids than other body organs/tissues.

Diseases and agents that interfere with the physiology of the liver may eventually result in the accumulation of lipid droplets in the hepatocytes resulting in hepatic steatosis, which may develop as a consequence of multiple dysfunctions such as alterations in beta-oxidation, defective very low density lipoprotein secretion, and pathways involved in the synthesis of fatty acids. In addition, an increased circulating pool of non-esterified fatty acid may be a major determinant in the pathogenesis of fatty liver disease among CHB patients (Williams, 2006).

2.8.1 The value of aminotransferases as a measure of functional integrity of liver

The two main enzymes whose levels are used to measure the functional integrity of the liver are – aspartate aminotransferase (AST) and alanine amino transferase (ALT).

a) Aspartate amino transferase (AST)

Aspartate aminotransferase (EC 2.6.1.1) was formerly called serum glutamic oxaloacetate transaminase (SGOT). It is a pyridoxal phosphate (PLP) – dependent enzyme, catalyzing the reversible transfer of an α-amino group between aspartate and glutamate and, as such is an important enzyme in amino acid metabolism. Apart from the liver, the AST can also be found in the heart, skeletal muscle, kidneys, brain, and red blood cells. Its level in serum is commonly measured as a marker for liver disease. Its normal range is about 6 – 40 (IU/L). Unlike ALT, the AST is a non specific diagnostic index for measuring the health of the liver. Because it occurs in multiple tissues other than the liver, its level is likely to rise even in non-

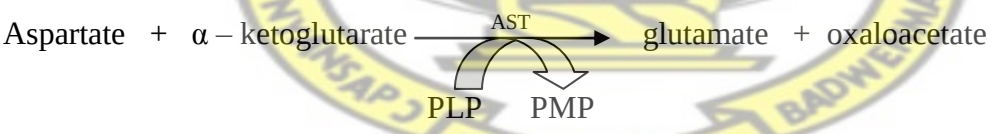
liver related diseases like myocardial infarction, acute renal failures, acute pancreatitis, acute hemolytic anemia, etc.

There are two isoenzyme forms of the AST namely:

cAST / GOT-1 - cytosolic isoenzyme derived mainly from red blood cells and heart.

mAST/ GOT-2 - mitochondrial isoenzyme present largely in the liver (Berg *et al.*, 2006)

As a prototypical transaminase, AST relies on PLP as a cofactor to transfer the amino group from aspartate or glutamate to the corresponding ketoacid. The amino group transfer catalyzed by this enzyme is crucial in both amino acid degradation and biosynthesis. In amino acid degradation, following the conversion of α- ketoglutarate to glutamate, the glutamate subsequently undergoes oxidative deamination to form ammonium ions, which are excreted as urea. In the reverse reaction, aspartate may be synthesized from oxaloacetate, which is a key intermediate in the Kreb's cycle. In the process, the cofactor shuttles between PLP and the pyridoxamine phosphate (PMP) (Kirsch *et al.*, 1984).



b) Alanine transaminase (ALT)

Also called serum glutamic pyruvic transaminase (SGPT) (EC 2.6.1.2) or alanine amino tranferase (ALAT), catalyzes the transfer of an amino group from alanine to α – ketoglutarate producing pyruvate and glutamate using the coenzyme pyridoxal phosphate (PLP). It is found in the serum and in various body tissues, but is most commonly associated with the liver. Clinically, serum ALT levels are useful in diagnostic evaluation of hepatocellular injury

(Ghouri *et al.*, 2010). Its normal range is about 10 – 40 IU/L. Values significantly higher than this range infers some level of liver damage. Unlike the AST, the serum ALT levels give a more specific diagnosis of the health of the liver.

c) AST/ALT Ratio

It is also known as the "De Ritis Ratio" (McClatchey, 2002). As a clinical index, it is useful together with the serum AST and ALT levels in distinguishing between the causes of liver diseases. Ideally, the ratio should be less than 1 with relatively normal values of AST and ALT. When the AST/ALT ratio is less than 1, but with extremely high levels of AST and ALT, it signifies viral hepatitis. When the ratio is greater than 2, it implies alcoholic hepatitis or hepatocellular carcinoma. When greater than 1 but less than 2, it is likely to be due to cirrhosis (Sorbi *et al.*, 1999).

The AST/ALT ratio is less useful in scenarios where the liver enzymes are not elevated, or where multiple diseases coexist.

2.9 METABOLISM OF LIPIDS

Fatty acids are usually ingested as large molecular weight neutral triglycerides, which cannot be absorbed by the intestine. Triacylglycerols are broken down into free fatty acids and monoglycerides by pancreatic lipases, which form a 1:1 complex with a protein called colipase which is necessary for its activity (Simopoulos, 1999). The activated complex can only work at a water-fat interface: it is therefore essential that lipids are emulsified by bile salts for optimal activity of these enzymes. People who have had their gallbladder removed due to gallstones have great difficulty digesting fats. Most are absorbed as free fatty acids (FFA) and 2-monoglycerides, but a small fraction is absorbed as free glycerol and as diglycerides. Once across the intestinal barrier, they are reformed into triglycerides and

packaged into chylomicrons which are released into the lacteals, the capillaries of the lymph system and then into the blood. Eventually, they bind to the membranes of hepatocytes, abdominal adipocytes or muscle fibers, where they are either stored or oxidized for energy.

Triglycerides present in the chylomicrons undergo lipolysis by the lipoprotein lipases of target tissues which yield glycerol and free fatty acids. Free fatty acids released into the blood are then available for cellular uptake. Free fatty acids not immediately taken up by cells may bind to albumin for transport to surrounding tissues that require energy. Serum albumin is the major carrier of free fatty acids in the blood. Hence, diseases of the liver such as hepatitis B infection that impair albumin synthesis by the liver often have the tendency of affecting circulating free fatty acid levels in the blood (Simopoulos, 1999). The glycerol fraction also enters the bloodstream and is absorbed by the liver or kidney where it is converted to glycerol-3-phosphate by the enzyme glycerol kinase. Hepatic glycerol-3-phosphate is mostly converted into dihydroxyacetone phosphate (DHAP) and then glyceraldehyde-3-phosphate to rejoin the glycolytic and gluconeogenetic pathways as an anaplerotic substrate.

2.10 TRANSPORT OF LIPIDS

Generally, lipids and fatty acid, by virtue of their non-polar nature, would cause a lot of harm if allowed to circulate freely. As poorly soluble molecules (solubility ≈ 1μM at 37°C), they would readily precipitate, settle or adhere themselves to a part of the circulatory system (Teruya, 1995). They could also diffuse to 'forbidden' areas of the body, creating several possible pathologies *(ibid)*. To avert this, several carrier molecules which are more soluble in the polar stream of blood and body fluids exist to transport lipids to their respective sites in the body. Carrier molecules help to improve solubility of the lipids (fatty acids) in the blood and also helps regulate lipid distribution through their apoprotein molecules. For instance

albumin – bound FFA has almost a 10^3 fold increase in solubility (1.0mM), compared to unbound FFA (1μM at 37°C) in the blood *(ibid).*

In the plasma, there are many vehicles that deliver fatty acids to needy cells. Among these are the fatty acid-containing phospholipids (PL), triglycerides (TG) and cholesteryl esters (CE) in the four major plasma lipoproteins, and a much smaller pool of free fatty acids bound to albumin. Thus, fatty acids can be delivered to cells from at least 13 different vehicles. Teruya *et al.,* (1995) demonstrated by their work that there exists a tangible correlation between the vehicle that delivers the fatty acid to the cells and intracellular fate of fatty acid so delivered. While some delivery forms promote storage of the fatty acid, others promote immediate oxidation of the fatty acid to generate energy.

2.10.1 Transport of lipids in extracellular fluid

Generally, the albumin and lipoproteins sereve as the principal carrier molecules for the tansport of lipids through the extracellular fluid matrix.

2. 10.1.1 Lipoproteins

Unlike the muscle and heart tissues, the liver mostly synthesizes lipids for distribution than it degrades them (Fielding, 1992). This permits it to serve as a central player in lipid homeostasis in the blood. Often, the lipids are packaged in smaller micelles called lipoproteins for transport to target tissues. Structurally, the lipoproteins are made up of apolipoproteins (A, B, C and E), cholesterol, triglycerides, and phospholipids in different amounts *(ibid).*

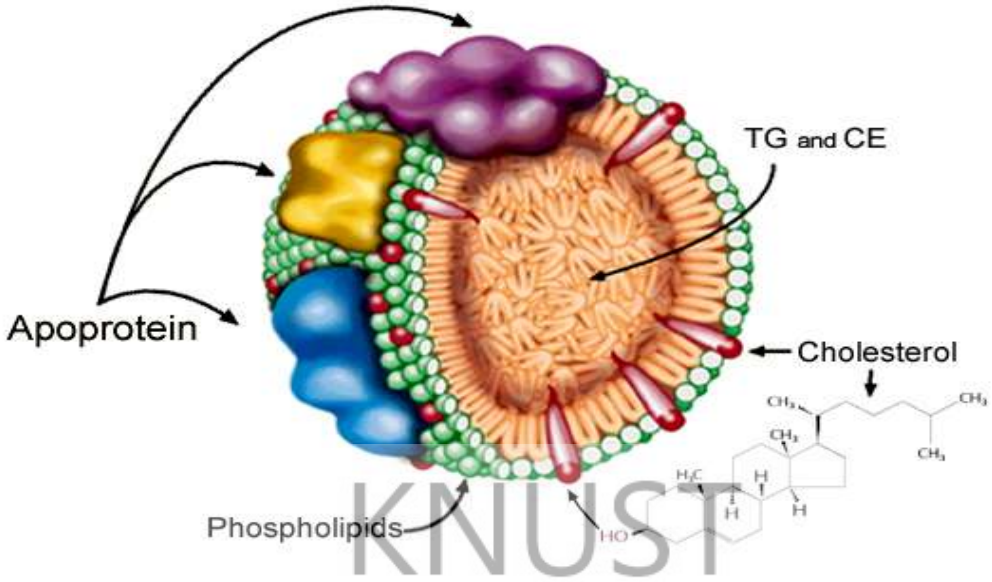


Fig. 2.10.1: Annotated structure of a typical lipoprotein (Hu *et al.*, 2001)

This variation in the amount of these constituent molecules tends to produce five different forms of physiological lipoprotein complexes, namely;

- VLDL (carries lipids synthesized by the liver to body cells)
- LDL (carries cholesterol around the body)
- IDL (carries fats and cholesterol through the polar bloodstream)
- HDL (carries cholesterol from the body back to the liver for breakdown and excretion).
- Chylomicrons (carry diet-derived lipids to body cells) (Fielding, 1992).

