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SEROEPIDEMIOLOGY AND VIROLOGICAL ANALYSIS OF HEPATITIS B VIRUS INFECTIONS IN KAYAYEI

BY

RAYMOND BIRIKORANG ASARE

(10805529)

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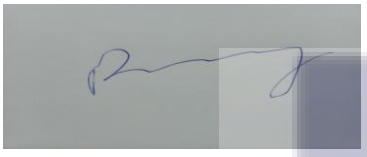
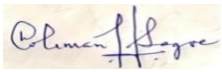
DECLARATION

I, Raymond Birikorang Asare do hereby declare that this thesis is the result of research undertaken by me towards the award of the Master of Philosophy in the Department of Medical Microbiology, Medical school, College of Health Sciences, University of Ghana. I certify that, with the exception of adequately referenced references to literature, this thesis has not been presented anywhere in whole or in part.



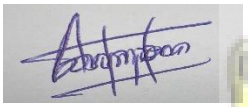
RAYMOND BIRIKORANG ASARE Date: 14/12/2023

(Candidate)



REV. PROFESSOR KWAMENA SAGOE Date: 14/12/2023

(Principal Supervisor)



DR. MILDRED ADUSEI-POKU Date: 14/12/2023

(Co-Supervisor)

DEDICATION

This thesis is dedicated to my parents Mr. and Mrs. Asare Koduah, my wife Mrs. Joyceline Addison Birikorang, my entire family, supervisors and colleagues at the Department of Medical Microbiology, University of Ghana Medical School. Without your supervision, effort, and support, this project would not have been a success.

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LIST OF ABBREVIATIONS

HBV: Hepatitis B virus

HCV: Hepatitis C virus

DNA: Deoxyribonucleic acid

WHO: World Health Organization

ORF: Open reading frame

HCC: Hepatocellular carcinoma

CHB: Chronic hepatitis B

CDC: Centre for Disease Control

OBI: Occult hepatitis B virus infection

HBsAg: Hepatitis B surface antigen

GHS: Ghana Health Service

anti-HBc: Hepatitis B core antibody

HBeAg: Hepatitis B envelope antigen

IgM: Immunoglobulin M

anti-HBs: Hepatitis B surface antibody

HBcAg: Hepatitis B core antigen

Th: T helper

IFN-γ: Interferon gamma

IL: Interleukin

CTL: Cytotoxic T lymphocyte

IgG: Immunoglobulin G

ELISA: Enzyme linked immunosorbent assay

PCR: Polymerase chain reaction

HIV: Human immunodeficiency virus

STIs: Sexually transmitted infections

UNFPA: United Nations Population Fund

RDT: Rapid diagnostic test

COVID-19: Coronavirus Disease 2019

EDTA: Ethylenediamine tetraacetic acid

RNA: Ribonucleic acid

US: United States

TBE: Tris borate ethylenediamine tetraacetic acid

bp: base pair

CHS: College of Health Sciences

EPRC: Ethics and Protocol Review Committee

GHS-ERC: Ghana Health Service Ethics Review Committee

HBcDNA: Hepatitis B core Deoxyribonucleic acid

ABSTRACT

Globally, hepatitis B virus is a leading cause of liver cirrhosis and hepatocellular carcinoma, with approximately 240 million persons living with chronic hepatitis B. In Sub-Saharan Africa, the disease is highly prevalent (≥8%), but little is known about the epidemiology and occult hepatitis B virus infection in migrant female head porters (Kayayei) in Ghana. Occult hepatitis B virus infection, defined as the presence of hepatitis B virus DNA in an individual negative for hepatitis B surface antigen with or without serological marker has become a global health threat. This study sought to provide information on the prevalence and nature of hepatitis B virus infection in migrant female head porters (Kayayei). The outcome is meant to promote health and inform policies aimed at curbing the spread of hepatitis B virus.

A simple random sampling technique was employed to enrol 390 Kayayei from the slum (Agbogbloshie and Madina). Blood samples were collected into EDTA tubes and subsequently separated into plasma. A rapid diagnostic test kit (Micropoint, Micropoint Diagnostics, China) was used to detect hepatitis B surface antigen and hepatitis B virus combo test kit (Diaspot (Serum/Plasma) Colloidal Gold, Zhejiang Orient Gene Biotech, China) was used to detect the serological profile. DNA was extracted and conventional PCR was used to detect hepatitis B virus core DNA.

Participants between the ages of 20-29 years, 44.1% (172/390) formed the highest age group recruited. The median age of study participants was 26 years. Majority of the Kayayei were married, 53.3% (208/390), 83.8% (327/390) had no formal education and 80.5% (314/390) were not aware of HBV infection. A total of 228 (58.5%) Kayayei were recruited from Madina and 162 (41.5%) from Agbogbloshie. There were some significant differences in the socio-demographic characteristics of study participants at both research sites.

The prevalence of HBsAg and occult hepatitis B virus infection were 6.4% (25/390) and 1.9% (7/365) respectively. There was no significant association between socio-demographics of participants and hepatitis B surface antigen positivity. Majority, 52.8% (66/125) of participants were susceptible to hepatitis B virus disease because they were naïve to the infection.

Prevalence of 6.4% indicates moderate endemicity of HBV infection among the Kayayei. No identifiable factor was found to be associated with HBV positivity. Occult hepatitis B virus infection has implications for blood donation and transfusion with vulnerable group as blood donors. Vaccines should be made accessible since majority of the Kayayei had no hepatitis B virus serological marker.

Keywords: Seroepidemiology, hepatitis B virus, Kayayei

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The term "hepatitis," which can refer to both infectious and non-infectious liver inflammation, is used generally. Non-infectious causes can be due to heavy alcohol use, toxins, and some medications whereas infectious causes can be due to some viruses and bacteria. However, a group of viruses known as hepatitis viruses is responsible for the majority of hepatitis cases in the world. Hepatitis A, B, C, D, and E are the five unrelated hepatotropic viruses that can affect the liver. While Hepatitis B and C (HBV and HCV) infections can become chronic and have long-term effects, Hepatitis A and E viruses normally produce acute, self-limiting infections.

Hepatitis B virus (HBV) is a hepadnavirus from a family of enveloped DNA viruses, and one of the smallest virus known to infect humans (WHO, 2015). The 3.2 kb, partly double-stranded circular DNA genome of this virus has 4 open reading frames (ORFs) that code for the proteins Core and preCore, PreS1, PreS2, and S surface antigens, Pol protein reverse transcriptase, and X protein (You *et al*., 2014). The non-cytopathic virus (HBV) damages or injures the liver through immunopathogenesis (Rehermann & Nascimbeni, 2005). After HBV infection, liver damage and viral clearance are thought to be caused by the immune system's response to viral antigens. HBV infection is predominantly acquired by vertical or horizontal transmission during infancy in highly endemic regions like Asia and Sub-Saharan Africa (Custer *et al*., 2004; WHO, 2009).

Early indications and symptoms of HBV infection include feeling ill generally, losing appetite, losing weight, vomiting, being weary, having dark urine, having yellowing of the skin (jaundice), and having right upper abdomen pain but, early signs and symptoms of HBV infection may not

occur (Connor *et al*., 2006). However, some symptoms, such as fluid retention, bruising, and persistent bleeding, may occur as the condition worsens (Parole Officers, 2013).

Every year, millions of individuals are affected by viral hepatitis, a threat to worldwide public health that results in disability and mortality. According to estimations, 257 million individuals worldwide, mostly in low- and middle-income countries, are infected (WHO, 2015). As per projections, 1 million individuals every year (2.7% of all deaths) pass away due to viral hepatitis-related conditions, mostly liver diseases including cirrhosis and liver cancer (WHO, 2012).

HBV and HCV are currently the most significant viral hepatitides in Ghana because they result in chronic infections, which lead to cirrhosis and hepatocellular carcinoma (HCC), one of the most common cancers in the nation with a high mortality rate (Ghana Health Service, 2017). HBV infection was identified as a significant risk factor for developing liver cirrhosis in Accra, Ghana, based on findings from Blankson et al. (2005) that the probability of getting cirrhosis increased 8-fold in individuals with HBV infections compared to those without (Blankson *et al*., 2005).

1.2 Problem statement

According to a systematic analysis of biomarker studies, 240 million people worldwide have chronic hepatitis B (CHB) (Ott *et al*., 2013). Hepatocellular carcinoma and other primary liver malignancies have been linked to chronic hepatitis B infection, according to studies (WHO, 2012).

Good data, which policymakers and health officials regularly need in order to plan and improve pertinent initiatives to stop the disease at an early stage of its discovery, is one crucial instrument in the fight against this disease. A major issue in the fight against the viral hepatitis epidemic,

according to WHO, is the dearth of data necessary to make judgments about policy that are supported by evidence (WHO, 2012). In Africa, accurate determination of the burden of HBV is difficult due to poor record keeping and under-reporting, but estimates put that about 70 – 90% of the adult population show evidence of past exposure to HBV infection and the HBsAg seroprevalence rate has been put around 6 - 20% (Ott *et al*., 2012). In addition, the World Health Assembly in 2016 adopted the first "Global Health Sector Strategy on Viral Hepatitis 2016–2021" (WHO, 2016). According to the policy, it aims to reduce viral hepatitis-related fatalities by 65% and new cases of the disease by 90% by 2030 (WHO, 2016). If there is comprehensive understanding of HBV studies, particularly in high risk groups, this vision may be feasible.

Ghana is considered a high prevalence country for chronic hepatitis B (CHB) [≥8%] worldwide (Abesig *et al*., 2020). According to research, the burden of the disease is known in Ghana. For instance, a study was conducted among a large population of pregnant women who accessed free antenatal services at a tertiary hospital in Ghana to determine an HBV prevalence of 6.0% (Antuamwine *et al*., 2022). Another study was conducted among HIV-1 patients on combination antiretroviral therapy (cART) to establish high frequency of HIV-hepatitis co-infections (Benjamin *et al*., 2021). Furthermore, a study investigated testing patterns for HBV and described the age, sex and region-specific prevalence of HBV infection in Ghana using hospital data from 2016-2021 (Nartey *et al*., 2022). Although there may have been significant research among some groups of the Ghanaian population into understanding the burden of HBV, the evidence available remains fragmented and more high risk groups stand at a chance of getting infected.

Moreover, there is a phenomenon called occult hepatitis B virus infection (OBI). This is due to events of modifications in the HBV surface antigen gene which results in the presence of HBV-

DNA in the liver tissue, serum or plasma among persons who do not have HBV surface antigen (HBsAg), with or without serological markers of prior viral exposure (Torbenson & Thomas, 2002; Chemin & Trépo, 2005). Occult HBV infection is a global issue of public health and a challenge for the clinical entity. These observations point out that there is a gap in the studies into understanding the burden of HBV in Ghana

1.3 Justification

HBV infection can lead to severe disease and death, affecting people worldwide. About 2 billion people worldwide are estimated to have been exposed to HBV, with almost one quarter of them having a chronic infection (Schweitzer et al., 2015; Global Hepatitis Report, 2017). Considering the grave consequences of HBV infection, it is very imperative to identify more sections of the Ghanaian population that are highly susceptible to contracting HBV since the prevalence and the level of endemicity among some sections of the Ghanaian people such as the Kayayei is not determined. There is no known data on the Kayayei in relation to HBV infection. According to research, the Kayayei are exposed to a number of threats to their physical, social, and mental health. Due to sexual abuse, upper respiratory infections, diarrheal diseases, and other hazards to their health and safety, they are at an increased risk of contracting HIV and other sexually transmitted infections (STIs) (UNFPA, 2011).

