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SERUM LEVELS OF INFLAMMATORY MARKERS IN HEPATITIS B VIRUS INFECTION

BY

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SERUM LEVELS OF INFLAMMATORY MARKERS IN HEPATITIS B VIRUS INFECTION

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BY

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MAY, 2016

DECLARATION

I hereby declare that this is my own work towards the Master of Philosophy in Immunology and that, to the best of my knowledge, it contains no material previously published by another person or materials which have been accepted for an award of any other degree in another University, except where due acknowledgement has been made in this thesis.

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Certified by

Prof. (Mrs.) Margaret T. Frempong (Supervisor)	Date

Prof. Francis Agyemang Yeboah (Head of Department)	Date

DEDICATION

This thesis is dedicated to my wonderful and supportive wife Mrs. Abenaa Ayisaa Domfeh for her spiritual support and encouragement throughout my programme. Further, to my lovely twins Ohene Agyei Domfeh and Ohenewaa Agyei Domfeh.

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ABBREVIATIONS AND DEFINITIONS

AAR	Aspartate-Alanine Transaminase Ratio
AHB	Acute Hepatitis B Virus Infected Persons
AIDS	Acquired Immunodeficiency Syndrome
ALAT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
Anti-HBc	Hepatitis B Virus Core Antibody
Anti-HBe	Hepatitis B Virus Envelope Antibody
Anti-HBs	Hepatitis B Virus Surface Antibody
AP	Age-Platelet
APRI	Aspartate Transaminase Platelet Ratio Index
ASAT/AAT	Aspartate Aminotransferase
AST	Aspartate Transaminase
AUROC	Area under Receiver Operating Characteristic Curve
CDC	Centre for Disease Control and Prevention
CHB	Chronic Hepatitis B Virus Infected Persons
CHB1	Chronic Hepatitis B Virus Infected Persons with Inactive Infection
CHB2	Chronic Hepatitis B Virus Infected Persons with Active Infection

CRP	C-Reactive Protein
DNA	Deoxyribonucleic Acid
DNPH-ine	Dinitrophenylhydrazine
DNPH-one	Dinitrophenylhydrazone
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
FIB-4	Fibrosis 4 Index
GGT	Gamma-Glutamyl Transferase
GOT	Glutamate Oxaloacetate Transaminase
HB	Haemoglobin
HBcAg	Hepatitis B Virus Core Antigen
HBeAg	Hepatitis B Virus Envelope Antigen
HBsAg	Hepatitis B Virus Surface Antigen
HBSP	Hepatitis B Virus Surface Protein
HBV	Hepatitis B Virus
HBx/HBxP	Hepatitis B Virus x Protein
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus

HIV	Human Immunodeficiency Virus
ID	Identification
Ig	Immunoglobulin
IL-6	Interleukin 6
LDH	Lactate Dehydrogenase
LFT	Liver Function Test
LYM	Lymphocyte
MDH	Malate Dehydrogenase
MHC	Major Histocompatibility Complex
MoH	Ministry of Health
Na^+	Sodium
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (Reduced)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Reduced)
NALFD	Non-Alcoholic Fatty Liver Disease
NEU	Neutrophil
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
PAMPs	Pathogen-Associated Molecular Patterns

PBS	Phosphate Buffered Saline
PLT	Platelet
PRRs	Pattern Recognition Receptors
RNA	Ribonucleic Acid
SEM	Standard Error of the Mean
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SPSS	Statistical Package for Social Scientist
STI	Sexually Transmitted Infection
TLRs	Toll-Like Receptors
TMB	Tetramethylbenzidine
USA	United States of America
WBC	White Blood Cell

ABSTRACT

Currently, the drugs used in the management of chronic hepatitis B virus (HBV) infection do not have anti-inflammatory properties. Hence, this study was relevant to establish the presence of liver inflammation in drug naive HBV infected persons in Ghana to support the proposal for the inclusion of anti-inflammatory drugs in the management of the infection to reduce the risk of liver fibrosis which may occur due to the chronic liver inflammatory process. A total of 210 participants were recruited for this cross-sectional study, comprising of 146 HBV infected persons (males: 74.7% and females: 25.3%) and 64 controls (males: 79.7% and females: 20.3%). The HBV infected persons and healthy controls were recruited from the clients visiting the Medilab Diagnostics Services Limited and Regional Blood Transfusion Centre, Kumasi respectively. Ethical clearance was obtained from the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. Each participant gave a written informed consent to take part in the study after verbal and written explanation of the methods and risks involved had been given. Information on socio-demographic characteristics and medical history were obtained with standardised questionnaires which was administered to them. Venous blood samples were collected from the participants and assayed for haematological parameters (haemoglobin, WBC, lymphocytes neutrophils and platelets), biochemical parameters (AST, ALT, ALP, GGT and albumin) and inflammatory makers (CRP and IL-6). Some non-invasive markers of liver fibrosis (AAR, APRI and FIB-4) were also calculated. Further, anti-HBc IgM and anti-HBc was estimated qualitatively in the HBV infected persons to determine the presence of acute and chronic HBV infection. Also, HBeAg was estimated qualitatively to determine the presence of active and inactive HBV infection among the participants. The data obtained was analysed using the Statistical Package for Social Scientist (SPSS) Statistical Software (version 16.0, SPSS Inc., Chicago, IL, USA). The mean age of the HBV infected individuals (38.17±0.78 years) was not significantly different ($p=0.115$) from those of the control group (36.13±0.76 years). Out of the 146 HBV infected participants, 81.5% (CI: 74.2 - 87.4) were having acute infection whereas 18.5% (CI: 12.6 - 25.8) were having chronic infection. Overall, the chronic HBV infected individuals with active infection was 5.5% (CI: 2.4 - 10.5). The levels of CRP in the HBV infected individuals were significantly ($p<0.001$) increased as compared to the control group. This suggests an increased in liver inflammation as the HBV infection progressed in the HBV infected individuals. The levels of

IL-6 pattern in the participants showed consistency with the CRP levels. The levels of AST ($p<0.001$) and ALT ($p<0.001$) in the HBV infected persons were markedly increased as compared to the control group, signifying an increase in liver injury as the infection progressed in the HBV infected persons. Further, the non-invasive markers of liver fibrosis scores in the HBV infected persons were significantly ($p<0.001$) increased as compared to the control group, suggestive of an increase in liver fibrosis as the infection progressed in the HBV infected persons. The haematological assays revealed significant ($p<0.001$) anaemia and leucocytosis in HBV infected persons compared to the control group, confirming some degree of anaemia and leucocytosis in the HBV infected persons. The levels of AST ($r=0.856$), ALT ($r=0.909$) and IL-6 ($r=0.959$) showed positive significant ($p<0.001$) associations with the levels of CRP in the chronic HBV infected persons. Similarly, levels of AST ($r=0.799$) and ALT ($r=0.855$) showed positive significant ($p<0.001$) associations with the levels of IL-6 in the chronic HBV infected individuals. The findings of this study support the assertion that chronic liver inflammation in HBV infected individuals is aggravated by increases in viral activities. The increase in the viral activities causes the release of IL-6 which in turn triggers the synthesis of CRP, and the damaged hepatocytes result in the leakage of transaminases into the bloodstream causing the serum levels of these markers to rise as observed in the study. The chronic liver inflammation, if left untreated, may lead to liver fibrosis. Therefore, the inclusion of antiinflammatory drugs in the management of HBV infection may be relevant to suppress the chronic liver inflammatory process and improve liver function.

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CHAPTER ONE INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Hepatitis B is an infectious disease of the liver which is caused by the hepatitis B virus (HBV) (Barker *et al.,* 1996). About one-third of the population worldwide has been infected at one point in their lives, including 350 million people who are chronic carriers (Schilsky, 2013). In Ghana, the reported sero-prevalence of the hepatitis B virus surface antigen (HBsAg) are 9.6% among blood donors visiting the Kintampo Municipal Hospital (Walana *et al*., 2014), 11.5% among women of childbearing age (Goldstein *et al*., 2005), 11.6% among replacement blood donors and 10.8% among voluntary blood donors visiting the Tamale Teaching Hospital (Dongdem *et al*., 2012). A study conducted by Amidu *et al.* (2012) in three sub-populations (Garrison, Aboabo and Tafo) in Kumasi, also showed an overall prevalence of 8.68%.

The virus is transmitted by an exposure to infected blood or body fluids such as vaginal fluids and semen (Kidd-Ljunggren *et al.,* 2006). Perinatal transmission is the major route of infection (Chang, 2007). Other risk factors associated with the hepatitis B viral infection include acupuncturing, working in a healthcare setting, dialysis, tattooing, transfusions, and sharing toothbrushes or sharp objects with an infected person (Pungpapong *et al.,* 2007).

The HBV infection has diverse clinical conditions depending on the age at which the individual is infected, and the immune status of the individual (Williams, 2006). During the incubation period of the infection (6 – 24 weeks), the individual may feel unwell with possible nausea, anorexia, headache, vomiting and diarrhoea. Most adults with the infection recover completely but others, about 5 – 10%, do not clear the virus and therefore become asymptomatic carriers or develop chronic hepatitis (Schilsky, 2013). Moreover, individuals with chronic infection have about 15 – 40% risk of developing conditions such as liver cirrhosis, liver failure, and/or

hepatocellular carcinoma (Lok and McMahon, 2007). During hepatitis (liver inflammation), serum levels of aspartate and alanine transaminases are raised (Nyblom *et al.,* 2006). These enzymes are associated with the liver parenchymal cells and are useful biomarkers of liver injury (Nyblom *et al.,* 2006).

Some proteins are often released during the inflammatory process and are readily identified in the blood, hence called inflammatory markers (Pepys and Hirschfield, 2003). The most frequently used biomarker of inflammation is the C-reactive protein (Loffreda *et al*., 1998). This acute phase protein is produced by the liver cells in response to pro-inflammatory cytokines mainly interleukin 6, which are released by macrophages and T cells to stimulate immune responses (Pepys and Hirschfield, 2003).

Anti-inflammatory drugs are designed for remedying pain and reducing inflammation thereby slowing the tissue-damaging process (Mathison *et al*., 1994). Non-steroidal anti-inflammatory drugs and most analgesics have anti-inflammatory properties and are suitable for the management of both acute and chronic inflammatory disorders (Mathison *et al*., 1994). Thus the appropriate administration of anti-inflammatory and/or hepato-protective medication would be significant in the management of hepatitis (Chen *et al*., 2002). The role of aspirin was studied by Sitia *et al.* (2012) in rodents with chronic necro - inflammatory liver disease, and their study showed that aspirin was more effective in the rodents with hepatitis B virus - related carcinogenesis as compared to those with chemical liver carcinogenesis.

1.2 RATIONALE OF THE STUDY

The HBV mainly affects the functions of the liver by multiplying in the liver cells leading to hepatitis (Glebe and Urban, 2007). The inflammatory process comprise of tissue breakdown

and repair attempts, therefore the high tissue turnover in chronic active hepatitis often lead to scarring and hepatocytes damage (Serhan and Savill, 2005). Hence if the liver inflammation is not managed well, it could lead to liver fibrosis and finally cirrhosis. In cirrhosis, the hepatocytes die and are progressively replaced with fibrotic tissues. Also, the internal structure of the liver is disturbed leading to the blockade of blood flow to the liver and decrease in the liver function (Trepo and Guillevin, 2001). Therefore, the treatment of the liver inflammation may be essential to decrease the risk of liver fibrosis and cirrhosis.

In the management of the HBV infection in Ghana, the Ministry of Health (MoH) recommends rest, plenty of fluids and any food that the patient can tolerate as a non-pharmacological treatment and vitamin B preparation for pharmacological treatment (Ministry of Health, 2010). The National HIV/AIDS/STI Control Programme also recommends the combination of Efavirenz (TDF), Lamivudine (3TC) and Tenofovir (EFV) as the preferred first line regimen for the management of HBV co-infection with HIV whereas the alternate combination is Efavirenz, Emtricitabine and Tenofovir (National HIV/AIDS/STI Control Programme, 2010).

The drugs recommend by the National HIV/AIDS/STI Control Programme and the Ministry of Health halt the virus from multiplying and also boost the immune system (Lai and Yuen, 2007). However, these drugs do not have anti-inflammatory properties. There is therefore the need to assess the presence of liver inflammation and liver injury in drug naive HBV infected persons in Ghana to support the proposal for the inclusion of anti-inflammatory drugs in the management of the infection to prevent liver fibrosis and subsequent cirrhosis.

1.3 PROBLEM STATEMENT

Based on the current research available, there is limited information on the serum levels of inflammatory markers and biomarkers of liver injury in drug naive HBV infected persons in

Ghana. It would be therefore relevant to analyse the serum levels of inflammatory markers to confirm the presence of liver inflammation, and finding their correlation with biomarkers of liver injury in Ghanaian drug naive HBV infected individuals to support the proposal for the inclusion of anti-inflammatory drugs in the management of the infection.

1.4 RESEARCH QUESTIONS

1.4.1 What are the percentages of acute and chronic infections among drug naive HBV infected individuals?

1.4.2 Is there any association between drug naive HBV infection and liver inflammation, liver fibrosis and anaemia?

14.3 Are there any associations between the markers of inflammation and liver injury among drug naive HBV infected individuals?

1.5 STUDY HYPOTHESIS

Both acute and chronic drug naive HBV infections are associated with liver inflammation, liver fibrosis and anaemia, therefore anti-inflammatory drugs should be included in the management of the HBV infection to reduce the risk of cirrhosis.

1.6 AIM AND OBJECTIVES OF THE STUDY

It is worth investigating the liver inflammation, connected to the pathogenesis of the HBV infection, which leads to liver fibrosis and cirrhosis. Currently, the definitive treatment for HBV infection do not suppress the liver inflammation. Therefore this study, seeks to analyse the

serum levels of inflammatory markers to confirm the existence of liver inflammation in HBV infected persons, and finding their correlation with biomarkers of liver injury to support the proposal for the inclusion of anti-inflammatory drugs in the management of the infection in Ghana. It also seeks to estimate the presence of liver fibrosis and anaemia, and establish the percentage of active infection among the drug naive chronic HBV infected persons in Ghana.

The specific objectives include the following:

1.6.1 To determine the percentages of acute and chronic infections as well as active infection among the drug naive HBV infected persons.

1.6.2 To assess relevant haematological and biochemical parameters in the progression of the HBV infection.

1.6.3 To evaluate the presence of liver inflammation and liver fibrosis, and find out if there are any associations between the biomarkers of liver injury and inflammation within the drug naive HBV infected persons.

CHAPTER TWO LITERATURE REVIEW

2.1 THE HEPATITIS B VIRUS

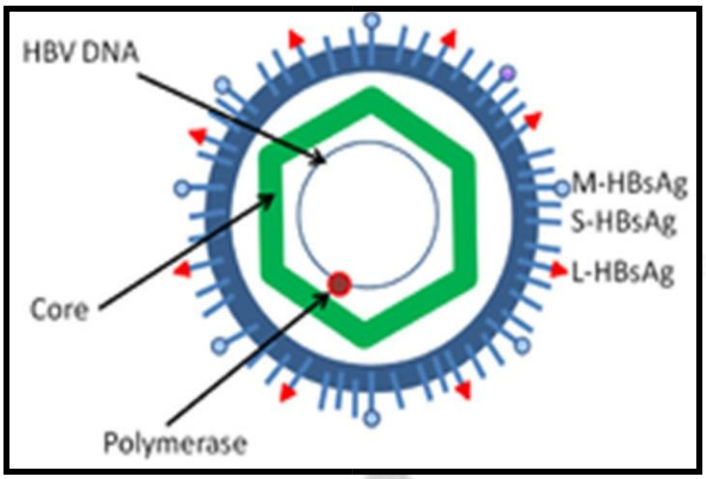

Figure 2.1: The structure of the hepatitis B virus (Courtesy: Locarnini, 2004)

The hepatitis B virus (HBV) belongs to the hepadnavirus family. The virus, which is 42 nm in diameter, consists of a partially double stranded DNA particle with a lipid envelope and an icosahedral nucleocapsid core composed of proteins (**Figure 2.1**). The nucleocapsid contains the viral DNA and a polymerase enzyme which has a reverse transcriptase function like that of the retroviruses (Locarnini, 2004). The embedded proteins in the outer envelope help in viral attachment and entry into susceptible cells (Locarnini, 2004). These embedded proteins are the hepatitis B virus surface antigens (HBsAg). Other viral proteins include the core antigen (HBcAg), envelope antigen (HBeAg), and the transcriptional trans-activating protein (HBx) (Guo *et al*., 2009). The function of the HBx is not well known (Guo *et al*., 2009), but a study had suggested that it plays a role in the activation of the viral transcription (Benhenda *et al*., 2013) (**Figure 2.2**).

