KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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SCHOOL OF MEDICAL SCIENCE

DEPARTMENT OF CLINICAL MICROBIOLOGY

HAEMATOLOGICAL PROFILE OF ACUTE AND CHRONIC HEPATITIS B POSITIVE PATIENTS ATTENDING KOMFO ANOKYE TEACHING HOSPITAL

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY, K N U S T, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF MPHIL. CLINICAL MICROBIOLOGY

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my mother, Afia Serwaa, and sisters, Aisha Abdallah and Christiana Agyapong.

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The race is neither for the swift nor for the strong, but the Lord who grants victory. I therefore gratefully thank the Almighty God for His abundant grace and guidance throughout my life and education.

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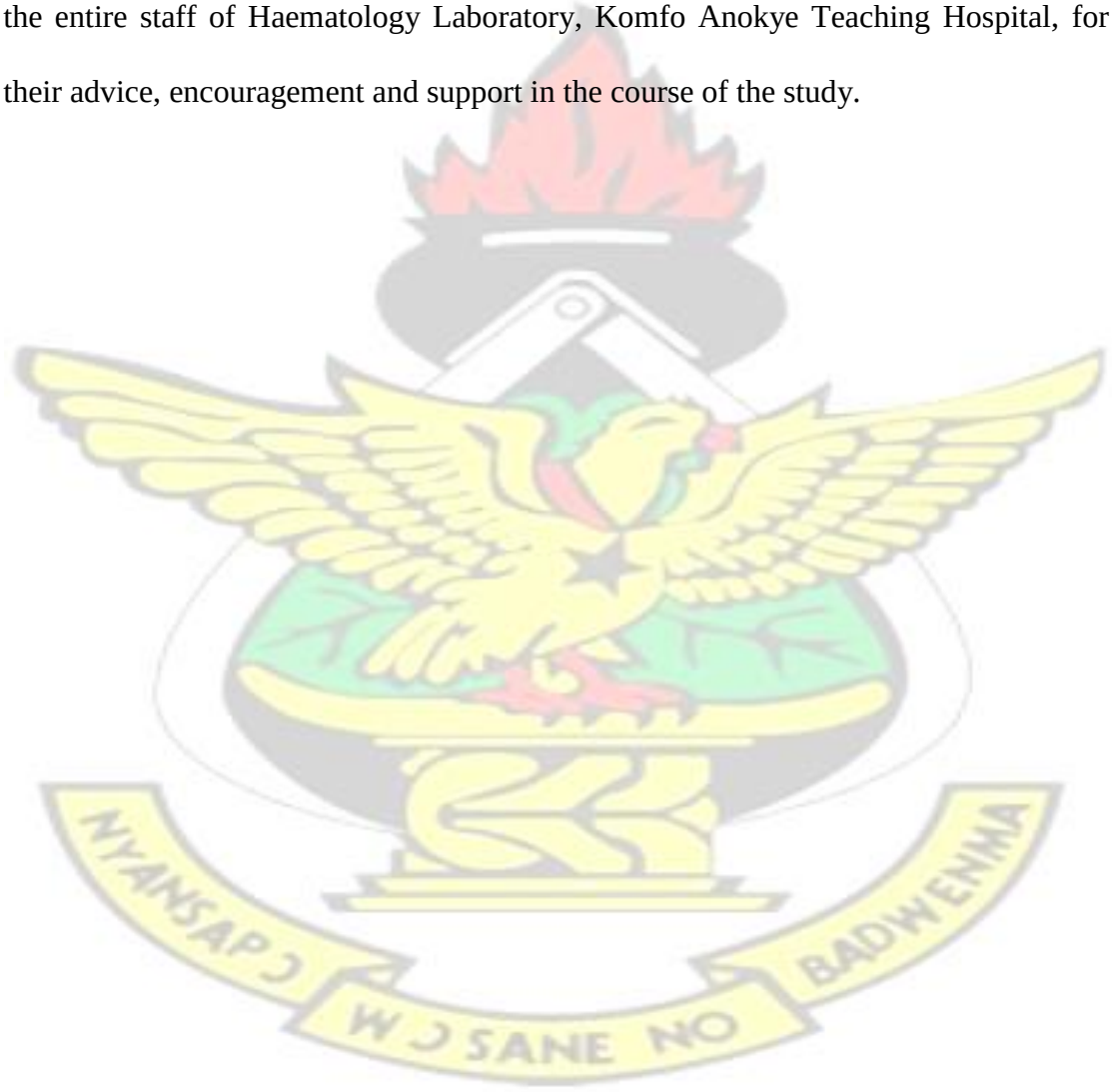

ABSTRACT

Hepatitis B Virus (HBV) infection still remains a major global health problem, especially in hyper-endemic countries like Ghana. The infection presents as acute infection which may develop into chronic infection, and may be symptomatic or asymptomatic. The study determined the haematological profile likely to present at the various stages of the infection, including the acute and chronic stages seen at Komfo Anokye Hospital. The study recruited 122 each of HBV infected and uninfected subjects from 218 HBV infected and 1351 HBV uninfected patients, from August 2015 to April 2016. The infected subjects were grouped into acute and chronic subjects by their positivity or negativity respectively to HBcAb-IgM test. The infected subjects were also grouped into symptomatic and asymptomatic subjects based on their clinical signs and symptoms and their ALT levels. The Complete Blood Count, Erythrocyte Sedimentation Rate (ESR) and Peripheral Blood Examination of all the subjects were determined by standard manual and automated methods. Hepatitis B prevalence of 13.8% was recorded by the study. The HBV infected as well as the symptomatic subjects recorded significant ($P<0.05$) abnormally high ESR but low RBC count, Hb concentration and HCT compared to the uninfected and asymptomatic subjects who recorded normal values of these parameters. Also the symptomatic subjects recorded abnormally high Total WBC and Neutrophil count compared to the normal values recorded for asymptomatic subjects. There was no significant difference in all the haematological parameters of the acute and chronic infected subjects, although both recorded abnormally high ESR, and WBC count; but low Hb concentration, RBC count and HCT value. No haematological malignant cells was recorded in the peripheral blood of the subjects, although the HBV infected subjects showed significant ($P<0.05$) peripheral blood picture of Atypical Mononuclear cells, Reactive Lymphocytes and RBC hypochromasia compared to the uninfected subjects. It is recommended that education on prevention of HBV infection be intensified to help reduce the high prevalence rate revealed by the study. Also as part of the management of HBV infected patients, routine haematological investigations should be carried out, particularly for symptomatic HBV infected patients as well as acute and chronic patients, to help remedy the likely haematological abnormalities that they may present, in order to enhance their overall effective management.

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LIST OF ABBREVIATIONS AND DEFINITIONS

ALT	Alanine aminotransferase
BASO	Basophil
CBC	Complete Blood Count
CTL	Cytotoxic T Lymphocytes.
CccDNA	covalent closed circular DNA
EO	Eosinophil
EPI	Expanded Programme on Immunisation.
ESR	Erythrocyte Sedimentation Rate.
DNA	Deoxyribonucleic Acid
Hb	Haemoglobin
HBV	Hepatitis B Virus
HBsAg	Hepatitis B Surface Antigen
HBeAg	Hepatitis B Envelope Antigen
HBsAb	Hepatitis B Surface Antibody
HBc-IgM	Hepatitis B Core Antibody Immunoglobulin M Type
HBc-IgG	Hepatitis B sCore Antibody Immunoglobulin G Type
HBeAb	Hepatitis B Envelope Antibody
HCT	Haematocrit
ISGs	Interferon inducible genes
LYMPH	Lymphocyte

Mrna	Messenger RNA
MONO	Monocyte
RNA	Ribonucleic Acid
RBC	Red blood cell
WBC	White blood cell
WHO	World Health Organisation

CHAPTER 1- INTRODUCTION

1.1 Background

Hepatitis B is a life-threatening infectious liver disease caused by the hepatitis B virus (HBV). The virus can be transmitted parenterally, perinatally and sexually, and it causes an acute or chronic liver infection which can put people at high risk of death from cirrhosis of the liver and liver cancer **(Ganem and Alfred, 2004).**

Hepatitis B viral (HBV) infection is a major global health problem as it has a worldwide distribution. It is estimated that more than 2 billion people world-wide have been infected with the virus. Of these, approximately 360 million individuals are chronically infected and at risk of serious illness and death, mainly from liver cirrhosis and hepatocellular carcinoma **(WHO, 2009).** Prevalence of hepatitis B infection is highest in sub-Saharan Africa and East Asia. Most people in these regions become infected during childhood and between 5–10% of the adult population are chronically infected **(WHO, 2013)**. In Ghana, the prevalence of the infection is reported to be 8% according to Ghana Health Service. Also a study conducted in three densely populated communities in Kumasi recorded an overall hepatitis B prevalence of 8.68% **(Amidu and Alhassan, 2012)**. These prevalence rates place Ghana and Kumasi in a high hepatitis B endemicity level (≥ 8%) **(WHO, 2009).**

Primary hepatitis B viral infection is often accompanied with subclinical illness or with acute liver inflammation which may be mild or severe and may result in chronic infection **(Robinson, 1995)**. About 90% of primary adult HBV infections result into acute hepatitis. The rest 5 - 10% of primary adult infections and more that 90% of new-born and infant HBV infections result into chronic hepatitis B, and may continue for the life span of the patient **(Hollinger and Liang, 2001)**.

The acute form of hepatitis B is associated with the sero-presence of HBcAb-IgM type and the presence of HBsAg and HBeAg. Whilst the sero-presence of HBcAbIgG type as well as HBsAg for more than six months is considered indicative of chronic hepatitis B **(Chisari and Ferrari, 1997**). Acute and chronic hepatitis B infection may cause liver disease of varying severity, evident in the levels of serum aminotransferases at these stages of the infection. In acute hepatitis B, there is usually increase in aminotransferases, especially alanine aminotransferase (ALT) level, from a moderate to an astronomical increase of more than 100 folds. Chronic hepatitis B infection however usually presents with normal or elevated ALT levels up to 200 U/L in about 90% of patients **(Hollinger and Liang, 2001).**

Liver diseases associated with hepatitis B infection may result in abnormal haematological parameters. A study conducted among pregnant women in Nigeria demonstrated a significant association of hepatitis B infection and reduction in Haematocrit (HCT), Haemoglobin concentration, platelets, total white blood cell, lymphocyte, eosinophil and monocyte counts **(Eze and Buseri, 2009).** Also Ajugwo *et al* and Bozkaya *et al* have demonstrated some association between hepatitis B infection and abnormal Haematocrit, Haemoglobin concentration and Erythrocyte Sedimentation Rate; and aplastic anaemia respectively **(Ajugwo and Ukaji, 2015; Bozkaya and Yurdaydin, 2002)**. Therefore the varying severity of hepatitis and other liver diseases associated with various stages of hepatitis B infection, including the acute and chronic stages, may also produce varying degree of abnormal haematological parameters.

As stated above, hepatitis B infection is considered highly endemic in Ghana and especially in Kumasi. However to the best of our knowledge the extent of haematological abnormalities that may be associated with various stages of the hepatitis B infection, including the acute or chronic stages, are not known in Ghana. We therefore undertook this study to determine the likely haematological profile that may be associated with the acute or chronic and other stages of the hepatitis B infection among hepatitis B positive patients attending Komfo Anokye Teaching Hospital in Kumasi.

1.2 Problem statement

Various stages of HBV infection including the acute and chronic stages may cause liver disease of varying severity, evidenced in the levels of serum aminotransferases at these stages. In acute HBV infection, there is usually increase in aminotransferases, especially alanine aminotransferase (ALT) level, from a moderate to an astronomical increase of more than 100 folds. Whilst chronic HBV infection usually presents with normal or slightly elevated ALT levels in about 90% of patients **(Hollinger and Liang, 2001)**. The varying severity of liver disease associated with acute and chronic HBV infections, may consequently result in varying degree of abnormal haematological parameters in HBV infected patients. However research in this area is scanty and little is known on the likely haematological picture that may present at different stages of the HBV infection, including the acute and chronic phases of the infection.