Undoubtedly, the several strategies adopted to slow down the transmission of HBV among groups of individuals across the country look promising. An example of such strategies include vaccination of HBV in infants as part of the expanded programme on immunization (EPI) which was introduced in 2002 (Owusu-Ansah, 2014). However, data on the positivity rate especially among the Kayayei in Ghana is not known.

Therefore, this study determined HBV seroepidemiology, serological profile and occult HBV infection in Kayayei, to aid policy making health officials design evidence based policies to help curb the spread of hepatitis B virus.

1.4 Aim of Study

To determine the prevalence and nature of hepatitis B virus (HBV) infections in Kayayei.

1.5 Specific Objectives

1. To determine the seroepidemiology of HBV infections.
2. To determine the serological profile of HBV infections.
3. To determine the prevalence of occult HBV infections.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of HBV infection

HBV infection can be acute or chronic, ranging from asymptomatic infection or mild disease to severe or, in rare cases, fulminant hepatitis (WHO, 2015). With a case fatality rate of 0.5–1%, acute hepatitis B is typically a self-limiting illness characterized by acute inflammation and hepatic necrosis (WHO, 2015). Chronic hepatitis B (CHB) infection encompasses a spectrum of disease, and is defined as persistent HBV infection with or without accompanying active viral infection, liver damage, and signs of inflammation (WHO, 2015).

Research states that age plays a significant role in determining the likelihood of persistent infection. In neonates, after acute infection, chronicity is common and 20–60% of young children under 5 years old, whereas only 5% of adults contract the infection (WHO, 2015).

It is estimated that globally, 2 billion people have evidence of past or present infection with HBV, and 240 million are chronic carriers of hepatitis B surface antigen (HBsAg) (Ott *et al*., 2012). Age-specific HBsAg seroprevalence varies significantly by geographic area, with the highest incidence (>5%) found in sub-Saharan Africa, East Asia, various Balkan regions, the Pacific Islands, and the Amazon Basin of South America (WHO, 2015).

The global prevalence of CHB infection is therefore categorized into high, intermediate and low endemicity. Since most childhood infections in the Western Pacific region are asymptomatic and there is minimal evidence of acute HBV-related disease, this leads to relatively high rates of chronic liver disease and liver cancer (Alter, 2003). Regions with intermediate endemicity include Eastern and Southern Europe, the Middle East, Japan, and a portion of South America. In

these populations, there is evidence of 10–16% HBV infections, and 2-7% of individuals are chronic carriers (Hou *et al*., 2005). There are more acute HBV infections than chronic carriers in low endemic regions like North America, Northern and Western Europe, and Australia because HBV infections occur in young adults and adolescents of well-defined risk populations (intravenous drug users, homosexual male, health workers etc.) (Hou *et al*., 2005). There have been studies that have often shown disparities in the levels of endemicity of HBV across the world, with Sub-Saharan Africa and East Asia among the high endemic areas where about 5% and 10% of the adult populace, respectively, is chronically infected (WHO, 2015; Yambasu, 2018)

In Africa, although the HBV infection rate prevalence is not precisely known, it is thought to be among the highest in the entire world: 8 percent in West Africa; 5 to 7 percent in Central, Eastern, and Southern Africa. Globally Ghana is considered a high prevalence country for chronic hepatitis B [≥8%] (Abesig *et al*., 2020).

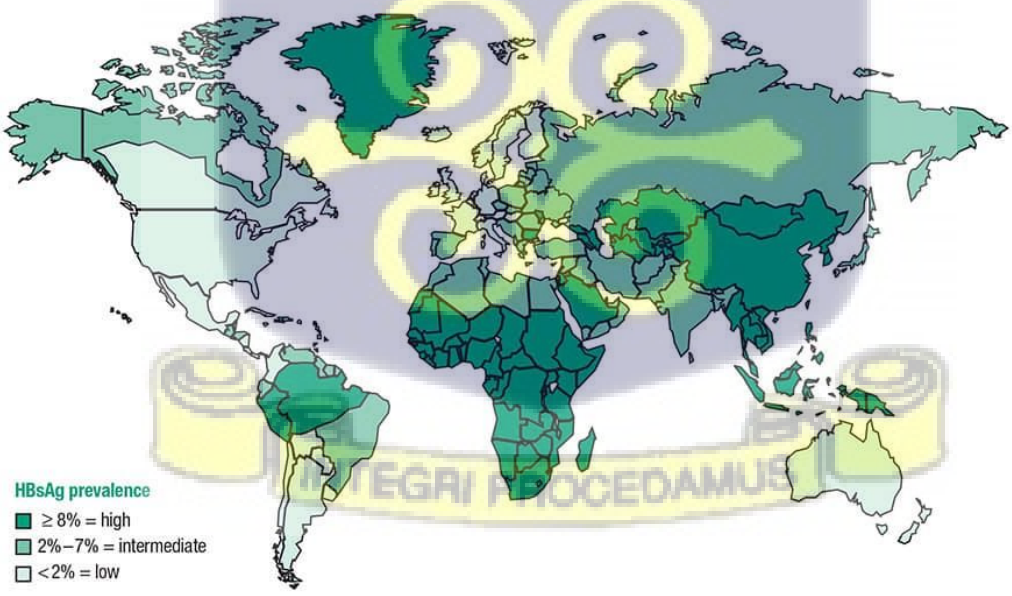

Fig 2.1: A map showing world distribution of HBV prevalence

In Ghana, viral hepatitis has come to be a significant public health issue. Based on the Disease Surveillance Department surveillance information on clinical viral hepatitis, there is an annual trend of growing clinical viral hepatitis cases reported from every region of Ghana (Disease Surveillance Department, GHS, 2015). According to research and statistics on hand establishes that the majority of the approximately four million Ghanaians who have Hepatitis B or C are unaware of their condition. Consequently, more individuals or some section of the Ghanaian people such as pregnant women, prison inmates, female head porters (Kayayei), scrap dealers, stand at high risk of getting infected with HBV.

However, some research has been done on some section of the Ghanaian people. A study done on pregnant women at HIV sentinel sites in Ghana estimated that, overall HBsAg prevalence among the pregnant women in Ghana was 14%, anti-HBc was 75.3% and HBeAg was 1.23% (Adade, 2016). Another study found that pregnant women receiving antenatal care at Korle- Bu Teaching Hospital had an HBsAg prevalence of 7.7% and there was no connection between the socio-demographic aspects of the participants and HBV infection (Dortey *et al*., 2019). More so, a research done on the transmission of HBV among prison inmates in Ghana established that HBeAg had a strong association based on the type of prison and the amount of time spent there (Ayiku, 2015). According to Ayiku (2015), out of 323 HBsAg positive samples analyzed; 91 (28.2%) tested positive for HBeAg, and 16 (5.0%) tested positive for anti-HBc IgM.

2.2 Structure of HBV

HBV belongs to the Hepadnaviridae family which infects exclusively hepatocytes of humans and some non-human primates. It is found in several forms in the blood. The infectious form is the Dane particle which has a diameter of 42nm and contains a partially double-stranded circular DNA genome (3.2 kilobases) linked to a polymerase surrounded by a nucleocapsid and three envelope proteins called the large (L), middle (M), and small (S) surface proteins (Fig 2.2) (Lamontagne et al., 2016). The viral polymerase is covalently attached to the partially double-stranded DNA genome. The core protein forms the capsid of the viral particles. The C-terminal S domain is common to all three envelope proteins. The M protein also contains an extra N-terminal preS2 domain, and the L protein contains a preS1 domain in addition to the preS2 and S domains (Herrscher et al., 2020). The envelope proteins contain domains essential for attachment to hepatocytes. Two other forms, secreted in large amounts and described as subviral particles (SVPs), are also present and contain only envelope proteins (Hu & Liu, 2017). Subviral particles are non-infectious, contain only envelope proteins, and are secreted in large excess relative to infectious Dane particles.

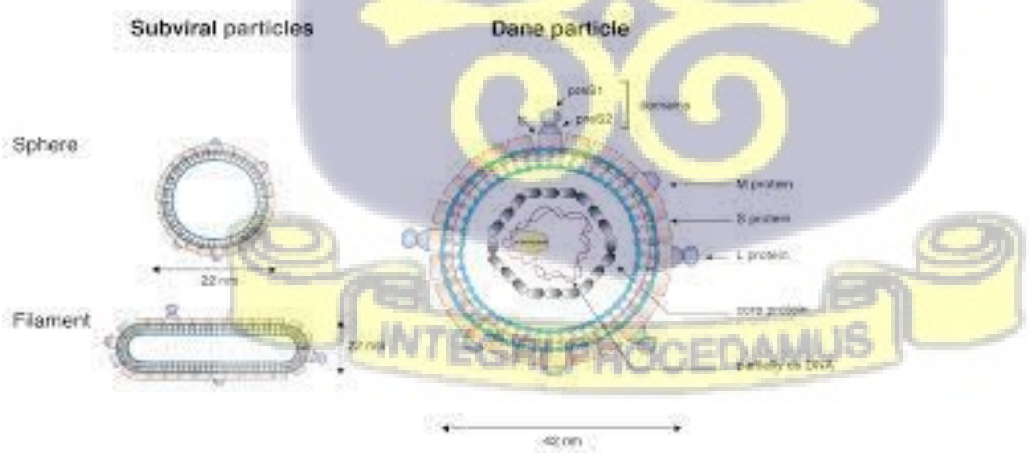

Fig 2.2: Schematic diagram of HBV particle (Herrscher *et al.*, 2020)

2.3 HBV Serological Markers

Different serological markers or combinations of markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection, whether the person is immune to HBV as a result of prior infection or vaccination, or he/she is susceptible to HBV infection (CDC, 2005). These HBV markers are;

- Hepatitis B Surface Antigen (HBsAg)
- Hepatitis B Core Antibody (anti-HBc)
- Hepatitis B Envelope Antigen (HBeAg)
- Hepatitis B Surface Antibody (anti-HBs)
- Hepatitis B Envelope Antibody (anti-HBe)

The first serologic marker to be detected is hepatitis B surface antigen (HBsAg). It is a protein on the surface of hepatitis B virus which can be detected in high levels in serum one to two weeks of infection (Hoofnagle, 1981). The presence of HBsAg shows that the person is contagious (CDC, 2005). Hepatitis B surface antibody (anti-HBs) is an antibody to HBsAg. Develops in response to HBV vaccination and while recovering from acute hepatitis B, indicating prior infection and immunity. Hepatitis B core antigen (HBcAg) is the HBV core protein. The core protein is coated with HBsAg and therefore not found free in serum. Antibody to hepatitis B core (capsid) protein, anti-HBc are not neutralizing antibodies and are detected in both acute and chronic infection. Appears at the onset of symptoms in acute hepatitis B and persists for life (CDC, 2005).

Hepatitis B e antigen (HBeAg) is the viral protein found in the high replicative phase of hepatitis B. HBeAg is usually a marker of high levels of replication with wildtype virus but is not essential for viral replication. Therefore, it is a sign of infectiousness. (Hoofnagle, 1981).

Positive IgM anti-HBc tests show recent (within six months) hepatitis B virus infection. Its presence suggests acute infection (CDC, 2005).

However, Robério (2012) identified atypical serologic profiles that are most frequently found in HBV serology. According to Robério (2012), despite the serologic findings that enable both the diagnosis of HBV infection as well as assessing of its clinical course are already well established, the dynamics of viral proteins expression and of the antibodies production may differ during the natural course of the infection (Robério, 2012).