2.2 HEPATITIS B VIRUS REPLICATION CYCLE

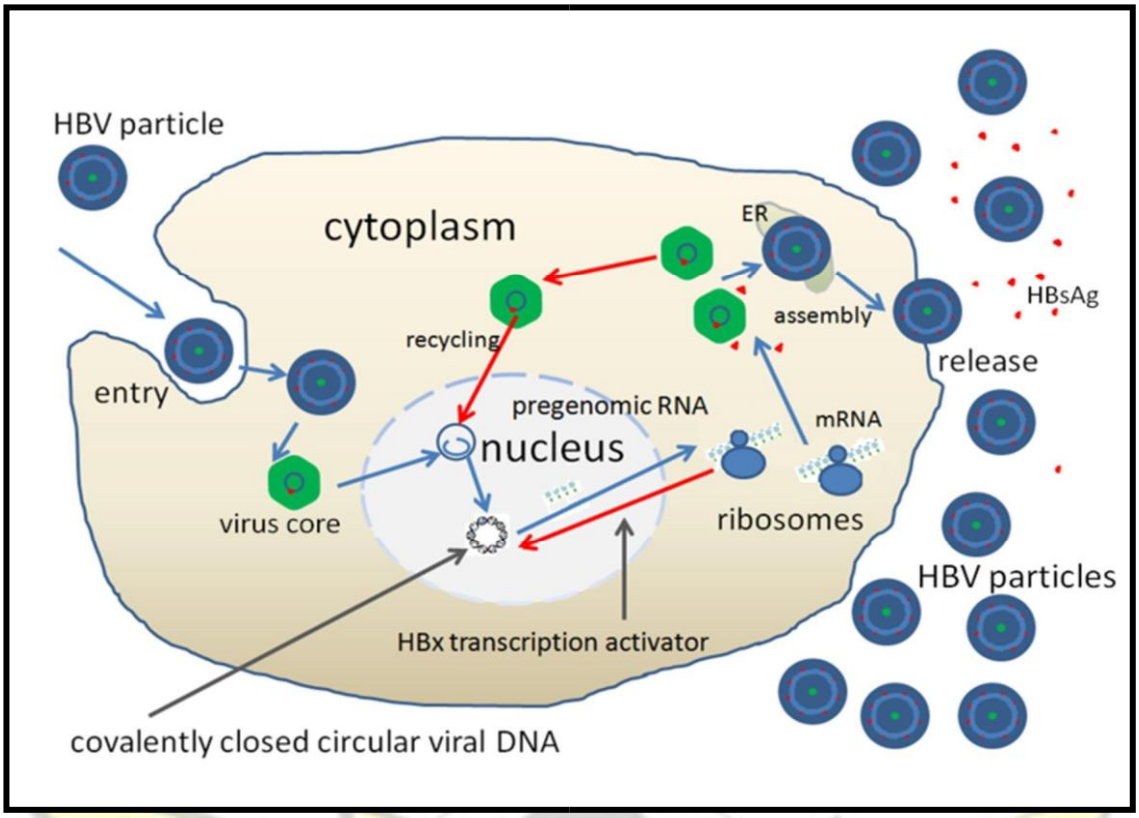

Figure 2.2: The schematic diagram of the hepatitis B virus life cycle
(Courtesy: Beck and Nassal, 2007)

The virus attaches to a receptor on the outside of the liver cell. Some of these hepatocyte surface receptors have been recognised which include the asialoglycoprotien receptor, human liver endonexin and transferrin receptor (Beck and Nassal, 2007). When the nucleocapsid of the virus enters the host cell, it moves to the nucleus where the genome of the virus is delivered. The viral genome then uncoils for the creation of a second-strand DNA by a reverse transcription (Bruss, 2007). The gaps in the old and new DNA strands are mended to produce a covalently closed circular super coiled DNA (**Figure 2.2**). This covalently closed circular DNA then aids as a pattern for the synthesis of four new viral RNAs (Bruss, 2007).

These new viral RNAs are then polyadenylated, and conveyed into the cytoplasm to be decoded into viral precore antigen and capsid (pre-C), DNA polymerase (P), envelope proteins, and

transcriptional trans-activating proteins (HBx) (Robinson, 1995). The envelope proteins are inserted as trans-membrane proteins in the lipid layer of the endoplasmic reticulum (ER) of the host cell. A pregenomic RNA, which is 3.5kb long, is enveloped together with the viral polymerase, protein kinase and HBx in the core. The core is made from the capsid and precore antigen. The pregenomic RNA serves as the pattern for reverse transcription of the negative-strand DNA during viral replication (Ganem and Schneider, 2001).

The new viral nucleocapsids can follow two diverse intracellular pathways. The first pathway leads to the development and release of new virions, while the other leads to the intensification of the viral genome inside the nucleus of the host cell (Mahoney and Kane, 1999). In the pathway of the assembly of the virions, when the nucleocapsids get to the endoplasmic reticulum of the host cell, they join together with the envelope proteins and sprout into the inner space of the endoplasmic reticulum. And from here, the new virions are then released by means of the Golgi apparatus by exocytosis from the host cell (Chisari and Ferrari, 1997).

2.3 THE LIVER AS A TARGET FOR THE HEPATITIS B VIRUS

The virus binds to the cell of the host through the preS domain of the surface antigen of the virus and are afterwards endocytosed by the host cell (Coffin *et al*., 2011). The hepatitis B virus-preS-specific receptors are found mainly on the liver cells; however, viral proteins and DNA have also been identified in some extra-hepatic sites, indicating that cellular receptors for the virus may also be found on extra-hepatic cells (Coffin *et al*., 2011). The cell surface receptor necessary for the entry of the hepatitis B virus into the hepatocytes is the sodium/bile acid cotransporter also known as the Na^+-taurocholate co-transporting polypeptide or the liver bile acid transporter (Yan *et al*., 2012).

2.4 HEPATITIS B VIRUS INFECTION

The hepatitis B virus (HBV) infection has diverse clinical presentations depending on the immune competency and age of the individuals at the point of infection, and the period at which the infection is detected (Williams, 2006). Through the incubation period of the infection (6 – 24 weeks), the person infected may feel unwell with symptoms like; nausea, anorexia, headache, vomiting and diarrhoea. The person may also present with jaundice though the loss of appetite and feverish condition could improve (Williams, 2006).

Occasionally, the infection neither present with jaundice nor noticeable signs and symptoms (Hollinger and Liang, 2001). This asymptomatic case can be recognised by identifying some specific viral serological alterations in the blood of the infected person. Asymptomatic HBV infected persons may become reservoirs and silent carriers of the virus for transmission (Hollinger and Liang, 2001).

2.5 THE TRANSMISSION OF THE HEPATITIS B VIRUS

The modes of transmission of the virus include: contact with an infected person (horizontal), exposure to blood or other infected fluids (parenteral), and mother to child at birth (perinatal) (Kidd-Ljunggren *et al.,* 2006). The surface antigens of the virus has been identified in some body excretions and secretions, but then, the antigens detected in the blood, semen and vaginal fluids have been shown to be infectious (Kidd-Ljunggren *et al*., 2006).

The virus can be transferred between household fomites, sexual partners and in toddler-aged children in groups with the infection (Kidd-Ljunggren *et al.,* 2006). The hepatitis B virus can be stable on inanimate surfaces for a week and therefore, inoculation of the virus can occur

through inert objects, like: toothbrushes, razors, toys, baby bottles, cutlery sets and hospital devices, coming in contact with the open skin or mucous membrane (Chang, 2007).

The persons who are at risk of being infected with the hepatitis B virus include: kids in schools or residential settings together with other infected kids, infants born to infected mothers, healthcare workers, sexual contacts with infected persons, people sharing unsterilised medical or dental devices (Kidd-Ljunggren *et al.,* 2006), people who provide or receive acupuncture or tattooing with unsterilised devices, and inhabitants or persons travelling to endemic countries (Pungpapong *et al.,* 2007).

2.6 PATHOGENESIS OF THE HEPATITIS B VIRUS INFECTION

The HBV infection when acquired earlier in life may possibly result in the chronic form of the infection, followed by liver fibrosis and eventually cirrhosis and/or hepatocellular carcinoma, usually after a period of 30 – 50 years (Coffin *et al*., 2011). The females are more possibly to be infected briefly and to produce antibodies to the hepatitis B surface antigen than males (Coffin *et al*., 2011).

The hepatitis B virus mainly obstructs the functions of the liver by multiplying in the hepatocytes. The hepatitis B virus itself might not be cancer-causing by a direct viral activity, but rather as a result of the host immune response to the virus, and the liver regeneration which continues for many years (Iannacone *et al*., 2005). These pathological mechanisms, particularly if it leads to cirrhosis, could be cancer-causing without the direct oncogenic activity of the virus (Coffin *et al*., 2011). The hepatitis B virus may not be directly cytopathic (Iannacone *et al*., 2005). This is because large amounts of the viral surface antigens could be found in the hepatocytes of some apparently healthy chronic carriers (Coffin *et al*., 2011).

During the pathogenesis of the infection, the immune reaction of the host leads to both liver injury and viral clearance (Iannacone *et al*., 2007). The adaptive immune reaction, specifically the virus-specific cytotoxic T lymphocytes, leads to most of the liver damage related to the HBV infection (Iannacone *et al*., 2007). Cytotoxic T lymphocytes eradicate the virus by killing virus-infected cells, and secreting antiviral cytokines which are used in flushing out the hepatitis B virus from the healthy hepatocytes (Iannacone *et al*., 2007).

2.7 CLINICAL FEATURES OF THE HEPATITIS B VIRUS INFECTION

The quantity of the viral inoculum as well as the age of the individual infected are the main factors that relate to the severity of the HBV infection (Terrault *et al*., 2005). Almost 10% of children as well as 30 – 50% of adults with acute infection are likely to develop icteric disease (El-Serag and Hashem, 2011).

Primary HBV infection may be related to either no or little liver disease, or acute hepatitis with severity varying from mild to fulminant hepatitis (El-Serag and Rudolph, 2007). Hepatitis B virus infection is brief in 90% of adults and about 10% of new-borns. Majority of the acute infection are sub-clinical, but the less than 1% of cases which are symptomatic lead to fulminant hepatitis (Terrault *et al*., 2005). Persistent HBV infection is occasionally linked with histologically normal liver with usual function (El-Serag and Hashem, 2011). But about onethird of persons with chronic HBV infection develop cirrhosis and hepatocellular carcinoma (Lok and McMahon, 2007).

2.7.1 Acute Hepatitis B Virus Infection

The acute hepatitis B virus infection frequently resolves naturally within 4 – 8 weeks. Some HBV infected persons recover without any major consequences and relapse (Terrault *et al.,* 2005). However, a positive prognosis is uncertain, particularly in adults who can develop lethal cases of acute hepatic necrosis (Pungpapong *et al.,* 2007). Children hardly develop acute clinical disease, but those who are infected before seven years of age could become chronic carriers. The incubation phase of the virus usually varies from 45 – 120 days, with a mean of 60 – 90 days. The disparity in the incubation duration is related to the quantity of the viral inoculum, the route of infection and the host immune reaction (Hollinger and Liang, 2001).

The characteristic feature of acute hepatitis B virus infection is the rise in serum transaminases activities. The increase in the serum transaminases activities, especially alanine transaminase (ALT), varies from a mild or moderate (3 – 10 fold) to a remarkable increase (above 100 fold) (Mahoney and Kane, 1999). The acute infection is also characterised by the existence of HBsAg and anti-HBc IgM, and the absence of an anti-HBs in the serum of HBV infected persons. In persons with clinical signs, the onset of the infection is generally characterised with abdominal discomfort, tiredness, headache, rash, nausea, vomiting, arthralgias and jaundice (Liang, 2009).

The icteric period of the acute viral hepatitis normally starts within 10 days of the early signs with the occurrence of dark urine tailed by pale stools and yellowish staining of the mucous membranes, conjunctiva, sclera and skin. The development of jaundice turn out to be clinically obvious when the total bilirubin levels in the blood surpasses 20 – 40 mg/L (Liang, 2009). After about 4 – 12 weeks, the jaundice and other symptoms resolve with the appearance of natural defensive antibodies (anti-HBs) in almost 95% of adults. Only a small proportion of persons die from acute hepatitis B virus infection (Mahoney and Kane, 1999).

2.7.2 Chronic Hepatitis B Virus Infection

Even though some HBV infected persons recover entirely from the acute infection, in others (5 – 10% of adults and 70 – 90% of infants) the virus persists in the body (Gan *et al*., 2005). Chronic HBV infection can cause critical destructive liver diseases, and normally develops over some years during which the patient will pass through many disease conditions (Trepo and Guillevin, 2001). Chronic HBV infection is a prolonged (more than 6 months) infection with the presence of serum HBsAg and anti-HBc IgG, and the absence of anti-HBc IgM and anti-HBs. The hepatitis B virus DNA and HBeAg are often noticeable at high amounts, but the HBeAg may wane if the replication of the virus stops or if mutation occurs that avert the production of the precore protein precursor of the HBeAg (Gan *et al*., 2005).

There are three stages of viral replication which occur during the progression of the HBV infection particularly in persons with chronic HBV infection, namely: high replication phase, low replication phase and non-replication phase (Gitlin, 1997). In the high replication phase, HBsAg, HBeAg and HBV DNA are detectable in the sera with increased levels of serum transaminases. Moderate inflammatory condition is histologically apparent and the risk of developing into cirrhosis is high (Gitlin, 1997). But in the low replicative period, there is the loss of HBeAg with the appearance of anti-HBe, and the loss of the HBV DNA levels. Histologically, a decline in inflammatory activities are obvious. The serologic alterations, such as the loss of HBeAg and HBV DNA, are known as sero-conversion (Gitlin, 1997). Also in the non-replicative phase, the markers of viral replication (HBeAg and HBV DNA) are either lost or below the detection level with reduced liver inflammation. Though, if cirrhosis has previously developed in the non-replicative phase, it persists indefinitely (Gitlin, 1997).

During the chronic HBV infection, the laboratory anomalies comprise of an increase in the serum levels of alanine transaminase varying from normal to 200 U/L in almost 90% of individuals (Hollinger and Liang, 2001). Serum levels of bilirubin and gamma globulin are mild to markedly raised, and autoimmune antibodies including anti-smooth muscle antibody, antinuclear antibody and anti-mitochondrial antibody could be present (Hollinger and Liang, 2001). The persistent increases in the serum transaminases, in addition to the existence of HBsAg for more than 6 months are suggestive of chronic HBV infection (Hollinger and Liang, 2001).

Cirrhosis is a serious liver disorder which is connected to chronic hepatitis and often extensive destruction of liver cells going on over for several years (Liang, 2009). In cirrhosis, the hepatocytes die and are gradually substituted with fibrotic tissues which leads to the formation of nodules. During cirrhosis, the liver internal arrangement is disturbed causing the blockade of blood flow and reduction in the liver function (Trepo and Guillevin, 2001). This injury is triggered by the persistent immune reactions which is incited by the existence of the hepatitis B virus. Since liver inflammation can be completely asymptomatic, the development of cirrhosis can happen without the consciousness of the patient (Liang, 2009).

2.8 DIAGNOSIS OF THE HEPATITIS B VIRUS INFECTION

The clinical identification of hepatitis is ascertained by the biochemical evaluation of the liver. The initial laboratory assessment include: alanine and aspartate transaminases, total protein, albumin, serum globulin, alkaline phosphatase, total and direct bilirubin, coagulation analysis and full blood count (Hollinger and Liang, 2001). The diagnosis of HBV infection is established by the existence of specific antigens and/or antibodies in the serum of the patient. The three clinical important antigen-antibody systems that have been recognised include:

hepatitis B virus surface antigen (HBsAg) and antibodies to the HBsAg (anti-HBs), antibodies (anti-HBc, either the IgM or IgG form) to hepatitis B virus core antigen (HBcAg), and hepatitis B virus envelope antigen (HBeAg) and the antibodies to the HBeAg (anti-HBe) (Hollinger and Liang, 2001).