2.10.1.2 Very low-density lipoprotein (VLDL)

Very-low-density lipoprotein (VLDL) is made by the liver. VLDL is assembled in the liver from cholesterol, cholesteryl ester, triglycerides, and apolipoproteins and is converted in the bloodstream to low-density lipoprotein (LDL). Functionally, the VLDL transports

endogenous triglycerides, phospholipids, cholesterol, and cholesteryl esters. It functions as the body's internal transporter of lipids.

2.10.1.3 Low density lipoprotein (LDL)

Low-density lipoprotein (LDL) transports cholesterol and triglycerides from the liver to peripheral tissues. Like all lipoproteins, LDL enables triglycerides and cholesterol to move within the water-based solution of the bloodstream. LDL also regulates cholesterol synthesis at these sites (peripheral tissues). Since a high level of LDL – cholesterol is a risk factor to conditions like cardiovascular disease, LDL is sometimes called *"bad cholesterol"*, (as opposed to HDL, which is frequently referred to as "*good cholesterol"* or healthy cholesterol).

LDL poses a risk for cardiovascular disease when it invades the endothelium and becomes oxidized, since the oxidized form is more easily retained by the proteoglycans. A complex set of biochemical reactions regulate the oxidation of LDL, chiefly stimulated by presence of free radicals in the endothelium (Toth, 2005).

2.10.1.4 High-density lipoprotein (HDL)

In healthy individuals, about thirty per cent of blood cholesterol is carried by HDL (Toth, 2005). It is hypothesized that HDL can remove cholesterol from atheroma within arteries and transport it back to the liver for excretion or re-utilization. A high level of HDL-C seems to protect against cardiovascular diseases, and low HDL cholesterol levels (less than 40 mg/dL or about 1mmol/L) increase the risk for heart disease (Fielding, 1992).

HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by direct and indirect pathways. HDL is removed by HDL receptors such as

scavenger receptor BI (SR-BI), which mediates the selective uptake of cholesterol from HDL. In humans, probably the most relevant pathway is the indirect one, which is mediated by cholesteryl ester transfer protein (CETP). This protein exchanges triglycerides of VLDL for cholesteryl esters of HDL. As a result, VLDLs are processed to LDL, which are removed from the circulation by the LDL receptor pathway. The triglycerides are not stable in HDL, but get degraded by hepatic lipase so that finally small HDL particles are left, which restart the uptake of cholesterol from cells (Toth, 2005).

The cholesterol delivered to the liver is excreted into the bile and, hence, intestine either directly or indirectly after conversion into bile acids. Delivery of HDL cholesterol to adrenals, ovaries, and testes is important for the synthesis of steroid hormones.

2.10.1.5 Intermediate-density lipoprotein (IDL)

Intermediate-density lipoproteins are formed from the degradation of very low-density lipoproteins. Each native IDL particle consists of protein that encircles various fatty acids, enabling the fatty acids to travel in the aqueous blood environment, as part of the fat transport system within the body. Their size is, in general, 25 to 35 nm in diameter, and they contain primarily a range of triacylglycerols and cholesterol esters (Brown and Goldstein, 1986). They are cleared from the plasma into the liver by receptor-mediated endocytosis, or further degraded to form LDL particles. Although one might intuitively assume that "intermediate-density" refers to a density between that of high- and low-density lipoproteins, it actually refers to a density between that of low-density and very low-density lipoproteins (Brown and Goldstein, 1986). In general, IDL, somewhat similar to low-density lipoprotein (LDL), transports a variety of triglycerides and cholesterol and, like LDL, can also promote the growth of atheroma (Grover *et al.*, 1994).

The IDL particles do lose most of their triglyceride, but they retain cholesteryl esters. Some of the IDL particles are rapidly taken up by the liver; others remain in circulation where they undergo further triglyceride hydrolysis and are converted to LDL. A distinguishing feature of the IDL particle is its content of multiple copies of the receptor ligand apolipoprotein E (ApoE), in addition to a single copy of apolipoprotein B-100 (ApoB-100) (Brown and Goldstein, 1986). The multiple copies of ApoE allow IDL to bind to the LDL receptor with a very high affinity. When IDL is converted to LDL, the ApoE leaves the particle and only the ApoB-100 remains. Thereafter, the affinity for the LDL receptor is much reduced *(ibid).*

2.10.1.6 Chylomicrons

Chylomicrons are large lipoprotein particles that consist of triglycerides (85-92%), phospholipids (6-12%), cholesterol (1-3%) and proteins (1-2%) (Mahmood Hussain, 2000). They transport dietary lipids from the intestines to other locations in the body. Chylomicrons transport exogenous lipids to liver, adipose, cardiac, and skeletal muscles, where their triglyceride components are unloaded by the activity of lipoprotein lipase. As a consequence, chylomicron remnants are formed and are taken up by the liver.

2.10.1.7 Serum albumin

Serum albumin is the major carrier of free fatty acids in the blood. It helps to solubilise the non-polar fatty acids in plasma. For instance, albumin-bound FFA has almost a 10^3 fold increase in solubility (1.0 mM) than unbound FFA (1μM at 37°C) in the blood. Fatty acids bound to albumin are often referred to as Non-esterified fatty acids (NEFA) or free fatty acids (FFA).

When blood sugar is low, glucagon signals the adipocytes to activate hormone sensitive lipase, and to hydrolyse triglycerides into free fatty acids. These have very low solubility in

the blood, typically about 1 μM. However, the most abundant protein in blood, serum albumin, binds free fatty acids, increasing their effective solubility to ~ 1 mM. Thus, serum albumin transports fatty acids to organs such as muscle and liver for oxidation when blood sugar is low.

2.10.1.8 Cholesterol ratio

This is also known as the Castelli or atherogenic index and is given as the total cholesterol/HDL-C (TC / HDL-c ratio) for a sample. The total cholesterol is the sum of all amounts of all the types of cholesterol in the blood. This includes the LDL and HDL cholesterol. As a ratio, it has no specific units of measurement.

The ratio shows how high HDL-C is, relative to the overall cholesterol levels and is thought to be a better clinical indicator of heart disease risk than the total cholesterol alone. Generally, a low ratio < 4.5 represents a lower cardiovascular risk and vice versa. However, for treatment purposes, it is often important to know the absolute values of the various forms of cholesterol; i.e. HDL and LDL. Patients with relatively low HDLs or high LDLs may still be at substantial risk of cardiovascular disease even though their atherogenic index may be lower. For instance, Jeppessen *et al.*, (1998), in one study for the common risk factors for cardiovascular diseases among 120 apparently healthy volunteers, observed that individuals with higher TC/HDL-c ratios higher serum VLDL and LDL level and lower HDL-c. In addition, those with higher TC/HDL-c ratio had higher systolic and diastolic blood pressures according to the same study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials and Recruitment of volunteers:

Subjects

Volunteers were selected from hepatitis B-infected patients attending a specialist clinic at the Oncology unit of the KATH. Inclusion into the study involved signing of an informed consent form by the volunteers and serological detection of HBsAg and HBcAb in serum of patients. A copy of the questionnaire for gathering information from volunteers is in appendix II.

Volunteers were recruited as they reported to the clinic. Recruits were excluded from the study based on either one or more of the following factors:

a) Evidence of smoking
b) Use of drugs that affect the lipid profile in blood such as statins, steroids, insulin, etc.
c) Evidence of alcoholism
d) Obesity (BMI > 30 kgm^{-2})
e) Presence of other liver complications and infections such as liver cancer, HCV infection, hepatic cirrhosis, etc.
f) Congenital defects associated with lipid metabolism.
g) Age outside the range of 18 - 60years

The minimum sample size was determined by the web-based Sample Size Calculator, using a confidence interval of 5% for a population of 1000 chronic hepatitis B patients attending the

hospital facility per annum, and at 95% confidence level. The calculation gave a minimum sample size of 41 patients (www.surveysystem.com/sscalc).

The recruited subjects were categorized accordingly as chronic symptomatic, chronic asymptomatic, or control using the combined outcomes of sserological tests, biochemical assays, and some avert clinical signs observed among the patients. The criteria used was as shown below;

a) Chronic symptomatic
   - Anti-HBcAg positive and HBsAg positive
   - Serum AST and ALT ≥ 40 IU/l
   - Presence of one or more of the three (3) major clinical symptoms – pallor, jaundice, hepatomegaly.

b) Chronic asymptomatic
   - Anti-HBcAg positive and HBsAg positive
   - Serum AST and ALT ≥ 40 IU/l
   - Absence of all of the three (3) major clinical symptoms – pallor, jaundice, hepatomegaly.

c) Healthy control subjects
   - Anti-HBcAg positive and HBsAg positive
   - Serum AST and ALT ≤ 40 IU/l
   - Absence of all of the three (3) major clinical symptoms – pallor, jaundice, hepatomegaly.