2.4 Immune response to HBV infection

The immune system plays a key part in determining how HBV infection will turn out. It had been reported that several HBV factors, including cytokine production, genotype, viral load, and genomic mutations are linked with various liver disease progression risks.

The immune response is shaped and amplified by interactions between antigen-presenting macrophage cells and CD4+T helper (Th) lymphocytes in the liver. Two distinct subsets of effector Th cells are produced from the Th precursors; Th1 (IFN- γ, IL-2) and Th2 (IL-4, IL-10 and IL-13) (Mosmann *et al*., 1986, Romagnani *et al*., 1997). The pathogenesis of both persistent viral infections in humans and nonviral infections appears to be influenced by a difference between Th1 and Th2 cells (Rossol, 1997).

Defects in T cell response are thought to be a crucial component in the pathophysiology of chronic hepatitis, despite the fact that the immune evasion methods used by HBV are still mostly

unknown (Chisari and Ferrari, 1995; Lohr *et al*., 1995). Secreted immunity of the Th1 kind might be viewed as the immune system's proper response to stop viral replication and eradicate HBV. Enhanced cytotoxic T lymphocyte (CTL) activation, direct antiviral activity, increased expression of major histocompatibility complex class I molecules on infected cells, and macrophage activation are some of the possible ways by which IFN-γ favors the eradication of HBV (Ando *et al*., 1993; Toyonaga *et al*., 1994). Research has shown that predominant Th1 (IFN-γ) cytokine profile of hepatitis B core antigen (HbcAg)-specific and hepatitis B surface antigen (HbsAg)-reactive T cells is associated with acute self-limited hepatitis B (Sun & Ran, 2004).

Progressive viral infections have been linked to a cytokine balance that favors Th2 type cytokine production (Sun & Ran, 2004). Th3 and Th2 cell co-activation has the potential to negatively influence immunological responses and may be related to the immune tolerance seen in chronic HBV infection (Jiang et al., 2000). Chronic HBV (CHB), a persistent infection, throws off the balance between immunostimulatory and inhibitory cytokines, prolonging inflammation and increasing the risk of necrosis, fibrosis, and chronic liver disease (Jacobson Brown & Neuman, 2001).

2.5 Mode of transmission

HBV is spread predominantly by percutaneous or mucosal exposure to infected blood, blood products or body fluids (vaginal fluids, seminal fluids, menstrual fluids). This can happen through un-protected sexual contact with an infected person, either heterosexual or homosexual; direct contact with infected or contaminated blood; sharing personal items such as toothbrushes, razors, syringes; direct contact with open sores of an infected person; intravenous and percutaneous drug abuse; tattooing; body piercing; and acupuncture (WHO, 2015; GHS, 2017).

Perinatal transmission (mother to child transmission) is the major route of HBV transmission in many parts of the world including Ghana.

Horizontal transmission (exposure to infected blood), including household, intrafamilial and especially child to-child, is also important. This may occur through biting and scratching when young children are playing together.

Transfusion with contaminated blood and other blood products is considered a mode of transfusion, although blood donors are screened and less likely to occur (GHS, 2017).

2.6 Diagnosis

The specimen of choice for laboratory diagnosis of HBV infection is blood. The main diagnostic techniques employed in the laboratory diagnosis of HBV infection are serological/immunological and nucleic-acid base test. Serological techniques employ the use of an antigen to detect antibodies or an antibody to detect an antigen of a pathogen in a specimen. Examples of serological techniques used are Immunochromatography, enzyme immune assays, counter current immunoelectrophoresis and agar gel diffusion (Adade, 2016). Enzyme linked immunosorbent assay (ELISA) a subtype of heterogeneous, solid phase enzyme immune assay is a very sensitive technique capable of detecting HBsAg at low concentrations. On the other hand, nucleic acid techniques are based on the detection of HBV DNA in a specimen. This can be detected with the real time PCR or conventional PCR.

For HBsAg – positive persons, routine assessment is needed to guide management and indicate the need for treatment (WHO, 2015). Generally, this assessment includes: additional serological markers of HBV infection (HBeAg); measuring aminotransferase levels to help determine liver inflammation; quantification of HBV DNA levels; and stage of liver fibrosis by non-invasive

tests (NITs) such as aspartate aminotransferase (AST)-to-platelet ratio index (APRI), transient elastography (FibroScan) or FibroTest (WHO, 2015).

2.7 Occult hepatitis B virus infection (OBI)

In the 1970s, a patient with acute hepatitis who tested positive for anti-hepatitis B core (anti-HBc) immunoglobulin G (IgG) but negative for HBsAg was found to have a unique type of clinical HBV infection (Tabor et al., 1979). Hence, the name occult hepatitis B virus infection (OBI) was adopted.

The presence of HBV-DNA in the liver tissue of HBsAg negative people with or without HBV serological indicators of prior exposure to the virus has been used to define occult hepatitis B virus infection (Torbenson & Thomas, 2002; Chemin & Trépo, 2005). The HBsAg gene's preS1, preS2, and S regions were revealed to have mutations, which cause the protein to be undetectable using an enzyme-linked immunosorbent assay (ELISA) (Raimondo *et al*., 2007; Giudice *et al*., 2008).

The gold standard for identifying occult HBV infection is liver HBV-DNA extract analysis. Patients with chronic liver disease who tested negative for HBsAg and anti-HCV had occult HBV infection in 13–71% of their liver tissue and 5–55% of their sera (Brechot *et al*., 2001; Hu, 2002; Torbenson & Thomas, 2002). But several real-time PCR-based tests for detecting HBV-DNA in serum or plasma are sensitive enough to catch many but not all OBI cases (Chan *et al*., 2000; Gilbert et al., 2002).

Prevalence of occult HBV varies from region to region worldwide. Even in some places where the HBV endemicity is low, there have been reports of occult HBV infection (Minuk *et al*., 2005). The frequency of detecting HBV DNA in HBsAg-negative individuals changes

significantly depending on the rate of HBV infection. For instance, in the Northern countries where the prevalence of the infection is less than 5% and the prevalence of chronic infection is less than 1% prevalence of occult HBV was ≤5% in blood donors who were anti-HBc positive (Allain *et al*., 1999; Kleinman *et al*., 2003). On the contrary, in places with high prevalence, HBV DNA detection was found in 4-24% of the population in India, Taiwan, Japan, and Sardinia (Wang *et al*., 1991; Iizuka *et al*., 1992). About 5% of all HBV DNA carriers in West Africa do not have HBsAg (Allain *et al*., 1999). In Egypt, the prevalence of OBI ranged from a low 4.1% to high 26.8% in hemodialysis patients (Abu El Makarem *et al*., 2012; Elgohry *et al*., 2012). Furthermore, in Moroccan HIV infected patients, the prevalence of occult HBV was 58% (Bajjou *et al*., 2015).

In Ghana, a study revealed the prevalence of occult HBV infection in patients with HIV and HBV co-infection in Korle-Bu teaching hospital. Four samples (30.8%) of the 13 without HBsAg tested had HBV DNA (Attiku *et al*., 2021).

2.8 Migrant Female Head Porters (Kayayei)

In Ghana, the term "Kayayei" refers to a well-known group of migrant female head porters. *Kaya* in the Hausa language means luggage, load or goods. *Yoo* means woman in Ga, the language of the indigenes of Accra. Given that yoo has the plural form yei, Kayayei are ladies who charge a fee to carry other people's loads on their heads (Opare, 2003).

The Kayayei can be found in areas within Accra such as Agbogbloshie, Mallam Atta, and Madina. In Ghana's huge urban marketplaces, head porterage is associated with a number of health problems. According to research, migrant female head porters who work with clients on a daily basis are exposed to a number of threats to their physical, social, and mental health. Due to sexual abuse, upper respiratory infections, diarrheal diseases, and other hazards to their health

and safety, they are at an increased risk of contracting HIV and other sexually transmitted infections (STIs) (UNFPA, 2011). They are extremely vulnerable to gender-based violence, including rape and its potential repercussions, such as unintended pregnancy and unsafe abortion, because they lack strong family ties or kinship networks in the slum (Streifel, 2017).

Kayayei frequently encounter significant obstacles to maintaining good health, including as prejudice, linguistic and cultural limitations, and other economic and social challenges (Van Landingham, 2003; Adepoju, 2010). When the health-seeking patterns of the Kayayei in the Kumasi metropolitan were analyzed, it was found that 67% of the 378 respondents picked over-the-counter drug vendors as their preferred healthcare provider (Boateng, 2020). Further research revealed that access to quality treatment was mostly hampered by affordability, with 76% of respondents lamenting the costs associated with healthcare services (Boateng, 2020). In addition, another study done in Accra on the health seeking behavior of the Kayayei established that 75.5% of 213 respondents sought healthcare outside orthodox health facilities (Ziblim & Yiadana, 2019).

Unfortunately, knowledge of sexual reproduction health including family planning and transmission routes for sexually transmitted infections (HIV, HBV, Syphilis etc.) is significantly low among the Kayayei (UNFPA, 2011).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

This research was cross-sectional involved with the use of primary data.

3.2 Study Site

The study was carried out in Ghana's Greater Accra region, which is located in the country's south-east. The focus was on the Agbogbloshie settlement and Madina, which are well known for Kayayei activities.

Agbogbloshie is one of Accra's major slums today, with an estimated 40,000 people (Streifel, 2017). It is carved into the Korle lagoon, which empties into the Gulf of Guinea, and is located northwest of the major business center.

Madina is an Accra suburb located in the La Nkwantanang Madina Municipal District of Greater Accra. With 137,162 residents, it is the twelfth most populated settlement in Ghana.

3.3 Study Population

Participants in this study included Kayayei who worked as head porters at the Madina market and Agbogbloshie market. A random sampling technique was used to select the Kayayei.

3.4 Sample Size

The sample size was calculated from the formula $n = Z^2 P (1-P) / d^2$ and was determined to be a minimum of 384. Where n denotes the sample size, Z denotes the statistic with a 95% confidence level of 1.96, P denotes expected prevalence of 50%: 0.5, and d denotes precision of 5%: 0.05 (Pourhoseingholi *et al*., 2013). The expected prevalence was 50% because it was assumed that

50% of the Kayayei were infected with HBV since the prevalence was not known. A total of 390 participants were enrolled. The duration for sample collection was 8 months, thus, from 26/09/2021 to 30/04/2022.

3.5 Enrolment of Participants

The leadership of the community and Kayayei was consulted before they were recruited to participate in the study. A free health screening was organized, where the Kayayei were made to go through four processes. A simple random sampling technique was used to enrol participants.

The first process was education. They were sensitized on HB infection which included; mode of transmission, health implications of CHB, prevention and treatment. This was done in batches as and when the participants arrived for the exercise. All participants went through pre-counselling.

The second process was registration. Their consent was sought before any data was collected from any of them. Participants below 18 years had their Kayayei association leader who consented on their behalf.

Furthermore, the third process was testing. Blood samples from participants were taken, and an HBsAg rapid diagnostic test (RDT) strip was used to conduct the test.

Lastly, the fourth process was results issuing and interpretation. Results were recorded on a result slip and given to the participants. Interpretation of results was done by a physician assistant present at the health screening exercise. Participants who were negative for HBsAg were advised to go for the hepatitis B vaccine whereas those who were positive for HBsAg were advised to go for further tests and treatment in a health facility.

The following COVID 19 prevention protocols were ensured during the health screening;

• Wearing of nose or face mask.

• Using soap and running water to wash hands.