1.3. Justification

Some studies have demonstrated an association between hepatitis B virus (HBV) infection and some abnormal haematological parameters among HBV infected patients **(Eze and Buseri, 2009; Ajugwo and Ukaji, 2015)**. However, since HBV infection usually manifest as an acute infection which may develop into chronic infection, it is also necessary to know the haematological picture that may present at the acute and chronic phases of the infection.

Also HBV infection is considered highly endemic in Ghana and especially in Kumasi (**Amidu and Alhassan, 2012).** Therefore the outcome of this study will serve as useful information to enhance the overall management of HBV infected patients, as clinicians may know the likely haematological abnormalities that may be associated with the various stages of the HBV infection including the acute and chronic stages of the infection.

1.4. Hypothesis

Various stages of the HBV infection including the acute and chronic stages, may present with varying degree of abnormal haematological parameters.

1.5 General Objective

To determine the haematological picture likely to present at the various stages of the hepatitis B virus (HBV) infection among HBV infected patients attending Komfo Anokye Teaching Hospital.

1.6. Specific Objectives

- To determine the prevalence of the HBV infection at Komfo Anokye Teaching Hospital.
- To determine the symptomatic, asymptomatic, acute and chronic stages of the HBV infection among study subjects.
- To determine and compare the haematological profile of HBV infected and uninfected study subjects.
- To determine and compare the haematological profile of acute and chronic HBV infected study subjects.
- To determine and compare the haematological profile of symptomatic and asymptomatic HBV infected study subjects.

CHAPTER 2- LITERATURE REVIEW

2.1. Epidemiology of HBV Infection

The hepatitis B virus (HBV) is a very common virus with a worldwide distribution and also highly infectious, i.e. about 100 times more infectious than HIV. It is estimated that about one-third of the world population has evidence of present or past HBV infection of which about 360 million people are chronic carrier, and more than one million people die annually as a result of HBV related liver disease such as cirrhosis, HCC and fulminant hepatitis **(WHO, 2009).**

HBV infection is highly endemic in all of Africa, some parts of South America, Alaska, Northern Canada and parts of Greenland, Eastern Europe, the Eastern Mediterranean area, south-east Asia, China, and the Pacific Islands, except Australia, New Zealand and Japan. About 5 to 15% of the population in most of these areas is chronically infected carriers of HBV **(WHO, 2009).** In low-risk areas of the world like North America and Western Europe, the highest incidence of the disease is seen in teenagers and young adults **(Mahoney, 1999)**. However in endemic areas of Africa and Asia, majority of HBV infections occur in neonates, infants and children due to mother to child transmission or close childhood contact with the virus. Also exposure with contaminated needles such as unsafe injection is also a possible means of transmission of HBV in these endemic countries **(Mahoney, 1999)**.

2.2. Prevalence of HBV Infection

The prevalence of hepatitis B varies across the world depending on the standard of living. Africa and Asia has the highest prevalence rates, with overall HBsAg carrier rate

of about 10 – 15%. Countries with the high standard of living such as the Great Britain, United states, Canada and some European countries is reported to have the lowest HBV prevalence rate **(Hou *et. al.*, 2005)**. WHO classifies areas of the world where the prevalence of HBsAg is >8% as highly endemic, areas with prevalence of HBsAg from 2%-7% and <2% as intermediate and low endemic areas respectively **(WHO, 2009)**. Figure 1 below shows the global distribution of HBsAg prevalence.

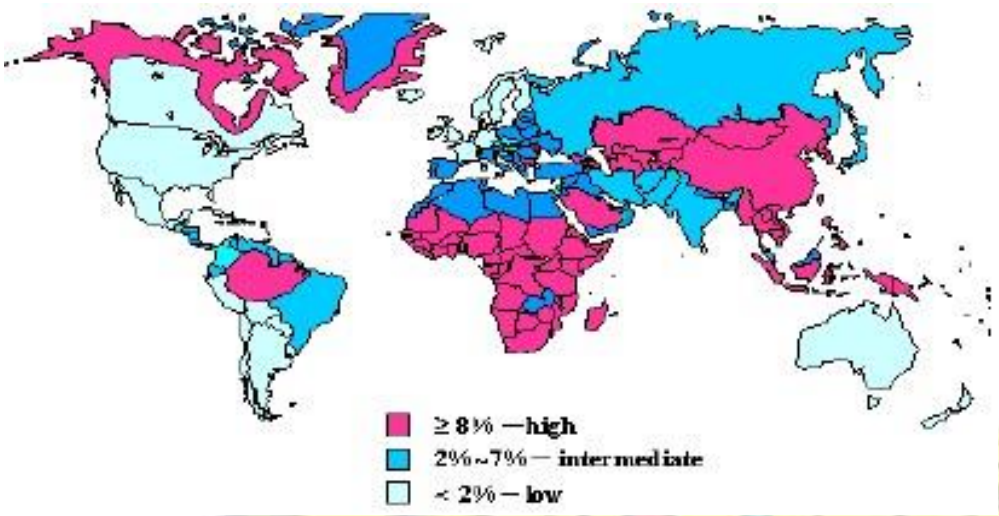

Figure 1: World distribution of HBsAg prevalence. Source: (**Hou *et. al.*, 2005**)

2.3. The Hepatitis B Virus

2.3.1 Classification and structure

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family. The virion is a double-shelled particles, 40 to 42 nm in diameter (Figure 2.). It has a lipoprotein covering (envelope) which contains three associated envelope glycoproteins, also known as the surface antigens. The envelope covers the viral nucleocapsid, also called the viral core. The nucleocapsid contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase enzyme that is responsible for the synthesis

of viral DNA in infected cells **(Ganem, 1991)**. In addition to virion, HBVinfected cells produce two distinct subviral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter (Figure 3**)**. These HBsAg particles contain only envelope glycoproteins and host-derived lipids and typically out number the virion by 1000:1 to 10,000:1 **(Ganem and Alfred, 2004).**

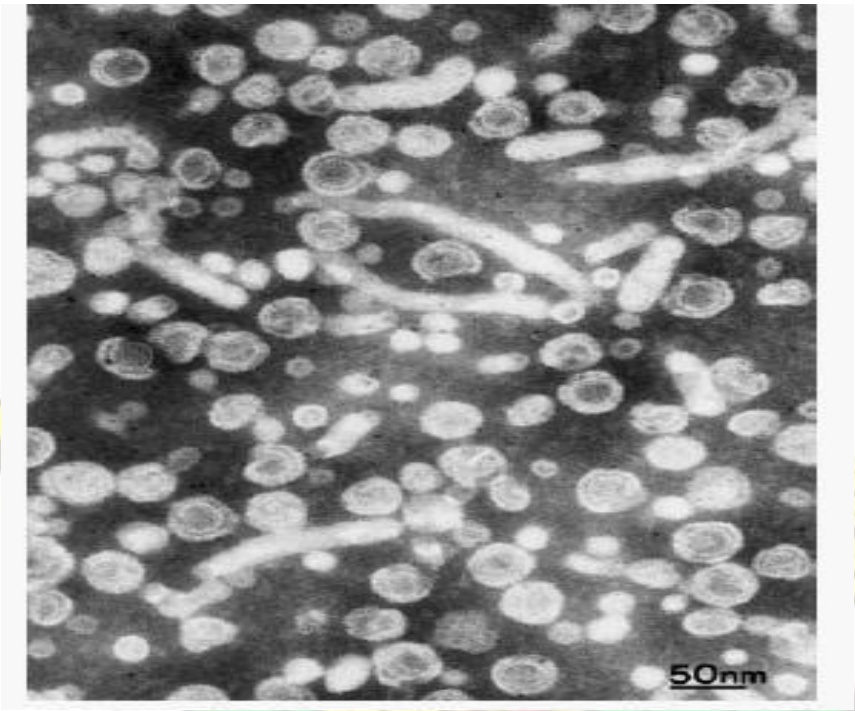

Figure 2. HBV virions (Danes particles and filamentous forms). Source**: (Ganem and Alfred, 2004**)

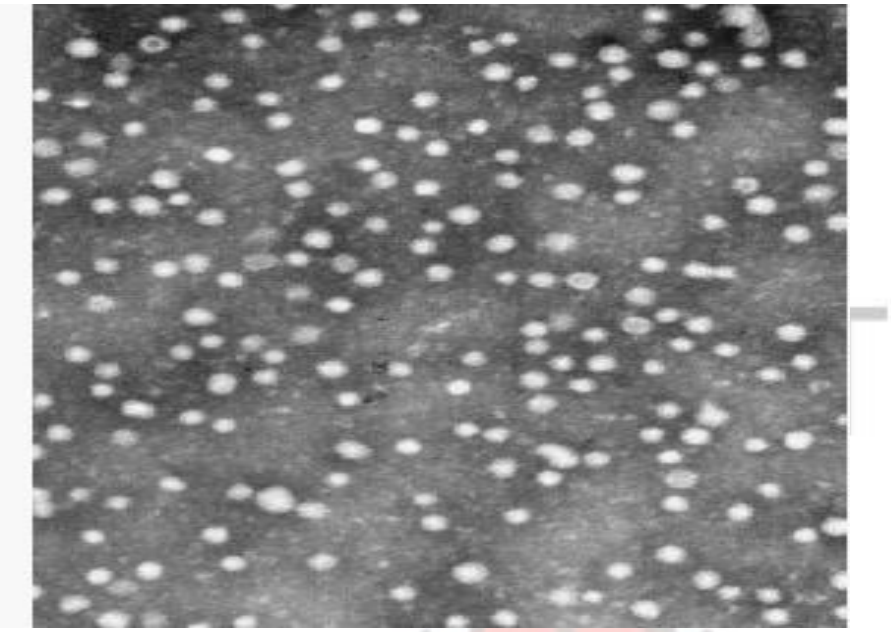

Figure 3. 20nm HBsAg particles. Source: **(Ganem and Alfred, 2004)**

2.3.2. HBV Genomic structure and Proteins

The HBV genome has four long open reading frames: The preS-S region, thepreC-C region, the P coding region and the X coding region. The preS-S region of the genome encodes the three viral surface antigens. This is achieved by differential initiation of translation at each of the three in-frame initiation codons. This give rise to the most abundant 24-kD S protein (known as HBsAg) **(Bruss and Ganem, 1991)**.

Initiation at the most upstream start codon generates the M (or preS2) protein and the L (or preS1) protein. The function of preS2 is unknown but preS1 play key roles in the binding of the virus to host-cell receptors and in the assembly of the virion and its release from the host cell **(Bruss and Ganem, 1991).**

The preC–C (precore–core) region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). These two proteins are also derived by alternative

initiation of translation at two in-frame AUG codons (**Ganem and Schneider, 2001**). The HBcAg which is a 21KD C protein and the structural polypeptide of the capsid, is encoded by the internal AUG whereas the preC protein (24 KD) production is encoded by the upstream AUG. the preC protein chain is then directed in to the secretory pathway by signal sequence encoded by the preC region. The chain is cleaved by cellular enzymes as it moves through the Golgi body, producing HBeAg (16 KD piece) which is secreted in to the blood.

The Viral polymerase is encoded by the P coding region. The viral polymerase is a multipurpose enzyme which helps in DNA synthesis and RNA encapsidation. The viral X protein (HBx) is encoded by the X open reading frame. HBx modulates host cell signal transduction. It can directly and indirectly affect the expression of the host cell genes and viral genes. HBx activity is a necessity for the replication and spread of the virus **(Ganem and Schneider, 2001)**. Figure 4 below shows HBV genomic organization.