Data on age, BMI, and presenting clinical signs and symptoms of subjects were acquired by the use of a questionnaire. Ethical approval for the work was granted by the Committee on

Human Research, Ethics, and Publication of the KATH and School of Medical Sciences, KNUST.

Eleven (11) apparently healthy individuals, with normal BMI of 23.0kg/m^2, aged between 23 – 40 years, and who were HBsAg –ve and HBsAb –ve, were selected randomly from the KNUST campus to serve as the control subjects.

3. 2. Sample collection and preparation:

A 6.0 ml venous blood was drawn from volunteers after a 12- hour overnight fast, using sterile syringes by a phlebotomist, at the serology laboratory, KATH into sterile vacutainers. Collected blood samples were centrifuged at 3.6 x 10^3g for 6 minutes and the serum collected for the required assays.

3.3. Laboratory analysis/assays

3.3.1 HBV serology

This was done using commercial HBV immune-panels manufactured by Fortress Diagnostics Ltd., Antrim BT14 1QS, UK, as shown below, to evaluate volunteers' status for HBV immunological factors – hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (HBeAb), and Hepatitis B core antibody (HBcAb).

This test works on the principle of enzyme immunoassay (EIA). The columns of the panels are lined internally with antibodies and antigens which specifically form visible immune complexes with different viral antigens and antibodies respectively in the patient sample.

During the test, the infected patient's serum sample is added as a drop to one end of the column and allowed to diffuse gradually through the column.

During the flow, antibodies or antigens lined in the column react with their respective ligands in the patient's serum sample forming visible complexes in the respective columns of the panel. The antibody-antigen complexes formed in the column appear as visible bands. According to the test protocols, the appearance of a single visible band in a column gives a negative test while the appearance of a double band indicates a positive result.

One of the HBV combination test cassettes used for the serological determinations is as shown in fig. 3.0.

Fig. 3.0: HBV combination test cassette

3.3.1 Liver Function Tests (LFTs)

The functional state of the liver was assessed by assaying for alanine transaminase (ALT) and aspartate transaminase (AST) levels in serum samples from respondents. These indices were determined by a semi-automated method using commercial kits (Fortress Diagnostic Ltd., Antrim BT41 1QS, UK). The procedure for the assay was according to the protocol as directed by the manufacturer. This is a spectrophotometric technique and measurements of the ALT and AST activity were carried out by monitoring the rate of NADH oxidation in coupled reaction, using lactate and malate dehydrogenases (LDH and MDH) respectively. The reduction in absorbance as NADH is oxidized is measured at 340 nm. From the Beer-Lambert's law, $A = \varepsilon b c$ where;

ε = molar absorptivity of solution ($dm^3mol^{-1}cm$)

b = path length of the sample (cuvette)

c = concentration of compound in solution analysed ($moldm^{-3}$)

A = absorbance measured

From the equation above, the absorbance of the sample can be correlated to the original amount of the ALT or AST in the sample used. The reagent consisted of lactate dehydrogenase (LDH), oxoglutarate, L-Alanine, and NADH in a Tris buffer at a pH of 7.8. Upon addition of the test sample (patient's serum), the ALT enzymes in the sample catalyses the reaction involving the 2-Oxoglutarate and L-Alanine to produce glutamate and pyruvate respectively. The pyruvate formed is next reduced by the LDH, using NADH.

$$\text{2-Oxoglutarate} + \text{L-Alanine} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

A similar protocol but with slight modification in the reaction substrates and enzyme was used for the assay for serum AST levels in the samples of the study population.

As a general rule, a drop in absorbance of the NADH due to its oxidation correlates with the concentration of the ALT or AST present in the test sample.

3.3.2 Serum lipid profile

Serum lipid profile, including HDL, total cholesterol, LDL, and triglycerides was determined by semi-automated colorimetric protocols, using commercial kits (Fortress Daignostics Ltd., Antrim BT41 1QS, UK). For HDL-C assay, the kit uses a specific detergent formulation containing anti-human lipoprotein antibody to selectively precipitate non-HDL lipoprotein particles (Chylomicrons (CM), LDL, and VLDL) while leaving HDL particles intact.

$$\text{CM, LDL, VLDL} \xrightarrow{\text{antihuman Lp-Ab}} \text{Ag – Ab Complex}$$

The agglutinated complex thus formed inhibits any further enzyme reactions. Next, a second detergent containing cholesterol esterase and oxidase is added to the sample to solubilize the remaining HDL particles. In this case, the cholesterol esterase hydrolyses the cholesterol ester to cholesterol. The dissolved cholesterol is oxidized by the cholesterol oxidase to produce hydrogen peroxide. The hydrogen peroxide product then reacts with bis (4-sulphobutyl)-m-toluidine (F-DOAS) and 4-aminoantipyrine to form a coloured product. The intensity of the resulting color change is measured at 550 nm is proportional to the amount of HDL-C originally present in the sample.

$$\text{HDL-C} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{apo-B lipoproteins} + \text{fatty acids}$$

$$\text{Cholesterol} + H_2O + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2 + \text{fatty acids}$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{HSDA*} + H^+ \xrightarrow{\text{peroxidase}} \text{purple-blue pigment} + H_2O$$

* HSDA – N - (2-hydroxy – 6- sulfopropyl) -3, 5-dimethoxyaniline

For the total cholesterol level, a similar protocol was followed, even though with a different reagent (commercial kit from Fortress Diagnostics Ltd., UK). The principle for the test is as summarized in the steps below;

- Cholesterol esters in the forms of HDL, LDL, VLDL, and chylomicrons are enzymatically hydrolysed by cholesterol esterase (CE) to release cholesterol and free fatty acids.
- The free cholesterol is then oxidized by cholesterol oxidase (CHOD) to cholest-4-ene-3-one and hydrogen peroxide.
- The hydrogen peroxide, in the presence of peroxidase (POD), effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine complex.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-ene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

The intensity of the red color produced is directly proportional to the total cholesterol concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

Levels of serum triglyceride were further determined by a semi – automated colorimetric method, using commercial test kits from Fortress Diagnostics, UK. The principle behind the protocol for the assay is as summarized according to the steps below.

- Triacylglycerides in the HDL, LDL, VLDL, and chylomicrons in sample are hydrolysed by lipoprotein lipase (PLP) in the reagent to produce free fatty acids and glycerol.
- The glycerol is further phosphorylated by glycerol kinase to produce glycerol-3-phosphate (G-3-P) which is subsequently oxidized by glycerol-3-phosphate oxidase (GPO) to release dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.

$$\text{Triglycerides} + H_2O \xrightarrow{PLP} \text{Glycerol} + \text{RCOOH (free fatty acids)}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{GK/Mg} \text{G} - 3 - \text{P} + \text{ADP}$$

$$\text{G} - 3 - \text{P} + O_2 \xrightarrow{GPO} \text{DHAP} + H_2O_2$$

$$H_2O_2 + \text{p-Chlorophenol} + \text{4-AAP} \xrightarrow{POD} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2H_2O + HCl$$

The intensity of the blue color produced by the 4-(p-benzoquinone-monoimino)-phenazone complex is directly proportional to the total triglyceride concentration. It is determined by measuring the increase in absorbance at 550 nm.

The LDL levels were determined by calculation using a modified form of the Friedewald equation (Friedewald *et al.*, 1972) as below;

$$\text{LDL} = (\text{Chol.}) - (\text{HDL-C}) - \left(\frac{Triglyceride}{2.2}\right)$$

This gives the LDL level in mmol/l. The only demerit of the above formula is that it is not applicable if total serum triglyceride level is more than 4.5 mmol/l (Friedewald *et al.*, 1972).

Quality control of assays was achieved by;

1. Optimizing the assay procedure using pre-determined standards.
2. Repetition of the procedures to obtain consistent data.

3.4.0 Data analysis

All values are expressed as means ± SE. Statistical analysis was performed using the GRAPHPAD® program (Version 5.0, SAS Institute, Cary, U.S). To examine the association between the measured indices, multivariate logistic regression was used. The results of the logistic regression analysis were expressed as odds ratios with 95% confidence intervals (CI). The Student's *t* – test was used to evaluate the measure of significance in the relationship between mean values of analytes. A *p* -value of less than 0.05 was considered significant.

CHAPTER FOUR

RESULTS

4.1 Age and gender distribution among the study population

The age and gender of the study population as determined are summarized in Table 4.1. There were 41 males and 16 females making up the chronic hepatitis B (CHB) infected subjects enrolled in the study. Of the 57 CHB infected patients, 18 were chronic symptomatic (33.9%) of whom there were 15 (83.3%) males and 3(16.7%) females. The remaining 39 (66.1%) patients who were chronic asymptomatic according to a combination of serological, biochemical, and clinical measurements consisted of 26 (66.9%) males and 13 (33.1%) females. The eleven (11) healthy control volunteers enrolled in the study were considerably younger, in comparison with the CHB-infected patients, even though the mean ages of these groups were found to be statistically insignificant (p=0.3679).