• Using of hand sanitizer.

• Maintaining social distance (1 metre apart).

3.6 Blood Sample Collection and Processing

The period of blood sample collection was from 26th September, 2021 to 30th April, 2022. About 4 millilitres (mls) of venous blood was collected with syringe and needle into an EDTA tube. All the blood samples were packaged and transported to Iran Clinic laboratory. At the laboratory, the samples were centrifuged at 3,000 rpm for 10 minutes. Red blood cells, buffy coat, and plasma were isolated from the blood samples. With the use of a micropipette, 2mls of plasma was transferred into a labelled Eppendorf tube. The buffy coat was also transferred into another labelled Eppendorf tube and both samples were stored in a -20°C freezer.

3.7 Detection of HBsAg using Rapid Diagnostic Test (RDT) kit

The HBsAg rapid test is designed to identify hepatitis B virus surface antigen (HBsAg) in human whole blood, serum, and plasma samples using chromatographic immunoassay. Blood samples collected from participants were screened with the rapid diagnostic test kit (Micropoint, Micropoint Diagnostics, China) to detect HBsAg. A drop of whole blood was placed on the test strip and a drop of buffer was added. It was then observed for 15 minutes and results recorded. Two lines, one at the Control (C) and the other at the Test (T) indicated a positive reaction but one line appearing at the C line only indicated negative reaction. Any reactions apart from the two were considered invalid and the test repeated. This procedure was done according to the manufacturer's instructions.

3.8 Detection of HBV serological markers using HBV combo test device

The hepatitis B virus combo test device (Diaspot (Serum/Plasma) Colloidal Gold, Zhejiang Orient Gene Biotech, China) is a rapid chromatographic immunoassay for the qualitative detection of HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc in human serum or plasma. The product uses the colloidal gold and membrane chromatography technology to measure HBsAg and HBeAg in serum or plasma. With dual-antibody sandwich method, it measures anti-HBs and with dual-antigen sandwich method, it measures anti-HBe. For measuring anti-HBc, the product uses neutralization competitive inhibition method.

The test board and the testing samples were allowed to attain room temperature. The right side of the test board was kept horizontal from the original package, from left to right, respectively corresponding to HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc. Two drops (70 µl) of the participants' plasma were added into the 5 sample wells of the test board.

The result was observed and recorded within 15 minutes. Positive and negative control samples were tested on each batch of the test kits used. For HBsAg, anti-HBs and HBeAg, negative result was recorded when merely single purple bar appeared in the control zone and no purple bar appeared in the test zone. Positive result was recorded when two purple bars appeared at the test zone and control zone. Likewise, results were reported invalid and repeated when single purple bar appeared at the test zone but no purple bar appeared at the control zone and vice versa. However, for anti-HBe and anti-HBc, negative result was recorded when double purple bars appeared in the test and control zones. Positive result was recorded when merely single purple bar appeared at the control zone. Similarly, results were reported invalid and repeated when no purple bar appeared at the control zone.

3.9 HBV Deoxyribonucleic acid (DNA) extraction

DNA molecule was extracted from participant plasma with the DaAnGene RNA/DNA Purification kit (Spin Column). The testing principle of this product is based on lysis solution (contains strong protein denaturant) which can quickly dissolve protein and make nucleic acid dissociated under the existence of lysis solution and ethyl alcohol. The dissociated nucleic acid compositions can be combined on the silicone membrane, then by actions of inhibitor remover and deionized solution, remove the protein, inorganic salt ions and many organic impurities. Then, an eluent is used to extract pure nucleic acid.

Two hundred microliters (200 µL) of the plasma samples were pipetted into a labeled 1.5 mL sterile centrifuge tube inside a biosafety cabinet (Airtech ClassIIA/B3, AirTech, Japan). Fifty microliters (50 µL) of proteinase K, 200 µL of lysis buffer (lysis solution containing Carrier RNA) were added to the samples. Following a 15-second pulse vortexing mixture, the lysate was placed on a heat block and incubated for 10 minutes at 72 ˚C.

After the heat incubation, 250 µL of molecular-grade absolute ethanol was added, and the lysate was pulse-vortexed to create a homogenous mixture. The lysate was then pipetted into a spin column (from the kit) that was appropriately labeled. The spin column was then centrifuged for one minute at 12000 g using the DHS NX-1 Centrifuge. A fresh collecting tube was used in lieu of the old one, which was discarded along with the filtrate.

A 500 µL dose of the inhibitor remover was introduced into the spin column, followed by a one-minute centrifugation at 12000 g. Then, a second wash using the same amount of deionized solution was added, and it was centrifuged for one minute at 12000 g. The spin column was then repositioned on the same collecting tube, the flow through was discarded, and the second wash was repeated.

After removing the spin column from the collecting tube and discarding the flow through, centrifugation at 14000 g for three minutes was performed to remove any remaining wash buffer and ethanol residues. The spin column was placed in a clean, labeled 1.5 mL centrifuge tube and placed on the heat block with the spin column opened for 2 minutes at 72 ˚C. The collection tube was discarded.

Following the dry heating procedure, 50 µL of elution buffer was poured into the spin column, which was then let to sit on the bench for one minute before being centrifuged at 14000 g for one minute. Prior to HBV DNA detection by conventional PCR, the labelled 1.5 mL centrifuge tube was temporarily stored at 4 ˚C and the spin column was discarded.

3.9.1 Amplification of HBV core Gene by Conventional PCR (Semi-Nested PCR)

Samples received were amplified using the Qiagen One-Step RT-PCR reagents. The RNase/DNase-free water, 5 X PCR buffer, Enzyme mix, 10 M each of the forward and reverse primers, and 10 mM dNTPs were the components of the PCR buffer cocktail. The core gene was amplified by semi-nested PCR utilizing three distinct primers. The conditions for amplification were modified from the US CDC procedure used to amplify the HBV S gene.

The primer sequences used to amplify and sequence the HBV core DNA are shown in the table below.

Table 3.1: Primer Sequences for the HBV Core Gene Amplification and Sequencing

Region	Primer name	Nucleotide sequence of the primers (5´–3´)
Round 1	2263 (+)	TTC GGA GTG TGG ATT CGC ACT CCT
	2958 (-)	GTT GGG ATT GAA GTC CCA ATC TGG AT
Round 2	2263 (+)	TTC GGA GTG TGG ATT CGC ACT CCT
	2908 (-)	CTG GTG GTC GGG AAA GAA TCC CAG

PCR first round

The reaction mixture for 1st round PCR was prepared by mixing 2.5 µL of 5 X PCR buffer, 0.5 µL Enzyme mix, 0.25 µL of sense primer (05 µM 2263 +), 0.25 µL of anti-sense primer (10 µM 2958 -), 0.5 µL of 10 mM dNTP, 6 µL of PCR water and 10 µL of template DNA, making a total volume of 20 µL.

The Taq DNA polymerase enzyme was initially denatured and activated for 5 minutes at 94 ˚C, followed by 35 cycles of denaturation for 45 seconds at 94 ˚C, annealing at 55 oC for 20 seconds, extension for 1 minute at 72 ˚C, and extended finally for 7 minutes at 72 ˚C. The Veriti thermal cycler (ThermoFisher Science) was used in the amplification process. Prior the second round amplification, the amplicons were maintained at 4 ˚C.

PCR second round

The reaction mixture for second round PCR was prepared by mixing 5 µL of 5 X PCR buffer, 1 µL Enzyme mix, 0.5 µL of sense primer (10 µM 2263 +), 0.5 µL of anti-sense primer (10 µM 2908 -), 1 µL of 10 mM dNTP, 12 µL of PCR water and 5 µL of template DNA, making a total volume of 25 µL. The Taq DNA polymerase enzyme was initially denatured and activated for 5 minutes at 94 ˚C, followed by 30 cycles of denaturation for 45 seconds at 94 ˚C, annealing at 55 ˚C for 30 seconds, extension for 1 minute at 72 ˚C, and extension finally for 7 minutes at 72 ˚C. The Veriti thermal cycler (ThermoFisher Science) was used in the amplification process. Prior to gel electrophoresis, the amplicons were maintained at 4 ˚C. Both positive and negative controls were used in the PCR for validation.

Agarose Gel Electrophoresis

Tris borate ethylenediamine tetraacetic acid (TBE) buffer was first prepared from a 10X stock solution by measuring 100 mL of the stock TBE buffer and adding 900 mL of distilled water in a 1 L reagent bottle, mixing the contents thoroughly. The results of the amplification of the HBcDNA on a 2.0% agarose in the TBE buffer were then viewed. Two grams of agarose were weighed into the conical flask containing the 1X TBE buffer after 100 mL of the 1X TBE buffer had been measured into it using a weighing scale.

A DNA staining dye, gel red, was added to the mixture after it had been swirled to mix the components and microwaved to melt the agarose. The mixture was then allowed to cool to about 45 ° C. Once the desired comb size and number were in place, the gel was poured into a casting tray and let around 15 minutes to solidify. A gel electrophoresis tank containing 1X TBE buffer as a running buffer was filled with the set gel. A 100 bp DNA ladder(Invitrogen, ThermoFisher Scientific, USA) and 5 microliters of the amplified PCR products were loaded into the wells of the gel along with 1 L of 6X Blue/Orange Trackit Loading Dye (Invitrogen by Thermo Fisher Scientific) and run at 100 volts (V) for 35 minutes. A UV-trans illuminator was used to see the gel.

3.9.2 Ethical Issues

The University of Ghana's College of Health Sciences (CHS), with protocol identification number CHS-Et/M5 -5.7/2020-2021, and the Ghana Health Service Ethics Review Committee (GHS-ERC), with protocol identification number GHS-ERC 052/05/21, were consulted for ethical approval.

3.9.3 Management of Data and Statistical Analysis

On the field and in the lab, data was gathered and recorded in data sheets and notebooks. Data were later entered, stored and managed in Microsoft Excel, 2013. The data was protected by a password. Proportions and percentages analysis was done using Microsoft Excel. To ascertain whether there was a statistically significant relationship between the categorical variables, the chi-square test was utilized. A p-value <0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS

Three hundred and ninety (390) Kayayei in all consented to take part in this research. Out of the 390 participants, 25 (6.4%) were positive for HBsAg. Prevalence of occult HBV infection was 1.9% (7/390). The most common HBV serological profile observed was interpreted to be susceptible according to the US CDC interpretation.

4.1 Prevalence and associated risk factors for HBsAg carriage

Out of the 390 study participants, 25(6.4%) were positive for HBsAg. Therefore, prevalence of HBsAg among the Kayayei was 6.4%. Details are shown in Fig 4.1 below.