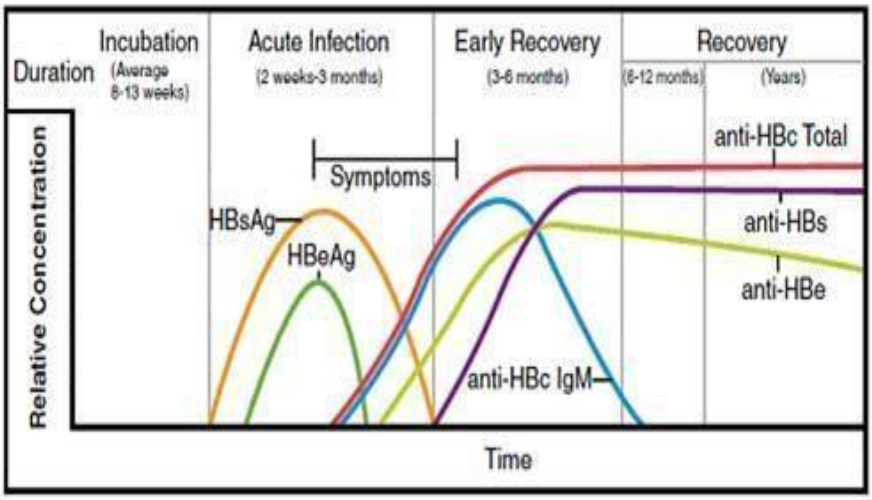

Figure 2.3: The serological forms of the acute hepatitis B virus infection with recovery (Courtesy: Hollinger and Lau, 2006).

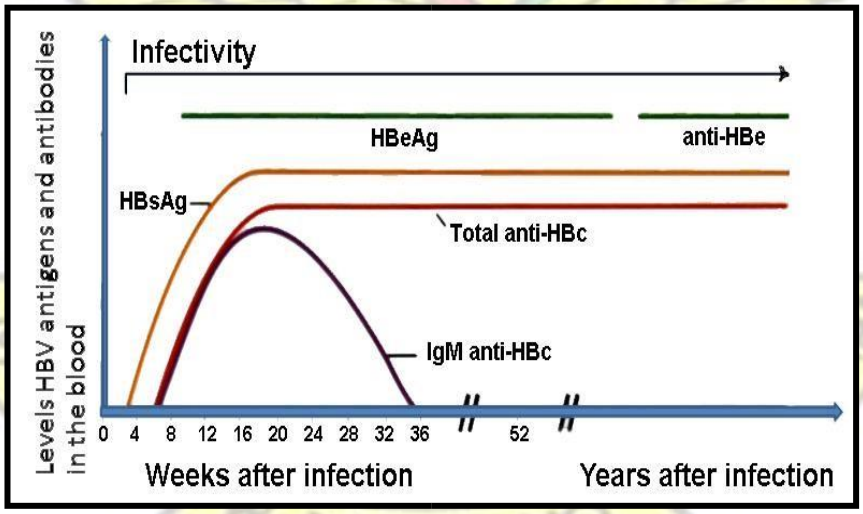

Figure 2.4: The serological forms of the chronic hepatitis B virus infection (Courtesy: Hollinger and Lau, 2006).

The HBsAg can be identified in the serum of infected individuals from some weeks to months before and after the inception of symptoms (**Figure 2.3**). The HBsAg exist in the serum during the acute infection and keeps on in the chronic infection. The existence of the HBsAg indicates

that the patient is potentially contagious (Robinson, 1995). The virions, viral DNA, and the HBeAg can likewise be identified in the serum. However, the existence of the HBeAg is linked to disease severity and high infectivity (Hollinger and Liang, 2001).

The anti-HBc is the first antibody to develop, and the presence of this antibody in the serum is indicative of current or past infection. The anti-HBc IgM is present in high concentration during the acute infection and normally declines within 6 months (**Figure 2.4**), even though it can exist in exceptional cases of chronic infection. The IgG form of the anti-HBc generally remains measurable for a lifetime (Hollinger and Liang, 2001). The anti-HBe appears after the antiHBc, and its existence relates to a reduced infectivity. The anti-HBe substitutes the HBeAg in the resolution of the disease. Also, the anti-HBs substitutes the HBsAg as the acute infection is resolving (**Figure 2.3**). The anti-HBs in general exists for a lifetime in more than 80% of persons which is an indicative of immunity (Hollinger and Liang, 2001). Persons with acute hepatitis B virus infection who maintain a persistent serum HBsAg levels, or whose serum HBeAg exists for 8 – 10 weeks even after the symptoms have resolved (**Figure 2.4**) are most likely to become carriers (Hollinger and Liang, 2001).

2.8.1 Classification Based on the Hepatitis B Viral Markers

Serologic testing for HBV infection involves measurement of several HBV-specific antigens and antibodies. Different serologic markers or combinations of markers are used to identify different phases of the HBV infection (**Table 2.1**).

Table 2.1: Combination of the hepatitis B viral markers and their interpretations

Marker	Presence	Interpretation
HBsAg	Negative	Susceptible
anti-HBc	Negative	

anti-HBs	Negative	
HBsAg anti-HBc anti-HBs	Negative Positive Positive	Immune due to natural infection
HBsAg anti-HBc anti-HBs	Negative Negative Positive	Immune due to hepatitis B vaccination
HBsAg anti-HBc IgM anti-HBc anti-HBs	Positive Positive Positive Negative	Acutely infected
HBsAg anti-HBc IgM anti-HBc anti-HBs	Positive Positive Negative Negative	Chronically infected

HBsAg: Hepatitis B virus surface antigen, **anti-HBs**: Antibody to the hepatitis B virus surface antigen, **anti-HBc**: Antibody to the hepatitis B virus core antigen, **IgM**: Immunoglobulin M (Courtesy: CDC, 2008)

2.9 EPIDEMIOLOGY OF THE HEPATITIS B VIRUS INFECTION

The HBV infection is among the most common infectious diseases (CDC, 2008) with a global distribution (**Figure 2.5**). It is been projected that about a third of the population worldwide has been infected at a stage in their lives, which includes 350 million people who are chronic carriers (Schilsky, 2013). The proportion of the HBsAg carrier status varies from 0.1 to 20% among different populations worldwide (Mahoney and Kane, 1999). The prevalence of the hepatitis B carrier status among these populations is associated most notably, to the prevalence of the infection and the age of the individual when infected. This infection causes not less than a million deaths annually (Custer *et al*., 2004).

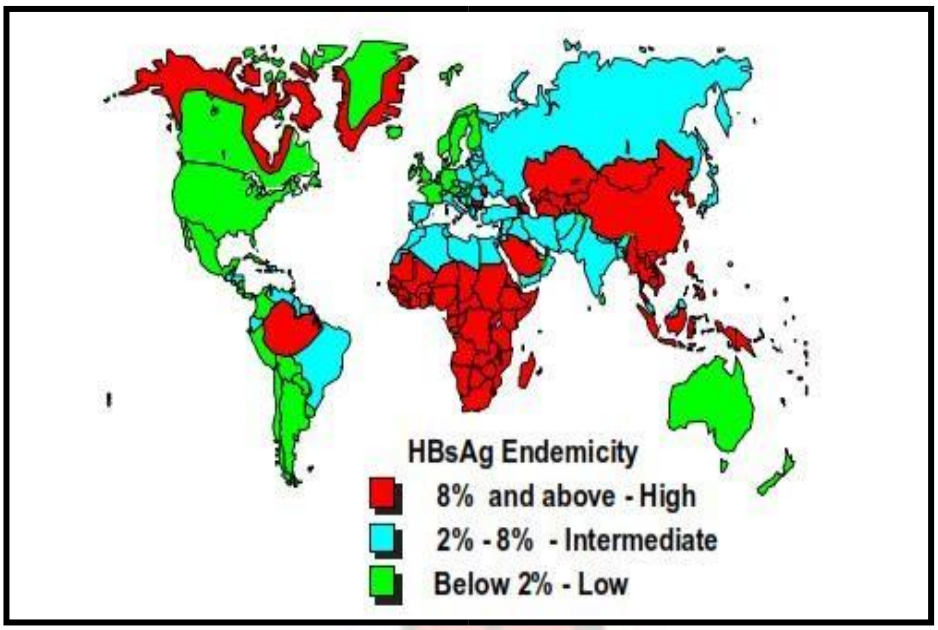

Figure 2.5: The geographical distribution of the hepatitis B virus infection (Courtesy: CDC, 2008)

In endemic areas of Asia and Africa, the epidemiological patterns vary from those seen in Western Europe and North America (CDC, 2013). In the endemic regions, most of the infections occur among infants and children due to mother to child transmission or close contact. However, percutaneous exposure to contaminated needles or following unsafe injections can be possibilities in these countries (Redd *et al*., 2007). The chronic liver disease such as cirrhosis and hepatocellular carcinoma related to the HBV infection are among the most important human health problems in the endemic regions (CDC, 2013).

The reduction in the age-related prevalence of the infection through the adoption of universal immunisation of infants in countries where hepatitis B is highly endemic is suggestive that it may be likely to eliminate HBV infection from humans (Dienstag, 2008). The availability of vaccines for hepatitis B have been dated since the 1982, and millions of persons have been immunised with an exceptional record of safety and effect on the disease (Alter, 2003). Hepatitis B carrier state has been decreased from high to low prevalence rates in immunised groups of children in several countries (Dienstag, 2008).

In Ghana, the reported sero-prevalence of the HBsAg is 15.3% among blood donors (Sarkodie *et al.*, 2001), 15.8% among children (Martinson *et al.*, 1996) and 20.9% among the general population (Martinson *et al.*, 1998) in the rural areas. A study conducted by Amidu *et al.* (2012) in three sub - populations (Garrison, Aboabo and Tafo) in Kumasi, Ashanti Region, showed an overall prevalence of 8.68%. Furthermore, the sero-prevalence of the HBsAg among women of childbearing age is estimated as 11.5% (Goldstein *et al*., 2005), and among replacement and voluntary blood donors visiting the Tamale Teaching Hospital in 2009 as 11.59% and 10.79% respectively (Dongdem *et al*., 2012). A study conducted by Walana *et al*. (2014) at the Kintampo Municipal Hospital in the Brong Ahafo Region also showed a seroprevalence of 9.6%.

2.10 MANAGEMENT OF THE HEPATITIS B VIRUS INFECTION

At the present, there is limited management for acute HBV infection in general (Mahoney and Kane, 1999), but symptomatic management of anorexia, nausea, vomiting and other symptoms may be indicated (Hollinger and Liang, 2001). The management of chronic HBV infection is targeted at reducing infectivity to prevent the spread of the virus, preventing the progress of the liver disease, enhancing the histologic and clinical picture of the liver, and preventing the development of hepatocellular carcinoma (Gitlin, 1997). Some of the antiviral drugs used in the treatment are interferons, lamivudine, entecavir, adefovir, telbivudine and tenofovir. Evidence of treatment is achieved by the loss of markers of the hepatitis B viral replication in the serum such as: HBeAg and HBV DNA (Mahoney and Kane, 1999). The loss of these markers in the serum is accompanied by the normalisation of the alanine transaminase activity and the resolution of the hepatic inflammation (Hollinger and Liang, 2001).

Non-steroidal anti-inflammatory drugs (NSAIDs) and most analgesics have anti-inflammatory properties and are suitable for the management of both acute and chronic inflammatory disorders (Mathison *et al*., 1994). The functions of aspirin and NSAIDs rely on a number of mechanisms, which are partly linked to the inhibition of cyclooxygenases and prostaglandins, such as a decline in angiogenesis and cancer cell multiplication, upsurge in apoptosis, and the decrease in pro-inflammatory cytokine production (Mathison *et al*., 1994). Thus the appropriate administration of anti-inflammatory and/or hepato-protective medication would be significant in the management of hepatitis (Chen *et al*., 2002).

The role of aspirin was studied by Sitia *et al.* (2012) in rodents with chronic necroinflammatory liver disease and they found out that the aspirin supressed the inflammatory process in the rodents. Furthermore, platelet inhibition by aspirin activity may block the release of vital growth factors which play various roles in the development and growth of hepatocellular carcinoma (Dube *et al*., 2007).

Also, a study conducted by Sahasrabuddhe *et al*. (2012) in participants with hepatocellular carcinoma and chronic liver disease who used aspirin or NSAIDs revealed that the use of aspirin alone was related to a 41% decreased risk of developing HCC and a 51% decreased risk of death from chronic liver diseases which are not related to HCC, whereas the use of only NSAIDs was related to only reduced death from chronic liver disease by 34%.

2.11 INFLAMMATORY MARKERS

Inflammation is the complex part of the response of vascular tissues to harmful stimuli, including damaged cells or pathogens, and it can be categorised as either acute or chronic (Ferrero-Miliani *et al*., 2007). Acute inflammation is the primary inflammatory reaction that happens almost rapidly after minor injuries such as burns and cuts, and major trauma like

myocardial infarction (Serhan and Savill, 2005). The mechanisms of the acute inflammation lead to the restoration of the damaged tissue. The inflammation is resolved when the irritant is removed (Serhan and Savill, 2005). The classical signs of acute inflammation are redness, swelling, pain, heat, and stiffness (Parakrama and Taylor, 2005).

Chronic inflammation on the other hand may occur after acute inflammation or occur distinctly. Chronic inflammation is a continuous process, as in the continuous tissue breakdown and repair which frequently results in tissue scarring and destruction. Chronic inflammation can happen after an infection or due to an autoimmune disease (Serhan and Savill, 2005).

Some proteins are often released during the inflammatory process and are readily identified in the blood, hence called inflammatory markers (Pepys and Hirschfield, 2003). The most frequently used marker of inflammation is the C-reactive protein (Loffreda *et al*., 1998). This acute phase protein is produced by the liver cells in response to pro-inflammatory cytokines mainly interleukin 6, which are released by macrophages and T cells to stimulate immune responses (Pepys and Hirschfield, 2003).

2.11.1 C-Reactive Protein

C - reactive protein is an acute phase protein which is synthesised by the hepatocytes in response to pro-inflammatory cytokines. C - reactive protein has 224 amino acids, a monomer molecular mass of 25106 Da, and with an annular pentameric discoid shape (Clyne and Olshaker, 1999).

The C-reactive protein (CRP) binds to the phosphocholine found on the surface of dead or dying cells and some bacteria (Thompson *et al*., 1999). This triggers the complement system, enhancing phagocytosis by macrophages, which clear necrotic and apoptotic cells, and

bacteria. C-reactive protein functions in the innate immunity as an early defence mechanism against infections (Danesh *et al*., 2004). This protein increases within two hours of the inception of inflammation up to a 50,000-fold, and peaks at 48 hours. It has a half-life of about 48 hours (Liu *et al*., 2013). The serum level of CRP is associated with the rate of production due to the severity of the precipitating stimulus. C-reactive protein is used in the screening for inflammation and therefore mainly used as a marker of inflammation (Liu *et al*., 2013).

In a study, CRP has been found to be associated with HBV replication, liver damage and fibrosis in individuals with chronic HBV infection, and therefore serum CRP may be a marker for the diagnosis of significant fibrosis in patients with chronic HBV infection (Ma *et al.,* 2015).

2.11.2 Interleukin 6

Interleukin 6 (IL-6) functions as both an anti-inflammatory myokine and a proinflammatory cytokine (Banks *et al*., 1994). It is secreted by the T cells and macrophages to stimulate immune responses which lead to inflammation (Kishimoto *et al*., 1995). Also, IL-6 are produced by the osteoblasts in response to osteoclast formation (Bastard *et al*., 1999).

Interleukin 6 can also be secreted by macrophages in response to some specific microbial molecules called pathogen-associated molecular patterns (PAMPs) (Benedict *et al*., 2009). These PAMPs bind to some molecules of the innate immune system, also called pattern recognition receptors (PRRs), which include the toll-like receptors (TLRs) (Heinrich *et al*., 2003). These receptors are found on the cell surface and intracellular compartments, and induce intracellular signalling cascades which give rise to inflammatory cytokine synthesis (Bastard *et al*., 1999). Interleukin 6 is responsible for the stimulation of CRP synthesis as well as the production of neutrophils in the bone marrow (Heinrich *et al*., 2003).