Table 4.1: Mean age and gender distribution of study population by sex

Parameter	Chronic symptomatic n=18	Chronic asymptomatic n=39	Healthy control n=11	*P*-value
Mean age (yrs)	34.8 ± 2.49[a]	33.0 ± 1.50	28.54 ± 1.90[a]	0.3679[a]
Gender				
Male (%)	15(83.3%)	26 (66.9%)	5(45.5%)	
Female (%)	3(16.70%)*	13(33.1%)*	6(54.5%)	0.0063*

** significant at p < 0.05* *[a] not significant at p ≥ 0.05*

4.2 Presenting clinical symptoms of the CHB-infected patients and control subjects

There were 57 CHB-infected patients enrolled in the study of which 39 (68.4%) were chronic asymptomatic and 18 (31.6%) were chronic symptomatic. The chronic asymptomatic patients appeared relatively healthier, compared to the chronic symptomatic individuals, as shown in the table 4.2 below. The chronic symptomatic appeared quite emaciated with a mean body-mass index (BMI) of 16.4 kg/m^2. Sixteen (16 (88.9%)) of the chronic symptomatic patients were jaundiced, 15 (83.4%) were visibly anemic, and 5 (27.8%) had enlarged abdomen due to inflammed liver, according to table 4.2. Most (94.4%) of the symptomatic CHB-patients enrolled in the study that showed any of the overt symptoms had AST/ALT ratio above 2.0.

Table 4.2 Distribution of some clinical symptoms among the CHB-infected population compared to the control subjects.

Presenting clinical signs among volunteers	Study population			*p* - value
	Chronic symptomatic n=18	Chronic asymptomatic n=39	Healthy control n=11	
BMI(kg/m^2)	16.4 ± 2.04*	20.1 ± 2.59	23.0 ± 1.67*	0.0121*
Pallor	15 (83.4%)	0	0	-
Jaundice	16 (88.9%)	0	0	-
Hepatomegaly	5 (27.8%)	0	0	-

* significant at $p < 0.05$

4.3 HBV serology among the population enrolled in the study

Table 4.3 shows the HBeAg status in the serum among the CHB-infected male and female volunteers. There were 10 patients (8 (80%) males and 2 (20%) females) out of the CHB-infected population who had active viral replication (HBeAg +ve) according to the serology. The remaining 47 patients (33 (70.2%) males and 14 (29.8%) females)) were carriers of inactive hepatitis B infection (HBeAg –ve) among the volunteers studied. Thus, majority of the patients studied (47/57 CHB-patients) (82.5%) were inactive carriers (HBeAg –ve).

Table 4.3: HBeAg status distribution among the CHB patients.

HbeAg status of subjects	**Gender**		
	Male n=41	**Female n=16**	***P*-value**
HBeAg (+ve)	8 (≈ 20.0%)	2 (≈12.5%)	0.1718
HBeAg (-ve)	33 (≈80.0%)	14 (≈ 87.5%)	

4.4 Serum AST levels among sample population

Figures 4.3a and b show variation of mean serum AST levels among the chronic hepatitis B–infected patients. Figure 4.3(a) shows that mean levels of serum AST was significantly higher (281(± 16.4) IU/l) among the chronic symptomatic hepatitis B infected patients compared to the asymptomatic patients (p=0.0001) and healthy volunteers (p=0.0001) at 95% confidence intervals. However, no significant difference was observed for the mean serum AST levels between chronic asymptomatic hepatitis B infected patients and healthy individuals (p=0.5460). The mean serum AST level in the chronic symptomatic stage was about 965% higher than that for patients in the chronic asymptomatic stage of the infection. The variation is further shown to be dependent on the HBeAg status of the CHB-infected patients studied

according to the fig. 4.3(b) ($p < 0.0001$). In all, 17.5% (10/57) of the CHB-infected patients had active chronic infection (HbeAg +ve) and the remaining 82.5% (47/57) had inactive chronic hepatitis B infection (HBeAg -ve). From fig. 4.3b, the mean serum AST levels among the patients with active chronic hepatitis B infection (HbeAg +ve) were almost twice as much as those for those with inactive infection (HbeAg –ve) ($p=0.0064$).

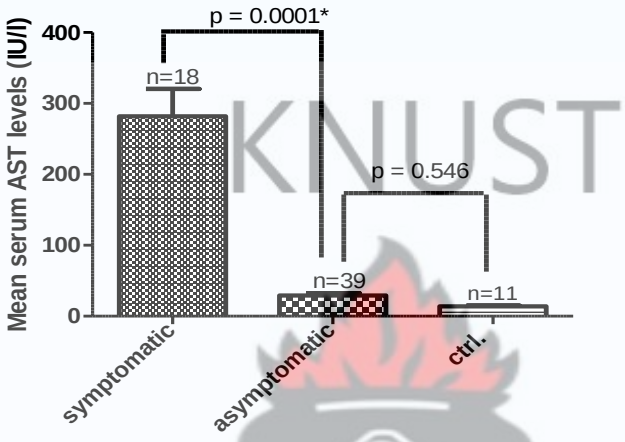


Fig 4.3a

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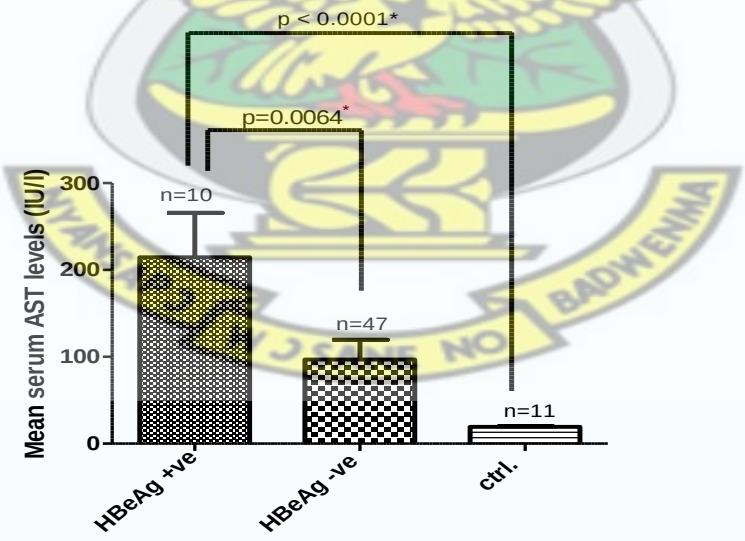

Fig. 4.3b

Variation of mean serum AST levels, disease stages, and HBeAg status of sample population

Values are expressed as mean ± S.D

* *p - value significant.*

4.4 Serum ALT levels among the studied population

Fig.4.4a shows a statistically significant increase in the mean serum ALT levels among the patients at the symptomatic stage of the infection, compared to the asymptomatic patients (p=0.000) and healthy volunteers (p=0.001) at 95% confidence interval. This variation was further shown to be dependent on the HBeAg status according to fig. 4.4(b) (p=0.0142). No significant differences in the mean serum ALT levels were observed among patients at the chronic asymptomatic stage and healthy individuals (p=0.702). Fig. 4.4a shows that the mean serum ALT levels in patients at the chronic symptomatic stage of the infection (161.9 ± 18.8 IU/l) is 630% and 1300% higher than those for individuals in the healthy control group (25.6 ± 5.21 IU/l) and chronic asymptomatic groups (12.3 ± 6.45 IU/l) respectively.

The mean AST/ALT ratio among the healthy control (0.365 ± 0. 117) was almost five to ten-folds less than the average ratio for those of the chronic hepatitis B-infected patients (4.18 ± 0.725) according to Figs. 4.4c and 4.4d. There was a significant difference (p = 0.002) in the mean AST/ALT ratio among the chronic asymptomatic (2.68 ± 0.248) and the chronic symptomatic patients (5.659 ± 0.601).

There was however, no significant correlation between the mean AST/ALT ratios and mean HDL/LDL ratios among the CHB-infected population and the control subjects as showed in the table 4.4.