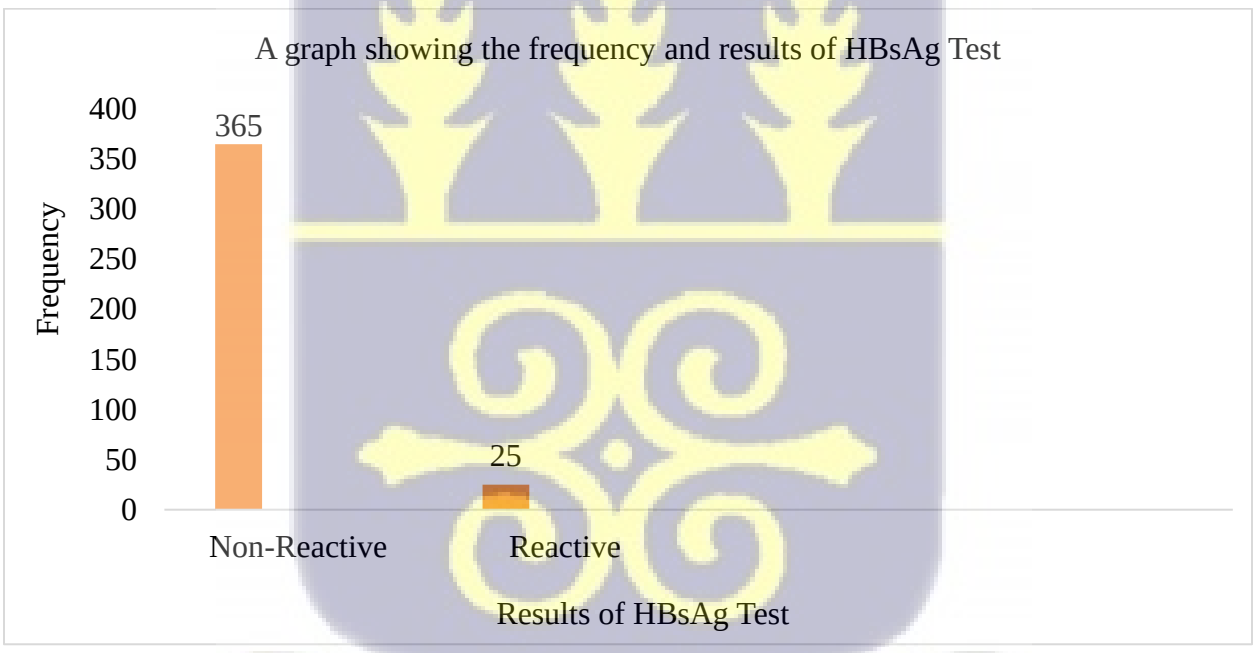

Fig 4.1: Prevalence of hepatitis B surface antigen (HBsAg)

The greatest number of samples was collected from the 20-29 year age range, 44.1% (172/390). The second highest was the 30-39 year age group, 26.2% (102/390), followed by the ≤ 19 year age group, 21.0% (82/390). The least quantity of samples was then collected from the 40-50 year age range, 8.7% (34/390). Majority of the participants were married, 53.3% (208/390). In addition, most of the study participants, 83.8% (327/390) had no formal education and 80.5% (314/390) were not aware of HBV infection. Details are shown Table 4.1 below.

Table 4.1: Socio-demographics of study participants

VARIABLE	FREQUENCY	PERCENT (%)
Age Group (Years)		
≤ 19	82	21
20 - 29	172	44.1
30 - 39	102	26.2
40-50	34	8.7
Residence		
Slum	380	97.4
Urban	10	2.6
Marital Status		
Single	141	38.2
Married	208	53.3
Divorced	7	1.8
Co-habiting	24	6.2
Widowed	2	0.5
Educational Level		
No formal education	327	83.8
Primary education	52	13.3
Junior high school	8	2.1
Senior high school	3	0.8
HBV Awareness		
Yes	76	19.5
No	314	80.5
HBV Mode of Transmission		
None	336	86.2
Unprotected sex	42	10.8

Mother-to-child	1	0.3
Kissing	6	1.5
Sharing personal items	4	1
Direct contact with infected blood	1	0.3
Vaccination Status		
Yes	5	1.3
No	385	98.7
Place of Healthcare		
Hospital	57	14.6
Clinic	51	13.1
Chemical shop	281	72.1
Herbalist	1	0.3

There was no statistically significant relationship found in between socio-demographic traits of study participants and HBsAg status. For instance, although majority of participants who were positive for HBsAg had no formal education, there was no significant association between educational level and HBV positivity since p value was set at 5%. Details are shown in Table 4.2 below.

Table 4.2: Association between socio-demographics of study participants and HBsAg status

Variables	**HBsAg Status**				
	Non-Reactive	**Reactive**	**Total**	**Chi Square**	**P-value**
Age Group (Years)				0.686	0.877
≤ 19	78 (95.1 %)	4 (4.9 %)	82		
20 – 29	161 (93.6 %)	11 (6.4 %)	172		
30 – 39	95 (93.1 %)	7 (6.9 %)	102		
40-50	31 (91.2 %)	3 (8.8 %)	34		
Residence				0.703	0.402
Slum	355 (93.4%)	25 (6.6%)	380		
Urban	10 (100.0%)	0 (0.0%)	10		
Marital Status				2.864	0.581
Single	140 (94.0 %)	9 (6.0%)	149		
Married	192 (92.3%)	16 (7.7%)	208		
Divorced	7 (100.0%)	0 (0.0%)	7		

Co-habiting	24 (100.0%)	0 (0.0%)	24		
Widowed	2 (100.0%)	0 (0.0%)	2		
Educational level				2.969	0.396
No formal education	303 (92.7%)	24 (7.3%)	327		
Primary education	51 (98.1%)	1 (1.9%)	52		
JHS	8 (100.0%)	0 (0.0%)	8		
SHS	3 (100.0%)	0 (0.0%)	3		
HBV Awareness				0.954	0.329
Yes	73 (96.1%)	3 (3.9%)	76		
No	292 (93.0%)	22 (7.0%)	314		
Mode of HBV transmission				4.64	0.461
None	314 (93.5%)	22 (6.5%)	336		
Unprotected sex	41 (97.6%)	1 (2.4%)	42		
Mother-to-child	1 (100.0%)	0 (0.0%)	1		
Kissing	5 (83.3%)	1 (16.7%)	6		
Sharing personal items	3 (75.0%)	1 (25.0%)	4		
Direct contact with infected blood	1 (100.0%)	0 (0.0%)	1		
Vaccination Status				0.347	0.556
Yes	5 (100.0%)	0 (0.0%)	5		
No	360 (93.5%)	25 (6.5%)	385		
Place of healthcare				0.339	0.953
Hospital	53 (93.0%)	4 (7.0%)	57		
Clinic	47 (92.2%)	4 (7.8%)	51		
Chemical Shop	264 (94.0%)	17 (6.0%)	281		
Herbalist	1 (100.0%)	0 (0.0%)	1		

A total of 228 (58.5%) Kayayei were recruited from Madina and 162 (41.5%) from Agbogbloshie. There were some significant differences in the socio-demographic characteristics (residence, marital status and HBV mode of transmission awareness) of study participants at both research sites. Below are details shown in Table 4.3.

Table 4.3: Association between socio-demographics and Research sites

Variables	Research Site				
	Agbogbloshie	Madina	Total	Chi Square	P-value
Age Group (Years)				6.731	0.081
≤ 19	24 (29.3%)	58 (70.7%)	82		
20 - 29	79 (45.9%)	93 (54.1%)	172		
30 - 39	45 (44.1%)	57 (55.9%)	102		
40-50	14 (41.2%)	20 (58.8%)	34		
Residence				7.292	0.007
Slum	162 (42.6%)	218 (57.4%)	380		
Urban	0 (0.0%)	10 (100%)	10		
Marital Status				13.393	0.01
Single	50 (33.6%)	99 (66.4%)	149		
Married	93 (44.7%)	115 (53.3%)	208		
Divorced	6 (85.7%)	1 (14.3%)	7		
Co-habiting	11 (45.8%)	13 (54.2%)	24		
Widowed	2 (100%)	0 (0.0%)	2		
Educational level				3.205	0.361
No formal education	137 (41.9%)	190 (58.1%)	327		
Primary education	23 (44.2%)	29 (55.8%)	52		
JHS	2 (25.0%)	6 (75.0%)	8		
SHS	0 (0.0%)	3 (100%)	3		
HBV Awareness				1.985	0.159
Yes	37 (48.7%)	39 (51.3%)	76		
No	125 (39.8%)	189 (60.2%)	314		
Mode of HBV transmission				12.045	0.034
None	137 (40.8%)	199 (59.2%)	336		
Unprotected sex	15 (35.7%)	27 (64.3%)	42		
Mother-to-child	1 (100%)	0 (0.0%)	1		
Kissing	6 (100%)	0 (0.0%)	6		
Sharing personal items	2 (50.0%)	2 (50.0%)	4		
Direct contact with infected blood	1 (100%)	0 (0.0%)	1		
Vaccination Status				0.711	0.399
Yes	3 (60.0%)	2 (40.4%)	5		
No	159 (41.3%)	226 (58.7%)	385		

Place of healthcare				6.386	0.094
Hospital	28 (49.1%)	29 (50.9%)	57		
Clinic	14 (27.5%)	37 (72.5%)	51		
Chemical Shop	120 (42.7%)	161 (57.3%)	281		
Herbalist	0 (0.0%)	1 (100%)	1		

4.2 Prevalence of occult hepatitis B virus infection

Occult HBV infection prevalence was 1.9%. Out of the 365 participants who were negative for HBsAg, 7 (1.9%) of the study participants had the HBcDNA present in their plasma, thus, they tested positive for HBcDNA while they were negative for HBsAg.

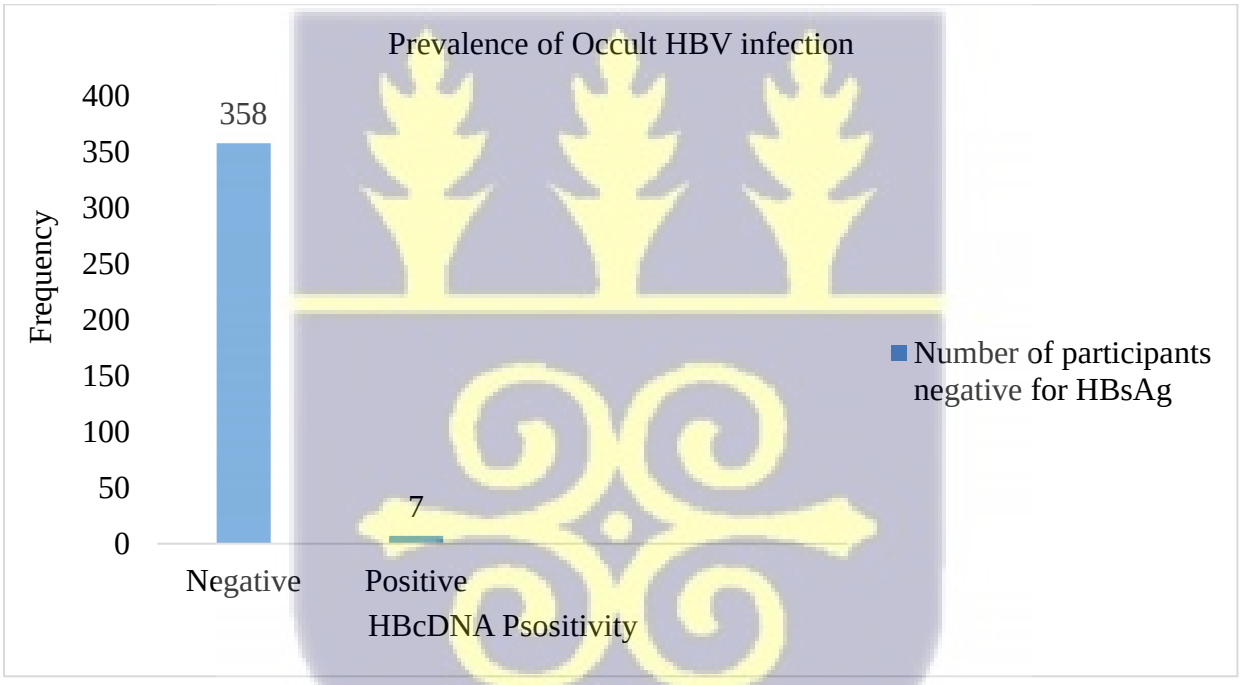

Fig 4.2: Prevalence of occult HBV infection

Participants who had the HBsAg present in their plasma as a marker of infection were 25. In addition, 10 participants had the HBcDNA in their plasma as a marker of infection. Three study participants had both HBsAg and HBcDNA present in their plasma. Details are shown in Fig 4.3 below.