Interleukin 6 had been demonstrated to be induced by the HBx protein (Benhenda *et al*., 2013). The HBx protein had been found to play a part in the activation of the hepatitis B viral transcription process (Benhenda *et al*., 2013). And it had been observed by Khan *et al*. (2011) that, there is a positive significant association between IL-6 and hepatitis B viral load. The HBx protein transactivates the human IL-6 promoter through the NF-κB binding site. The upregulation of the IL-6 by the HBx protein can induce hepatitis, and this can play an essential function in the pathogenesis of liver fibrosis, which can develop into cirrhosis and/or hepatocellular carcinoma (Lee *et al*., 1998).

2.12 MARKERS OF LIVER INJURY

Majority of liver diseases cause mild symptoms initially, but it would be significant if these diseases are diagnosed earlier. This is because the hepatic involvement in some of these diseases can be of clinical importance (Johnston, 1999). Liver function test is collection of clinical chemistry assays intended to give information about the status of the liver of a patient. Among the markers measured in a liver function test include: aspartate and alanine transaminases, prothrombin time, protein (albumin and globulin), bilirubin (direct and indirect), gamma-glutamyl transferase, and alkaline phosphatase (Tonya *et al*., 2007).

The levels of serum albumin are related to the functionality of the hepatocytes whereas the serum levels of alkaline phosphatase and gamma-glutamyl transferase are linked to conditions of the biliary tract (Tonya *et al*., 2007). The alanine and aspartate transaminases are known to be valuable markers of liver injury in individuals with some grade of intact liver function (Nyblom *et al.,* 2006; Tonya *et al*., 2007).

2.12.1 Alanine Transaminase

The alanine transaminase (ALT), also called alanine aminotransferase (ALAT) or serum glutamic-pyruvic transaminase (SGPT), is a pyridoxal dependent enzyme (Wang *et al*., 2012). The ALT had been found in the plasma and in various bodily tissues, but is mostly associated with the liver (Ghouri *et al*., 2010). It catalyses the transfer of an amino group from alanine to αketoglutarate in a reversible reaction forming pyruvate and glutamate (Wang *et al*., 2012). This transaminase is generally measured clinically as a part of the diagnostic assessment of the liver to determine the presence of hepatocellular injury (Ghouri *et al*., 2010).

2.12.2 Aspartate Transaminase

The aspartate transaminase (AST), also known as aspartate aminotransferase (ASAT/AAT) or serum glutamic-oxaloacetic transaminase (SGOT), is also a pyridoxal phosphate-dependent transaminase (Hayashi *et al*., 1990). It catalyses the reversible reaction which involves the transfer of an amino group between aspartate and glutamate and, as such, is a significant enzyme in amino acid metabolism (Almo *et al*., 1994).

Aspartate transaminase is similar to ALT because both enzymes are associated with the function of the liver parenchymal cells (Nyblom *et al.,* 2006). However the difference is that; ALT is found predominantly in the liver, with clinically negligible quantities found in the heart, kidneys and skeletal muscle, whereas the AST is found in the liver, brain, red blood cells, heart (cardiac muscle), skeletal muscle and kidneys (Gaze, 2007). Therefore, ALT is a more specific indicator of hepatocellular injury as compared to AST (Nyblom *et al.,* 2006).

2.13 HAEMATOLOGICAL ABNORMALITIES IN THE HEPATITIS B VIRUS INFECTION

The liver has undeniable effect on several critical functions of many organs in the body which include the haematopoietic system (Fasola *et al*., 2009). Apart from its role as an extravascular haemopoietic organ in early foetal life and bone marrow infiltrative diseases, the liver produces and stores many of the factors necessary in blood production. It also synthesises some factors required in haemostasis (Fasola *et al*., 2009).

The causes of low haemoglobin in chronic liver disease include: iron deficiency, aplastic anaemia, hypersplenism, autoimmune haemolytic disease, folate deficiency, and effects of antiviral drugs (Little *et al*., 2012). Anaemia of chronic disease is common in persons with chronic hepatitis. Anaemia of chronic disease has a complex aetiology involving, shortened red cell life span, erythropoietin hyposecretion, tissue hyporesponsiveness to erythropoietin and impaired iron reutilisation (Little *et al*., 2012).

The leukocytes (white blood cells) are cells of the immune system and are involved in protecting the body against both contagious diseases and foreign invaders. Hence, the total number of the leukocytes in the blood is often an indicator of disease or immune response (Akarsu *et al*., 2008). The development of thrombocytopenia in persons with chronic liver disease is multifactorial. Thrombocytopenia is possibly due to the suppression of platelets in the bone marrow, splenic sequestration of platelets, and the decreased activity of the haematopoietic growth factor thrombopoietin (Afdhal *et al*., 2008).

2.14 NON-INVASIVE MARKERS OF LIVER FIBROSIS

Various factors such as drugs, toxins, and infection with the hepatitis virus are able to affect the functions of the liver (Bedossa and Carrat, 2009). These factors cause gradual necrosis of active hepatocytes which can result in liver fibrosis, cirrhosis and in the end death (Martinez *et al.,*

2011). There are non-invasive markers for the estimation of liver fibrosis apart from using liver biopsy. These markers are serum markers, separately or in algorithm models, used in the estimation of liver fibrosis (Papastergiou *et al*., 2012). There are many of these non-invasive markers however Aspartate Transaminase Platelet Ratio Index (APRI), Fibrosis 4 Index (FIB4), and Aspartate-Alanine Transaminase Ratio (AAR) were used in this study.

2.14.1 Aspartate-Alanine Transaminase Ratio (AAR)

The AAR is a simple index used in the evaluation of liver fibrosis. It is calculated from two routine enzymes: AST and ALT (Williams and Hoofnagle, 1988). It is calculated from the formula:

$$AAR = AST / ALT$$

In a study that was carried out among persons with non-alcoholic fatty liver disease (NAFLD), it was found that the area under receiver operating curve (AUROC) was 0.61 with AAR of 0.8 for the prediction of advance fibrosis (Kruger *et al*., 2011). Also, a study reported that AAR ≥1 is specific for liver fibrosis in persons with hepatitis (Park *et al*., 2000).

2.14.2 Aspartate Transaminase Platelet Ratio Index (APRI)

The APRI was initially described by Wai *et al*. (2003). It is dependent on two routine tests: AST and platelet count. It is calculated from the formula:

$$APRI = [(AST / Upper\ Limit\ of\ Normal) / Platelet\ Count\ (\times 10^9/L)] \times 100$$

A study conducted by Shin *et al*. (2008) showed that in persons with chronic hepatitis B virus infection, the predictive power of detecting significant fibrosis is based on the AUROC of 0.850 – 0.950 and APRI ≥ 1.5. They also concluded that this index is a useful indirect marker for estimating significant fibrosis.

2.14.3 Fibrosis 4 Index (FIB-4)

The FIB-4 is related to ALT, AST, platelet count and age (years) (Sterling *et al*., 2006). It is calculated from the formula:

$$FIB\text{-}4 = [age\ (years) \times AST\ (U/L)] / [(Platelet\ Count\ (\times 10^9/L) \times ALT\ (U/L))^{1/2}]$$

A study reported that FIB-4 had the significant power of differentiating between persons with mild and significant fibrosis in non-alcoholic fatty liver disease with the AUROC as 0.081 and FIB-4 > 1.45 (Yang *et al*., 2012). Shah *et al*. (2009) in another study revealed that among the different serum based algorithms, FIB-4 had a better diagnostic accuracy for the assessment of liver fibrosis. This index can also be used in the estimation of the stage of the liver fibrosis: FIB-4 < 1.45 = F0 – F1 while FIB-4 > 3.25 = F3 – F4 (Martinez *et al.,* 2011).

CHAPTER THREE MATERIALS AND METHODS

3.1 STUDY DESIGN

A cross-sectional study design was used in this research, and the study period was from June to October, 2014. Each participant who was enrolled in the study was given a participant's

information sheet after informing him or her of the study protocol at the appropriate literacy level. The participant was then asked to sign or thumbprint a consent form. All participants were informed that the study was confidential, and that the information provided by them will not affect their laboratory results or the management of their infection. Questionnaires were administered for demographic data of participants, and to exclude other forms of inflammatory conditions and liver diseases (**Appendix III**). Blood samples were then collected from the participants for laboratory analyses.

3.2 STUDY SITE

The Medilab Diagnostics Services Limited was used as the study site. The company is among the private medical laboratories in Ghana. It has branches in Accra, Sunyani, Obuasi, Techiman and Kumasi. The laboratories in the company are equipped with state of the art equipment and perform various laboratory tests in clinical chemistry, haematology, microbiology, endocrinology, among others. Medilab Diagnostics Services Limited also serves as the referral centre for most of the clinical laboratory investigations in Kumasi.

3.3 STUDY POPULATION

3.3.1 Selection of the Hepatitis B Virus Infected Participants

The HBV infected persons were selected from the clients of the Medilab Diagnostics Services Limited, Kumasi. The inclusion criteria was persons diagnosed as HBsAg sero-positive, and all newly diagnosed drug naive hepatitis B virus infected persons between 18 and 60 years were considered and included in this study. After the written informed consent was sought, the participants were screened for the presence of the HBsAg in their serum, and those who were

positive were recruited for this study. A total number of one hundred and forty-six (146) HBV infected persons were recruited for this study from the study population.

3.3.2 Selection of the Control Participants

Sixty-four (64) blood donors with similar age and sex distribution as the HBV infected persons were recruited as the control group from the Regional Blood Transfusion Centre, Kumasi.

3.3.3 Exclusion Criteria

The participants were screened for hepatitis C, HIV and malaria, and persons with these infections were excluded from the study. Also participants with liver diseases, diabetes mellitus, hypertension, rheumatoid arthritis, other forms of inflammatory diseases, and HBV infected persons on therapy were excluded from this study.

3.4 BLOOD SAMPLE COLLECTION

The blood samples were obtained from the ante-cubital vein of all the participants. A rubber tourniquet was applied for less than one minute. The site to be punctured was disinfected with 70% methylated spirit and five millilitres (5mls) of blood sample was collected from each participant. The blood sample was divided into two: 2mls was dispensed into a BD Vacutainer® K_2EDTA Tube while 3mls was dispensed into a BD Vacutainer® SST™ Gel Separator Tube.

Haematological analyses were performed on the blood samples in the BD Vacutainer® K_2EDTA Tubes within two (2) hours after the sample collection. However, the blood samples in the BD

Vacutainer® SST™ Gel Separator Tubes were allowed to clot and centrifuged at 3000 revolutions per minute (rpm) for five (5) mins to obtain the serum. The serum samples were then pipetted into cryogenic storage tubes and stored for a month at -20˚C prior to biochemical and immunological analyses.

3.5 HAEMATOLOGICAL ANALYSES

The haematological parameters that were analysed were: total white blood cell count, lymphocyte count, neutrophil count, haemoglobin concentration and platelet count. The Mindray Auto Haematology Analyser (BC-3200, Shenzhen Mindray BioMedical Electronics Co. Ltd., China) was used in the determination of these parameters.

3.5.1 Determination of the Full Blood Count

The Mindray Auto Haematology Analyser (BC-3200, Shenzhen Mindray BioMedical Electronics Co. Ltd., China) determines the haemoglobin concentration by the colorimetric method while the platelets and the WBCs are counted and sized by the impedance method. A control specimen was run before the samples of the participants. The tube containing the control specimen was inverted about 5 – 10 times to ensure that the content of the tube was well mixed. The tube was uncapped and then placed in the sample holder of the analyser. The aspirate button was pressed for the analyser to aspirate the required volume of blood for the analyses. After about 20 seconds, the result was ready and printed from the analyser. This procedure was followed for all the samples of the participants.

3.6 BIOCHEMICAL ANALYSES

The biochemical parameters that were analysed included: aspartate and alanine transaminases, gamma-glutamyl transferase, alkaline phosphatase and albumin. These parameters in the serum samples were assayed using a semi-automatic biochemistry analyser (BC-3000M, Sinnowa Medical Science and Technology Co. Ltd., China) with commercial kits (Labcare Diagnostics Pvt. Ltd., India).

3.6.1 Determination of Aspartate and Alanine Transaminases, Gamma-Glutamyl Transferase and Alkaline Phosphatase Activities

Principle for the Determination of Aspartate Transaminase

In this procedure, the aspartate or glutamic-oxaloacetic transaminase in a specimen catalyses the transfer of an amino group from aspartate to α-ketoglutarate in a reaction forming glutamate and oxaloacetate. The oxaloacetate, in the presence of malate dehydrogenase reacts with NADH forming malate and NAD. The rate of NADH consumption is measured photometrically and is directly proportional to the AST activity in the specimen.

Principle for the Determination of Alanine Transaminase

In this procedure, the alanine or glutamic-pyruvic transaminase in a specimen catalyses a reaction involving the transfer of an amino group from alanine to α-ketoglutarate forming glutamic and pyruvic acids. The pyruvic acid, in the presence of lactate dehydrogenase reacts with NADH forming lactate and NAD. The rate of NADH consumption is measured photometrically and is directly proportional to the ALT activity in the specimen.

Principle for the Determination of Gamma-Glutamyl Transferase Activity

In this procedure, the gamma-glutamyl transferase in a specimen transfers gamma-glutamyl from gamma-glutamyl p-nitroanilide to glycyl glycine. The p-nitroaniline formed absorbs light at 405nm and is directly proportional to the gamma-glutamyl transferase activity.

Principle for the Determination of Alkaline Phosphatase Activity

In this procedure, the alkaline phosphatase in a specimen converts p-nitrophenyl phosphate to p-nitrophenol and phosphate. The increase in absorption of the colour formed by p-nitrophenol at 405nm is proportional to the alkaline phosphatase activity in the specimen.

Methodology for the Determination of the Activities of the Enzymes

The commercial kits composed of buffers and enzyme reagents. The reagents were brought to room temperature before use. A working reagent was prepared by mixing 4ml of the buffer reagent with 1ml of the enzyme reagent. A control specimen was run before the samples of the participants. One thousand microlitres (1000µl) of the working reagent was transferred into a labelled control test tube and incubated at 37°C in a water bath for 10 mins. After the 10 mins, 100µl of the control specimen (20µ in the case of the alkaline phosphatase) was added to the corresponding test tube. The control test tube with its content was mixed and incubated at 37°C in a water bath for a minute. Exactly after this stage, the content of the test tube was aspirated by the semi-automatic analyser for analysis. After 2 minutes, the result was read from the analyser. This procedure was followed for all the samples of the participants which were run in duplicates. The linearity for the methods are up 200U/L, 440U/L and 2000U/L for

gammaglutamyl transferase, aspartate and alanine transaminases, and alkaline phosphatase determinations respectively.

3.6.2 Determination of Albumin Concentration

Principle and Methodology

In this procedure, the measurement of the albumin in the specimen is due to its ability to bind quantitatively to the indicator 3, 3', 5, 5' - tetrabromo-m-cresol sulphophthalein (bromocresol green, BCG). The albumin-BCG complex absorbs light at 578nm and is directly proportional to the albumin concentration in the specimen.

The commercial kit which composed of bromocresol green (BCG) reagent was brought to room temperature before use. A control specimen was run before the samples of the participants. One thousand microlitres (1000µl) of the working reagent was transferred into a labelled control test tube and incubated at 37°C in a water bath for 10 minutes, after which 5µl of the control specimen was added to the corresponding test tube. The control test tube with its content was mixed and incubated at 37°C in a water bath for 5 minutes. The content of the test tube was then aspirated by the semi-automatic analyser for analysis. After 15 seconds, the result was read from the analyser. This procedure was followed for all the samples of the participants which were run in duplicates.

3.7 IMMUNOLOGICAL ANALYSES

The immunological parameters that were analysed included: HBsAg, anti-HBc, anti-HBc IgM, HBeAg, IL-6 and CRP. The CRP and IL-6 concentrations were quantified based on the

sandwich enzyme linked immunosorbent assay principle whereas the hepatitis B viral markers were detected in the serum based on the line immunoassay principle using commercial kits.

3.7.1 Detection of Hepatitis B Virus Surface Antigen and Core Antibody (IgM)

Principle for the Hepatitis B Virus Surface Antigen Detection (Sandwich Immunoassay)

The ABON™ One Step Hepatitis B Surface Antigen Test Strip (Abon Biopharm Co. Ltd., China) was qualitatively used to detect the presence of HBsAg in the serum of the participants. The strip is a lateral flow chromatographic immunoassay consisting of a colloid gold conjugate sample pad, and a nitrocellulose membrane pre-coated with control and test regions. The strip employs the sandwich immunoassay method to detect the HBsAg in the specimen.