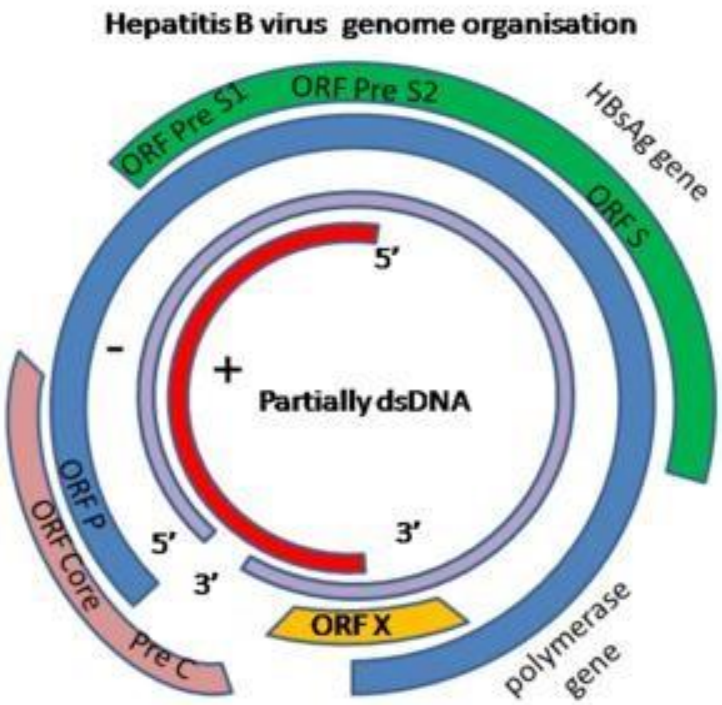

Figure 4: Genomic structure of HBV. Source: (Kay and Zoulim, 2007)

2.3.3 HBV Genotypes and Distribution

There are eight viral genotypes of HBV in existence confirmed by DNA sequencing of many isolates of the virus. Each genotype has characteristic geographic distribution (Table 1.). The genotypes are A, B, C, D, E, F, G and H **(Calvin and Jin, 2005)**.

Of these eight known HBV genotypes which have distinct geographical distributions, genotype A and E prevail in Africa. While genotype A can be found in sub Saharan Africa and elsewhere in the world, genotype E is mainly found in Africa **(Kramvis *et. al.*, 2005)**. Some research in Ghana has shown varying percentages of the prevalence of HBV genotype E ranging from 87% to 100% of mostly chronically infected persons **(Geretti *et. al.*, 2010; Candotti *et al.*, 2006)**. However, 10% and 3% of individuals in one of the studies expressed genotype A and genotype D, respectively

(**Candotti *et. al.*, 2006**).

Genotype	Geographic Distribution
A	Africa, India, Northern Europe, United States
B	Asia, United States
C	Asia, United States
D	India, Middle East, Southern Europe, United States
E	West and South Africa
F	Central and South America
G	Europe, United States
H	Central and South America, California in United States

Table 1. Geographical distribution of HBV Genotypes. Source: **(Calvin and Jin, 2005)**

2.3.4. HBV Replication cycle

HBV replicate through pregenomic RNA intermediate reverse transcription **(Beck and Nassal, 2007)**. Infectious virion interacts with specific receptors sites on hepatocytes leading to attachment and binding by means of the PreS1 domain, and subsequently penetrates the hepatocytes. It then uncoats, releasing partially doublestranded relax circular DNA into the cytoplasm. This is then transported into the nucleus and cellular enzymes synthesize DNA to complete the uncompleted strand converting it to covalent closed circular DNA (ccc DNA). The cccDNA serves as the template for the production of HBV messenger ribosomal nucleic acids (mRNAs) including a 3.5-kb RNA pregenome. The pregenome and a viral polymerase protein (with HBV reverse transcriptase and RNase H activity) are encapsidated forming newly synthesized core particles. Using the RNA pregenome as template, the reverse transcriptase synthesise

the negative strand within the capsule while the RNase removes the RNA pregenome template **(Beck and Nassal, 2007)**.

A complementary strand to the negative strand (positive DNA strand) is synthesised but the synthesis does not proceed to completion within the core, resulting in replicative intermediates consisting of full-length minus DNA strand and a positive DNA strand of variable-length about 20%–80% complete. Core particles (nucleocapsids) containing these DNA replicative intermediates with a relaxed circular DNA may bud from pre-Golgi membranes (acquiring HBsAg in the process), exiting the cell as a virion **(Ganem and Alfred, 2004)**.

The nucleocapsid may re-enter the intracellular infection cycle migrating to the nucleus. There, it will deliver the freshly produced viral nucleic acid into the nucleus of the host hepatocytes causing a great increase in the cccDNA leading to the production of more nucleocapsids. Amplification of cccDNA resulting in new and repetitive replication cycles produces varied hepatitis B viral genotypes, sub-genotype and strains **(Ganem and Alfred, 2004)**. Figure 5 below shows the replication cycle of HBV.

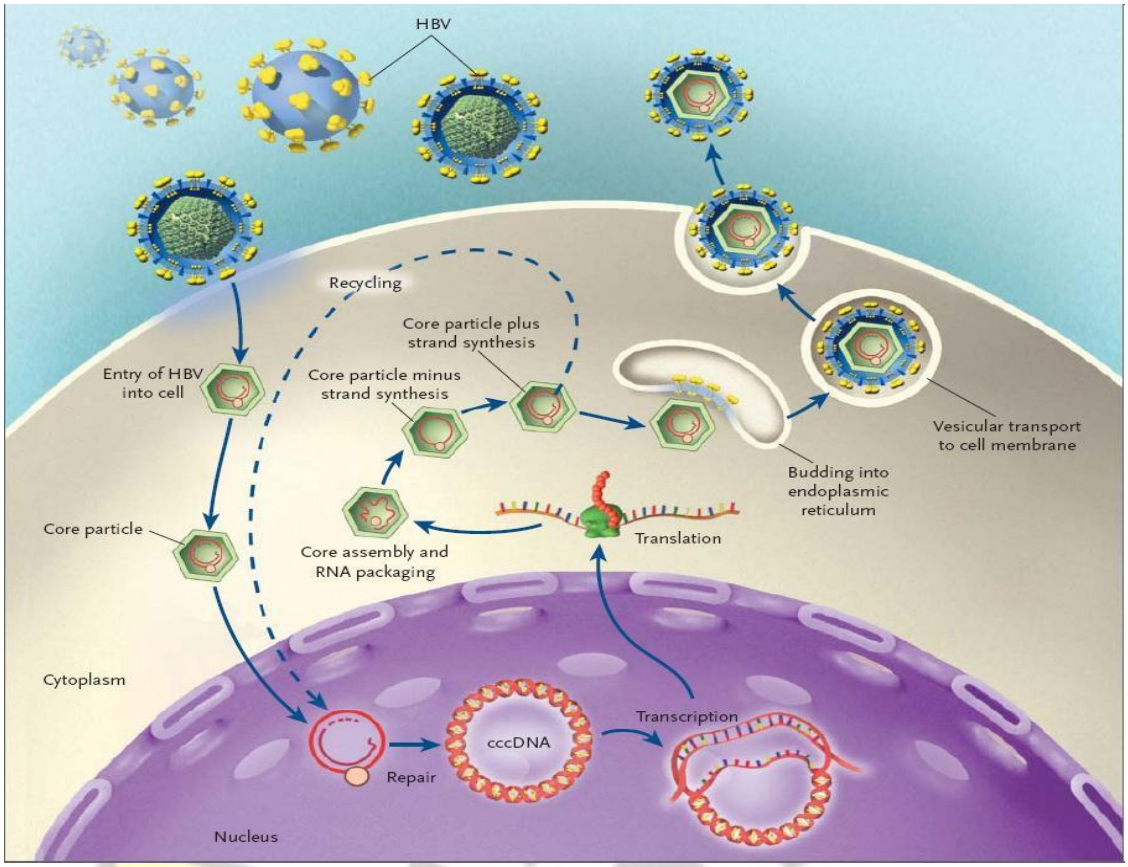

Figure 5. The Replication Cycle of HBV. Source: **(Ganem and Alfred, 2004)**

2.4. Pathogenesis of HBV infection

HBV after entering the body through a broken skin or mucous membrane, is transported to the liver which is the preferred site to cause infection. Hepatic damage as a result of HBV infection is reported to be due to the response of the immune system of the body to the infection, as the virus itself is not directly cytopathic to infected liver cells **(Xuanyong, 2011)**.

Generally there are two types of immune response which occur as a response to HBV infection. These are the innate and the adaptive immune response. The innate immune is an immediate and the first line response to infections. The innate immune response

often result in the stimulated production of interferon alpha and beta by the infected cells. The interferon stimulate the expression of large quantities of interferon inducible genes (ISGs), which in turn initiate different kinds of intracellular antiviral pathways capable of limiting viral production and spread and ultimately minimising pathogenic processes **(Alexopoulou, *et. al.*, 2001)**. Shockingly, the hepatitis B virus is able to evade innate immune response shortly after infection of host cells by acting as a stealth virus as it spreads through the liver. It is able to do so by not stimulating any cellular gene expression including ISGs **(Wieland *et. al.*, 2004)**.

The adaptive immune response is responsible for the elimination of infected viruses during HBV infection. In the process of clearing infected viruses from the liver, it causes collateral damage to hepatocytes leading to hepatic injury and damage. The adaptive immune response is more pronounced in acute HBV infections and to a lesser extent in some chronic infections. Hepatic injury and damage in acute HBV infection is due to the attack of T lymphocytes on infected hepatocytes, in an attempt to clear the infecting viruses. Both the T helper lymphocytes (Th) and the cytotoxic T lymphocyte (CTL) play an active role in the adaptive immune response to HBV infections **(Guidotti and Chisari, 2006)**. The T helper cells produce cytokines which help in the stimulation of CTL and B lymphocytes. The stimulated B lymphocytes produce antibodies which help to inactivate and reduce the number of circulating viruses **(Guidotti and Chisari, 2006)**. Although the T helper cells do not have direct effect on elimination of infecting viruses and the occurrence of hepatic damage, they are required to stimulate and maintain the levels of CTLs **(Xuanyong, 2011)**. The elimination of infecting viruses and ensuing hepatic damage (hepatitis) is largely attributed to CTL response. Studies in chimpanzees has shown that the reduction of CTLs at high viraemia level delays the

initiation of hepatic damage and the elimination of infecting viruses **(Xuanyong, 2011)**. Activated CTL and Natural killer (NK) cells kill their target infected cells by releasing cytolytic granules to these cells. The granules then stimulate cell death process (apoptosis) in the target infected cells, as depicted in Figure 6 below. The granules are made up of perforin which is a membrane-disrupting protein and granzymes (Gzms) which are serine proteases

(Kagi *et. al.*, 1994).

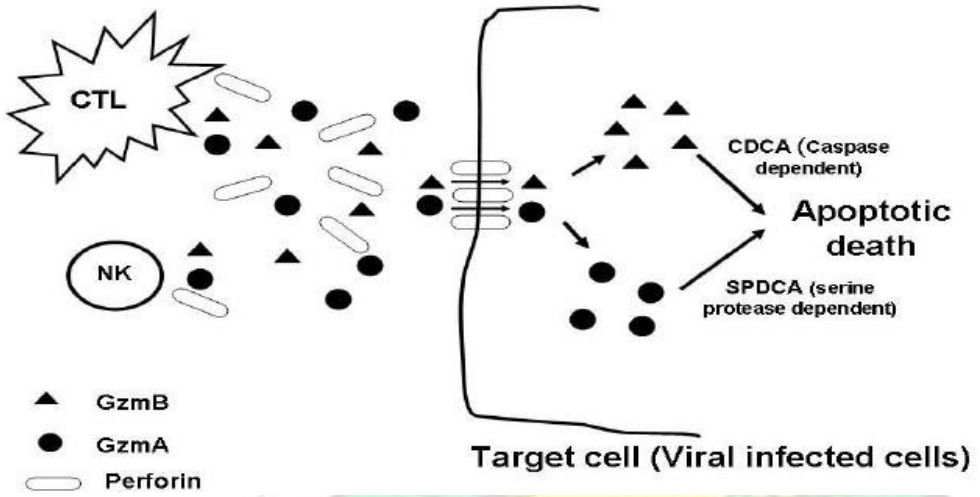

Figure 6**.** Immune killing of infected cell mediated by CTL and NK cells. Source: **(Kagi *et. al.*, 1994)**.