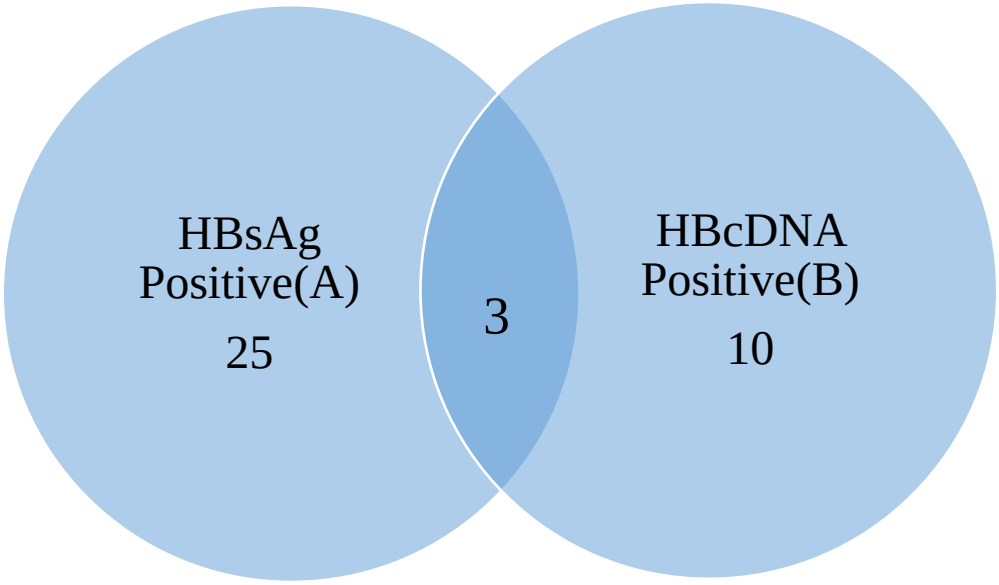

Fig 4.3: Identification of HBV markers of Infection

HBV infections were considered as HBsAg positive only and either HBsAg positive and/or HBV DNA (as measured by the HBcDNA) positive. An analysis was done to determine an association between HBV infection and socio-demographic characteristics of study participants. However, no statistically significant association was established. Details are shown in Table 4.4 below.

Table 4.4: Association between socio-demographics and HBV Infection (Both HBsAg and HBV DNA positives)

Variables	HBV Infection				
	Negative	Positive	Total	Chi Square	P-value
Age Group (Years)				1.215	0.749
≤ 19	73 (89.0%)	9 (11.0%)	82		
20 – 29	160 (93.0%)	12 (7.0%)	172		
30 – 39	94 (92.2%)	8 (7.8%)	102		
40-50	31 (91.2%)	3 (8.8%)	34		
Residence				0.917	0.338
Slum	348 (91.6%)	32 (8.4%)	380		
Urban	10 (100.0%)	0 (0.0%)	10		
Marital Status				3.636	0.458
Single	134 (98.9%)	15 (10.1%)	149		
Married	191 (91.8%)	17 (8.2%)	208		
Divorced	7 (100.0%)	0 (0.0%)	7		
Co-habiting	24 (100.0%)	0 (0.0%)	24		
Widowed	2 (100.0%)	0 (0.0%)	2		
Educational level				4.414	0.22
No formal education	296 (90.5%)	31 (9.5%)	327		
Primary education	51 (98.1%)	1 (1.9%)	52		
JHS	8 (100.0%)	0 (0.0%)	8		
SHS	3 (100.0%)	0 (0.0%)	3		
HBV Awareness				2.272	0.132
Yes	73 (96.1%)	3 (3.9%)	76		
No	285 (90.8%)	29 (9.2%)	314		
Mode of HBV transmission				4.22	0.518
None	307 (91.4%)	29 (8.6%)	336		
Unprotected sex	41 (97.6%)	1 (2.4%)	42		
Mother-to-child	1 (100.0%)	0 (0.0%)	1		
Kissing	5 (83.3%)	1 (16.7%)	6		
Sharing personal items	3 (75.0%)	1 (25.0%)	4		
Direct contact with infected blood	1 (100.0%)	0 (0.0%)	1		
Vaccination Status				0.453	0.501
Yes	5 (100.0%)	0 (0.0%)	5		
No	353 (91.7%)	32 (8.3%)	385		
Place of healthcare				0.123	0.989

Hospital	52 (91.2%)	5 (8.8%)	57
Clinic	47 (92.2%)	4 (7.8%)	51
Chemical Shop	258 (91.8%)	23 (8.2%)	281
Herbalist	1 (100.0%)	0 (0.0%)	1

4.3 Serological profile of HBV infection

A total of 125 samples were selected for serological profiling. The 25 positive samples and 100 samples were structurally selected at random. Details are shown in Table 4.5 below.

Table 4.5: HBV serological profile

VARIABLE	FREQUENCY	PERCENT (%)
HBsAg		
Non-Reactive	98	78.4
Reactive	27	21.6
anti-HBs		
Non-Reactive	95	76
Reactive	30	24
HBeAg		
Non-Reactive	122	97.6
Reactive	3	2.4
anti-HBe		
Non-Reactive	103	82.4
Reactive	22	17.6
anti-HBc		
Non-Reactive	93	74.4
Reactive	32	25.6

The HBV serological profile was classified based on the United States Centre for Disease Control (CDC) interpretation of HBV serological profile. According to the CDC interpretation, majority (52.8%, 66/125) were susceptible to HBV infection since they had no HBV serological marker. Details are shown in Table 4.6 below.

Table 4.6: Classification of HBV serological profile

HBV PROFILE/ INTERPRETATION	FREQUENCY	PERCENT (%)
Susceptible	66	52.8
Immune due to vaccination	25	20
Immune due to natural infection	5	4
Interpretation unclear (four possibilities; Resolved infection, false positive to anti- HBc, low level chronic infection, resolving acute infection)	2	1.6
Acutely infected if IgM anti-HBc is positive but chronically infected if IgM anti-HBc is negative.	23	18.4
Active viral replication	2	1.6
Unclassified	2	1.6

CHAPTER FIVE

5.0 DISCUSSION

Hepatitis B surface antigen (HBsAg) prevalence in Ghana has been estimated by a comprehensive evaluation of hepatitis B research to be between 3.5% and 22.1%, with 86.6% of these studies reporting a prevalence of 8% or above (Ofori-Asenso & Agyeman; 2015). However, there is no literature on the epidemiology of the disease among Kayayei. The overall prevalence of HBV infection in this study was 6.4% and 1.9% for occult HBV infection. There was no statistically significant association between socio-demographics and HBsAg positivity. Majority (52.8%) of the Kayayei were susceptible to HBV infection.

According to World Health Organizations standards for HBV severity, (≥ 8%; high, 2–7%; moderate and < 2%; low), the prevalence of 6.4% suggests a moderate level of endemicity. This finding is similar to the results of earlier research done among pregnant women. For instance, a recent study of HBV infection in pregnant women receiving ante-natal care at Korle-Bu Teaching Hospital found a prevalence of 7.7% (Dortey *et al.*, 2019). Another investigation in northern Ghana discovered a prevalence of 7.5% among pregnant women (Anabire *et al.*, 2019). These results indicate that HBV infection is still a significant public health issue and threat in Ghana. Prevalence from an HIV sentinel location is lower than that discovered in this study. A study done among pregnant women in HIV sentinel sites in Ghana found a prevalence of 14.0% (Adade, 2016). The differences in findings might be due to the presence of HIV infection in these sites, and both HIV and HBV having the same route of transmission. To add to this, in Ghana, the prevalence of HBV seems to be decreasing with time. For instance, research from 1995 to 2002 showed the highest prevalence, 17.3%, followed by studies from 2003 to 2009, 14.7%, and studies from 2010 to 2015, 10.2% (Ofori-Asenso & Agyeman; 2015), and 6.4% in

this study. These findings suggest that many Ghanaians are vaccinating against HBV or the introduction of the vaccination programme in Ghana has had a positive impact on the HBV infection in Ghana. Moreover, variations in sample sizes, HBV screening techniques, or risky socio-cultural and behavioral practices across generations may all contribute to the decline in prevalence.

Another finding from this study showed that there was no connection between HBV infection and the socio-demographic traits of study participants. This finding is supported by previous studies in Ghana. In the Northern region, Anabire (2019) discovered that socio-demographic traits were not risk factors for HBV infection among pregnant women. Another study conducted in the Ashanti region confirmed that socio-demographic characteristics had no bearing on pregnant women's HBV infection (Ephraim *et al*., 2019). Contrarily, comparable investigations in Ethiopia, Uganda, and Cameroon discovered a strong relationship between socio-demographic elements like marital status, age, and occupation with HBV infection in pregnant women (Bayo *et al*., 2014; Tanga *et al*., 2019; Eyong *et al*., 2019). These data imply that country-specific risk factors for HBV exist. Therefore, the disparities in results could be explained by the widespread lack of understanding and awareness of HBV among Ghanaians, regardless of social level.

HBV prevalence at research sites did not vary greatly since both indicated moderate endemicity. Prevalence of HBV at Agbogbloshie and Madina were 5.5% and 7% respectively. The difference can be due to the number of samples tested at each site. This finding agrees with the work of Adade (2016) where he found HBV prevalence in Ghana's northern, middle, and southern regions to be 14.8, 13.20% and 14.70% respectively. Moreover, there were significant differences in some socio-demographic characteristics (residence, marital status and HBV mode of transmission awareness) of study participants at both research sites as determined by the test

statistic. This suggests that the same approach cannot be used in both research sites to curb the spread of the disease.

It was discovered that 1.9% of Kayayei had occult HBV infection (OBI).This finding differs from previous studies. An investigation carried out at Korle-Bu Teaching Hospital among HIV infected persons revealed a prevalence of 30.8% (Attiku *et al*., 2021). Also, in Moroccan HIV infected patients, the prevalence of occult HBV infection was found to be 58% (Bajjou *et al*., 2015). Differences in the sensitivity of the PCR techniques utilized and the amount of samples analyzed account for these variations. Additionally, the prevalence of occult HBV infection is influenced by the characteristics of the research population as well as the prevalence of HBV (Cohen *et al*., 2009). Not only these but also, the two studies were conducted among HIV patients who are immunosuppressed and these are people who are likely to be transfused with blood and other blood products, hence, the high prevalence of occult HBV infection as compared to the prevalence of this study. Furthermore, the diagnosis of occult HBV infection is based on the identification of HBV DNA in liver samples, which is thought to be the gold standard (Samal *et al*., 2012) but HBcDNA was detected from plasma samples in this study. In persons with occult HBV infection, testing for numerous sites on the HBV genome increases the rates and chances of HBV DNA detection (Hassan *et al*., 2011).