The conjugate sample pad contains polyclonal anti-HBsAg conjugated with colloid gold, and the nitrocellulose membrane is pre-coated with a monoclonal anti-HBsAg antibody at the test region. During testing, the HBsAg present in the sample bind to the anti-HBsAg gold conjugate. This immunocomplex moves along the strip chromatographically by a capillary action and reacts with the pre-coated anti-HBsAg antibody, forming a coloured line at the test region indicative of a positive test result.

Principle for the Hepatitis B Core Antibody (IgM) Detection (Sandwich Immunoassay)

The iCARE ™ One Step anti-HBc IgM Rapid Test Strip (JAL Innovation, Pte, Ltd., Singapore) was qualitatively used to detect the presence of anti-HBc IgM in the serum of the participants. The strip is a lateral flow chromatographic immunoassay consisting of a colloid gold conjugate sample pad, and a nitrocellulose membrane pre-coated with control and test regions. The strip employs the sandwich immunoassay method.

The conjugate sample pad contains anti-HBc IgM antibody conjugated with colloid gold, and the nitrocellulose membrane is pre-coated with IgM μ chain antibody on the test region. During testing, the anti-HBc IgM present in the sample bind to the gold conjugate. This immunocomplex moves along the strip chromatographically by a capillary action and reacts with the IgM μ chain antibody, forming a coloured line at the test region indicative of a positive test result. Absence of this coloured line at the test region suggests a negative result.

Methodology for the Hepatitis B Virus Surface Antigen and Core Antibody (IgM) Detection

The sample was brought to room temperature and mixed well prior to assay. The strips were removed from their protective pouches and placed on a clean flat surface and labelled with the sample ID. Fifty microlitres (10μL in the case of anti-HBc IgM) of the serum was added to the sample pad (which was immediately followed by the addition of 100μl of sample diluent in the case of anti-HBc IgM). The result was visually read after 15 mins. If red lines appeared both at the control and test regions, it indicates positive result. But if only a red line appeared at the control region, it indicates negative. To serve as a control in the procedure, a coloured line appears at the control region signifying that an adequate volume of sample has been added and wicking of the strip had occurred.

3.7.2 Detection of Hepatitis B Virus Core Antibody (total) and Envelope Antigen

The Wondfo™ Five Step Rapid Test Kit (Wondfo Biotech Co. Ltd., China) was qualitatively used to detect the anti-HBc and HBeAg in the serum of the persons. The test kit is a lateral flow chromatographic immunoassay consisting of a test panel strip assembled in a cassette. The strip composes of a colloid gold conjugate sample pad, and a nitrocellulose membrane

precoated with control and test regions. The strip for the anti-HBc employs the competitive immunoassay method while the HBeAg strip employs the sandwich immunoassay method.

Principle for the Hepatitis B Virus Core Antibody Detection (Competitive Immunoassay)

The conjugate sample pad contains anti-HBc conjugated with colloid gold, and the nitrocellulose membrane is pre-coated with HBcAg at the test region. During testing, if antiHBc level in the specimen is below the test sensitivity (1ng/dL), the anti-HBc conjugates will have enough binding sites to bind to the HBcAg coated on the test region, therefore forming anti-HBc conjugates-HBcAg immunocomplex and leading to a coloured line at the test region indicative of a negative result. However, if the anti-HBc level in the specimen is higher than or equal to the test sensitivity, it will bind to the HBcAg on the test region, competing with the anti-HBc conjugates. Since the anti-HBc in the serum is unconjugated with gold, the absence of the coloured line at the test region indicates a positive result.

Principle for the Hepatitis B Virus Envelope Antigen Detection (Sandwich Immunoassay)

The conjugate sample pad contains polyclonal anti-HBe conjugated with colloid gold, and the nitrocellulose membrane is pre-coated with a monoclonal anti-HBe antibody at the test region. During testing, the HBeAg present in the sample bind to the anti-HBe gold conjugate. This immunocomplex moves through on the strip chromatographically by capillary action and reacts with the pre-coated anti-HBe antibody, forming a coloured line at the test region indicative of a positive test result.

Methodology for the Hepatitis B Virus Core Antibody and Envelope Antigen Detection

The sample was brought to room temperature and mixed well prior to assay. The test cassette was placed on a clean, flat surface and labelled with the sample ID. A pipette dropper was filled with the sample and holding the dropper vertically, 2 – 3 drops of the sample were placed into the sample well of the cassette making sure that there were no air bubbles. The cassette with the sample was left for 15mins before visually reading the results. With the anti-HBc strip, if coloured lines appeared both at the control and test regions, the test indicates a negative result whereas only a coloured line at the control region indicates a positive result. But with the HBeAg strip, if coloured lines appeared both at the control and test regions, the test is indicative of a positive result while only a coloured line at the control region indicates a negative result. To serve as a control in the procedure, a coloured line appears at the control region signifying that an adequate volume of sample has been added and wicking of the strip had occurred.

Classification of the Participants based on the Hepatitis B Viral Markers

The participants were identified as having HBV infection when they tested positive for the HBsAg. Also, participants were grouped into acute HBV infection when they tested positive for both the anti-HBc IgM and anti-HBc or chronic HBV infection when they tested negative for the anti-HBc IgM but positive for the anti-HBc. The chronic HBV infected persons were further identified as having active infection when they tested positive for the HBeAg or inactive infection when they tested negative for the HBeAg.

3.7.3 Determination of Interleukin 6 and C-Reactive Protein Concentrations

Principle for the Determination of C-Reactive Protein

The CRP ELISA Assay Kit (Eagle BioSciences, Inc., Nashua, USA) was used to measure the level of CRP in the serum. The kit employs a monoclonal antibody to human CRP immobilised on a microtiter plate to bind the human CRP in the sample or standard. After incubation with a specimen, the excess specimen is washed out and a horseradish peroxidase conjugated antihuman CRP is added. This antibody binds to the human CRP arrested on the plate. After another incubation, the extra antibodies are washed out and a tetramethylbenzidine (TMB)peroxide substrate colour developer is added. The peroxidase present will react with the peroxide substrate and TMB chromogen, resulting in blue colour development. The enzymatic reaction is stopped by the addition of an acidic stop solution (sulphuric acid) which turns the blue colour to yellow. The absorbance of the solution, measured at 450nm, correlates to the concentration of the human CRP bound to the well.

Principle for the Determination of Interleukin 6 Concentration

The IL-6 ELISA Assay Kit (Eagle BioSciences, Inc., Nashua, USA) was used to quantify the level of IL-6 in the serum. The kit employs a monoclonal antibody to human IL-6 immobilised on a microtiter plate to bind the human IL-6 in the sample or standard. After incubation with a specimen, the excess specimen is washed out and a horseradish peroxidase conjugated antihuman IL-6 is added. This antibody binds to the human IL-6 arrested on the plate. After another incubation, the extra antibodies are washed out and a tetramethylbenzidine (TMB)-peroxide substrate colour developer is added. The peroxidase present will react with the peroxide substrate and TMB chromogen, resulting in blue colour development. The enzymatic reaction is stopped by the addition of an acidic stop solution (sulphuric acid) which turns the

blue colour to yellow. The absorbance of the solution, measured at 450nm, correlates to the concentration of the human IL-6 bound to the well.

Methodology for the Determination of both C-Reactive Protein and Interleukin 6

All the reagents were brought to 25°C using a water bath before use. The standards were provided pre-diluted 1:10,000. All serum samples were diluted by initially diluting with phosphate buffered saline (PBS), 10µl sample was pipetted into 10 ml PBS to make 1:10 with the sample diluent provided by the manufacturer for a final dilution of 1:10,000. After the dilution, 100µl of the standards, diluted control and diluted patient sample were transferred into the respective wells. To achieve blanking on the plate reader, 100µl of sample diluent was added to the first two wells. This acted as the zero point for the curve.

The wells were incubated at 25°C for one (1) hour and then washed five (5) times with diluted wash buffer. This was done manually with a multichannel pipette. The wells were emptied and tapped dry on a paper towel. After drying, 100µl of ready-to-use horseradish peroxidase conjugate was added to each well. The wells were incubated at 25°C for one (1) hour and washed as above. After that, 100µl of ready-to-use tetramethylbenzidine (TMB)-peroxide substrate colour developer was added to each well.

The wells were incubated at 25°C for 30 mins. After the incubation, 100µl of the stop solution was added to each well and tapped gently to ensure uniform colour distribution and read at 450nm using an ELISA plate reader (EMR-500, Sinnowa Medical Science and Technology Co. Ltd., China) within 15 mins. The samples of the participants were run in duplicates**.**

3.8 DATA ANALYSIS

The data obtained was analysed with the help of the Statistical Package for Social Scientist (SPSS) Statistical Software (version 16.0, SPSS Inc., Chicago, IL, USA). The categorical variables were expressed in percentages whereas the continuous variables were expressed as mean ± standard error of the mean (SEM). The confidence intervals of the percentages were calculated using the binomial test. Comparison of means were carried out using the t-test. Moreover, correlations were evaluated using the Pearson's correlation analysis. Multiple linear and logistic regressions were also done. In all comparisons, $p<0.05$ was considered significant.

3.9 ETHICAL ISSUES

The Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi approved the study. Permission was also sought from the Managing Director, Medilab Diagnostic Services Limited, to undertake the study with their company as the study site. A written informed consent was sought before enrolling each participant.

CHAPTER FOUR RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

Two hundred and ten (210) participants were recruited for the study comprising of 146 HBV infected participants (males: 74.7% and females: 25.3%), and 64 control participants (males: 79.7% and females: 20.3%). There was no substantial difference (p=0.430) between the proportions of males and females in both the HBV infected participants and the controls, as shown in **Table 4.1**. Also, there was no substantial variation (p=0.064) between the average ages of the HBV infected participants (38.17±0.78 years) and the controls (36.13±0.76 years), as shown in **Table 4.1**. However with regards to the females, there was a considerable difference (p=0.024) between the average ages of the HBV infected participants (39.40±1.57 years) and the controls (32.77±1.94 years), as shown in **Table 4.1**.

Table 4.1: Demographic characteristics of the participants

Parameter	Healthy Controls n = 64	HBsAg Sero-positive n = 146	p-value
Age (years)			
Male	36.98±0.78	37.79±0.91	0.580
Female	32.77±1.94	39.40±1.57	0.024*
Total	36.13±0.76	38.17±0.78	0.064
Gender			
Male	51 (79.7)	109 (74.7)	0.430
Female	13 (20.3)	37 (25.3)	0.430

Age is expressed as mean±SEM, gender is expressed as frequency (percentage) and compared using the two-sample proportion z-test, **n:** number of participants, ***:** difference in the parameters statistically significant ($p<0.05$)

4.2 PERCENTAGES OF ACUTE AND CHRONIC HEPATITI B VIRUS

INFECTIONS AMONG THE PARTICIPANTS

The HBV infected participants were grouped into acute and chronic infections based on the presence or absence of the anti-HBc IgM in their serum respectively. Also, individuals were considered to be having active or inactive infection based on the presence or absence of the HBeAg in their serum respectively. Out of the 146 HBV infected participants, 81.5% (CI: 74.2-87.4) were having acute HBV infection whereas 18.5% (CI: 12.6 - 25.8) were having the chronic HBV infection, comprising of 13.0% (CI: 8.0 - 19.6) with inactive infection and 5.5% (CI: 2.4 - 10.5) active infection, as shown in **Table 4.2**.

Table 4.2: Percentages of acute and chronic hepatitis B virus infections among the participants

	Gender		Total
	Male	Female	
Parameter			
AHB	92 (63.0; 54.6-70.8)	27 (18.5; 12.6-25.8)	119 (81.5; 74.2-87.4)
CHB	17 (11.6; 6.9-18.0)	10 (6.9; 3.3-12.2)	27 (18.5; 12.6-25.8)
CHB1	11(7.5; 3.8-13.1)	8 (5.5; 2.4-10.5)	19 (13.0; 8.0-19.6)
CHB2	6 (4.1; 1.5-8.7)	2 (1.4; 0.2-4.9)	8 (5.5; 2.4-10.5)

The data are expressed as frequencies (percentages; 95% confidence interval), **AHB:** Acute HBV Infected Persons, **CHB:** Chronic HBV Infected Persons, **CHB1:** Chronic HBV Infected Persons with Inactive Infection, **CHB2:** Chronic HBV Infected Persons with Active Infection

4.3 HAEMATOLOGICAL PARAMETERS

4.3.1 Haemoglobin (HB) Concentration

There was a substantial variation ($p<0.001$) in the HB levels between the HBV infected participants (12.89±0.20 g/dL) and the controls (14.76±0.20 g/dL) (**Table 4.3**). Further, there was a substantial variation ($p<0.001$) between the HB levels in the participants with acute HBV infection (13.68±0.16 g/dL) as compared to those with chronic infection (9.41±0.40 g/dl) (**Table 4.4**). However, there was no considerable difference ($p=0.345$) between the HB levels in the chronic HBV infected individuals with active infection (8.85±0.65 g/dL) as compared to those with inactive infection (9.65±0.49 g/dL), as shown in **Table 4.4**.

4.3.2 White Blood Cell (WBC) Count

There was a considerable variation ($p<0.001$) in the WBC count between the HBV infected participants (12.33±0.34 $x10^9$/L) and the controls (6.78±0.18 $x10^9$/L) (**Table 4.3**). Also, there was a considerable variation ($p<0.001$) between the WBC count in the persons with acute HBV infection (11.09±0.25 $x10^9$/L) as compared to those with chronic infection (17.80±0.88 $x10^9$/L) (**Table 4.4**). However, there was no substantial distinction ($p=0.070$) between the WBC count in the chronic HBV infected persons with active infection (20.32±1.52 $x10^9$/L) as compared to those with inactive infection (16.74±1.00 $x10^9$/L), as shown in **Table 4.4**.

4.3.3 Lymphocyte Count

There was a considerable difference ($p<0.001$) in the lymphocyte count between the HBV infected participants (6.09±0.22 $x10^9$/L) and the controls (2.67±0.14 $x10^9$/L) (**Table 4.3**).

However, there was no considerable distinction (p=0.052) between the lymphocyte count in the persons with acute HBV infection (5.83±0.21 $x10^9$/L) as compared to those with chronic infection (7.27±0.68 $x10^9$/L) (**Table 4.4**). Also, there was no substantial difference (p=0.245) between the lymphocytes in the chronic HBV infected persons with active infection (8.49±1.16 $x10^9$/L) as compared to those with inactive infection (6.76±0.83 $x10^9$/L) (**Table 4.4**).

4.3.4 Neutrophil Count

There was a considerable difference (p<0.001) in the neutrophil count between the HBV infected participants (5.62±0.23 $x10^9$/L) and the controls (3.53±0.0.12 $x10^9$/L) (**Table 4.3**). Likewise, there was a considerable variation (p<0.001) between the neutrophil count in the participants with acute HBV infection (4.67±0.16 $x10^9$/L) as compared to those with chronic infection (9.80±0.55 $x10^9$/L) (**Table 4.4**). But there was no considerable disparity (p=0.097) between the neutrophils in the chronic HBV infected persons with active infection (11.14±0.84 $x10^9$/L) as compared to those with chronic inactive infection (9.24±0.67 $x10^9$/L) (**Table 4.4)**.

4.3.5 Platelet Count

There was a considerable variation (p<0.001) in the platelet count between the HBV infected persons (225.24±6.61 $x10^9$/L) and the controls (268.95±5.90 $x10^9$/L) (**Table 4.3**). Similarly, there was a considerable variation (p<0.001) between the platelet count in the persons with acute HBV infection (236.96±7.23 $x10^9$/L) as compared to those persons with chronic infection (173.59±12.12 $x10^9$/L) (**Table 4.4)**. But there was no considerable distinction (p=0.782) between the platelet count in the chronic HBV infected persons with active infection (168.75± 18.81 $x10^9$/L) as compared to those with inactive infection (175.63±15.58 $x10^9$/L) (**Table 4.4**).