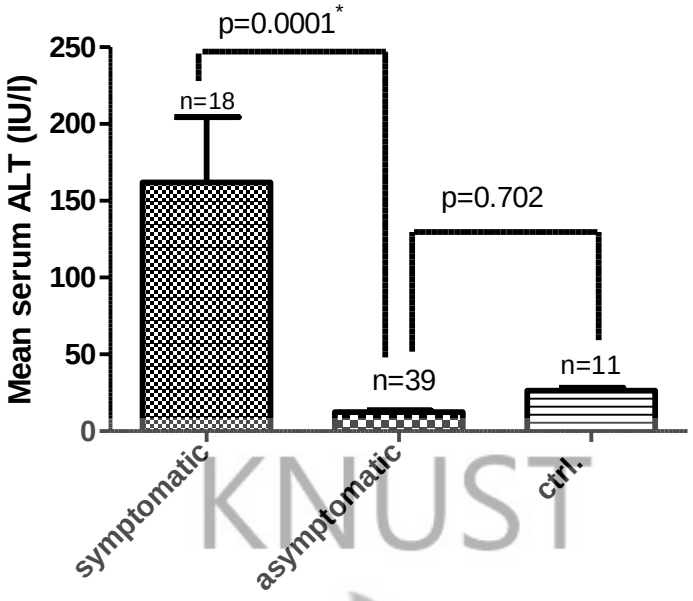

Fig 4.4a

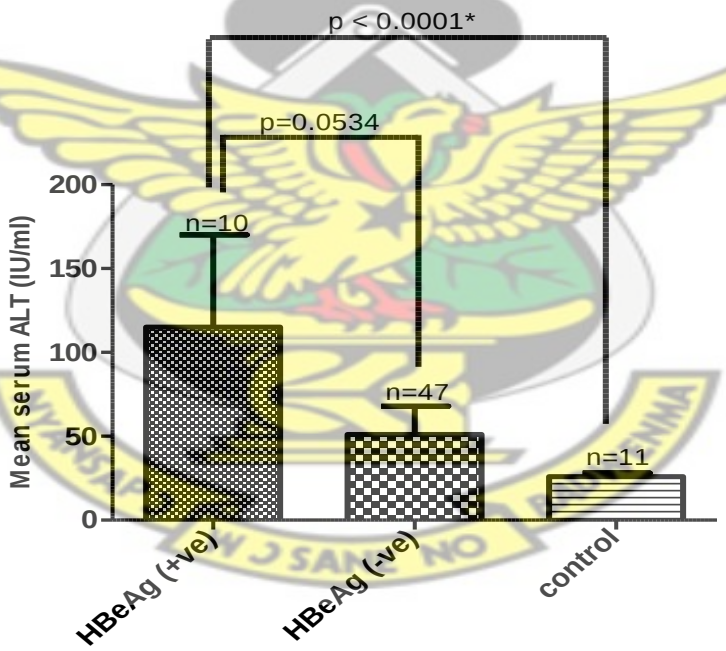

Fig. 4.4b

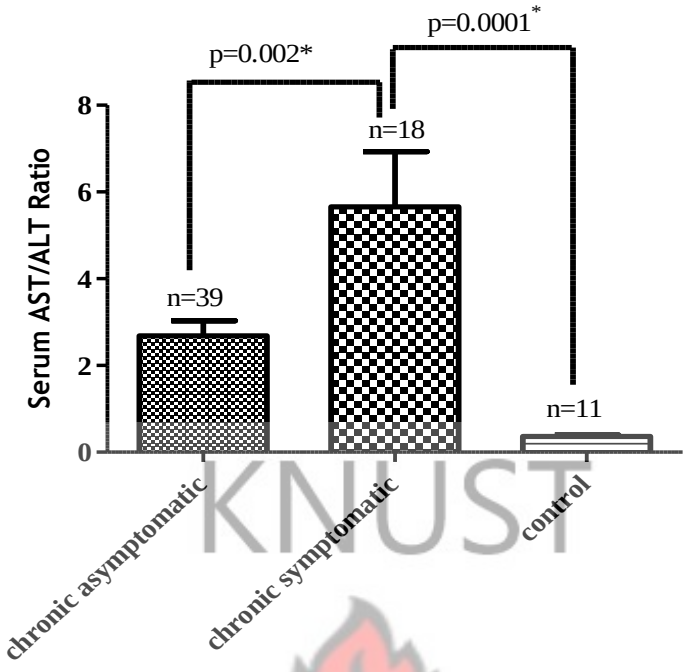

Fig. 4.4c

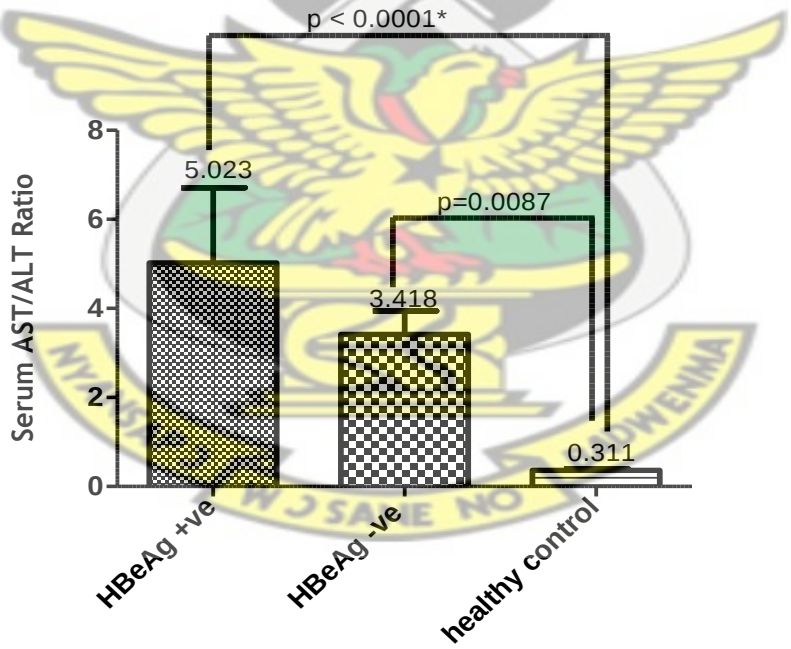

Fig. 4.4d

Mean serum AST/ALT ratio among patients with active and inactive hepatitis B infection compared to healthy individuals.

values are expressed as mean ± S.D

** p-value significant at 95% CI*

Table 4.4 Mean AST/ALT ratio and HDL/LDL ratio among the studied population

Disease stage	Clinical indices		
	AST/ALT ratio	HDL/LDL ratio	*p-value*
Chronic symptomatic	5.93 ± 1.15	0.34 ± 0.120	0.109
Chronic asymptomatic	2.58± 0.58	0.49 ± 0.144	0.548
Control subjects	0.36 ± 0.18	0.51 ± 0.015	0.114

4.5 Serum total cholesterol levels among study population

Mean serum total cholesterol levels in population are shown in figures 4.5a and b. The mean serum total cholesterol levels were 5.19 ± 3.06, 5.38 ± 1.22, and 4.70 ± 0.71 mmol/l for subjects in the chronic symptomatic, chronic asymptomatic and healthy control groups, respectively. Fig.4.5a shows that the mean serum levels of total cholesterol was highest among patients with chronic asymptomatic stage of the infection, though not statistically different (p= 0.6810), compared to that of those patients in the chronic symptomatic stage and healthy control subjects at 95% confidence intervals (fig. 4.5a). Fig. 4.5b shows higher levels of mean serum total cholesterol for the group of patients who are HBeAg –ve, as compared to those patients who are HBeAg +ve (p=0.4766) and healthy controls (p=0.0245). Fig. 4.5c compares the mean cholesterol ratio (total cholesterol/HDL) among the study population. The chronic symptomatic population had the highest mean cholesterol ratio (5.172 ± 0.843), then the chronic asymptomatic (3.530 ± 0.187), and the healthy control had the least (2.673 ± 0.885).

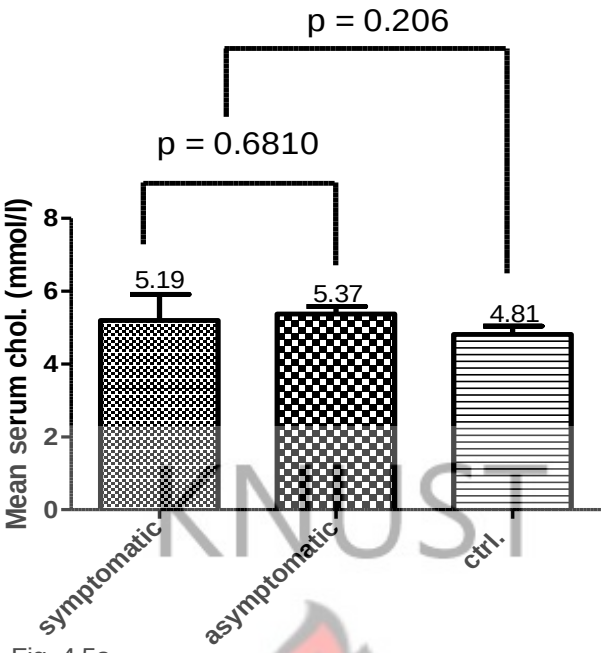

Fig. 4.5a

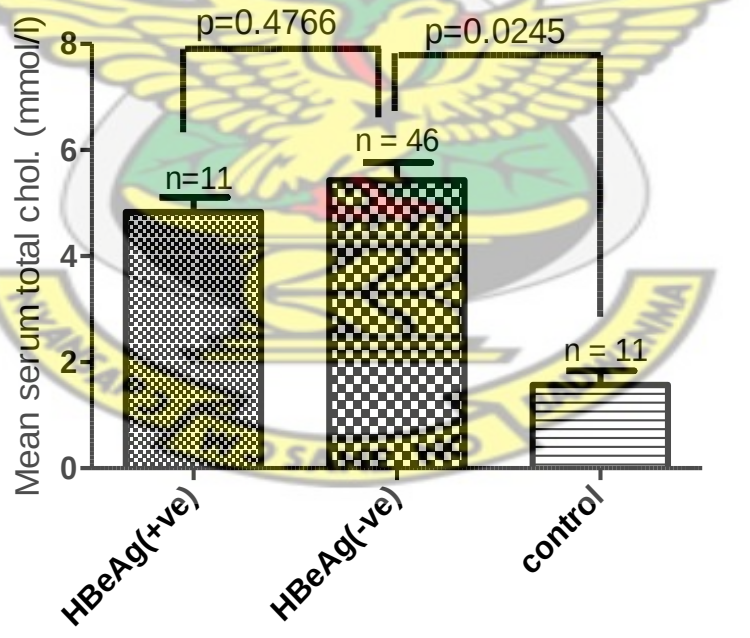

Fig 4.5b

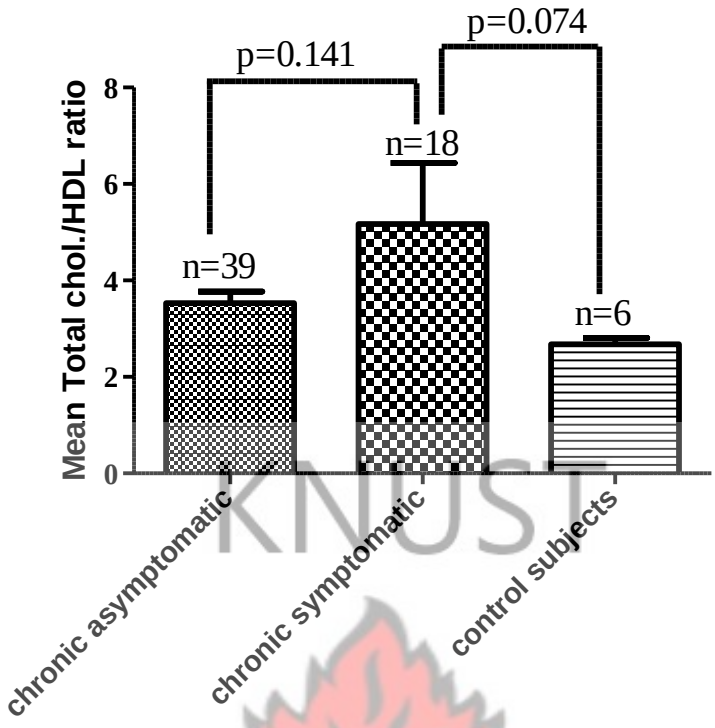

Fig 4.5c

Mean serum total cholesterol, HbeAg status, and total cholesterol / HDL ratio among the chronic hepatitis B-infected patients and healthy control.