2.5. Clinical Presentation of HBV Infection

Primary hepatitis B viral infection is often accompanied with subclinical illness or with acute liver inflammation which may be mild or severe and may result in chronic infection **(Robinson, 1995)**. About 90% of primary adult HBV infections result into acute hepatitis. The rest 5 - 10% of primary adult infections and more that 90% of new-born and infant HBV infections result into chronic hepatitis B, which may live with the

patient for his/her entire life (**Hollinger and Liang, 2001**). Figure 7 below shows the schematic progress of liver disease after HBV infection in humans

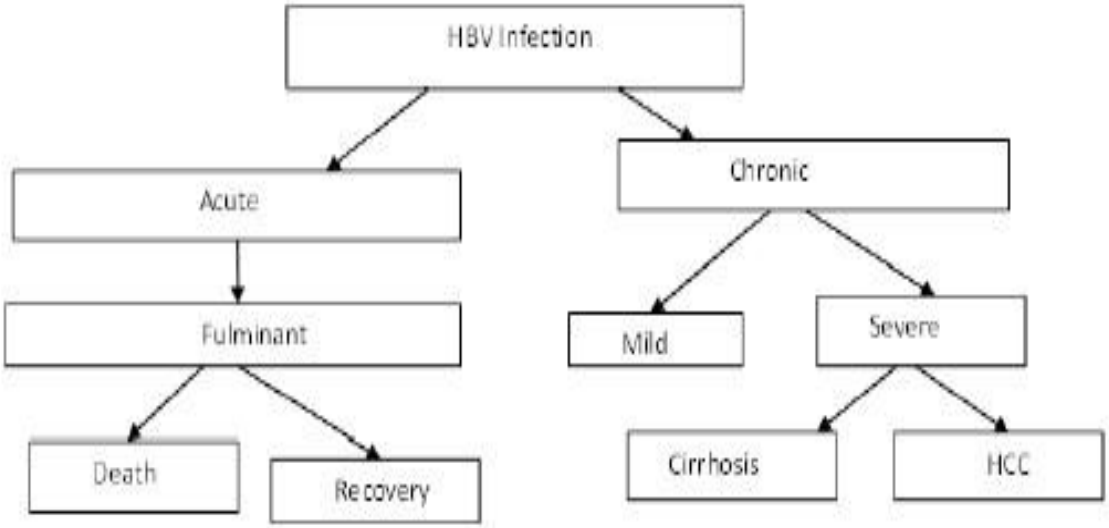

Figure 7: Schematic progress of liver disease after HBV infection in humans.

Source: **(Guidotti and Chisari, 2006**).

2.5.1. Acute HBV infection

Acute hepatitis B virus infection is mostly characterised asymptomatic short mild illness which mostly resolves undetected **(Jake, 2009)**. Only about 1/3 of adults with acute hepatitis B show mild clinical signs and symptoms such as nausea and fatigue. After exposure to HBV, acute hepatitis usually manifest in two to three months. The amount of the virus one is exposed to, to some extent, determines the duration of the incubation time of acute hepatitis **(Jake, 2009)**. A short pre-jaundice symptoms like body aches, fatigue, fever, nausea, anorexia etc. follow the incubation period. This period is accompanied with a striking rise in alanine aminotransferase (ALT) levels and also high levels of HBV DNA and HBsAg (Figure 8.a). This preicteric period is

followed by jaundice, after lasting for a few days to about one week **(Jake, 2009)**. The jaundice period last for about one to two weeks. During this period the amount of the virus decline, and as the jaundice resolves HBsAg and viral DNA disappear from the serum, although symptoms may continue for about a week or month **(Jake, 2009)**.

2.5.2 Chronic Hepatitis B

Chronic hepatitis B has a dynamic and a variable clinical course. Early during infection, HBeAg, HBsAg and HBV DNA are usually present in high titers, and there are mild to moderate elevations in serum aminotransferase levels (Figure 8.b). However the disease activity can resolve with time, either with persistence of high levels of HBeAg and HBV DNA (the ―immune tolerance phase‖) or with loss of
HBeAg and fall of HBV DNA to low or undetectable levels (―inactive carrier state‖). Other patients continue to have chronic hepatitis B, although some lose HBeAg and develop anti-HBe (HBeAg-negative chronic hepatitis B) **(Jake, 2009)**.
Chronic HBV infections are mostly asymptomatic or may present with symptoms such as tiredness, right upper quadrant discomfort etc. However severe infection or disease may present with symptoms such as jaundice, spider angiomata,
splenomegaly, palmar arythaema, fetor hepaticus etc.

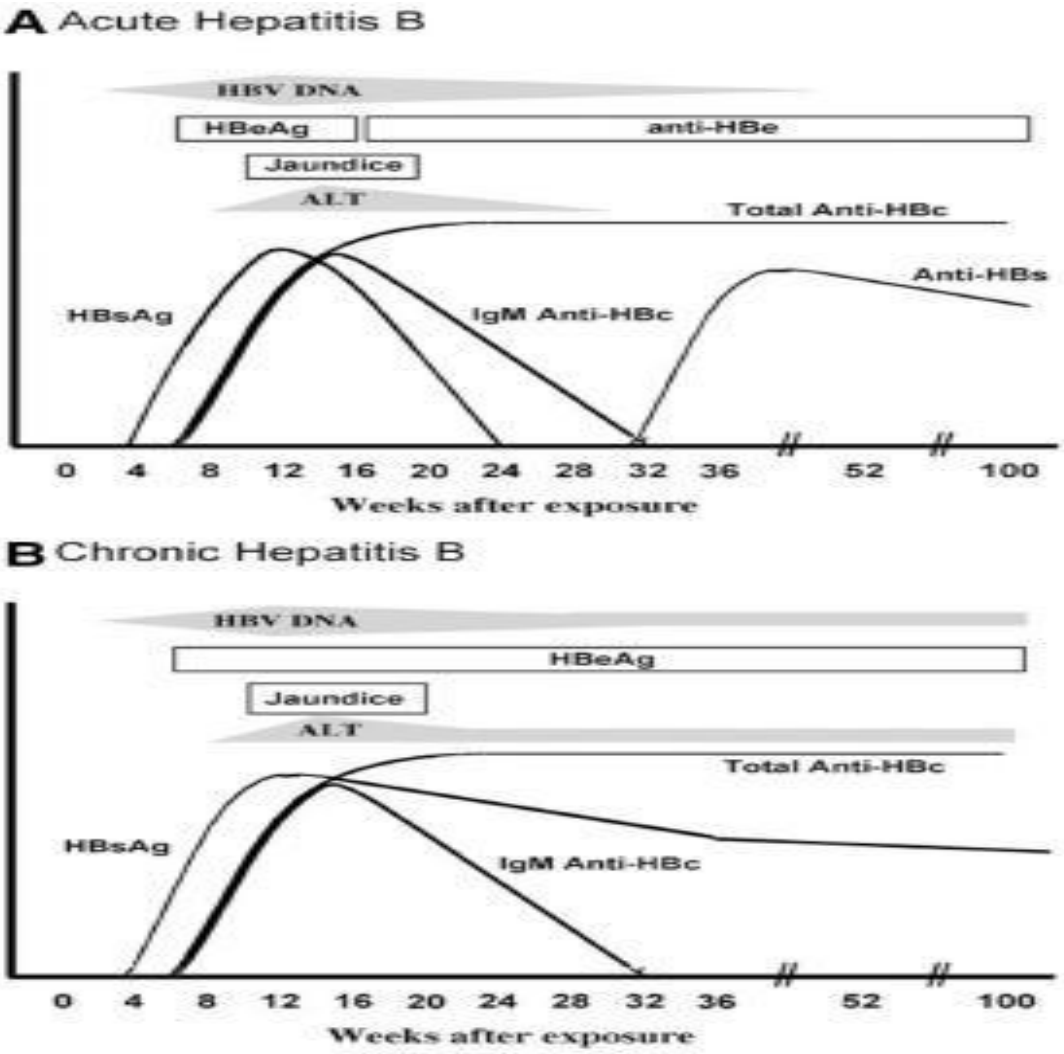

Figure 8: the clinical course and serological profile of acute (A) and chronic (B) hepatitis B. Source: **(Jake, 2009)**.

2.6.1. Diagnosis of Hepatitis B

The diagnosis of HBV infection and its associated disease is based on a ccombination of clinical, biochemical, histological, and serologic findings **(Jake, 2009)**. Diagnosis of HBV infection is not based on clinical signs and symptoms only because other hepatitis viruses have similar symptoms. Also HBV infections especially chronic infections usually present as asymptomatic and diagnosis based on clinical signs and symptoms

could be missed. Acute hepatitis B presents symptoms of fatigue, nausea, abdominal pain, darkening of urine, skin rashes, arthralgias etc. Later, it develops into jaundice just like the other viral hepatitis. Chronic hepatitis B patients do not show many of the symptoms of acute HBV infection including jaundice until liver damage is advanced, hence such patients can remain undiagnosed for a very long time (**WHO, 2002**).

Biochemical test can detect elevated liver enzymes level, although it not specific to Hepatitis B only. Test for liver enzymes aminotransferases that i.e. Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALT) is sensitive and one of the widely used blood tests for evaluating patients with hepatitis B. These enzymes are usually contained within the hepatocytes and are spilled into the blood stream when the liver is injured or scarred raising the levels of the enzymes in the blood indicating liver damage **(Pan and Zhang, 2005)**. Patients with acute hepatitis B can present very high AST and ALT levels in serum which fall to normal levels in succeeding weeks or months as the patient clears the virus and seroconvert. Patients with chronic hepatitis B however typically have normal to mild elevation of AST and ALT levels which could last for years in the immune tolerant phase of chronic infection **(Pan and Zhang, 2005)**.

Molecular test can detect hepatitis B virus DNA. Molecular technique is the most sensitive and specific test for detection of HBV genome **(Hollinger and Liang, 2001)**.

Histological techniques helps in the examination of diseased specimen for the detection of antigens and components associated the hepatitis B virus **(Hollinger and**

Liang, 2001).

Also serological detection of HBV markers has proven to be rapid and useful for large scale screening of HBV infection. Clinically useful serological markers used in the diagnosis of HBV infection include: hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs); antibody (anti-HBc IgM and anti-HBc IgG) to hepatitis B core antigen (HBcAg); and hepatitis B envelope antigen (HBeAg) and antibody to HBeAg (anti-HBe) **(WHO, 2002).** HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections **(Hollinger and Liang, 2001)**.

2.6.1.1. Hepatitis B Virus Serological Markers

The clinical course of HBV infection is accompanied with different serological viral markers in the blood. Depending on whether the infection is acute or chronic, these serological markers vary. The markers include the surface, core, and e antigen and their corresponding antibodies.

The hepatitis B surface antigen (HBsAg): this is the major component of the coat of the hepatitis B viral particle, coded for by the S gene weighing about 24 kilo Daltons and may or may not be glycosylated. The product of the S gene constitutes the major protein of HBsAg. Products of the PreS1 and PreS2 incorporated into the HBsAg are in small quantities in the shells of the non-infectious HBV particles **(Mahoney, 1999)**. HBsAg can be detected in the serum from several weeks before onset of symptoms to

months after onset. This marker can be detected early in the serum and it stays for a long time during the course of the HBV infection. **(Hollinger and Liang, 2001)**. It has served as a useful diagnostic marker for rapid detection and screening on large scale for HBV infection.

The Hepatitis B core antigen: this antigen is a component of the nucleocapsid, essential for the packaging of the virus and coded for by the C gene in the L- strand and weighing 18-19 KD. When transcribed, it usually moves into the endoplasmic reticulum where it gets cleaved. It is usually difficult to locate this antigen in serum by conventional methods but can be easily identified in liver biopsy of patients where it appears in the nuclei of infected hepatocytes **(Eligouhari *et. al.*, 2008)**.