Table 4.3: Mean of the parameters analysed among the participants

Parameter	Total participants n = 210	Controls participants n = 64	HBV infected participants n = 146	pvalue
Haematological Parameters				
HB (g/dL)	13.46±0.76	14.76±0.20	12.89±0.20	0.000*
WBC (x10^9/L)	10.64±1.37	6.78±0.18	12.33±0.34	0.000*
LYM (x10^9/L)	5.05±0.88	2.67±0.14	6.09±0.22	0.000*
NEU (x10^9/L)	4.98±0.82	3.53±0.12	5.62±0.23	0.000*
PLT (x10^9/L)	238.56±23.47	268.95±5.90	225.24±6.61	0.000*
Biochemical Parameters				
AST (U/L)	45.85±15.40	11.78±0.46	60.79±4.28	0.000*
ALT (U/L)	31.18±7.44	15.36±0.59	38.11±2.08	0.000*
ALP (U/L)	69.02±12.76	44.81±3.47	79.64±3.35	0.000*
GGT (U/L)	43.65±8.98	32.65±2.32	48.48±2.53	0.000*
ALB (g/dL)	3.82±0.37	4.32±0.18	3.61±0.08	0.000*
Inflammatory Parameters	18.52±5.89			
CRP (mg/L)		5.14±0.26	24.38±1.62	0.000*
IL - 6 (pg/mL)	21.44±5.84	6.60±0.23	27.94±1.55	0.000*
Inflammatory Parameters	1.36±0.25			
AAR		0.83±0.06	1.60±0.07	0.000*
APRI	0.59±0.25	0.11±0.01	0.80±0.07	0.000*
FIB-4	1.42±0.44	0.42±0.02	1.86±0.12	0.000*

The data are expressed as mean±SEM, **n:** number of participants, ***:** difference in the parameters statistically significant ($p<0.05$), **HB**: Haemoglobin, **WBC**: White Blood Cell, **LYM**: Lymphocyte, **NEU:** Neutrophil, **PLT:** Platelet, **AST:** Aspartate Transaminase, **ALT:** Alanine Transaminase, **ALB:** Albumin, **GGT:** Gamma Glutamyl Transferase, **ALP:** Alkaline Phosphatase, **CRP:** C-Reactive Protein, **IL-6**: Interleukin 6, **APRI**: AST Platelet Ratio Index, **FIB-4:** Fibrosis 4 Index, **AAR:** AST ALT Ratio, **HBV**: Hepatitis B Virus

Table 4.4: Mean of the parameters analysed among the hepatitis B virus infected participants

Parameter	AHB n = 119	CHB n = 27	pvalue	CHB1 n = 19	CHB2 n = 8	pvalue
Haematological Paramet						
HB (g/dL)	13.68±0.16	9.41±0.40	0.000*	9.65±0.49	8.85±0.65	0.345
WBC (x10^9/L)	11.09±0.25	17.80±0.88	0.000*	16.74±1.00	20.32±1.52	0.070
LYM(x10^9/L)	5.83±0.21	7.27±0.68	0.052	6.76±0.83	8.49±1.16	0.245
NEU (x10^9/L)	4.67±0.16	9.80±0.55	0.000*	9.24±0.67	11.14±0.84	0.097
PLT (x10^9/L)	236.96±7.23	173.59±12.12	0.000*	175.63±15.58	168.75±18.81	0.782
Biochemical Param ters						
AST (U/L)	51.70±3.75	100.85±14.00	0.002*	56.48±4.52	206.23±8.86	0.000*
ALT (U/L)	33.70±2.04	57.57±5.40	0.000*	42.50±4.06	93.35±2.21	0.000*
ALP (U/L)	69.45±2.93	124.53±8.49	0.000*	121.72±9.99	131.20±16.91	0.638
GGT (U/L)	43.11±2.14	72.14±8.69	0.003*	66.09±11.67	86.49±8.56	0.171
ALB (g/dL)	3.69±0.09	3.23±0.12	0.003*	3.38±0.15	2.88±0.15	0.027*
Inflammatory Parameters						
CRP (mg/L)	18.48±0.59	50.40±6.37	0.000*	32.45±4.28	93.03±5.24	0.000*
IL - 6 (pg/mL)	22.37±0.57	52.49±6.13	0.000*	36.27±4.94	91.03±4.45	0.000*
Markers of Liver Fib rosis						
AAR	1.57±0.07	1.74±0.16	0.323	1.54±0.21	2.21±0.09	0.007*
APRI	0.62±0.05	1.62±0.25	0.001*	0.90±0.11	3.31±0.35	0.000*
FIB-4	1.60±0.11	2.97±0.36	0.001*	2.18±0.25	4.83±0.71	0.007*

The data are expressed as mean±SEM, **n:** number of participants, ***:** difference in the parameters statistically significant ($p<0.05$), **AHB:** Acute HBV Infected Persons, **CHB:** Chronic HBV Infected Persons, **CHB1:** Chronic HBV Infected Persons with Inactive Infection, **CHB2:** Chronic HBV Infected Persons with Active Infection , **HB**: Haemoglobin, **WBC**: White Blood Cell, **LYM**: Lymphocyte, **NEU:** Neutrophil, **PLT:** Platelet, **AST:** Aspartate Transaminase, **ALT:** Alanine Transaminase, **ALB:** Albumin, **GGT:** Gamma Glutamyl Transferase, **ALP:** Alkaline Phosphatase, **CRP:** C-Reactive Protein, **IL-6**: Interleukin 6, **APRI**: AST Platelet Ratio Index, **FIB-4:** Fibrosis 4 Index, **AAR:** AST ALT Ratio

4.4 BIOCHEMICAL PARAMETERS

4.4.1 Aspartate Transaminase (AST)

There was a substantial variation ($p<0.001$) in the AST levels between the HBV infected participants (60.79±4.28 U/L) and the controls (11.78±0.46 U/L) (**Table 4.3**). There was also a substantial variation ($p=0.002$) between the AST levels in the participants with acute HBV infection (51.70±3.75 U/L) as compared to those with chronic infection (100.85±14.00 U/L), as shown in **Table 4.4**. Likewise, there was also a considerable variation ($p<0.001$) between the AST levels in the chronic HBV infected persons with active infection (206.23±8.86 U/L) as compared to those with inactive infection (56.48±4.52 U/L) (**Table 4.4**).

4.4.2 Alanine Transaminase (ALT)

There was a considerable variation ($p<0.001$) in the ALT levels between the HBV infected persons (38.11±2.08 U/L) as compared to the controls (15.36±0.59 U/L) (**Table 4.3**). There was also a considerable variation ($p<0.001$) between the levels of ALT in the persons with acute HBV infection (33.70±2.04 U/L) as compared to those with chronic infection (57.57±5.40 U/L), (**Table 4.4**). Further, there was a substantial variation ($p<0.001$) between the ALT levels in the chronic HBV infected individuals with active infection (93.35±2.21 U/L) as compared to those with inactive infection (42.50±4.06 U/L), as shown in **Table 4.4**.

4.4.3 Alkaline Phosphatase (ALP)

There was a considerable variation ($p<0.001$) in the ALP levels between the HBV infected persona (79.64±3.35 U/L) and the controls (44.81±3.47 U/L) (**Table 4.3**). There was also a considerable variation ($p<0.001$) between the levels of ALP in the persons with acute HBV infection (69.45 ± 31.92 U/L) as compared to those with chronic infection (124.53±8.49 U/L), (**Table 4.3**). There was no considerable distinction ($p=0.638$) between the levels of ALP in the chronic HBV infected persons with active infection (131.20±16.91 U/L) as compared to those with inactive infection (121.72±9.99 U/L) (**Table 4.4**). However, all ALP values were within the normal reference range (35.0 – 150.0 U/L).

4.4.4 Gamma-Glutamyl Transferase (GGT)

There was a substantial variation ($p<0.001$) in the GGT levels between the HBV infected persons (48.48±2.53 U/L) and the controls (32.65±2.32 U/L) (**Table 4.3**). There was also a substantial variation ($p=0.003$) in the GGT levels in the persons with acute HBV infection (43.11±2.14 U/L) and those with chronic infection (72.13±8.69 U/L) (**Table 4.4**). But there was no substantial variation ($p=0.171$) between the GGT levels in the chronic HBV infected persons with active infection (86.49±8.56 U/L) as compared to those with inactive infection (66.09±11.67 U/L) (**Table 4.4**).

4.4.5 Albumin

There was a considerable disparity ($p<0.001$) in the albumin concentration between the HBV infected persons (3.61±0.08 g/dL) and the controls (4.31±0.18 g/dL) (**Table 4.3**). There was

also a considerable disparity (p=0.003) between the albumin concentration in the participants with acute HBV infection (3.70±0.09 g/dL) as compared to those with chronic infection (3.23±0.12 g/dL) (**Table 4.4**). Further, there was a substantial disparity (p=0.027) between the albumin concentration in the chronic HBV infected individuals with active infection (2.88± 0.15 g/dL) as compared to those with inactive infection (3.38±0.15 g/dL) (**Table 4.4**).

4.5 IMMUNOLOGICAL PARAMETERS

4.5.1 C-Reactive Protein (CRP)

There was a considerable difference (p<0.001) in the CRP levels between the HBV infected participants (24.38±1.62 mg/L) and the controls (5.14±0.26 mg/L) (**Table 4.3**). There was also a substantial variation (p<0.001) between the levels of CRP in the participants with acute HBV infection (18.48±0.59 mg/L) as compared to those with chronic infection (50.40±6.37 mg/L), as shown in **Table 4.4**. Further, there was a considerable variation (p<0.001) between the levels of CRP in the chronic HBV infected persons with active infection (93.03±5.24 mg/L) as compared to those with inactive infection (32.45±4.28 mg/L) (**Table 4.4**).

4.5.2 Interleukin 6

There was a substantial difference (p<0.001) in the IL-6 levels between the HBV infected participants (27.94±1.55 pg/mL) and the controls (6.60±0.23 pg/mL) (**Table 4.3**). There was also a considerable disparity (p<0.001) between the levels of IL-6 in the participants with acute HBV infection (22.37±0.57 pg/mL) as compared to those with chronic infection (52.49±6.13 pg/mL), as shown in **Table 4.4**. Further, there was a considerable disparity (p<0.001) between

the levels of IL-6 in the chronic HBV infected individuals with active infection (91.03±4.45 pg/mL) as compared to those with inactive infection (36.27±4.94 pg/mL) (**Table 4.4)**.

4.6 NON-INVASIVE MARKERS OF LIVER FIBROSIS

4.6.1 Aspartate-Alanine Transaminase Ratio (AAR)

There was a substantial variation ($p<0.001$) in the AAR between the HBV infected participants (1.60±0.07) and the controls (0.83±0.06) (**Table 4.3**). However, there was no significant disparity ($p=0.323$) between the AAR in the persons with acute HBV infection (1.57±0.07) as compared to those with chronic infection (1.74±0.16) (**Table 4.4**). There was a considerable variation ($p=0.007$) between the AAR in the chronic HBV infected persons with active infection (2.21±0.09) as compared to those with inactive infection (1.54± 0.21) (**Table 4.4**).

4.6.2 Aspartate Transaminase-Platelet Ratio Index (APRI)

There was a considerable variation ($p<0.001$) in the APRI between the HBV infected participants (0.80±0.07) and the controls (0.11±0.01) (**Table 4.3**). Likewise, there was a considerable variation ($p=0.001$) between the APRI in the participants with acute HBV infection (0.62± 0.05) as compared to those with chronic infection (1.62±0.25) (**Table 4.4**). Further, there was a considerable variation ($p<0.001$) between the APRI in the chronic HBV infected participants with active infection (3.31±0.35) as compared to those participants wit inactive infection (0.90±0.11), as shown in **Table 4.4**.

4.6.3 Fibrosis 4 (FIB-4) Index

There was a significant variation ($p<0.001$) in the FIB-4 index between the HBV infected participants (1.86±0.12) and the controls (0.42±0.02) (**Table 4.3**). There was also a significant variation ($p=0.001$) between the FIB-4 index in the participants with acute HBV infection (1.60±0.11) as compared to the persons with chronic infection (2.97±0.36) (**Table 4.4**).

Similarly, there was a considerable disparity ($p=0.007$) between the FIB-4 index in the chronic HBV with active infection (4.83±0.71) as compared to those with chronic inactive infection (2.18±0.25), as shown in **Table 4.4**.

4.7 CORRELATION BETWEEN THE BIOMARKERS OF LIVER INJURY AND INFLAMMATION

4.7.1 Participants with Acute Hepatitis B Virus Infection

From the Pearson's correlation analysis, there were positive significant associations between: AST and ALT levels ($r=0.744$, $p<0.001$), and CRP and IL-6 levels ($r=0.820$, $p<0.001$). However, there was a positive insignificant association between ALT and CRP levels ($r=0.005$, $p=0.958$). There were negative insignificant correlations between: AST and IL-6 levels ($r=0.067$, $p=0.469$), AST and CRP levels ($r=-0.057$, $p=0.535$), and ALT and IL-6 levels ($r=-0.041$, $p=0.658$) within the participants with acute HBV infection, as presented in **Table 4.5**.

4.7.2 Participants with Chronic Hepatitis B Virus Infection

The association between the biomarkers of liver injury and inflammation within the participants with chronic HBV infection were analysed by means of the Pearson's correlation analysis. From the correlation analysis, there were positive significant associations between:

AST and ALT levels($r=0.863$, $p<0.001$), CRP and IL-6 ($r=0.959$, $p<0.001$), IL-6 and ALT ($r=0.855$, $p<0.001$), IL-6 and AST ($r=0.799$, $p<0.001$), CRP and ALT ($r=0.909$, $p<0.001$), and CRP and AST ($r=0.856$, $p<0.001$), as demonstrated in **Table 4.5**.

Table 4.5: Correlation between some of the parameters within the participants with acute (Upper-Right Hand Side) and chronic (Lower-Left Hand Side) hepatitis B virus infections

Parameters	HB (g/dL)	AST (U/L)	ALT (U/L)	ALB (g/dL)	CRP (mg/L)	IL-6 (pg/mL)
HB (g/dL)		0.020	-0.024	0.097	0.000	-0.025
		(0.828)	(0.793)	(0.294)	(1.000)	(0.789)
AST (U/L)	-0.204		**0.744***	0.058	-0.057	-0.067
	(0.309)		(0.000)	(0.528)	(0.535)	(0.469)
ALT (U/L)	-0.115	**0.863***		0.066	0.005	-0.041
	(0.568)	(0.000)		(0.479)	(0.958)	(0.658)
ALB (g/dL)	0.268	-0.366	**-0.404***		-0.072	0.110
	(0.177)	(0.061)	(0.037)		(0.435)	(0.235)
CRP (mg/L)	-0.128	**0.856***	**0.909***	-0.240		**0.820***
	(0.524)	(0.000)	(0.000)	(0.227)		(0.000)
IL-6 (pg/mL)	-0.168	**0.799***	**0.855***	-0.275	**0.959***	
	(0.401)	(0.000)	(0.000)	(0.166)	(0.000)	

The data are expressed as correlation coefficients (p-values), ***:** correlation coefficient statistically significant ($p<0.05$), **HB**: Haemoglobin, **WBC**: White Blood Cell, **PLT: P**latelet, **AST:** Aspartate Transaminase, **ALT:** Alanine Transaminase, **ALB**: Albumin, **IL-6**: Interleukin 6, **CRP:** C-Reactive Protein

4.7.3 Chronic Hepatitis B Virus Infected Participants with Inactive Infection

The association between the biomarkers of liver injury and inflammation within the chronic HBV infected persons with inactive infection were analysed by means of the Pearson's correlation analysis. From the analysis, there were positive significant associations between: ALT and CRP levels (r=0.682, p=0.001), CRP and IL-6 levels (r=0.887, p<0.001), and ALT and IL-6 levels (r=0.550, p=0.015). There were also positive associations between: AST and CRP levels (r=0.202, p=0.406), AST and ALT levels (r=0.420, p=0.073), and AST and IL-6 levels (r=0.047, p=0.0849) but statistically insignificant, as revealed in **Table 4.6**.

4.7.4 Chronic Hepatitis B Virus Infected Participants with Active Infection

The correlation between the biomarkers of liver injury and inflammation within the chronic HBV infected individuals with active infection were analysed by means of the Pearson's correlation analysis. From the analysis, there were positive significant associations between: CRP and ALT levels (r=0.854, p=0.007), ALT and IL-6 levels (r=0.716, p=0.046), IL-6 and CRP levels (r=0.898, p=0.002), and AST and IL-6 levels (r=0.713, p=0.047). However, there were positive insignificant associations between: CRP and AST levels (r=0.458, p=0.253), and AST and ALT levels (r=0.300, p=0.471), as shown in **Table 4.6**.