values are expressed as mean ± S.D

4.6 Serum triglyceride levels among the respondents

Figures 4.6a and b compare the serum triglyceride levels in HBV-infected patients. There was an observed lower level of serum triglyceride among the infected patients than the healthy patients.The mean serum triglyceride level was significantly reduced in the CHB-infected subjects, in relation to the healthy control subjects (p=0.0041). However, between the patients at the symptomatic and asymptomatic stages, there was no significant difference (p=0.2692). A similar pattern is observed in Fig.4.6b, which shows a reduction in mean serum triglyceride levels between the HBV-infected patients and the healthy control population (p=0.0051). this observed reduction in serum triglyceride among the HBV-infected patients was further found to be HbeAg independent (p=0.4018) (Fig.4.6b).

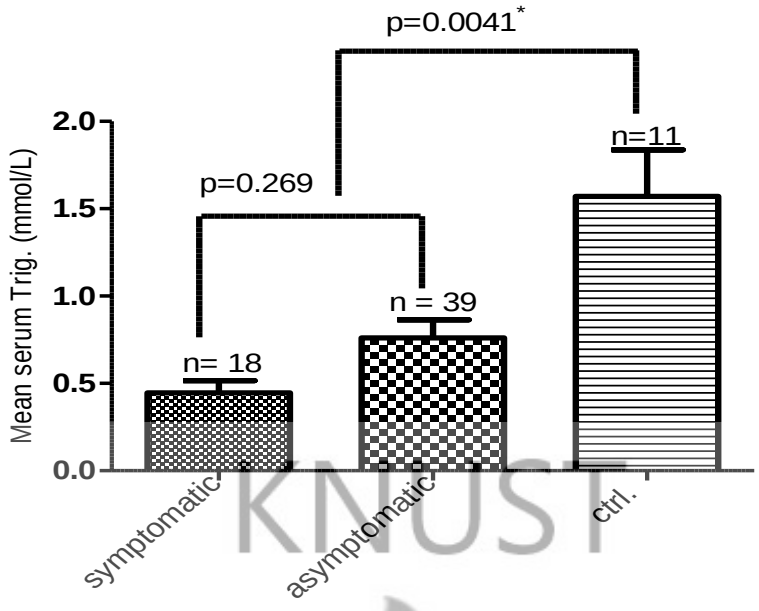

Fig. 4.6a

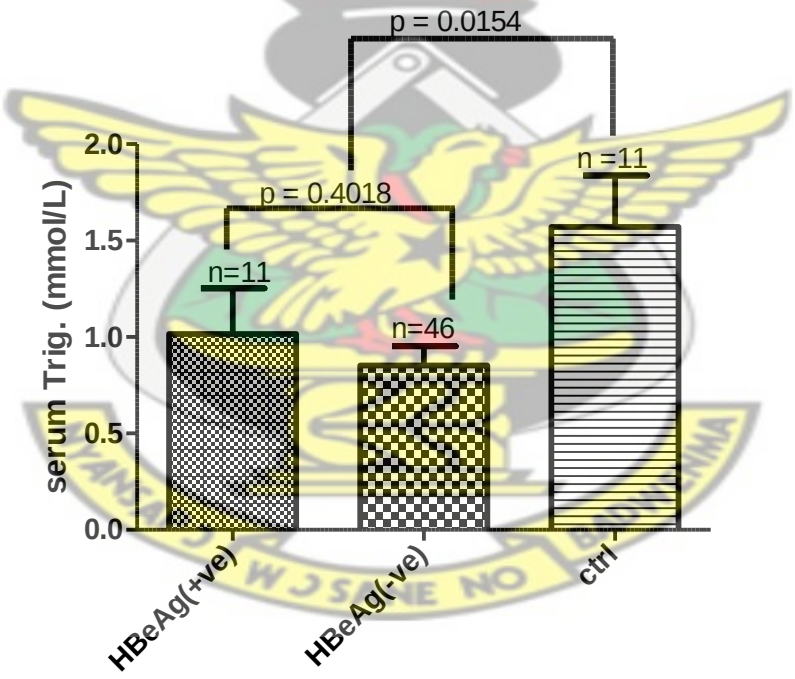

Fig. 4.6b

Mean serum triglyceride levels, disease stages, and HBeAg status of chronic hepatitis B - infected patients and control population.

Values expressed as mean ± S.D

** p-value significant*

4.7 Serum levels of HDL, LDL, and HDL/LDL ratio among sample population

From figure 4.7a, the mean serum HDL levels were lower among the HBV-infected subjects than the healthy control subjects, even though this is not statistically significant (p=0.0820). Fig. 4.7b shows marginal increase in mean serum LDL among the CHB-infected subjects in relation to the healthy control subjects though not statistically significant (p=0.7373). The mean serum LDL levels were 4.83 ± 3.86, 4.39 ± 1.01, and 3.98 ± 1.12mmol/l for the chronic symptomatic, chronic asymptomatic and healthy control subjects, respectively. The figure 4.7c compares the respective cardiovascular risk (HDL/LDL ratio) among the study population. The CHB-patients had lower coronary risk measured as compared to the healthy control even though this was not significant statistically (p = 0.191).