The Hepatitis B e antigen: This antigen is coded for by the C gene and translated from the same gene as HBcAg weighing 15.5 *k*D. This is a soluble antigen in the blood created by the pre-mature proteolytic cleavage of the core antigen. The presence of this antigen indicates that the virus is rapidly replicating in the patient and thus high numbers of virions are present in the blood; hence, the patient at this stage is highly infectious **(Eligouhari *et. al.*, 2008)**.

Hepatitis B surface antibody: this is a protecting and neutralizing antibody produced in response to infection with the HBsAg. The presence of this antibody against the common epitope ―a‖ of one HBsAg subtype confers immunity against re-infection or new infections with other subtypes **(Zukerman, 2006)**. When this antibody develops

after the HBsAg has been cleared from the serum at about six months after primary or acute infection, the antibodies persist for life **(Mahoney, 1999)**.

Hepatitis B core antibody: this antibody is produced by the body in response to infection with the Dane particle and can be detected just after the appearance of HBsAg. Detection of the hepatitis B core Immune globulin M (HBc IgM) is an indication of acute infection with Dane particle but the absence of anti-HBc-IgM and the presence of hepatitis B core Immune globulin G (HBc-IgG) together with HBsAg is an indication of chronic infection **(Zukerman, 2006)**.

Hepatitis B e antibody: this marker develops after HBeAg a soluble protein in the core has been cleared from the blood of infected person. The presence of HBeAb in the blood of persons infected with HBV indicates that the person in less infective; thus, the number of virions in the blood are fewer **(Zukerman, 2006).**

2.6.2. Transmission of HBV Infection

Human is the only natural reservoir of human HBV **(Robinson, 1995)**. There are four major contact modes of transmission of HBV which includes: from mother to child (vertical/perinatal), around the time of birth; non-sexual contact with an infected person (horizontal), including household transmission; sexual contact, of which male to male sexual practices are associated with higher risk; and parenteral exposure to infected blood and other bodily fluids **(Hollinger and Liang, 2001)**.

HBV can survive outside the body for about seven days and hence can also be transmitted via contaminated inanimate objects like toothbrushes, baby bottles, toys,

razors, eating utensils, hospital equipment and other objects, by contact with mucous membranes or open skin breaks. No evidence exist of an air-borne infections of HBV **(Hollinger and Liang, 2001).**

2.6.3. Treatment of Hepatitis B

Treatment for acute HBV infection is generally supportive. However there is antiviral therapy for chronic hepatitis B but these antiviral treatment do not eradicate the HBV. The aim of treatment for chronic HBV infection is to reduce the risk of developing chronic liver disease by sustained suppression of HBV replication in the liver **(Lok and McMahon, 2007)**. Long-term treatment with antiviral drugs has been shown to be effective in reducing the risk of both disease progression and of developing hepatocellular carcinoma, by up to 50% **(Lok and McMahon, 2007)**.

The usual markers of successful therapy are the loss of HBeAg, seroconversion to anti-HBe antibodies, and reduction of circulating viral load. These are useful indicators, since patients with stable seroconversion to anti-HBe positive status typically have improved histologic findings in the liver, and this improvement tends to be maintained over the long term (**Ganem and Alfred, 2004)**. Treatment regimens include administration of Interferon alfa which act as an immunomodulator; Lamivudine and Adefovir which are nucleotide analogues and directly block replication of the HBV genome **(Ganem and Alfred, 2004)**.

2.7.1. Haematological Disorders

Haematological disorders are a wide range of abnormal conditions which primarily affect the blood. The abnormal conditions may affect the White blood cells, the Red blood cells, the Platelet, the Plasma and the entire coagulation system. These abnormal blood disorders could range in severity from subclinical, needing less attention, to critical clinical situations which could be live threatening. There are diverse etiologic causes of abnormal haematological conditions, which include but a few such as nutritional imbalances, genetic factors, infections including hepatitis B infection, side effects of some medications, idiopathic causes etc. **(Chesbrough, 2006)**.

2.7.1.1. Red Blood Cell Disorders

RBCs are responsible for carrying transporting oxygen from the lungs to the body‘s organs and tissues. RBC disorders affect the oxygen carrying capacity of the RBCs and results in a number of clinical conditions. RBC disorders include: Anaemia, Haemoglobinopathies (thalassaemias, abnormal Haemoglobins), Disorders due to red cell enzyme defects, e.g. G6PD deficiency, Disorders due to red cell membrane defects, e.g. hereditary spherocytosis and Polycythaemia **(Chesbrough, 2006)**.

Anaemia is the most common red cell disorder worldwide. It occurs when the concentration of haemoglobin falls below what is normal for a person‘s age, gender, and environment, resulting in the oxygen carrying capacity of the blood being reduced **(Drew, 2003)**. The main causes of anaemia are: Malnutrition, associated particularly with: Iron deficiency, Folate deficiency, vitamin B12 deficiency and Protein deficiency;

Parasitic, bacterial, and viral infections; Inherited haemoglobinopathies such as Sickle cell disease, Thalassaemia syndromes; Glucose 6 phosphate dehydrogenase (G6PD) deficiency; and Obstetrical complications causing abnormal blood loss. Often these causes overlap with several factors contributing to a person becoming anaemic (**Chesbrough, 2006**).

2.7.1.2. White Blood Cell Disorder

The white blood cells primarily help the body to fight off infections. The five different types of white cells namely neutrophils, monocytes, eosinophils lymphocytes and basophiles, help to fight off bacteria, parasitic and viral infection as well as initiate inflammatory and hypersensitivity reactions. The most common disorders of white cells include: absolute increases in leukocyte numbers, involving neutrophils, lymphocytes and eosinophils in response to bacterial, viral, or parasitic infections, tissue injury, and inflammation. And absolute decreases in leukocyte numbers involving neutrophils and lymphocytes, caused by some infections, hypersplenism, immune destruction of cells, treatment with cytotoxic drugs, bone marrow dysfunction, megaloblastic anaemia, collagen disorders, and malignant conditions **(Drew, 2003)**. However less common forms of WBC disorders include the haematological malignancies such as Lymphoma: a form of blood cancer that develops in the lymph system; Leukaemia : a form of blood cancer in which a white blood cell becomes malignant and multiplies inside the bone marrow. Multiple myeloma: a blood cancer in which plasma cell becomes malignant **(Drew, 2003)**.

2.7.1.3. Platelets and Plasma Disorders

The blood platelets together with the vascular endothelium help to achieve effective cessation of bleeding. Also the blood plasma contains factors that help to achieve blood clot to prevent excessive bleeding. Therefore defects or disorders of the blood platelets and plasma factors result in uncontrolled and excessive bleeding. Damage to vascular endothelium, reduction in platelet numbers, or defective platelet function can result in purpura with bleeding from or into superficial tissues, e.g. mucous membranes. Defective platelet function may occur as a complication of viral haemorrhagic fevers, liver cirrhosis, alcoholism, leukaemias, paraproteinaemias, uraemia and treatment with certain drugs (e.g. aspirin and nonsteroidal antiinflammatory drugs) **(Chesbrough, 2006)**.

2.7.2 Hepatitis B Associated Haematological Disorders

Liver diseases associated with hepatitis B infection may result in abnormal haematological parameters. A study conducted among pregnant women in Nigeria demonstrated a significant association of hepatitis B infection and reduction in Pack cell volume (PCV), Haemoglobin concentration, total white blood cell, lymphocyte and eosinophil count **(Eze and Buseri, 2009)**. Also a study by Ajugwo and Ukaji has demonstrated some association between hepatitis B infection and reduction in Pack Cell Volume, Haemoglobin concentration and an increased Erythrocyte Sedimentation Rate (**Ajugwo and Ukaji, 2015**). The above studies reveals Hepatitis B infection is associated with Pancytopaenia, a reduction of all the blood cells types.

Again some studies have established an association between hepatitis B and Aplastic

Anaemia (AA), which is a severe form of Pancytopaenia **(Bisma and Muhammad, 2011; Bozkaya and Yurdaydin, 2002)**. The onset of pancytopenia usually takes two to three months after attack of acute hepatitis **(Brown *et. al.*, 1997)**. Hepatitis associated with aplastic anemia may be acute or chronic, mild or transient, selflimiting or fulminant and the development of aplastic anaemia is always fatal if not treated on time **(Gonzalez *et. al.*, 2009)**. Majority of the cases have been found as fulminant where the mortality rate reaches up to 85% **(Gonzalez *et. al.*, 2009**).

Various Immunological mechanisms have been reported to be accountable, in part, for the development of aplastic anemia following hepatitis. CD8+ kupffer cells detected by liver biopsies has been shown as a mediator of hepatitis associated aplastic anaemia. It has been reported that patient with hepatitis associated aplastic anaemia showed a decreased ratio of CD4/CD8 cells and a high percentage of CD8 cells, which appeared to be cytotoxic and myleopoietic during an *in vitro* study of aplastic anemia **(Cengiz *et. al.*, 2007)**.

Hepatitis associated aplastic anaemia was first reported in two cases by Lorenz and Quaiser in 1955. However, a more recent epidemiological report has indicated 2-5% prevalence of hepatitis associated aplastic anaemia among western countries and 410% prevalence in area more prevalent to hepatitis and Human Immunodeficiency Viruses (HIV), such as the far East and Africa (Savage et. al., 2007). The syndrome is more common in areas of low socioeconomic status **(Safadi et. al., 2001)**.

Also Hepatitis B virus does not only infect hepatocytes. To a lesser extent the virus can be detected in tissues other than hepatocytes, including circulating mononuclear cells, bone marrow cells, and the spleen **(Yasushi *et. al.*, 2002)**. Infection of bone marrow

cells by HBV culminates in the reduction of blood cell produced by the bone marrow. In an *in vitro* study in which bone marrow was exposed to hepatitis B virus, there was a dose-dependent inhibition of hematopoietic stem cells **(Yasushi *et. al*., 2002).** Furthermore, some studies have implicated a possible etiologic role of hepatitis B virus in hematopoietic malignancies, such as myelodysplastic syndrome and acute myeloid leukemia **(Yasushi *et. al*., 2002).**

CHAPTER 3 – METHODOLOGY

3.1. Study Site and location

The study was carried out at the Komfo Anokye Teaching Hospital in Kumasi. Kumasi is the capital of Ashanti region and the second largest city in Ghana. It covers about 254 sq km (97.6 sq miles) in the southern central part of Ghana. Kumasi has a unique central placement on the map of Ghana making it a major hub for commercial activities and traversing point for travellers from all parts of Ghana and other parts of West Africa countries (**Amidu and Alhassan, 2012).** Komfo Anokye Teaching Hospital is a tertiary and a referral hospital, serving mostly the northern and some part of the southern sectors of the country. The central placement of Kumasi on the map of Ghana and the referral status of Komfo Anokye Teaching Hospital introduced us to a study population of diverse socio-economic background

3.2 Study population

Patients who attended the serology department of Komfo Anokye Teaching Hospital for HBV and other viral investigations were the target study population.

3.3. Study Design

The study was a prospective hospital based study.

3.4. Sample Size

A study conducted in Kumasi among three densely inhabited communities recorded an overall hepatitis B prevalence of 8.68% (**Amidu and Alhassan, 2012).** With this prevalence of 8.68%, a standard score (z) of 1.96 at 95% confidence interval and 5%

absolute precision was considered for the study. Using the equation, $\mathbf{n = [z^2\ p(100-p)]/d^2}$: where **n**- is the minimum sample size, **z**-the standard score, **p**-the hepatitis B prevalence and **d**-the desired absolute precision, the minimum number of HBV infected subjects that were to be enrolled for the study was **122**. The study recruited 122 HBV infected and 122 HBV uninfected subjects from 1,569 patients.