Table 4.6: Correlation between some of the parameters within the chronic hepatitis B virus infected participants with inactive (Upper-Right Hand Side) and active (LowerLeft Hand Side) infections

Parameters	HB (g/dL)	AST (U/L)	ALT (U/L)	ALB (g/dL)	CRP (mg/L)	IL-6 (pg/mL)
HB (g/dL)		0.071	0.108	0.254	0.176	0.052
		(0.774)	(0.659)	(0.294)	(0.472)	(0.833)

AST (U/L)	-0.528		0.420	-0.003	0.202	0.047
	(0.179)		(0.073)	(0.991)	(0.406)	(0.849)
ALT (U/L)	-0.218	0.300		-0.231	**0.682***	**0.550***
	(0.604)	(0.471)		(0.34)	(0.001)	(0.015)
ALB (g/dL)	0.071	-0.246	0.250		0.123	0.044
	(0.867)	(0.557)	(0.551)		(0.617)	(0.859)
CRP (mg/L)	-0.417	0.458	**0.854***	0.271		**0.887***
	(0.304)	(0.253)	(0.007)	(0.516)		(0.000)
IL-6 (pg/mL)	-0.510	**0.713***	**0.716***	-0.046	**0.898***	
	(0.196)	(0.047)	(0.046)	(0.914)	(0.002)	

The data are expressed as correlation coefficients (p-values), ***:** correlation coefficient statistically significant ($p<0.05$), **HB**: Haemoglobin, **WBC**: White Blood Cell, **PLT: P**latelet, **AST:** Aspartate Transaminase, **ALT:** Alanine Transaminase, **ALB**: Albumin, **IL-6**: Interleukin 6, **CRP:** C-Reactive Protein

4.8 CORRELATION BETWEEN THE BIOMARKERS OF INFLAMMATION, ANAEMIA AND HEPATOCYTES FUNCTION

4.8.1 Correlation between C-Reactive Protein and Haemoglobin Concentrations

Using the Pearson's correlation analysis, there was no association between CRP and HB ($r=0.000$, $p=1.000$) within the participants with acute HBV infection. However, there was a negative insignificant association between CRP and HB ($r=-0.128$, $p=0.524$) within the participants with chronic HBV infection (**Table 4.5**).

There was a positive insignificant association between CRP and HB ($r=0.176$, $p=0.472$) within the chronic HBV infected participants with inactive infection. Also, there was a negative insignificant association between CRP and HB ($r=-0.417$, $p=0.304$) within the chronic HBV infected participants with inactive infection, as revealed in **Table 4.6**.

4.8.2 Correlation between C-Reactive Protein and Albumin Concentrations

Using the Pearson's correlation analysis there was a negative insignificant association between CRP and albumin (r=-0.072, p=0.435) within the participants with acute HBV infection. Also there was a negative insignificant association between CRP and albumin (r=-0.240, p=0.227) within the participants with chronic HBV infection, as presented in **Table 4.5**.

There was a positive insignificant association between CRP and albumin (r=123, p=0.617) within the chronic HBV infected persons with inactive infection. Also, there was a positive insignificant association between CRP and albumin (r=0.271, p=0.516) within the chronic HBV infected persons with inactive infection (**Table 4.6**)

4.9 PREDICTORS OF HIGH LEVELS OF C-REACTIVE PROTEIN AMONG THE HEPATITIS B VIRUS INFECTED PARTICIPANTS

The multiple linear regression analysis was used to determine whether haemoglobin, albumin and white blood cell levels could be used to predict high levels of CRP among the HBV infected participants.

There was a decrease of approximately 0.94g/dL (p=0.009) in the haemoglobin levels for each unit rise in the CRP levels in the HBV infected participants. There was also a decrease of approximately 0.49 g/dL (p=0.580) in the albumin levels for each unit rise in the CRP levels in the HBV infected participants but statistically insignificant (**Table 4.7**).

However, there was an increase of approximately 0.5 $x10^9$/L (p=0.024) in the WBC levels for each unit rise in the CRP levels in the HBV infected participants (**Table 4.7**).

Table 4.7: Predictors of high levels of C-reactive protein among the hepatitis B virus infected participants

	Estimate	Std. Error	T - value	p - value	L95%	U95%
Intercept	28.665	6.456	4.440	0.000	15.902	41.427
HB	-0.944	0.349	-2.708	**0.008**	-1.633	-0.255
ALB	-0.488	0.879	-0.555	0.580	-2.225	1.249
WBC	0.501	0.248	2.024	**0.024**	0.012	0.991

HB: Haemoglobin, **ALB**: Albumin, **WBC**: White Blood Cell, **CRP:** C-Reactive Protein, ***:** estimate statistically significant ($p<0.05$)

4.10 PREDICTORS OF SIGNIFICANT LIVER FIBROSIS AMONG THE HEPATITIS B VIRUS INFECTED PARTICIPANTS

The multivariate logistic regression analysis was used to determine whether age in years, gender, and the presence of viral markers (anti-HBc and HBeAg) were independent predictors of significant liver fibrosis. From the analysis, only the presence of HBeAg was an independent predictive factor (OR=115.33, $p<0.001$), as presented in **Table 4.8**.

Table 4.8: Predictors of liver fibrosis among the hepatitis B virus infected participants OR 95% CI for the OR p-value

		Lower	Upper	
Intercept	0.057	0.002	1.061	0.070
Age	0.987	0.917	1.062	0.715
Gender: Male	1.599	0.331	12.499	0.597
anti-HBc: Positive	2.908	0.565	12.294	0.160
HBeAg: Positive	115.327	15.970	2463.982	**0.000***

***OR**: Odd Ratio, **CI:** Confidence Interval, * OR statistically significant (p<0.05)*

CHAPTER FIVE

DISCUSSION

5.1 THE PERCENTAGES OF ACUTE AND CHRONIC INFECTIONS AMONG THE HEPATITIS B VIRUS INFECTED PARTICIPANTS

From this study, the proportion of males among the HBV infected participants was higher than the females. This finding is comparable to another study (Amidu *et al*., 2012) which reported a higher proportion of HBV infection among males (48/407: 11.79%) as compared to the females (20/375: 5.33%). Also, a study conducted by Baig (2009) showed a gender disparity among HBV infected individuals with a higher proportion of males (375: 79.5%) as compared to the females (97: 20.5%) among 472 participants. This disparity may be as a result of females producing antibodies at a higher frequency against the HBsAg and HBeAg than males due to high estrogen levels in females (Baig, 2009).

There was a higher percentage of acute HBV infection than those with chronic HBV infection. Male dominance was recorded in these clinical categories of HBV infected persons with acute and chronic infections. These findings are similar to those of Bello *et al*. (2013) who reported a high percentage (27: 64.3%) of acute infection among 42 HBV infected persons. Also, a study conducted by Baig (2009) revealed a higher percentage of acute HBV acute infection (75%) as compared to those persons with chronic HBV infection (25%). The lower proportion of the chronic HBV infection chronic infection may be due to the fact that most adults infected with hepatitis B virus recover entirely from the infection with about 5 – 10% who do not clear the infection and progress to chronic HBV infection (Schilsky, 2013). There was a higher percentage of chronic HBV infected persons with inactive infection as compared to those with chronic active infection. Male dominance was also recorded in these clinical categories. A similar outcome with male dominance has been found with chronic HBV infection-associated hepatocellular carcinomas (McMahon *et al.,* 1990; Yu and Chen*.,* 1994).

The male dominance recorded in these clinical categories of chronic HBV infected persons with active infection may be due to the fact that the male gender may be more vulnerable to developing symptomatic HBV infection 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 (Shimizu *et al*., 1998). The fact that hepatocellular carcinoma is more dominant in men than in women is suggestive that estrogen may play a substantial role in the progression of hepatocellular carcinoma (Lengi *et al*., 2006). De Maria *et al*. (2002) observed the expression of more variant estrogen receptors in males than in females with hepatocellular carcinoma. Also, an experimental study by Shimizu *et al*. (1998) demonstrated a suppressive influence of estradiol on the hepatocarcinogenesis in induced carcinomas. Furthermore, estradiol has also been reported to inhibit reactive oxygen species production and antioxidant enzyme loss through the suppression of NADH/NADPH oxidase activity (Lengi *et al*., 2006).

5.2 HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN THE PROGRESSION OF THE HEPATITIS B VIRUS INFECTION

Haematological Parameters

The haemoglobin (HB) concentration was lower among the HBV infected participants as compared to the controls. This finding is similar to previous studies (Fasola *et al*., 2009; Eze *et al*., 2009) which also reported a lower HB concentrations in HBV infection individuals as compared to control individuals.

Also, there was a lower HB concentration among the persons with chronic HBV infection than those with acute infection. This finding is similar to a previous study (Lin *et al*., 1991) which also reported a lower HB concentration among the persons with chronic HBV infection. Again, there was a lower HB concentration among the chronic HBV infected individuals with active infection than those with inactive infection. Most of the participants with chronic HBV infection were anaemic (mean HB < 10.0 g/dL).

The haemoglobin concentration in most individuals with viral hepatitis declines steadily during the acute phase of the disease (Connrad *et al*., 1965). This may be attributed to the suppression of the bone marrow and autoimmune haemolytic anaemia which may accompany viral hepatitis (Gumba and Chopra, 1995). The shared epitopes of human and viral antigens that are presented by the antigen presenting cells have been found to be able to cause an autoimmune trigger leading to autoimmune haemolysis (Fellermann and Stange, 2000).

Increased haemolysis has also been detected in many individuals with acute hepatitis due to the extravascular defect in the red cells which leads to decreased red cell life span (Katz *et al*., 1964). The decrease in the haemoglobin concentration in the participants with chronic HBV

infection may be due to anaemia of chronic disease or aplastic anaemia (Little *et al*., 2012). Also, dilutional anaemia can be another possible explanation for this observed anaemia in the persons with chronic HBV active infection. In dilutional anaemia, the plasma volume is normally increased in active hepatic disease causing pseudo-anaemia (Conrad *et al*., 1964).

There were higher total white blood cell (WBC), neutrophil and Lymphocyte counts among HBV infected participants as compared to the controls. This finding is similar to previous studies (Lin *et al*., 1991; Fasola *et al*., 2009) which also reported higher WBC, lymphocyte and neutrophil counts in HBV infected persons as compared to control individuals.

Further, there were higher WBC, lymphocyte and neutrophil counts among the persons with chronic HBV infection than those with acute infection. These findings are similar to a previous study (Akarsu *et al*., 2008) which also reported higher WBC, lymphocyte and neutrophil counts among the persons with chronic HBV infection. Also, there were higher WBC, lymphocyte and neutrophil counts among the chronic HBV infected persons with active infection than those with inactive infection. Overall, most of the participants with chronic HBV infection have leucocytosis with their mean lymphocyte, neutrophil and WBC counts greater than 4.0 $x10^9$/L, 8.0 $x10^9$/L and 11.0 $x10^9$/L respectively.

The white blood cells are cellular components of the immune system and they play a role in the defence of the body against communicable diseases and foreign invaders. Therefore, the amount of WBCs in the blood is normally used as an indicator of infectious disease (bacterial or viral) or immune response (Akarsu *et al*., 2008). The presence of leucocytosis in the persons with chronic HBV infection may possibly be due to the viral infection, inflammation and tissue necrosis (Cheesbrough, 2006). The lymphocytosis in the persons is as a result of the body's immune defence against the hepatitis B virus whereas the neutrophilia is an indication of tissue damage due to the viral replication in the hepatocytes (Hoffbrand *et al*., 2006).

There was a lower platelet count among the HBV infected participants as compared to the controls. This finding is similar to previous study (Eze *et al*., 2009) which also reported a lower platelet count in HBV infected persons as compared to control individuals. Also, there was a lower platelet count among the persons chronic HBV infection than those with acute infection. This finding is similar to a previous study (Xiao *et al*., 2014) which also reported a lower platelet count among the persons with chronic HBV infection. However, there was an insignificant difference in the platelet count among the chronic HBV infected participants with active infection as compared to those with inactive infection. Most of the persons with chronic HBV infection have lower but normal platelet count (mean platelet count > 150.0 $x10^9$/L).

The lower platelet count in the HBV infected persons with chronic infection predicts varying grade of liver fibrosis, and this is buttressed by a study (Xiao *et al*., 2014) which showed that lower platelet count is independently associated with significant liver fibrosis. The lower platelet count may also account for the anaemia in the HBV infected persons with chronic infection since individuals with serious hepatocellular disease can develop defects of blood coagulation as consequences of deficiencies of coagulation factors, thrombocytopenia, and endothelial dysfunction (Caldwell *et al*., 2006).

Biochemical Parameters

The liver contains transaminases which play critical roles in the synthesis, breakdown, and recycling of endogenous nitrogenous compounds such as amino acids (Sorbi *et al.,* 1999). These enzymes include aspartate and alanine transaminases. Even though the serum level of ALT is a more specific marker for liver damage, the combination of the basal serum AST and ALT levels provides a better precision for indicating the aetiology of the liver injury (Sheth *et al*., 1999; Sorbi *et al*., 1999).

These transaminases are found in lower concentrations in the serum but in a diseased liver, the liver tissue degenerate due to a localised autoimmune reaction mediated by the Major Histocompatibility Complex Class I (MHC - I) and Hepatitis B virus Surface Protein (HBSP) complex (Philip *et al.,* 2006). During this process, the cell membranes of the hepatocytes 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).

The serum levels of ALT and AST were higher in the HBV infected participants as compared to the controls. This finding is similar to a previous study (Al-Ajeeli, 2011) which also reported higher transaminases levels in HBV infected persons as compared to control individuals. There was an increase in the serum levels of these transaminases among the HBV infected persons with chronic infection than those with acute infection.

Also, there was a substantial rise in the levels of ALT and AST among the HBV infected participants with chronic active infection than those with chronic inactive infection. The higher serum levels of these transaminases in the persons with the chronic active infection may possibly be due to the fact that persons in this stage of the infection are supposedly to be having a more profound damage of the liver as a result of the spread of the viral infection and replication (Nyblom *et al.,* 2006). In the inactive infection, there is an initial aggressive host immune response and viral adaptation which may likely reduce the rate of viral replication and activity in these persons which helps to keep the liver in a stable condition (Philip *et al.,* 2006).

In the persons with chronic HBV infection, the changes in the serum transaminases were dependent on the occurrence of the hepatitis B virus envelope antigen (HBeAg) in their serum. Chronic HBV infection is characterised by the persistent serum level of HBsAg, anti-HBc IgG and detectable viral DNA. The HBeAg is often detectable but may disappear after

seroconversion to its antibody (anti-HBe). The presence of the HBeAg in serum is suggestive of an active viral infection (Hadziyannis and Vassilopoulos, 2001).

It has been reported that hepatitis B viral replication is highly related to cell death, which is contrary to the non-cytopathic characteristics of the hepatitis B virus (Tsai *et al.,* 1992). This was further validated by Milich *et al.* (1998) whose research revealed that two viral proteins (HBxP and HBsP) produced in higher titres during viral replication actively induce apoptosis in host hepatocytes. Therefore, it can be inferred that higher liver damage can occur during the active viral replicative stage. This is supported by the relatively elevated serum levels of ALT and AST in the chronic HBV infected persons with active infection as observed in this study. Comparatively, the serum levels of AST were relatively higher than ALT among all the classifications of the HBV infected participants. This is because generally, AST enzymes occur both in the cytoplasm and mitochondria unlike ALT which occurs mainly in the cytoplasm (Nyblom *et al.,* 2006). In cases of liver diseases, AST leak from both the cytoplasm and mitochondria hence their levels are higher than that of the alanine transaminase which occur in the cytoplasm as observed in this study.

Serum levels of ALP and GGT were higher among the HBV infected participants than the controls. This finding is similar to previous studies (Shaha *et al*., 2004; Asghar *et al*., 2011) which also suggested higher ALP and GGT levels in HBV infected persons as compared to control individuals. There was an increase in the levels of serum ALP and GGT among the HBV infected participants with chronic infection than those with acute infection. This finding is similar to previous studies (Hill and Sammons, 1987; Xiao *et al*., 2014) which also reported higher ALP and GGT levels in persons with chronic HBV infection. Also, there was a substantial upsurge in the levels of serum ALP and GGT among the HBV infected participants with chronic active than those with chronic inactive infection.