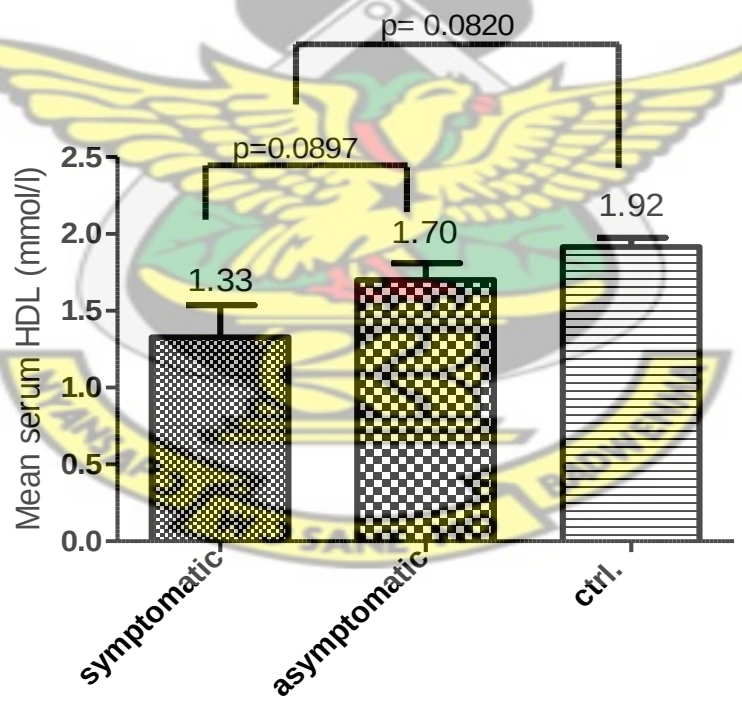

Fig. 4.7a

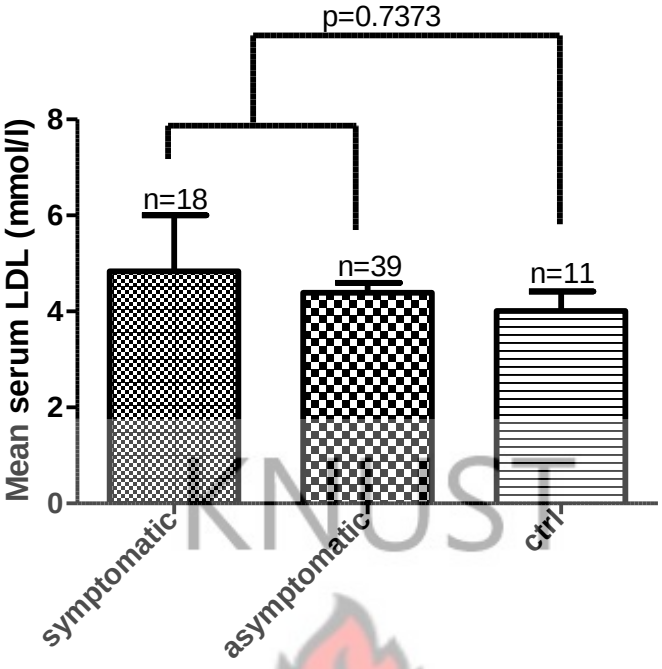

Fig. 4.7b

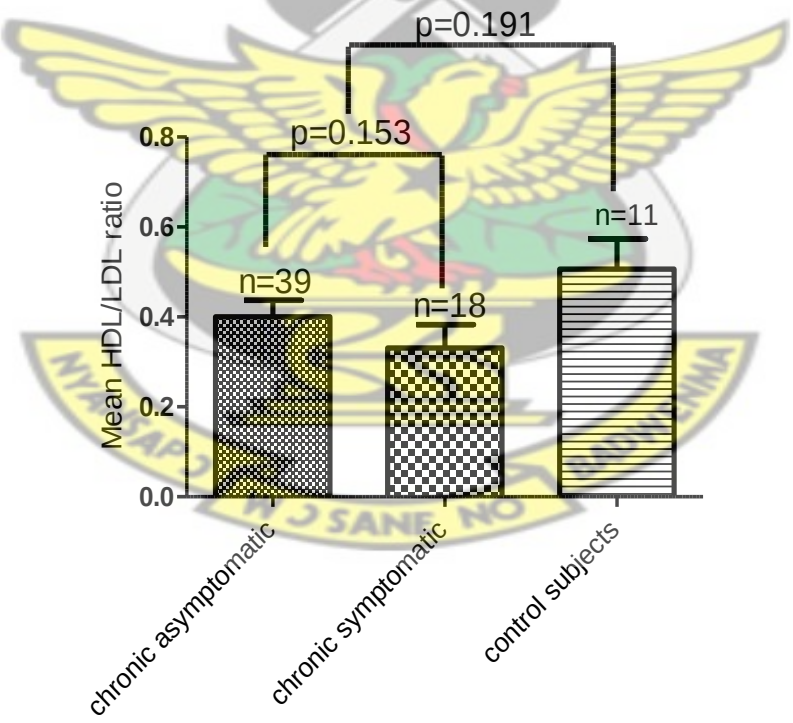

Fig. 4.7c

Measures of mean serum HDL, LDL, and HDL/LDL ratio (± S.D) among chronic hepatitis B-infected patients compared with the healthy control

Values are expressed as mean ± S.D

CHAPTER FIVE

DISCUSSION

5.1 Gender, HBeAg status, and distribution of some selected clinical symptoms among the CHB-infected population

Gender differences prevail in the infections caused by the Hepatitis B virus. Male dominance in chronic HBV – infection and disease type is shown in Tables 4.1 and 4.3. These results show that the male gender may be more vulnerable to developing symptomatic hepatitis than females, suggesting that the Hepatitis B virus may interact with the male and female host tissues in different ways and consequently to different degrees. Similar outcome has been found with CHB-related hepatocellular carcinomas (HCC) (McMahon *et al.*, 1990; Yu and Chen., 1994) and even a more pronounced gender disparity was revealed by work of Baig Saeeda (2009). From a study involving 472 patients with HBV infection, Baig reported that the frequency of hepatic infection in males was 79.5% (n=375) and in females 20.5% (n=97), with a male to female ratio of 3.8:1. Out of the 472 patients, 49% had acute hepatitis, 26% were carriers, 18% had chronic hepatitis, 6% had cirrhosis and 3% patients had hepatocellular carcinoma. Male dominance was found to be consistent in all categories of patients. When the patients were divided into groups according to age, the male to female ratio increased during the reproductive years, suggesting a probable influence of estrogen in the protection and defence of hepatic cells against the development of chronic liver disease (*ibid*).

In the advanced stage of the disease, significant inflammation and damage of the liver occurs. This constrains the ability of the liver to recycle bilirubin and iron (Fe) from hemoglobin for erythropoiesis, resulting in the net accumulation of bilirubin and reduction in erythrocyte level in the blood of the CHB-infected patients. This may account for the high incidence of

pallor, jaundice, and hepatomegaly among the chronic symptomatic patients compared to the chronic asymptomatic and healthy control subjects, as observed from table 4.2.

5.2 Serum ALT and AST levels in CHB infected patients

The main site of infection by the hepatitis viruses, the liver, contains the transaminases which play critical roles in synthesis, breakdown, and recycling of endogenous nitrogenous compounds, such as amino acids. These enzymes are compartmentalised in different locations in the hepatic parenchymal cells. They include alanine transaminase (ALT) and aspartate transaminase (AST). They are found in the serum at low concentrations. However, in diseased state, as with HBV infection, a localized autoimmune reaction mediated by MHC-1/Hepatitis B surface Protein (HBSP) complex leads to degeneration of the liver tissue (Philip *et al.,* 2006). During the apoptotic process, the cell membranes become more permeable and these enzymes leak into circulation, via nearby vascular vessels and thus increasing the serum levels of these transaminases (Altiparmak, *et al.,* 2005). Even though the serum levels of ALT is a more specific biomarker for liver damage *(ibid),* a combination of the basal serum AST and ALT levels provides a better precision at indicating the etiology of liver damage as in HBV infection (Sheth *et al.,* 1999; Sorbi *et al.,* 1999). As evident from Figs. 4.3a and Fig 4.4a, there was an observed significant increase in the serum levels of ALT and AST between the symptomatic and asymptomatic chronic hepatitis B patients ($p=0.0001$) and ($p < 0.0001$) respectively. The serum levels of these transaminases were higher in the chronic symptomatic stage; so patients in this stage of the infection supposedly have a more profound damage of the liver due to the spread of the viral infection, replication, and other cytopathic processes. There was however, no statistically significant difference in the mean serum levels of AST and ALT occurring between the chronic asymptomatic and the healthy volunteers ($p = 0.546$) and ($p = 0.702$) respectively. For the chronic asymptomatic carriers, an initial aggressive host

immune response and viral adaptation may likely reduce the rate of viral replication and activity in host which helps to keep the liver in stable condition as with the healthy non-infected hosts (Philip *et al.,* 2006).

These observed changes in serum transaminases were dependent on the viral serology (HBeAg status) as shown by Fig. 4.3b and Fig. 4.4b. Chronic hepatitis B virus (HBV) infection is characterized by persistent serum level of HBV surface antigen (HBsAg), IgGanti-core antibody (anti-HBcAb), and detectable HBV DNA. Hepatitis B envelope antigen (HBeAg) is often detectable but may disappear after seroconversion to its antibody (anti-HBe) (Hadziyannis and Vassilopoulos, 2001). The serological presence of HBeAg in serum is suggestive of an active HBV infection. The HBeAg is often associated with active and progressing liver disease, whereas seroconversion to anti-HBeAg often coincides with considerable decline of viral DNA, normalization of alanine aminotransferase (ALT), and clinical remission *(ibid).* It has been reported that HBV replication is highly related to cell death, which is different from the widely accepted non-cytopathic characteristics of HBV (Tsai *et al.,* 1992). This was further validated by Milich *et al.,* (1997) whose work revealed that two viral proteins (HBxP and HBsP) produced in higher titers during replication actively induce apoptosis in host hepatocytes. It can be inferred that higher liver damage occurs during the active viral replicative stage, as evident by the relatively elevated mean serum ALT and AST levels in the HBeAg (+ve) state of the infection, in relation to those who were HBeAg (-ve) and healthy volunteers. Comparatively, the levels of AST were relatively higher than ALT among all the categories of patient populations. Generally, the AST enzymes occur both in the cytosol and mitochondria unlike ALT which occur mainly in the cytosol. In cases of chronic liver abscesses, AST leak from both the cytosol and mitochondria hence their levels are higher than that of the ALT enzymes as observed in our studies.

Whereas the serum levels of the enzymes AST and ALT are predictive of the presence or absence of liver disease, their ratio (AST/ALT) gives more clue to the functional integrity of the diseased liver. It has been suggested that, irrespective of the aetiology, the AST/ALT ratio increases above one in cases of liver cirrhosis (William and Hoofnagle, 1988). Even though the reason for this observation is unknown, it has been suggested that sinusoidal clearance of serum AST decreases in cirrhosis patients (Nyblom *et al.,* 2004; Nyblom *et al.,* 2006; Park *et al.,* 2000; Alempijevic *et al.,* 2009). Our study showed averagely higher AST/ALT ratio among the CHB patients compared to the healthy control perhaps due in part to mild to significant hepatic cirrhosis among the CHB-infected cohorts. Indeed, several studies have shown that AST/ALT ratio increases above 1 in most cases of chronic liver diseases including chronic active hepatitis B (McClatchey, 2002).

Most (94.4%) of the symptomatic CHB-patients who showed any of three overt clinical symptoms had AST/ALT ratio above 2.0 according to fig. 4.1. This observation suggests that at least a significant liver damage may have to occur during the infection before the manifestation of any of the clinical symptoms. This finding is quite novel since not much has been done with regard to hepatitis research in relating serum biochemical indices with clinical symptoms of the disease. There was no statistically significant relationship between the mean serum AST/ALT ratios and HDL/LDL ratio, according to table 4.4, despite the strong relationship between the AST/ALT ratio and liver disease observed earlier from fig. 4.4c.