3.5. Ethical Considerations

Ethical clearance was sought for the study from the committee on Human Research Publication and Ethics (CHRPE), KNUST. Also permission to conduct the study at Komfo Anokye Teaching Hospital was sought from the Research and Development Unit of the Hospital. Signed or thumb-printed informed consent was obtained from each study volunteer after the procedure of the study, including the risks and benefits, were explained in details to them.

3.6. Inclusion and Exclusion Criteria

Inclusion Criteria

To qualify to be selected for the study, participants had to meet the following criteria:

- Should undergo HBV screening test at the Serology Department of Komfo Anokye Teaching Hospital.
- Should give an informed consent.

Exclusion Criteria

Study subjects who met any of the criteria below were excluded from the study:

- Subjects who fell outside 18 to 60 years.
- HBV infected subjects who had other medical conditions including HIV and HCV infections.
- HBV uninfected subjects who had medical conditions including HIV and HCV infections.
- Subjects who refused to have their blood samples drawn for laboratory analysis.

3.7. Study Procedure

The study proceeded with patients (1,569) who attended the Serology Department of Komfo Anokye Teaching Hospital for HBsAg screening test from August 2015 to April 2016. Most of the patients also underwent HIV and/or HCV antibody screening in addition to the HBsAg test screening at the department.

The study recruited 122 HBsAg positive (infected) and 122 HBsAg negative (uninfected) subjects from 218 HBsAg positive and 1,351 HBsAg negative patients out of the total of 1,569 patients.

The 122 HBV infected and 122 HBV uninfected subjects were recruited based on their negativity to HIV and HCV antibody tests; and also on the basis that they had no known clinical conditions which could have adverse effect on their haematological indices. This was done through the assessment of their medical folders/ OPD cards and questionnaire administration.

The 122 HBV infected subjects were categorised into symptomatic and asymptomatic subjects through the administration of questionnaire which specified signs and symptoms of HBV infection, and also through physical observation.

The 122 HBV infected subjects were also grouped into whether they had acute or chronic HBV infection through HBV serological profile investigations.

Haematological investigations including complete blood count (CBC), Erythrocyte Sedimentation Rate (ESR) and Peripheral blood morphological examination were then performed for both the recruited HBV infected and uninfected subjects.

3.7.1. Study Questionnaire

The study subjects were engaged with questionnaire in the form of a case report form, to record their personal information like age, sex, occupation etc; hepatitis B information such as duration of infection and signs and symptoms; and other known medical conditions they had.

3.7.2. Sample collection and Storage

The eligible HBV infected and uninfected subjects had 5ml of their venous blood samples taken aseptically. Two (2) and three (3) ml of the whole blood samples were dispensed into well labelled Serum separator and EDTA anticoagulant tubes respectively. The samples were then labelled with identification codes only to ensure anonymity of the subjects. Blood samples in the serum separator tubes were centrifuged at 1800 rpm for 10 minutes and the sera were pipetted into labelled

Eppendorf tubes. The EDTA anticoagulated as well as the serum samples were analysed within 24 hours, however serum samples which could not be analysed within

24 hours were stored in the freezer at -80^{0}C not exceeding one week of storage.

3.7.3. Laboratory Investigation

In the Laboratory, the EDTA anticoagulated samples were used for Peripheral blood film examination, complete blood count (CBC) and Erythrocyte sedimentation rate (ESR) estimation. The sera samples were also used for HBV serological profile and ALT test.

3.7.3.1. Peripheral Blood Examination/ Film comment

Each EDTA anticoagulated whole blood sample was well mixed and 5µl of it used to prepare a thin blood film, which was allowed to air-dry. The dried smear was then flooded with Leishmann stain for 5 minutes and double diluted with buffered distilled water and allowed for another 10 minutes. The slide was then properly rinsed under running water, and the underside was wiped with cotton wool to remove excess stain. The stained slide was placed on a rack with the feathered end sloping upwards to dry and then observed under the microscope with the X100 Objective lens using a drop of oil immersion. The stained slide were compared with a standard control slide to check stain quality.

3.7.3.2. Erythrocyte Sedimentation Rate (ESR)

Each EDTA anticoagulated sample was again well mixed and 1.6ml of it taken with a graduated Pasteur pipette and dispensed into an ESR tube containing 0.4ml of Sodium citrate. The content of the ESR tube was well mixed and the tube placed in the ESR

stand with the cap removed. Westergren pipette was then inserted into the tube and positioned vertically. Using a rubber bulb, the citrate blood mixture was drawn into the Westergren pipette to the zero (0) mark, avoiding air bubbles. The level at which the plasma met the red cells in mm was then recorded after exactly one (1) hour.

3.7.3.3. Complete Blood Count (CBC) Test

A fully automated machine, Sysmex XT4000i, was used for the analysis of the CBC of the study subjects. The remaining EDTA anticoagulated blood samples were well mixed and introduced to the probe of the machine, after the subjects ID, age, sex and the appropriate test had been keyed into the machine. The machine then analysed and generated a printed report for each of the samples. The machine was well calibrated and an external quality control material/sample consisting of high values, normal values and low values were run before batches of subjects‘ samples were analysed.

3.7.3.4. HBV Serological Profile testing

The Diagnostic Kit® for HBV Infection (Colloidal Gold) test kits were used for the determination of the HBV serological markers of the HBV infected subjects.

The product uses the colloidal gold and membrane chromatography technology. It measures HBsAg, HBeAg in whole blood or serum with dual-antibody sandwich method and measures HBsAb with dual-antigen sandwich method. It also measures HbeAb and HBcAb with neutralisation competitive inhibition method.

Part of the serum samples in Eppendorf tubes were used for the HBV serological makers test. The test cards and the samples were allowed to attain room temperature. 25 µl of

each sample was pipetted into 5 different wells of the test card, each well bearing the corresponding marker (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb). The results of the test was then observed and recorded in 15 minutes. Positive and negative control samples were tested on each batch of the test kits used. For the HBsAg, HBsAb and HBeAg test which uses the sandwich method, negative result was reported when only one purple bar (control line) appeared in the control zone and no line appeared in the test zone. Positive results were reported when two purple bars appeared at the test zone and the control zone. Also results were reported invalid and repeated, when one purple line appeared at the test zone but no line appeared at the test zone. However for HBeAb and HBcAb test which uses the competitive inhibition method, negative results was recorded when two purple lines appeared in the test and control zone. Positive results were recorded when only one purple line appeared at the control zone. Also results were reported invalid and repeated when no purple line appeared at the control zone.

3.7.3.5. HBcAb-IgM test

HBV infected subjects who were positive for HBcAb were further tested for HBcAbIgM, which is a marker of acute HBV infection. Subjects who tested positive for HBcAb-IgM were classified as having acute infection. However subjects who tested negative for HBcAb-IgM were classified as having chronic infection. The Advance Quality ^{TM} One Step HBc-IgM test kit (InTec Products, China) was used for the HBcAb-IgM test.

The test kit is a qualitative rapid immunochromatographic assay for the detection of IgM type antibody to the HBcAg. A captured antibody made up of IgMμ chain is immobilised on the membrane at the test zone. HBcAb-IgM in the sample binds to antigen-colloidal gold complex at the sample region and then migrates along the

membrane. At the test zone, the HBcAb-IgM bound to the antigen colloidal gold complex prevents the immobilised IgMμ from also binding to the antigen colloidal gold complex by competitive inhibition, and no purple line appears at the test zone.

Each subject serum that initially tested positive for HBcAb had 25μL pipetted and dispensed into the sample region of the Advance Quality ™ One Step HBcIgM test kit. A drop of the kit's buffer was added to the sample and the results were observed and recorded in 15 minutes. After the stipulated 15 minutes, negative results were recorded when two purple lines appeared at the test and control zone. Positive results were recorded when only one purple line appeared at the control zone. Also results were reported invalid and repeated when no purple line appeared at the control zone. Stored HBcAb-IgM positive and negative samples were tested on the batch of the test kit.

3.7.3.6. Alanine aminotransferase (ALT) Test

A fully automated machine, Cobas Integra 400® from Roche, was used for the analysis of the study subjects' sera samples to determine their ALT levels. The frozen sera samples were left on the bench to thaw and attain room temperature. Portions of the samples were poured into the sample container of the machine to the appropriate mark. The sample containers with the samples were placed in the sample container rack and fed into the machine. The appropriate test ALT was selected on the screen and the subjects ID, age and sex were also keyed in. The run test button was pressed and the machine analysed the samples and generated a printed report of each test. The Machine was well calibrated with Cobas Integra calibration reagents for ALT.

3.8. Statistical Analysis

The data obtained from the laboratory as well as the case report forms were well documented and transferred onto Microsoft Excel® spread sheet. The data were then imported and analysed using IBM SPSS Version 22 statistical tool. The Independent Sample t-Test was used to analyse and compare parametric variable and Independent Sample Mann-Whitney or Kruskal Wallis test was used to analyse and compare nonparametric variables. Significance in the difference between results was inferred at $p<0.05$. Sex dependent haematological parameters (ESR, RBC count, Hb concentration and HCT) were analysed separate from the sex independent parameters (PLT count, Total WBC count ,WBC Differential count and Peripheral blood film examination).

CHAPTER 4 - RESULTS ANALYSIS

4.1. HBsAg Prevalence

A total of 1,569 patients were screened for HBsAg and other viral makers such as HIV and HCV antibodies at the Serology Department of Komfo Anokye Teaching Hospital from August 2015 to April 2016. 218 patients were HBsAg positive and 1,351 patients were HBsAg negative, representing HBsAg prevalence of 13.89% as shown in Table 2.

Table 2. HBsAg Test Results

Total Patients	HBsAg Positive	HBsAg Negative
1,569	**218**	**1,351**
	13.89%	**86.11%**

4.2. Description of Recruited Study subjects

Of the 1,569 patients, a total of 244 study subjects comprising of 122 each of HBsAg positive (Infected) and HBsAg negative (Uninfected) subjects were recruited from 218 HBsAg positive and 1,351 HBsAg negative patients, based on the inclusion and exclusion criteria specified at Section 3.6 of the study methodology. The hepatitis B infected group were categorised into symptomatic and asymptomatic groups and acute and chronic infection groups. Table 3 below shows the frequency and the male and female distribution among these groups.

Table 3. Descriptive Statistics of Recruited Study Subjects

	Frequency	Female	Male
HBV Status	244 (100%)		
Infected	122 (50%)	48 (39%)	74 (61%)
Uninfected	122 (50%)	26 (21%)	96 (79%)
Symptoms	122 (100%)		
No	57 (47%)	28 (49%)	29 (51%)
Yes	65 (53%)	20 (31%)	45 (69%)
Hepatitis B profile remarks	122 (100%)		
Acute infection	7 (6%)	3 (43%)	4 (57%)
Chronic infection	115 (94%)	45 (39%)	70 (61%)

4.3. Results of Haematological parameters of HBV Infected and Uninfected Subjects

The female and male HBV infected subjects recorded significantly high ESR and low RBC count, Hb concentration and HCT values compared to the uninfected female and male subjects. With the sex independent parameters, the HBV infected subjects recorded significantly high Total WBC, Neutrophil, Monocyte and Basophil counts; and low Platelet, Lymphocyte and Eosinophil counts, compared to the uninfected subjects. Also from the peripheral blood film examination, the study identified four (4) distinct morphological blood picture among the infected and uninfected subjects. Thus the Normal blood picture, RBC hypochromasia blood picture, Predominant atypical mononuclear cell blood picture and Predominant reactive Lymphocyte blood picture.

Higher percentage of HBV infected subjects showed peripheral blood picture of Atypical mononuclear cells, RBC hypochromasia and Reactive lymphocytes compared to the uninfected subjects.

Tables 4 and 5; and Figure 9 below respectively show the sex dependent, the sex independent and the Peripheral blood film examination results of the HBV infected and uninfected subjects.

Table 4. Results of Sex dependent Haematological Parameters of HBV Infected and Uninfected Males and Females.