In this study, among the 3 HBsAg positive carriers who were also positive for HBcDNA, only 1 out of the 3 had HBeAg present or as another marker of HBV infection. This finding suggests a low HBV viremia among the HBsAg positive carriers. Previous studies suggest that, the appearance of HBeAg in the serum of HBsAg positive carriers has in general been considered an indication of active viral replication, often correlating with infectivity. Additionally, in chronic HBV infection, the presence of HBeAg substantially corresponds with HBV DNA levels and an

aggressive course of liver disease (Feng *et al*., 2018). According to some research, infants of HBeAg-positive mothers are 80% to 90% likely to contract HBV (Zhang, 1993; Zhang, 1995). In a previous study, HBV DNA was found in the serum of all 14 HBeAg positive mothers, and the new-borns' HBV DNA detection rate reached 92.9% (13/14) (Zhang *et al*., 1998). However, only 25.9% of HBeAg negative mothers had HBV DNA positivity, and 14.8% (4/27) of their new-borns had HBV DNA present in serum (Zhang *et al*., 1998).

Furthermore, the sensitivity of the PCR method used could be an aspect affecting the identification of HBV DNA among the HBsAg positive carriers. This study made use of the conventional PCR (semi- nested) to detect HBV DNA. Comparing nested PCR (n-PCR) to semi-nested conventional PCR, n-PCR boosts sensitivity and specificity while preventing the "plateau" of one-time amplification by switching the primers and plates (Yang & Wang, 1992), whereas semi- nested PCR uses the same primers in two rounds of PCR to increase the sensitivity and specificity.

Finally, this study characterized the HBV serological profile of the study participants according to US CDC interpretation of HBV serological profile. Majority (52.8%) of the study participants were classified as susceptible. They had no HBV serological marker in their plasma. This finding suggests that most of the study participants stand at a high risk of getting infected with HBV. This could be as a result of the study participants' dearth of information and education regarding HBV infection. To add to this, Kayayei are unable to access the hepatitis B vaccines, although effective vaccines are available. Since there are no studies to compare with and little information on the HBV serological profile in Kayayei, it is likely that other factors also contributed to this finding.

5.1 Limitations of study

This study had some limitations. First, the inability to employ ELIZA for sero- analysis might have resulted in the small number of seropositive cases, although the screening method (immunochromatography) used in this study has a relative sensitivity and specificity of 100% and can give accurate results. In addition, this study was skewed towards socio-demographic determinants of HBV infection. Some possible factors such as medical and family history, sexual behaviours were not controlled. Lastly, the use of real time PCR would have been superior for HBV – DNA detection as compared to the semi nested PCR method used in this study.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

Prevalence of 6.4% indicates moderate endemicity of HBV infection among the Kayayei. No significant association was established between socio-demographics and HBV positivity. Occult HBV infection prevalence was 1.9%, which has consequences for blood donation and transfusion. Vaccines should be made accessible since majority of the Kayayei were susceptible to HBV infection. The results of this study therefore give empirical information that will further understanding of HBV epidemiology in Ghana and will also inspire additional research on the condition, particularly among the vulnerable.

6.2. RECOMMENDATION

According to this study's findings, the following are strongly advised;

1. Future studies should explore other possible risk factors of HBV infection.
2. Screening for occult HBV infection should be adopted in blood bank units.
3. More education and awareness of HBV infection should be made known to the Kayayei.
4. HBV vaccines should be made very affordable and accessible to the Kayayei.

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APPENDICES

APPENDIX I: Chi-square test analysis between socio-demographics and HBsAg Status

a. Age and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
age group (years)	≤ 19	Count	78	4	82
		% within age group	95.1%	4.9%	100.0%
	20 – 29	Count	161	11	172
		% within age group	93.6%	6.4%	100.0%
	30 – 39	Count	95	7	102
		% within age group	93.1%	6.9%	100.0%
	40-50	Count	31	3	34
		% within age group	91.2%	8.8%	100.0%
Total		Count	365	25	390
		% within age group	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.686[a]	3	.877
Likelihood Ratio	.680	3	.878
Linear-by-Linear Association	.625	1	.429
N of Valid Cases	390		

a. 1 cells (12.5%) have expected count less than 5. The minimum expected count is 2.18.

b. Residence and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
Where do you stay	Slum	Count	355	25	380
		% within Where do you stay	93.4%	6.6%	100.0%
	Urban	Count	10	0	10
		% within Where do you stay	100.0%	0.0%	100.0%
Total		Count	365	25	390
		% within Where do you stay	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.703[a]	1	.402		
Continuity Correction[b]	.034	1	.854		
Likelihood Ratio	1.343	1	.247		
Fisher's Exact Test				1.000	.511
Linear-by-Linear Association	.701	1	.402		
N of Valid Cases	390				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is .64.
b. Computed only for a 2x2 table

c. Marital status and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
Are you married	Single	Count	140	9	149
		% within Are you married	94.0%	6.0%	100.0%
	Married	Count	192	16	208
		% within Are you married	92.3%	7.7%	100.0%
	Divorced	Count	7	0	7
		% within Are you married	100.0%	0.0%	100.0%
	Co-habiting	Count	24	0	24
		% within Are you married	100.0%	0.0%	100.0%
	Widowed	Count	2	0	2
		% within Are you married	100.0%	0.0%	100.0%
Total		Count	365	25	390
		% within Are you married	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.864[a]	4	.581
Likelihood Ratio	4.945	4	.293
Linear-by-Linear Association	.750	1	.387
N of Valid Cases	390		

a. 4 cells (40.0%) have expected count less than 5. The minimum expected count is .13.

d. Educational level and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
What is your educational level	No formal education	Count	303	24	327
		% within What is your educational level	92.7%	7.3%	100.0%
	Primary education	Count	51	1	52
		% within What is your educational level	98.1%	1.9%	100.0%
	Junior high school	Count	8	0	8
		% within What is your educational level	100.0%	0.0%	100.0%
	Senior high school	Count	3	0	3
		% within What is your educational level	100.0%	0.0%	100.0%
Total		Count	365	25	390
		% within What is your educational level	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.969[a]	3	.396
Likelihood Ratio	4.277	3	.233
Linear-by-Linear Association	2.689	1	.101
N of Valid Cases	390		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .19.

e. HBV awareness and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
Are you aware of HBV	Yes	Count	73	3	76
		% within Are you aware of HBV	96.1%	3.9%	100.0%
	No	Count	292	22	314
		% within Are you aware of HBV	93.0%	7.0%	100.0%
Total		Count	365	25	390
		% within Are you aware of HBV	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.954[a]	1	.329		
Continuity Correction[b]	.513	1	.474		
Likelihood Ratio	1.064	1	.302		
Fisher's Exact Test				.439	.245
Linear-by-Linear Association	.952	1	.329		
N of Valid Cases	390				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.87.
b. Computed only for a 2x2 table

f. HBV mode of transmission and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
What is the mode of transmission of HBV	None	Count	314	22	336
		% within What is the mode of transmission of HBV	93.5%	6.5%	100.0%
	Unprotected Sex	Count	41	1	42
		% within What is the mode of transmission of HBV	97.6%	2.4%	100.0%
	Mother-to-child	Count	1	0	1
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0%
	Kissing	Count	5	1	6
		% within What is the mode of transmission of HBV	83.3%	16.7%	100.0%
	Sharing personal items	Count	3	1	4
		% within What is the mode of transmission of HBV	75.0%	25.0%	100.0%
	Direct contact with infected blood	Count	1	0	1
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0%
Total		Count	365	25	390
		% within What is the mode of transmission of HBV	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.640[a]	5	.461
Likelihood Ratio	3.895	5	.565
Linear-by-Linear Association	1.028	1	.311
N of Valid Cases	390		

a. 8 cells (66.7%) have expected count less than 5. The minimum expected count is .06.

g. Vaccination status and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
Have you been vaccinated against HBV	Yes	Count	5	0	5
		% within Have you been vaccinated against HBV	100.0%	0.0%	100.0%
	No	Count	360	25	385
		% within Have you been vaccinated against HBV	93.5%	6.5%	100.0%
Total		Count	365	25	390
		% within Have you been vaccinated against HBV	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.347[a]	1	.556		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.667	1	.414		
Fisher's Exact Test				1.000	.717
Linear-by-Linear Association	.346	1	.556		
N of Valid Cases	390				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .32.
b. Computed only for a 2x2 table

h. Place of healthcare and HBsAg status

Crosstab

			HBsAg Testing		Total
			Non-Reactive	Reactive	
Where do you seek healthcare	Hospital	Count	53	4	57
		% within Where do you seek healthcare	93.0%	7.0%	100.0%
	Clinic	Count	47	4	51
		% within Where do you seek healthcare	92.2%	7.8%	100.0%
	Chemical shop	Count	264	17	281
		% within Where do you seek healthcare	94.0%	6.0%	100.0%
	Herbalist	Count	1	0	1
		% within Where do you seek healthcare	100.0%	0.0%	100.0%
Total		Count	365	25	390
		% within Where do you seek healthcare	93.6%	6.4%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.339[a]	3	.953
Likelihood Ratio	.392	3	.942
Linear-by-Linear Association	.174	1	.676
N of Valid Cases	390		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .06.

APPENDIX II: Chi-square test analysis between socio-demographics and research sites

a. Age and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
age group (years)	≤ 19	Count	24	58	82
		% within age group	29.3%	70.7%	100.0%
	20-29	Count	79	93	172
		% within age group	45.9%	54.1%	100.0%
	30-39	Count	45	57	102
		% within age group	44.1%	55.9%	100.0%
	40-50	Count	14	20	34
		% within age group	41.2%	58.8%	100.0%
Total		Count	162	228	390
		% within age group	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.731[a]	3	.081
Likelihood Ratio	6.929	3	.074
Linear-by-Linear Association	2.120	1	.145
N of Valid Cases	390		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 14.12.

b. Residence and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
Where do you stay	Slum	Count	162	218	380
		% within Where do you stay	42.6%	57.4%	100.0%
	Urban	Count	0	10	10
		% within Where do you stay	0.0%	100.0%	100.0%
Total		Count	162	228	390
		% within Where do you stay	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.292[a]	1	.007		
Continuity Correction[b]	5.642	1	.018		
Likelihood Ratio	10.923	1	.001		
Fisher's Exact Test				.006	.004
Linear-by-Linear Association	7.274	1	.007		
N of Valid Cases	390				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.15.

b. Computed only for a 2x2 table

c. Marital status and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
Are you married	Single	Count	50	99	149
		% within Are you married	33.6%	66.4%	100.0%
	Married	Count	93	115	208
		% within Are you married	44.7%	55.3%	100.0%
	Divorced	Count	6	1	7
		% within Are you married	85.7%	14.3%	100.0%
	Co-habiting	Count	11	13	24
		% within Are you married	45.8%	54.2%	100.0%
	Widowed	Count	2	0	2
		% within Are you married	100.0%	0.0%	100.0%
Total		Count	162	228	390
		% within Are you married	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.393[a]	4	.010
Likelihood Ratio	14.428	4	.006
Linear-by-Linear Association	6.937	1	.008
N of Valid Cases	390		

a. 4 cells (40.0%) have expected count less than 5. The minimum expected count is .83.

d. Educational level and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
What is your educational level	No formal education	Count	137	190	327
		% within What is your educational level	41.9%	58.1%	100.0%
	Primary education	Count	23	29	52
		% within What is your educational level	44.2%	55.8%	100.0%
	Junior high school	Count	2	6	8
		% within What is your educational level	25.0%	75.0%	100.0%
	Senior high school	Count	0	3	3
		% within What is your educational level	0.0%	100.0%	100.0%
Total		Count	162	228	390
		% within What is your educational level	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.205[a]	3	.361
Likelihood Ratio	4.351	3	.226
Linear-by-Linear Association	1.065	1	.302
N of Valid Cases	390		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 1.25.