5.3 Serum triglyceride distribution in CHB-infected patients

Fig. 4.6a shows a significant difference ($p = 0.0041$) between the triglyceride level in sera of patients in the various stages of the disease progression. There is a more marked decrease in serum triglyceride among HBV-infected patients than healthy volunteers. This observation shows that chronic HBV hepatitis is inversely associated with serum triglyceride as has also been reported in the work of Shlomai and Shaul; (2009). This could be due to an interplay of several factors, including: a) interference in hepatic fatty acid synthesis, as a result of an active interaction of the hepatitis B X protein (HBxP) and host hepatic metabolic genes such as SREBP, ChREBP, PGC-1 alpha, which switch hepatic metabolism from fatty acid synthesis and secretion via lipoproteins to increased hepatic lipid synthesis and storage - a condition required to facilitate HBV replication in the host (Shlomai andShaul, 2009; Kim *et al.*, 2007), b) decreased secretion of the triglycerides by the liver following the infection. Thus, the liver becomes more adipogenic, sequestering most of the lipids that diffuse into it, thereby creating a steatotic condition as has been reported by some researchers (Altiparmak *et al.*, 2005; Thormopuolos *et al.*, 2006; Tsochatzis *et al.*, 2007; and Cindoruk *et al.*, 2007). Alternatively, the decreased basal serum triglyceride may be due in part, to interference by overload of toxins or other agents such as endogenous mercapatans produced by the cirrhotic liver as have been recorded in patients with liver diseases (Al Mardini *et al.*, 1984). In part, this variation in serum triglyceride levels could also be due to the host physiological response to the infection, owing to the prolonged surge in pro-inflammatory cytokines, such as TNF-α, IL-1, IL-2, IL-6, etc. following the chronic state of the viral infection (Grunfeld *et al.*, 1991; Naeem *et al.*, 2001; Khovidhunkit *et al.*, 2004). These factors may have worked to different extents at the various stages of the disease progression and thus accounting for the disjointed pattern of triglyceride distribution among patients.

According to Figs. 4.6a, and Fig. 4.6b serum triglyceride of the CHB patients was independent of HBeAg status (HBeAg –ve and HBeAg +ve) (p=0.4018). This practically eliminates the effect of the HBeAg protein in the triglyceride metabolism of CHB hosts. However, further studies into this observation are recommended to validate this observation. There was a significant decrease in mean serum triglyceride levels among the CHB patients (chronic symptomatic and chronic asymptomatic) compared to those of the healthy control subjects (p=0.0154). However, the trend showed an increase in the serum triglyceride levels among the symptomatic, through asymptomatic to the healthy control subjects according to fig. 4.6a.

5.4 Serum total cholesterol, lipoproteins (LDL and HDL) levels, and HDL/LDL ratio for the chronic hepatitis B – infected patients.

Generally, most factors or disease conditions that affect serum lipoprotein distribution in hosts would invariably affect serum total cholesterol level and vice versa. This explains the observed unchanged serum total cholesterol and lipoprotein levels with the pathological stages of the CHB disease, even in comparison with the healthy control. Fig.4.4a, together with Figs. 4.6 and 4.7 show no significant differences in the mean serum total cholesterol levels (p=0.6810), LDL (p= 0.7373), and HDL (p= 0.0820) respectively among patients in either stages of the CHB disease, in comparison to the healthy control. This may be due to the absence of an appreciable interaction of viral processes in host with the metabolic machinery involved in cholesterol and lipoprotein repackaging and release by infected- hepatic tissues in host. This finding was contrary to that observed by Su *et al*, (2004) who reported lowered serum total cholesterol and HDL among the chronic asymptomatic hepatitis B – infected patients from Taiwan.

In the fasting state, cholesterol in liver is packaged with triglycerides and apoproteins into very-low density lipoproteins (VLDL) which are released into circulation. At target sites, the VLDL offloads its triglycerides and become LDLs in the bloodstream. Free circulating cholesterol from tissues are mopped up by HDL and transported to the liver for recycling into bile salts and secreted in bile for excretion. Thus, the bulk of serum total cholesterol comes from circulating lipoproteins (LDL and HDL). Hence, the higher the levels of these circulating lipoproteins, the more elevated the serum level of total cholesterol. Nonetheless, serum total cholesterol offers not much diagnostic value until it is complemented with the serum profile of lipoproteins, to show which is contributing more or less to the total serum cholesterol. While elevated serum cholesterol with elevated HDL mean wellness, elevated LDL and serum cholesterol could pose danger and increased risk to several cardiovascular-related complications (Naeem *et al.,* 2001).

Estimation of cardiovascular risk has become the cornerstone of cardiovascular disease prevention. Although atherogenesis is a multifactorial process, abnormalities in lipoprotein metabolism are one of the key factors, representing around 50% of the population-attributable risk of developing cardiovascular disease (Yusuf *et al.,* 2004). The total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the HDL/LDL cholesterol ratio are two important components and indicators of cardiovascular risk, the predictive value of which is greater than the isolated parameters (Millán *et al.,* 2009). The evidence derived from large observational studies, including the Framingham Study (Castelli *et al.,* 1986), the LRCP (Grover *et al.,* 1994), and the PROCAM (Assmann *et al.,* 1989; Assmann *et al.,* 1998) suggests that the total/HDL cholesterol ratio is a more powerful cardiovascular risk predictor than independently used total cholesterol, LDL cholesterol and HDL cholesterol. As a rule, the ideal total cholesterol/HDL should be 4 – 4.5

unlike the HDL/LDL ratio (coronary risk) which should ideally be 0.4 – 0.5. Above these range of values, one is said to be more prone to cardiovascular disease. Our study did not find any significant differences in the measure of HDL/LDL (coronary risk) (p= 0.153) and Total/HDL cholesterol ratios (p=0.141) among the CHB-infected patients in comparison with the healthy control population. However, the CHB-patients had higher mean total/HDL cholesterol ratio and lower mean HDL/LDL ratio, compared to those of the control subjects. Hence these ratios would offer a poor prognostic value in monitoring the course of chronic HBV-infection in host.

CHAPTER SIX

CONCLUSIONS

Altogether, the study has revealed the following;

a) serum transaminases (ALT and AST) and their ratio (AST/ALT ratio) are elevated in CHB-infected patients with active infection, as compared to those with inactive infection and can be used together with other diagnostic indicators in measuring extent of liver damage in such group of patients as a reliable diagnostic index.

b) serum HDL, LDL, total cholesterol, HDL/LDL and total cholesterol/HDL ratios are not significantly affected by the presence or absence of the infection.

c) there is decrease in serum triglyceride levels among CHB infected patients, but this is HBeAg-independent.

6.1 RECOMMENDATIONS

Despite the reasonably elaborate nature of this study, it still failed to answer certain questions concerning lipid metabolism and the CHB disease. For instance, it lacks enough data on the pattern of fatty acid constituents of the triglyceride in diseased hosts. It is therefore, recommended that further studies should go into the serum fatty acid distribution among the different stages of hepatitis B infection in hosts.

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APPENDIXONE

TABLES OF RESULTS

Table A1.: HBeAg serology and gender of chronic HBV- infected population

	Chronic symptomatic		Chronic asymptomatic	
	HBeAg (+ve)	HBeAg (-ve)	HBeAg (+ve)	HBeAg (-ve)
Males	5	13	3	19
Females	1	3	1	12
Total	6	16	4	31

Table A2:Mean values of some selected clinical indices of liver function and lipid profile of the chronic HBV-infected patients vrs healthy controls.

Indices	***Chronic symptomatic***		***Chronic asymptomatic***		***Control***	***p-value***
	HBeAg(+ve)	HBeAg (-ve)	HBeAg(+ve)	HBeAg(-ve)		
	N=6	N= 16	N=4	N=31	N=11	
AST (IU/l)	322.7 (±110.2)[a]	261.5 (±186.1)[a]	32.7 (±18.9)[a]	28.7 (±19.9)[a]	9.4 (± 2.8)	0.0030[a]
ALT (IU/l)	183.0 (±201.7)[b]	151.4 (±178.0)[b]	13.5 (± 6.7)[b]	12.2 (± 6.5)[b]	26.5 (± 5.7)	0.0001[b]
Chol. (mmol/l)	4.55 (± 0.99)	5.52 (± 3.69)	5.25 (± 0.54)	5.39 (± 1.28)	4.70 (± 0.71)	0.7290
Trig. (mmol/l)	1.21 (± 0.91)[c]	1.07 (± 0.66)[c]	0.72 (± 0.31)[c]	0.76 (± 0.63)[c]	1.69 (± 0.83)	0.0100[c]
HDL (mmol/l)	1.43 (± 1.07)	1.28 (± 0.59)	2.00 (± 0.52)	1.66 (± 0.54)	1.92 (± 0.16)	0.2270
LDL (mmol/l)	3.60 (± 0.94)	5.30 (± 4.49)	3.82 (± 1.51)	4.47 (± 0.94)	3.98 (± 1.12)	0.7170

Values in table above are presented in the form (Mean ± S.D). [a,b,c] *Significant at p<0.05*

APPENDIX TWO

QUESTIONNAIRE FOR STUDY

STUDY ON THE CHANGES IN LIPID METABOLISM DURING PROGRESSIVE LIVER DAMAGE IN HEPATITIS B INFECTION.

QUESTIONNAIRE FOR VOLUNTEERS

CODE:……
DATE: ……..

NAME ……

AGE: ……years

OCCUPATION ……

RESIDENCIAL ADRESS: ……..

……..

Personal Phone no. ……..

Email address (if applicable) ……

HEIGHT: ……..m **WEIGHT**: ……..kg **BMI**: ……..kg/m^2

CLINICAL DETAILS

a) HBV SEROLOGY

INDICES	STATUS
HBsAg	
HBsAb	
HBeAg	
Anti-HBeAg (HBeAb)	
HBcAb	

b) Liver ALT …….IU/ml

c) Viral Load (HBVDNA) …….. IU/ml

- **FOR OFFICIAL USE ONLY**

CATEGORY OF DISEASE STATE: ……..