Parameter	**HBV Status, Females**	**Mean**	**Independent Samples MannWhitney U Test Sig.**	**P Values**
ESR mmfall/hr	Infected females	42.229	0.000	P<0.05
	Uninfected females	3.731		
RBC $X10^6/\mu L$	Infected females	3.8431	0.000	P<0.05
	Uninfected females	4.5758		
Hb g/dL	Infected females	10.921	0.000	P<0.05
	Uninfected females	12.658		
HCT %	Infected females	31.408	0.000	P<0.05
	Uninfected females	38.192		
	HBV Status, Males			
ESR mmfall/hr	Infected males	47.730	0.000	P<0.05
	Uninfected males	4.615		
RBC $X10^6/\mu L$	Infected males	4.1766	0.000	P<0.05
	Uninfected males	5.2865		
Hb g/dL	Infected males	11.485	0.000	P<0.05
	Uninfected males	14.293		
HCT %	Infected males	33.284	0.000	P<0.05
	Uninfected males	41.887		

Table 5. Results of Sex independent Haematological Parameters of HBV infected and Uninfected Subjects.

Parameter	**HBV Status**	**Mean**	**Independent Samples Mann-Whitney U Test Sig.**	**P value**
PLT $X10^3/\mu L$	Infected Uninfected	174.672 198.754	0.007	P<0.05
WBC $X10^3/\mu L$	Infected Uninfected	8.7292 5.6912	0.000	P<0.05
NEUT $X10^3/\mu L$	Infected Uninfected	5.7193 2.4097	0.000	P<0.05
LYMPH $X10^3/\mu L$	Infected Uninfected	1.9807 2.3998	0.000	P<0.05
MONO $X10^3/\mu L$	Infected Uninfected	0.8018 0.5878	0.001	P<0.05
EO $X10^3/\mu L$	Infected Uninfected	0.1471 0.2464	0.000	P<0.05
BASO $X10^3/\mu L$	Infected Uninfected	0.0541 0.0304	0.038	P<0.05

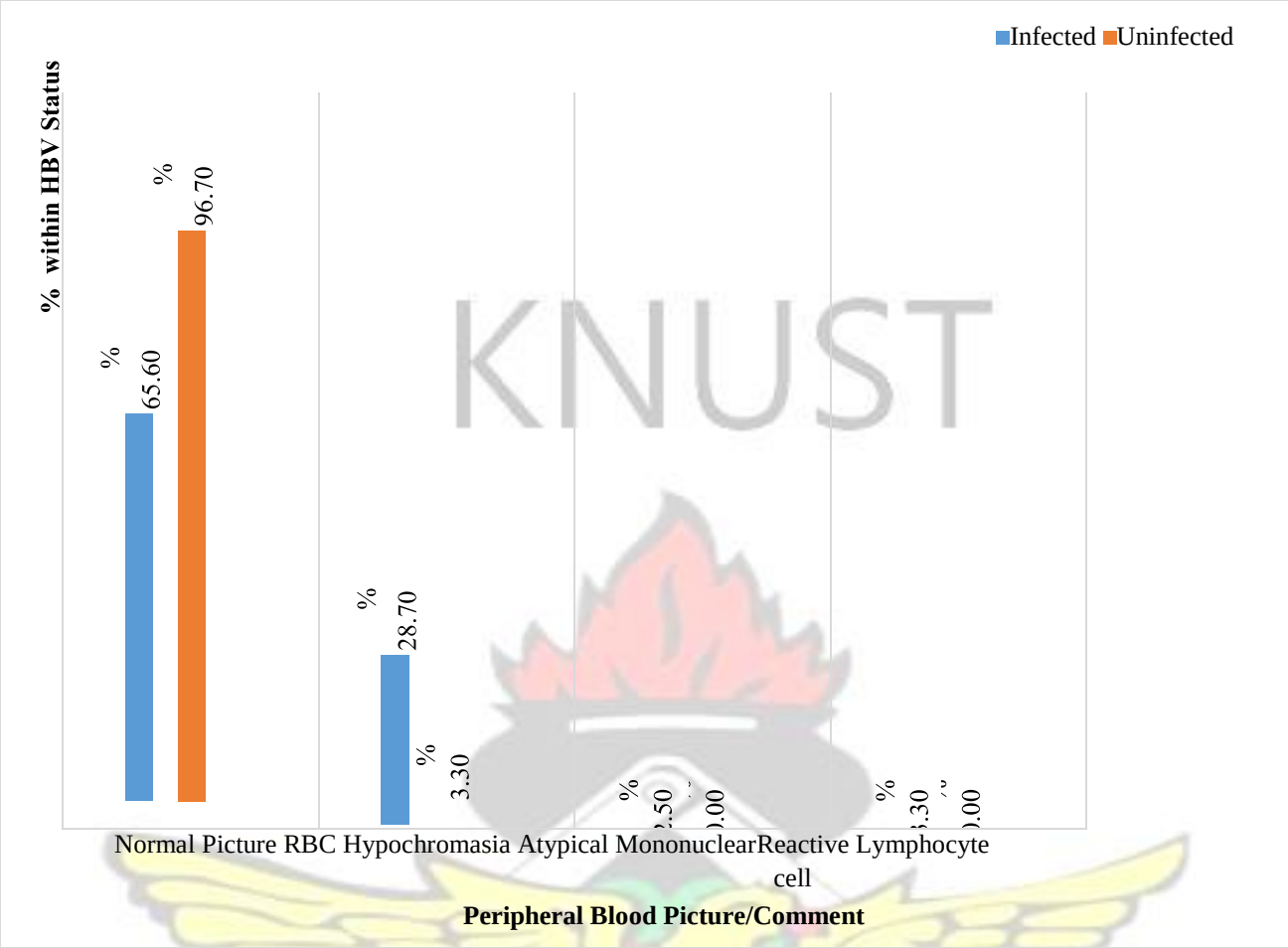

Figure 9: A Graph of Peripheral blood film comment of the HBV Infected and Uninfected Subjects.

4.4. Haematological parameters of Symptomatic and Asymptomatic Study subjects

The female and male symptomatic subjects recorded significantly high ESR and low RBC count, Hb concentration, and HCT values compared to the asymptomatic female and male subjects. With the sex independent haematological parameters, the symptomatic subjects recorded significantly high Total WBC, Neutrophil and Monocyte count compared to the asymptomatic subjects. However there were no significant differences in Platelet, Lymphocyte, Basophil and eosinophil counts of the symptomatic and asymptomatic subjects. Table 6 and 7 below respectively show the

sex dependent and sex independent haematological results of the symptomatic and asymptomatic subjects.

Table 6. Results of Sex Dependent Haematological Parameters of Symptomatic and Asymptomatic HBV Infected Female and Male Subjects.

Parameter	**Symptoms Females**	**Mean**	**Independent Samples t-Test Sig.**	**P Values**
ESR mmfall/hr	Yes females No females	74.85 18.929	0.000	P<0.05
RBC X106/µL	Yes females No females	3.288 4.2396	0.000	P<0.05
Hb g/dL	Yes females No females	9.73 11.771	0.001	P<0.05
HCT %	Yes females No females	26.915 34.658	0.000	P<0.05
	Symptoms Males			
ESR mmfall/hr	No males Yes males	10.345 65.378	0.000	P<0.05
RBC X106/µL	No males Yes males	4.9276 3.6927	0.000	P<0.05
Hb g/dL	No males Yes males	13.883 9.94	0.000	P<0.05
HCT %	No males Yes males	40.134 28.869	0.000	P<0.05

Table 7. Results of Sex Independent Haematological Parameters of Symptomatic and Asymptomatic HBV Infected Subjects.

Parameter	**Symptoms**	**Mean**	**Independent Samples t-Test Sig.**	**P value**
PLT $X10^3/\mu L$	NO YES	183.036 167.576	0.364	P>0.05

WBC $X10^3/\mu L$	NO YES	5.9975 11.1246	0.000	P< 0.05
NEUT $X10^3/\mu L$	NO YES	3.1848 8.4697	0.000	P<0.05
LYMPH $X10^3/\mu L$	NO YES	2.0648 1.9092	0.384	P>0.05
MONO $X10^3/\mu L$	NO YES	0.5755 0.7938	0.007	P<0.05
EO $X10^3/\mu L$	NO YES	0.1512 0.1436	0.835	P>0.05
BASO $X10^3/\mu L$	NO YES	0.0345 0.0708	0.126	P>0.05

4.5. Haematological profile of acute and chronic HBV infected subjects

There were no significant differences in all the sex dependent haematological parameters among the female and male subjects with acute and chronic infections. Also there were no significant differences in all the sex independent parameters among subjects with acute and chronic infections. Tables 8 and 9 below respectively show the sex dependent and sex independent haematological results of the subjects.

Table 8. Results of Sex dependent Haematological Parameters of Female and Male Subjects with Acute and Chronic HBV infections.

Parameter	**HBV Profile Remarks, Females**	**Mean**	**Independent Samples Mann-Whitney U test Sig.**	**P Values**
ESR mmfall/hr	Acute Females Chronic females	50.667 41.667	0.463	P>0.05
RBC $X10^6/\mu L$	Acute Females Chronic females	3.4433 3.7698	1.000	P>0.05

Hb g/dL	Acute Females Chronic females	10.967 10.918	0.904	P>0.05
HCT %	Acute Females Chronic females	29.700 31.522	0.779	P>0.05
	HBV Profile Remarks, Males			
ESR mmfall/hr	Acute Males Chronic Males	64.250 46.786	0.466	P>0.05
RBC $X10^6/\mu L$	Acute Males Chronic Males	3.7125 4.2031	0.588	P>0.05
Hb g/dL	Acute Males Chronic Males	11.125 11.506	0.899	P>0.05
HCT %	Acute Males Chronic Males	32.200 33.346	0.954	P>0.05

Table 9. Results of Sex Independent Haematological Parameters of Subjects with Acute and Chronic HBV infections.

Parameter	HBV Profile Remark	Mean	Independent Samples Mann Whitney U test. Sig.	P values
PLT $X10^3/\mu L$	Acute Chronic	148.571 176.261	0.534	P>0.05
WBC $X10^3/\mu L$	Acute Chronic	8.6886 8.7317	0.067	P>0.05

NEUT X10^3/µL	Acute Chronic	5.8971 5.7084	0.605	P>0.05
LYMPH X10^3/µL	Acute Chronic	1.9729 1.9811	0.758	P>0.05
MONO X10^3/µL	Acute Chronic	0.6786 0.8093	0.987	P>0.05
EO X10^3/µL	Acute Chronic	0.1100 0.1494	0.917	P>0.05
BASO X10^3/µL	Acute Chronic	0.0300 0.0556	0.592	P>0.05

4.6. Alanine Aminotransferase (ALT) Results of Subjects

Estimation of the amount of the liver enzyme ALT was done to assess the liver function of the study subjects. There were significant differences in ALT levels among the HBV infected and uninfected subjects; symptomatic and asymptomatic subjects; and the acute and chronic subjects.

Table 10 below shows the ALT results of the subjects.