Alkaline phosphatase originates predominately from the liver and bone. Persistently elevated ALP levels, accompanied by elevated GGT levels, indicate chronic cholestasis or infiltrative liver disease (Gopal and Rosen, 2000). In this study, majority of the participants with chronic HBV infection have normal ALP levels with their mean levels less than 150.0 U/L, hence the exclusion of chronic cholestasis. Gamma glutamyl transferase is best used to evaluate the origin of other serum enzymes (Pratt and Kaplan, 2000). Hence, the elevated GGT confirms the hepatic origin of the serum transaminases and alkaline phosphatase.

The albumin concentration was lower among the HBV infected participants as compared to the controls. This finding is similar to a previous study (Mehde, 2013) which also reported a lower albumin concentration in HBV infected persons as compared to control individuals. There was a lower albumin concentration among the HBV infected participants with chronic infection than those with acute infection. This finding is similar to a previous study (Rebar, 1999) which also reported a lower albumin concentration in HBV infected persons with chronic infection. Likewise, there was a significant reduction in the albumin concentration among the HBV infected participants with chronic active infection than those with chronic inactive infection.

The levels of serum albumin are normally related to the functionality of the hepatocytes (Tonya *et al*., 2007). A study conducted by Rebar (1999) showed that low serum albumin levels is indicative of poor liver function. Therefore, the decrease in the albumin concentration may be as result of the gradual loss of hepatocytes function due to the viral replication and the body's immune response (Iannacone *et al*., 2005; Coffin *et al*., 2011). The concentration of albumin in the serum is generally normal in chronic liver disease until a significant liver damage is apparent (Rebar, 1999), as seen in the HBV infected participants of this study.

5.3 THE PRESENCE OF LIVER INFLAMMATION AND LIVER FIBROSIS, AND

THE ASSOCIATIONS BETWEEN THE BIOMARKERS OF LIVER INJURY AND INFLAMMATION

Markers of Inflammation

Most inflammatory markers for instance CRP and IL-6 are found in lower concentrations in the serum but are raised in inflammatory conditions. The serum levels of CRP and IL-6 were higher among the HBV infected participants than the controls. These findings are similar to previous studies which reported higher levels of CRP (Amah *et al*., 2011) and IL-6 (Karumus and Shinagawa 1993) in HBV infected persons than control individuals.

There was a substantial upsurge in the serum levels of CRP and IL-6 among the HBV infected participants with chronic infection than those with acute infection. Also, there was a significant rise in the levels of these inflammatory markers among the HBV infected participants with chronic active infection than those with chronic inactive infection. These findings are concurring with a previous study (Karabassi *et al*., 2005) which reported elevated levels of CRP in HBV infected persons with chronic infection. Another study (Khan *et al*., 2011) also showed higher serum levels of CRP and IL-6 in chronic HBV infected persons. It was observed by Khan *et al*. (2011) that, there was a positive significant association between IL-6 and hepatitis B viral load. C-reactive protein has also been found to be associated with HBV replication, liver damage and fibrosis in individuals with chronic HBV infection, and therefore serum CRP may be a marker for the diagnosis of significant fibrosis in patients with chronic HBV infection (Ma *et al.,* 2015).

Interleukin 6 had been demonstrated to be induced by the HBx protein (Benhenda *et al*., 2013). The HBx protein had been found to play a part in the activation of the hepatitis B viral transcription process (Benhenda *et al*., 2013). The HBx protein transactivates the human IL-6 promoter through the NF-κB binding site. The up-regulation of the IL-6 by the HBx protein

can induce hepatitis, and this can play an essential function in the pathogenesis of liver fibrosis, which can develop into cirrhosis and/or hepatocellular carcinoma (Lee *et al*., 1998).

C- reactive protein is produced in a wide array of acute and chronic inflammatory conditions; viral, bacterial or fungal infections, tissue injury or necrosis, and other inflammatory diseases (Pepys and Hirschfield, 2003). These disorders cause the release of cytokines including interleukin-6 from the T cells and macrophages to activate the production of CRP and fibrinogen by the liver (Lau *et al.,* 2005). The classical function of CRP, which is part of the innate immunity, is its ability to bind to phosphocholine and thus recognise certain foreign pathogens as well as phospholipid components of damaged cells (Volariakis, 1997). The variations in concentration of this acute phase protein are largely due to the changes in their synthesis by the hepatocytes (Cem and Irving, 1999; Bonnete *et al*., 2003). This study suggested that inflammatory response was more intense in the HBV infected participants especially those with chronic active infection.

This study also showed that decrease in haemoglobin levels as well as increase in WBC levels were independent predictive factors for high levels of CRP in the HBV infected individuals. Therefore the combination of these two parameters could serve as a simple marker to predict high CRP levels in HBV infected individuals.

Non-Invasive Markers of Liver Fibrosis

The non-invasive markers for the estimation of significant fibrosis of the liver have been compared to the use of liver biopsy in the detection and staging of liver fibrosis, and have been found to be cheaper, simpler and useful (Shin *et al*., 2008; Bedossa and Carrat, 2009; Martinez *et al*., 2011; Yang *et al*., 2012; Papastergiou *et al*., 2012). Three of these simple markers were used in the estimation of liver fibrosis in this study: AAR, FIB-4 and APRI. To assume the

existence of significant liver fibrosis, at least two of these markers must have their calculated scores above their cut-off scores: AAR>1.0, APRI>1.5 and FIB-4>1.45.

In the study, the scores of AAR, APRI and FIB-4 were averagely higher among the HBV infected participants than the control individuals. This may be due to the mild to significant liver fibrosis among the HBV infected cohorts, with AAR and FIB-4 above the cut-off scores. A similar study also found significant liver fibrosis among HBV infected persons (Achmad *et al*., 2014), and the fibrosis was measured based on APRI, FIB-4, AAR, age-platelet (AP) index and Pohl score. Estimating the stage of fibrosis with the mean FIB-4 = 1.86, most of the HBV infected participants could be classified into F2.

Also, there were higher scores of AAR, APRI and FIB-4 among the HBV infected participants with chronic infection than those with acute infection. In the HBV infected persons with acute infection, only AAR was above its cut-off score supporting insignificant liver fibrosis in these persons. However in the HBV infected participants with chronic infection, all the markers were above their cut-off scores confirming mild to significant fibrosis in these category of persons. A study conducted by Fung *et al.* (2008) also found liver fibrosis among HBV infected persons with chronic infection with higher rates in males, and persons with higher ALT levels. Estimating the stage of fibrosis with the mean FIB-4 = 2.97, most of the persons with chronic HBV infection could be classified into F2.

Again, there were higher scores of AAR, APRI and FIB-4 among the chronic HBV infected persons with active infection than those with inactive infection. However among the persons with both active and inactive infection, there was the presence of liver fibrosis among them. Estimating the stage of fibrosis with the mean FIB-4 = 4.83, most of the chronic HBV infected persons especially those with active infection could be classified into F3 – F4.

This study showed that the presence of the hepatitis B virus envelope antigen (HBeAg), which is a marker of active hepatitis B viral replication, is an independent predictive factor for the development of significant liver fibrosis. It had also recognised that the presence of the hepatitis B virus envelope antigen is associated with relatively high infectivity and severity of the hepatitis B infection (Hollinger and Liang, 2001).

Correlation between the Biomarkers of Liver Injury and Inflammation

This study showed significant correlations between: CRP and IL-6 levels, and AST and ALT levels within the HBV infected participants with acute infection. However, there were insignificant correlations between: ALT and CRP levels, AST and CRP levels, AST and IL-6 levels, and ALT and IL-6 levels. Within the HBV infected participants with chronic infection, there were significant correlations between all the biomarkers. These findings agree with a previous study (Karabassi *et al*., 2005) which reported a significant association between elevated levels of serum CRP and ALT in the individuals with chronic HBV infection.

There were significant associations between: ALT and CRP levels, ALT and IL-6 levels, and CRP and IL-6 levels, but insignificant correlations between: AST and CRP levels, AST and IL-6 levels, and AST and ALT levels among the HBV infected participants with chronic inactive infection. In the chronic HBV infected individuals with active infection, there were significant correlations between: CRP and IL-6 levels, ALT and CRP levels, AST and IL-6 levels, and ALT and IL-6 levels while no significant correlations were seen between: AST and ALT levels, and CRP and AST levels.

The significant associations may be as a result of the hepatitis B viral activity in the hepatocytes which causes the release of IL-6 from the T cells and macrophages which also triggers the

production of CRP by the liver (Lau *et al.,* 2005), and the release of the transaminases from these damaged hepatocytes into the blood (Nyblom *et al.,* 2006).

Correlation between Biomarkers of Inflammation, Anaemia and Hepatocytes Function

This study showed no correlation between CRP and HB concentration, and a negative insignificant correlation between CRP and albumin concentration within the HBV infected participants with acute infection. However, there were negative insignificant correlations between: CRP and HB concentration, and CRP and albumin concentration within the persons with chronic HBV infection. Among the chronic HBV infected persons with active infection, there was a negative insignificant correlation between CRP and HB concentration and a positive insignificant association between CRP and albumin concentration.

In the individuals with chronic HBV infection, the negative correlations means that as the CRP levels increases, the concentration of haemoglobin and albumin decreases. The decreased in the albumin concentration may be as a result of the gradual loss of hepatocytes function due to the viral replication and immune response of the body (Iannacone *et al*., 2005; Coffin *et al*., 2011). However the rise in the CRP levels in the presence of the impaired hepatocytes function may be as a result of the fact that the increase IL-6 and other cytokines, caused by chronic inflammatory conditions, initiate the production of CRP by the liver (Pepys and Hirschfield, 2003; Lau *et al.,* 2005).

CHAPTER SIX

CONCLUSION

6.1 GENERAL CONCLUSION

The percentages of acute and chronic infections were 81.5% (CI: 74.2 - 87.4) and 18.5% (CI: 12.6 - 25.8) among the HBV infected participants respectively. Overall, the percentage of the chronic HBV infected persons with active infection was 5.5% (CI: 2.4 - 10.5). There was male dominance among all the clinical categories of the HBV infected persons.

The CRP and IL-6 were significantly increase in the persons with chronic HBV infection especially those with active infection. The changes in these inflammatory markers in the serum reflect the presence as well as the intensity of the liver inflammation. Therefore, these inflammatory markers may be useful as a clinical guide in the diagnosis and controlling of chronic liver inflammation in the HBV infected participants over the current use of only serum transaminases especially ALT which is only an indicator of liver injury.

This study further showed that decrease in haemoglobin levels as well as increase in WBC levels were independent predictive factors for high levels of CRP in the HBV infected

participants. Therefore the combination of these two parameters could serve as a simple marker for high CRP levels in the HBV infected individuals.

The study also showed significant anaemia, impaired hepatocytes function and liver fibrosis in the chronic HBV infected participants with active infection. The presence of the HBeAg, which is a marker of active hepatitis B viral replication, was an independent predictive factor for the development of significant liver fibrosis among the HBV infected participants.

This study showed a significant correlation between elevated levels of serum CRP and ALT in the chronic HBV infected persons with active infection, and therefore the combination of these biomarkers could be an important marker for the monitoring of the progression of the infection in these individuals.

6.2 RECOMMENDATIONS AND SUGGESTIONS

- Further studies should be conducted to establish the effectiveness of anti-inflammatory drugs in the management of the liver inflammation in HBV infected persons.
- Persons with chronic HBV infection should be screened for the presence of anaemia and liver fibrosis, and those affected should be managed appropriately to prevent further complications.

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APPENDIX I

ETHICAL CLEARANCE

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/336/14

29th September, 2014.

Mr. Seth Agyei Domfeh
Department of Molecular Medicine
School of Medical Sciences
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title: "Assessing Serum Levels of Inflammatory Markers as an Aid in the Management of Hepatitis B Infection."

Proposed Site: Medilab Diagnostic Services Limited.

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 6th February, 2014 from the Medilab Diagnostic Services Limited (study site) indicating approval for the conduct of the study in the laboratory.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Proposal.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Rev. Prof. John Appiah-Poku.
Honorary Secretary
For: CHAIRMAN

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APPENDIX II

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

PARTICIPANT INFORMATION LEAFLET

Title of the Research:

SERUM LEVELS OF INFLAMMATORY MARKERS IN HEPATITIS B VIRUS INFECTION

Names and Affiliations of Researchers:

This research is being conducted by Mr. Seth Agyei Domfeh and Prof. (Mrs.) Margret T. Frempong, Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi.

Background:

This research is about hepatitis B virus (HBV) infection. Hepatitis B is an infectious disease of the liver which is caused by the hepatitis B virus. Hepatitis B virus primarily interferes with the functions of the liver by multiplying in the liver cells. Currently, the management of hepatitis B virus infection reduces the multiplication of the virus in the liver cells, thereby reducing the amount of virus particles in the blood of patients. But the drugs used in the management of hepatitis B do not target the swelling conditions of the liver. In Ghana, there is no information on the levels of substances that can be used to determine the swelling conditions of the liver in patients with hepatitis B; therefore this research has become necessary to assess the levels of substances that can be used to determine the swelling conditions of the liver in hepatitis B virus infected persons so as to manage the swelling conditions.

Purpose of the Research:

The purpose of this research is to assess the levels of substances that can be used to determine the swelling conditions of the liver in hepatitis B virus infected persons to help in the management of the swelling conditions of the liver due to the infection in the future.

Procedure of the Research:
To accomplish the purpose of this research, about a teaspoon (approx. 5ml) of blood will be drawn from your arm using a syringe and needle for the assessment of the substances that can be used to determine the swelling conditions of the liver. Two hundred and ten (210) participants would be recruited for this research.

Risk:
The blood sample taking can be inconvenient to you. You may develop burning sensation or swelling at the site where the blood will be drawn from since a syringe and needle would be used.

Benefit:
You would get to know of your blood levels of the substances that can be used to determine the swelling conditions of the liver. The outcome of this research in Ghana would also help in the management of the hepatitis B in the future.

Confidentiality:
All information which will be collected from you in this research will be given code numbers. Data collected will not be linked to you in anyway. No name or identifier will be used in any publication or reports from this research. However, as part of our responsibility to conduct this research properly, we may allow officials from the ethics committee to have access to your records.

Voluntariness:
Taking part in this research should be out of your own free will. You are not under any obligation to take part in this research. This research is entirely voluntary.

Alternatives to Participation:
If you choose not to participate in this research, services provided to you in this company will not be affected in any way.

Withdrawal from the Research:
You may choose to withdraw from the research at any time without having to explain yourself to the researchers. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:
There will be no consequence, loss of benefit or care to you if you choose to withdraw from this research. Please note however that, some of the information that may have been obtained from you, before you chose to withdraw, may have been modified or used in analysis of reports and publications. These cannot be removed anymore. We do promise to comply with your wishes as much as practicable.

Contacts:
If you have any question concerning this research, please do not hesitate to contact Mr. Seth Agyei Domfeh (0547 267 635) and Prof. (Mrs.) Margret T. Frempong (0208 186 136).

Further, if you have any concern about the conduct of this research, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman Committee on Human Research and Publication Ethics Kumasi Tel: 0322 063 248 or 0205 453 785

APPENDIX III

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the research, including that on procedures, risks and benefits, to enable the prospective participant to make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research to decide that I want to take part in it.

I understand that I may freely stop being part of this research at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.
NAME: _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants): I

_____ (Name of Witness) certify that information

given to _____ (Name of Participant), in the local language, is a true reflection of what I have read from the research Participant Information Leaflet, attached.

WITNESS' SIGNATURE: _____

APPENDIX IV

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF MOLECULAR MEDICINE

RESEARCH QUESTIONNAIRE

Title of the Research:

SERUM LEVELS OF INFLAMMATORY MARKERS IN HEPATITIS B VIRUS INFECTION

Demographic Data:

Subject Number: _____ Date: _____

Age: _____ Sex: Male [ ] Female [ ]

Questions:

1. Have you been diagnosed with hepatitis C? Yes [ ] No [ ]
2. Have you been diagnosed with HIV? Yes [ ] No [ ]
3. Have you been diagnosed with any liver disease? Yes [ ] No [ ]
4. Have you been diagnosed with diabetics? Yes [ ] No [ ]
5. Have you been diagnosed with hypertension? Yes [ ] No [ ]
6. Have you been diagnosed with renal disease? Yes [ ] No [ ]
7. Have you been diagnosed with any inflammatory disease? Yes [ ] No [ ]

Remarks:

Included in the study [ ] Excluded from the study [ ]