e. HBV awareness and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
Are you aware of HBV	Yes	Count	37	39	76
		% within Are you aware of HBV	48.7%	51.3%	100.0%
	No	Count	125	189	314
		% within Are you aware of HBV	39.8%	60.2%	100.0%
Total		Count	162	228	390
		% within Are you aware of HBV	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.985[a]	1	.159		
Continuity Correction[b]	1.636	1	.201		
Likelihood Ratio	1.966	1	.161		
Fisher's Exact Test				.194	.101
Linear-by-Linear Association	1.980	1	.159		
N of Valid Cases	390				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 31.57.

b. Computed only for a 2x2 table

f. HBV mode of transmission and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
What is the mode of transmission of HBV	None	Count	137	199	336
		% within What is the mode of transmission of HBV	40.8%	59.2%	100.0%
	Unprotected Sex	Count	15	27	42
		% within What is the mode of transmission of HBV	35.7%	64.3%	100.0%
	Mother-to-child	Count	1	0	1
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0%
	Kissing	Count	6	0	6
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0%
	Sharing personal items	Count	2	2	4
		% within What is the mode of transmission of HBV	50.0%	50.0%	100.0%
	Direct contact with infected blood	Count	1	0	1
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0%
Total		Count	162	228	390
		% within What is the mode of transmission of HBV	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.045[a]	5	.034
Likelihood Ratio	14.850	5	.011
Linear-by-Linear Association	5.185	1	.023
N of Valid Cases	390		

a. 8 cells (66.7%) have expected count less than 5. The minimum expected count is .42.

g. Vaccination status and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
Have you been vaccinated against HBV	Yes	Count	3	2	5
		% within Have you been vaccinated against HBV	60.0%	40.0%	100.0%
	No	Count	159	226	385
		% within Have you been vaccinated against HBV	41.3%	58.7%	100.0%
Total		Count	162	228	390
		% within Have you been vaccinated against HBV	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.711[a]	1	.399		
Continuity Correction[b]	.149	1	.699		
Likelihood Ratio	.698	1	.404		
Fisher's Exact Test				.653	.344

Linear-by-Linear Association	.709	1	.400		
N of Valid Cases	390				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.08.
b. Computed only for a 2x2 table

h. Place of healthcare and research site

Crosstab

			Research Site		Total
			Agbogbloshie	Madina	
Where do you seek healthcare	Hospital	Count	28	29	57
		% within Where do you seek healthcare	49.1%	50.9%	100.0%
	Clinic	Count	14	37	51
		% within Where do you seek healthcare	27.5%	72.5%	100.0%
	Chemical shop	Count	120	161	281
		% within Where do you seek healthcare	42.7%	57.3%	100.0%
	Herbalist	Count	0	1	1
		% within Where do you seek healthcare	0.0%	100.0%	100.0%
Total		Count	162	228	390
		% within Where do you seek healthcare	41.5%	58.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.386[a]	3	.094
Likelihood Ratio	6.941	3	.074
Linear-by-Linear Association	.069	1	.793
N of Valid Cases	390		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is .42.

APPENDIX III: Chi-square test analysis between socio-demographics and HBV infection (both HBsAg and HBcDNA)

a. Age and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
age group (years)	≤ 19	Count	73	9	82
		% within age group	89.0%	11.0%	100.0%
	20-29	Count	160	12	172
		% within age group	93.0%	7.0%	100.0%
	30-39	Count	94	8	102
		% within age group	92.2%	7.8%	100.0%
	40-50	Count	31	3	34
		% within age group	91.2%	8.8%	100.0%
Total		Count	358	32	390
		% within age group	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.215[a]	3	.749
Likelihood Ratio	1.159	3	.763
Linear-by-Linear Association	.218	1	.641
N of Valid Cases	390		

a. 1 cells (12.5%) have expected count less than 5. The minimum expected count is 2.79.

b. Residence and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
Where do you stay	Slum	Count	348	32	380
		% within Where do you stay	91.6%	8.4%	100.0%
	Urban	Count	10	0	10
		% within Where do you stay	100.0%	0.0%	100.0%
Total		Count	358	32	390
		% within Where do you stay	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.917[a]	1	.338		
Continuity Correction[b]	.140	1	.708		
Likelihood Ratio	1.736	1	.188		
Fisher's Exact Test				1.000	.420
Linear-by-Linear Association	.915	1	.339		
N of Valid Cases	390				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is .82.

b. Computed only for a 2x2 table

c. Marital status and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
Are you married	Single	Count	134	15	149
		% within Are you married	89.9%	10.1%	100.0%
	Married	Count	191	17	208
		% within Are you married	91.8%	8.2%	100.0%
	Divorced	Count	7	0	7
		% within Are you married	100.0%	0.0%	100.0%
	Co-habiting	Count	24	0	24
		% within Are you married	100.0%	0.0%	100.0%
	Widowed	Count	2	0	2
		% within Are you married	100.0%	0.0%	100.0%
Total		Count	358	32	390
		% within Are you married	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.636[a]	4	.458
Likelihood Ratio	6.294	4	.178
Linear-by-Linear Association	3.204	1	.073
N of Valid Cases	390		

a. 4 cells (40.0%) have expected count less than 5. The minimum expected count is .16.

d. Educational level and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
What is your educational level	No formal education	Count	296	31	327
		% within What is your educational level	90.5%	9.5%	100.0%
	Primary education	Count	51	1	52
		% within What is your educational level	98.1%	1.9%	100.0%
	Junior high school	Count	8	0	8
		% within What is your educational level	100.0%	0.0%	100.0%
	Senior high school	Count	3	0	3
		% within What is your educational level	100.0%	0.0%	100.0%
Total		Count	358	32	390
		% within What is your educational level	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.414[a]	3	.220
Likelihood Ratio	6.409	3	.093
Linear-by-Linear Association	3.909	1	.048
N of Valid Cases	390		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .25.

e. HBV awareness and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
Are you aware of HBV	Yes	Count	73	3	76
		% within Are you aware of HBV	96.1%	3.9%	100.0%
	No	Count	285	29	314
		% within Are you aware of HBV	90.8%	9.2%	100.0%
Total		Count	358	32	390
		% within Are you aware of HBV	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.272[a]	1	.132		
Continuity Correction[b]	1.624	1	.203		
Likelihood Ratio	2.656	1	.103		
Fisher's Exact Test				.165	.095
Linear-by-Linear Association	2.266	1	.132		
N of Valid Cases	390				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.24.

b. Computed only for a 2x2 table

f. HBV mode of transmission and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
What is the mode of transmission of HBV	None	Count	307	29	336
		% within What is the mode of transmission of HBV	91.4%	8.6%	100.0 %
	Unprotected Sex	Count	41	1	42
		% within What is the mode of transmission of HBV	97.6%	2.4%	100.0 %
	Mother-to-child	Count	1	0	1
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0 %
	Kissing	Count	5	1	6
		% within What is the mode of transmission of HBV	83.3%	16.7%	100.0 %
	Sharing personal items	Count	3	1	4
		% within What is the mode of transmission of HBV	75.0%	25.0%	100.0 %
	Direct contact with infected blood	Count	1	0	1
		% within What is the mode of transmission of HBV	100.0%	0.0%	100.0 %
Total		Count	358	32	390
		% within What is the mode of transmission of HBV	91.8%	8.2%	100.0 %

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.220[a]	5	.518
Likelihood Ratio	4.458	5	.486
Linear-by-Linear Association	.271	1	.602
N of Valid Cases	390		

a. 8 cells (66.7%) have expected count less than 5. The minimum expected count is .08.

g. Vaccination status and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
Have you been vaccinated against HBV	Yes	Count	5	0	5
		% within Have you been vaccinated against HBV	100.0%	0.0%	100.0%
	No	Count	353	32	385
		% within Have you been vaccinated against HBV	91.7%	8.3%	100.0%
Total		Count	358	32	390
		% within Have you been vaccinated against HBV	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.453[a]	1	.501		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.862	1	.353		
Fisher's Exact Test				1.000	.650
Linear-by-Linear Association	.452	1	.502		
N of Valid Cases	390				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .41.
b. Computed only for a 2x2 table

h. Place of healthcare and HBV infection

Crosstab

			HBV Infection		Total
			Negative	Positive	
Where do you seek healthcare	Hospital	Count	52	5	57
		% within Where do you seek healthcare	91.2%	8.8%	100.0%
	Clinic	Count	47	4	51
		% within Where do you seek healthcare	92.2%	7.8%	100.0%
	Chemical shop	Count	258	23	281
		% within Where do you seek healthcare	91.8%	8.2%	100.0%
	Herbalist	Count	1	0	1
		% within Where do you seek healthcare	100.0%	0.0%	100.0%
Total		Count	358	32	390
		% within Where do you seek healthcare	91.8%	8.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.123[a]	3	.989
Likelihood Ratio	.204	3	.977
Linear-by-Linear Association	.019	1	.892
N of Valid Cases	390		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .08.

APPENDIX IV: College Ethical Clearance form

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/JUNE/2020 June 23, 2021

Mr. Raymond Birikorang Asare
Dept. of Medical Microbiology
University of Ghana Medical School
Korle Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M5 –5.7 /2020-2021***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on June 23, 2021 reviewed and approved your research protocol.

Title of Protocol: **"Seroepidemiology and Immunovirological Analysis of HBV Infections in Kayayei"**

Principal Investigator: Mr. Raymond Birikorang Asare

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid until June 16, 2022.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, UGMS
Head, Medical Microbiology

INTEGRI PROCEDAMUS

• P. O. Box KB 52, Korle Bu, Accra, Ghana. Telephone: +233 (0) 302 665103/4 • Fax: +233 (0) 302 660762
• Email: admin.chs@ug.edu.gh /provost.chs@ug.edu.gh • Website: www.chs.ug.edu.gh

APPENDIX V: Ghana Health Service Ethical Clearance form

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. GHS/RDD/ERC/Admin/App (21|297
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Fax + 233-302-685424
Email: ethics.research@ghsmail.org
26th July, 2021

Raymond Birikorang Asare
University of Ghana, Legon
P.O Box AN 6044, Accra North

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC 052/05/21**
Project Title	Seroepidemiology and Immunovirological Analysis of HBV Infections in Kayayei.
Approval Date	26th July, 2021
Expiry Date	25th July, 2022
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.
- Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
Dr. James Akazili
(Head, Ethics & Research Management Department)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX VI: Research Questionnaire

Research Data Entry Form

Socio-demographic Information

1. Date: ……..
2. Study ID: ……
3. Telephone Number: ……..
4. Age: …….. (Years)
5. Gender: Male [ ] =1 Female [ ] =2
6. Marital status, single [ ] =1 married [ ] =2 divorced [ ] =3 co-habiting [ ] =4 widowed [ ] =5 separated [ ] =6 remarried [ ] =7
7. Religion: Christianity [ ] =1 Islam [ ] =2 traditional [ ] =3
8. Ethnicity: Dagomba [ ] =1 Mamprusi [ ] =2 Others [ ] =3
9. Educational level: no formal education [ ] =1 primary education [ ] =2 secondary education
10. [ ] =3 vocational [ ] =4 tertiary [ ] =5
11. Residence: Rural [ ] =1 Slum [ ] =2 Urban [ ] =3

Knowledge on HBV infection

12. Have you heard of hepatitis B virus? Yes [ ] =1 No [ ] =2
13. Have you being vaccinated against hepatitis B virus? Yes [ ] =1 No [ ] =2