Table 10. ALT Results of Subjects

Parameter	**HBV Status**	**NO.**	**Mean**	**Test**	**P Values**
ALT U/L	Infected	122	119.004	Independent Samples Mann-Whitney U test Sig = 0.000	P<0.05
	Uninfected	122	8.572		
	Symptoms				
ALT U/L	No	57	17.726	Independent Samples tTest Sig = 0.002	P<0.05
	Yes	65	207.817		
	Hepatitis B Profile Remark				

ALT U/L	Acute Infection Chronic Infection	7 115	191.586 114.586	Independent Samples Kruskal Wallis test Sig = 0.000	P<0.05

CHAPTER 5 - DISCUSSION

Hepatitis B virus infection is hyper-endemic in Ghana and especially in Kumasi **(Amidu and Alhassan., 2012)**. The study sought to find the prevalence trend of the infection at Komfo Anokye Teaching Hospital. Of the 1,569 patients who were screened for HBsAg, HIV and/or HCV antibodies at the Serology department of the hospital from August 2015 to April 2016, HBsAg sero prevalence rate of 13.89% was recorded. The hepatitis B prevalence revealed by the study is equivalent to prevalence rate in the hyper endemic category (≥ 8%). The prevalence rate is also quite high

compared to a study conducted among three densely populated communities in Kumasi in 2012 by Amidu and Alhassan, which recorded a prevalence rate of 8.68% (**Amidu and Alhassan, 2012**). The high prevalence rate could partly be attributed to the referral status of Komfo Anokye Teaching Hospital which receive referred clinical cases from hospitals and clinics across the Northern and some part of the Southern sector of the country. Also the prevalence rate revealed by the study is quite close to the prevalence rate of 14.6% recorded among blood donors at Techiman Holy Family Hospital in the Brong Ahafo region by Mutocheluh *et. al*. in 2014 (**Mutocheluh *et. al.*, 2014**).

The study grouped the HBV infected subjects into subjects with acute and chronic infections; and symptomatic and asymptomatic subjects. Of the122 hepatitis B infected subjects recruited for the study, 7 (6%) had acute infection and 115 (94%) had chronic infection (Table 3). The relatively low number of HBV infected subjects with acute infection compared to subjects with chronic infection could be attributed to the fact that about 90% of primary adult HBV infections become acute but most patients recover after a 4 to 8 week illness, and only about 1/3 of these patients develop fulminant liver condition or become chronic carriers (**Jake, 2009**). However 5 to 10% of primary adult infections and over 90% of cases of new born and infant infections result in to chronic infection, and may live with the patient for his/her entire life **(Mahoney *et al*., 1999)**.

The results of the study with regards to the haematological parameters of the HBV infected and uninfected subjects, indicated that the infected subjects (both males and females) recorded significantly ($P<0.05$) low Hb concentration and HCT but high ESR compared to the uninfected subjects (Table 4). This finding is in tune with the study by Ajugwo and Ukaji in 2015 in Nigeria, which also recorded significantly low Hb

concentration and HCT value but high ESR among infected subjects compared to uninfected subjects (**Ajugwo and Ukaji, 2015**). Also a study by Eze and Buseri in 2009 among pregnant women in Port Harcourt, Nigeria shows a similar trend among infected and uninfected pregnant women (**Eze and Buseri, 2009**)

With reference to the normal values of CBC, ALT and ESR specified by Sysmex®, Cobas Integra® (Roche Diagnostics) and Chesbrough, 2006 respectively (Appendix 1, 2 and 3), the HBV infected subjects recorded abnormally high mean ESR and ALT values; but low RBC count, HCT value and Hb concentration compared to the uninfected subjects who recorded normal values of these parameters (Tables 4 and 10). The abnormally high ESR and ALT values of the infected subjects is indicative of the inflammatory liver condition (hepatitis) of these subjects, which could indirectly be linked to the abnormal haematological parameters recorded by them. The liver is responsible for the production of proteins needed for the synthesis of haemoglobin and other blood components **(Jelkmann, 2001).** Therefore the events of hepatic damage (Hepatitis) which result in hepatic malfunction, could ultimately have a negative effect on haematopoiesis. Also the abnormal haematological parameters recorded by the HBV infected subjects could directly be attributed to the negative effect of HBV on haematopoiesis. Apart from hepatocytes, HBV to a lesser extent infect other cells such as circulating mononuclear cells, bone marrow cells and the **spleen (Yasushi *et. al*., 2002)**. In an *in vitro* study in which bone marrow was exposed to HBV, there was a dose-dependent inhibition of hematopoietic stem cells **(Yasushi *et. al*., 2002)**.

From the above explanation, it is therefore not surprising that according to the study's results, the HBV infected subjects also showed significant peripheral blood picture of

atypical mononuclear cells, reactive lymphocytes and RBC hypochromasia (Figure 9), indicating viral infection and low RBC haemoglobin content, compared to the uninfected subjects. Although some studies have suggested a possible causative role of HBV in haematological malignancies such as myelodysplastic syndrome, acute myeloid leukaemia and B-cell Non-Hodgkin Lymphoma (**Yasushi *et. al.*, 2002; Fabrizio *et. al.*, 2012)**, the study did not find any haematological malignant cells in the peripheral blood of the HBV infected subjects.

Also the results of the study with regards to the haematological parameters of the symptomatic and asymptomatic HBV infected subjects, indicated that the symptomatic subjects recorded significantly high ESR, Total WBC, Neutrophil and Monocyte count; and low RBC count, Hb concentration and HCT compared to the asymptomatic subjects. The finding of the study with regards to ESR, Total WBC count, Hb concentration and HCT value of the symptomatic and asymptomatic subjects is inconsistent with the findings of Ajugwo and Ukaji, who found no significant differences of these parameters among the symptomatic and asymptomatic subjects (**Ajugwo and Ukaji, 2015**).

With reference to the normal values of CBC, ALT and ESR specified by Sysmex®, Cobas Integra® (Roche Diagnostics) and Chesbrough, 2006 respectively (Appendix 1, 2 and 3), the symptomatic subjects recorded abnormally high mean ESR, Total WBC count, Neutrophil count and ALT value; and abnormally low mean RBC count, Hb concentration and HCT value compared to the asymptomatic subjects who recorded normal values of these parameters (Tables 6, 7 and 10). These abnormal

haematological values recorded by the symptomatic subjects could be attributed to the relatively high degree of hepatic damage (hepatitis) suffered by these subjects, evident in their abnormally high mean ESR and ALT values, compared to the asymptomatic subjects who recorded normal mean ESR and ALT values. The symptomatic infected subjects also showed evidence of a non-viral co-infection, i.e. abnormally high mean Total WBC and Neutrophil counts which is suggestive of a bacterial infection. This give an indication that symptomatic hepatitis B infected subjects could be susceptible to acquiring non-viral opportunistic infections due to the systemic effect of hepatic damage, which could eventually compromise the immune system of these subjects **(Jakab and Orv, 2015)**.

Furthermore the results of the study concerning the haematological parameters of the acute and chronic HBV infected subjects indicated that there was no significant difference in all the haematological parameters between these groups, although they recorded significant differences in their ALT levels, with the acute infected subjects having a much higher mean ALT value compared to the ALT value of the chronic subjects (Table 10). Again with reference to the normal values of CBC, ALT and ESR specified by Sysmex®, Cobas Integra® (Roche Diagnostics) and Chesbrough, 2006 respectively (Appendix 1, 2 and 3), both the acute and chronic subjects recorded abnormal values of ESR, RBC count, Total WBC count, Hb concentration and HCT (Tables 8 and 9). These abnormal haematological parameters recorded by both the acute and chronic subjects could also be attributed to the indirect consequences of hepatic damage (hepatitis) of both subjects, evident in their abnormally high mean ALT values, and directly to the ability of HBV to inhibit haematopoiesis among these subjects **(Yasushi *et. al*., 2002)** .

CHAPTER 6 – CONCLUSION, RECOMMENDATIONS AND LIMITATIONS

6.1. Conclusion

The study revealed hepatitis B prevalence rate of 13.89 % at Komfo Anokye Teaching Hospital. This high prevalence rate places the catchment area, mostly the Northern and some part of the southern sector of the country, served by the hospital in the hyper-endemic HBV infection category.

The study also revealed that HBV infected subjects are likely to present with abnormally high ESR but low RBC count, Hb concentration and HCT compared to uninfected subjects.

Again the study revealed that symptomatic HBV infected subjects are likely to present with abnormally high ESR, Total WBC count and Neutrophil count but low RBC count, Hb concentration and HCT compared to asymptomatic subjects. Also the symptomatic HBV subjects are likely to be susceptible to opportunistic bacterial infections, evident in the abnormally high Total WBC count and Neutrophil count.

Furthermore the study revealed that acute and chronic HBV infected subjects are both likely to present with abnormally high ESR and WBC count but low RBC count, Hb concentration and HCT. However the acute and chronic HBV infected subjects may not show any significant variations in their haematological parameters.

6.2. Recommendation

From the findings of the study, it is highly recommended that public education on HBV and its mode of transmission be intensified to create the necessary awareness among

the populace particularly the youth, since infants and children are captured under EPI vaccination programmes. Also awareness creation on the availability of HBV vaccines should be created among the populace to enable them take advantage of the vaccines to prevent HBV infection. These measures would go a long way to help reduce the high prevalence of HBV infection as revealed by the study.

Also from the findings of the study, it is highly recommended that as part of the management of HBV infected patients, routine haematological investigations be carried out for them, particularly symptomatic HBV infected patients as well as acute and chronic patients. This will help to remedy the likely haematological abnormalities that they may present, in order to enhance their overall effective management.

Furthermore, studies about the haematological abnormalities associated with HBV infection is scanty especially in Ghana. We there recommend that more studies be conducted using a much larger sample size to provide sufficient reports and data on the subject matter.

6.3. Limitation

The major challenge the study faced was resource constraint, hence the minimum required sample size used for the study. Also the lack of existing documentation and data on HBV and associated haematological abnormalities in Ghana served as a challenge in the Literature Review.

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APPENDIX

1. **Sysmex® 4000i normal haematological values for adult 18 to 60 years**

Parameter		Male Normal values	Female Normal values
Hb	(g/dL)	13.6 – 18.0	11.5 – 16
HCT	(%)	39.8 – 52.0	34.7 – 46.0
RBC	(x 10^6 /µL)	4.31 – 6.40	3.85 – 5.20
WBC	(x 10^3 /µL)	4.00 – 10.00	4.00 – 10.00
Platelet	(x 10^3 /µL)	140 – 440	140 – 440
Neutrophil	(x 10^3 /µL)	1.50 – 8.00	1.50 – 8.00
Lymphocyte	(x 10^3 /µL)	0.80 – 4.00	0.80 – 4.00
Monocyte	(x 10^3 /µL)	0.00 – 1.20	0.00 – 1.20
Eosinophil	(x 10^3 /µL)	0.00 – 0.30	0.00 – 0.30
Basophil	(x 10^3 /µL)	0.00 – 0.30	0.00 – 0.30

2. **ESR Normal Adult Value. Source:** (Chesbrough, 2006)

   Male: 0 mmfall/hr – 15 mmfall/hr

   Female: 0mmfall/hr – 20 mmfall/hr

3. **Cobas Integra® 400 Normal ALT Values of Adults**.

   Females: 0 – 31 U/L

   Males: 0 – 41 U/L

4. **Study Questionnaire/Case Report Form**

CASE REPORT FORM/ DATA CAPTURING FORM

Date:……

Personal Data

1. Code of participant:……..

   Age:……. Sex……

2. Educational level of participant: [ ] Primary. [ ] Secondary. [ ] Tertiary.

3. Occupation of participants ……..

Hepatitis B Data

5. Hepatitis B Surface Antigen sero-status of participant: [ ] Positive. [ ] Negative.
6. When did you get to know of your hepatitis B infection status?

   [ ] < a month. [ ] < 3 months. [ ] < 6 months. [ ] > 6 months.

Signs and Symptoms of Hepatitis B infection

Signs	**Yes, Often**	**Yes, Occasional**	**No**
7. Tiredness			
8. Anorexia/loss of appetite			
9. Abdominal discomfort			
10. Nausea			
11. Vomiting			
12. Joint pain			
13. Body aches			
14. Rashes			
15.Yellowish sclera/ eye			
16. Yellowish skin and palm			

Other medical conditions

21. Do you have sickle cell anaemia or a bleeding disorder? [ ] Yes. [ ] No. [ ] Don‘t know
22. Have you ever been diagnosed of tuberculosis or Kidney disease before? [ ] Yes. [ ] No

23. Do you have any other chronic disease? [ ]No. [ ] Yes Specify…… 24. Other medical conditions apart from hepatitis B and its related diseases recorded in participant‘s folder/card……

Date of blood sample collection:……..

Name of examiner…… Signature of examiner…….