# PREVALENCE OF HBSAg AMONG FEBRILE PATIENTS ATTENDING LAG CLINIC IN LAGOS

By

**OLUFOWOBI,** ABIMBOLA JULIET

**17010101005**

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES,

MOUNTAIN TOP UNIVERSITY.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) IN MICROBIOLOGY

SEPTEMBER, 2021

## DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other university. All citations and sources of information are clearly acknowledged by means of references.

……..

OLUFOWOBI, ABIMBOLA JULIET

…….

Date

## CERTIFICATION

This is to certify that the content of this project entitled **"PREVALENCE OF HBsAg AMONG FEBRILE PATIENTS ATTENDING LAG CLINIC IN LAGOS"** was prepared and submitted by **OLUFOWOBI, Abimbola Juliet** in partial fulfillment of the requirements for the degree of BACHELOR OF SCIENCE IN MICROBIOLOGY.

The original research work was carried out by her under my supervision and is hereby accepted.

…… (Signature and date)

Dr. C.I. Ayolabi

Supervisor

……. (Signature and date)

Dr. O.T Kayode

Head of Department

## DEDICATION

I dedicate this work to God Almighty for his divine strength, wisdom and for his guidance and also to my mother Mrs. OLUFOWOBI, Adetokunbo Abimbola for her love and support.

## ACKNOWLEDGEMENTS

My sincere and utmost appreciation goes to my Lord and Redeemer, who in His infinite mercies has given me the wisdom, knowledge, assistance, support and protection to successfully complete this project.

My profound gratitude goes to my awesome supervisor, Dr. C.I. Ayolabi. This project would not have been feasible without your proper guidance and constants supervision.

To the Head of Department of biological sciences, Dr O.T Kayode, thank you very much for your advice and your unending support throughout my course of study.

To those who helped me in my project, Dr O.E Fayemi, Dr G.E Adebami andMr. T.S. Ogunbiyi, thank you for your supervision.

To my irreplaceable mother, Mrs Olufowobi Tokunbo, I am highly indebted to you for your surreal, unconditional, and consistent support, prayers and encouragement throughout my stay n he university. I would not have made it this far without you. I love you so much and may God blessings always cover you.

To my amazing, wonderful siblings, Alfred, Comfort, Alexander and Andrew thank you all for4 your love and supports, I am grateful and I love you.

To all my friends; Ogunpitan Tofunmi, Aluko Joy, Olugbenro Oyinkansola, Ojo Deborah, Akpabio Daniel, Oluwodamilare Simileoluwa, Moyege, Daudu Precious, Udeaga Chidera, Gerry, Kayode Blessing, Akintoye Omishakin, Adeboye Tolulope, Daniel, you all are the best. Thanks for caring, tolerating and listening to me. For the laughter and the tears and for being a huge part of my school life, and for the consistent love and encouragement, I'm extremely grateful for the friendship. To Gbadebo Abiola, thanks for giving me a reason to smile when I'm down, you inspire me a lot. To all my course mates, I'll miss you all, thanks for being a part of this journey. To everyone who helped me one way or another has knowingly helped me out with their abilities, thank you.

**TABLE OF CONTENT**

## LIST OF TABLES

## LIST OF FIGURES

## ABREVATIONS

AHB- Acute Hepatitis B

ALT- Alanine Aminotransferase

ART- Antiretroviral Therapy

AST-Aspartate Aminotransferase

cccDNA- Covalently Closed Circular DNA

CD4- Cluster of Differentiation 4

CHB- Chronic Hepatitis B

CLT- Cytotoxic Lymphocyte T Cells

DAA- Dose Administration Aid

DNA- Deoxyribo Nucleic Acid

EDTA- Ethylene Diamine Tetra Acetic Acid

ELISA- Enzyme Linked Immuno Assay

HAV- Hepatitis A Virus

HBc- Hepatitis B Core

HBcAg- Hepatitis B Core Antigen

HBeAg- Hepatitis B e Antigen

HBsAg- Hepatitis B Surface Antigen

HBV- Hepatitis B Virus

HCC- Hepatocellular Carcinoma

HCV- Hepatitis C Virus

HDV- Hepatitis D Virus

HEV- Hepatitis E Virus

HIV- Human Immunodeficiency Virus

IFN- Interferon

IgG- Immunoglobin G

IgM- Immunoglobin M

LAM- Lactation Amenorrhea Method

LTD- Laboratory Developed Test

NK- Natural Killer

RNA- Ribonucleic Acid

TAF- Tumor Angiogenic Factor

TDF- Testis Determining Factor

**ABSTRACT**

Hepatitis B virus belongs to a family of closely related DNA viruses called the hepadnaviruses which is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma, accounting for 1 million deaths annually. This study was aimed at investigating the prevalence of HBsAg viral marker among patients attending LAG Clinic with febrile illness. The study population includes 92 outpatients consisting of 32 males and 60 females. The screening of HBsAg was carried out using the rapid labs ELISA (Enzyme Linked Immunosorbent Assay) test kit. The result of this study showed that, 2 male patients were positive for HBsAg giving the prevalence of 6.3%, within the age limit of 21-30year and 41-50year, none of the female patients were positive for HBsAg. Overall, a prevalence of 2.17% was recorded in this study. The low prevalence observed can be attributed to the small number of samples recruited for this study. Increased sensitization and screening of HBV is recommended in other to further our understanding regarding the burden of Hepatitis B and to strengthen current public health interventions on management of this disease.

Key words: Hepatitis B, ELISA, Febrile, Prevalence, Patients.

# CHAPTER ONE

## 1.0 INTRODUCTION

Approximately a third of the world's population has been infected at one point in their lives. As of 2017, at least 391 million people, or 5% of the global population, were infected with chronic HBV, with another 145 million cases of acute HBV infection. Hepatitis B kills around 750,000 people each year, with about 300,000 of those dying from liver cancer. Hepatitis B is an infectious disease that damages the liver and is caused by the Hepatitis B virus (HBV)(Logan and Rice, 1987). It is a type of viral Hepatitis and it can occur in both acute and chronic phases. Most infected patients are usually asymptomatic while some are symptomatic, symptoms associated with acute infection include; vomiting, yellowish skin, tiredness, dark urine, and abdominal pain. These symptoms usually persist for few weeks, and only a small percentage of people die from the initial illness. (Rubin and Strayer, 2007). The incubation period of Hepatitis is usually from 30 to 180 days. In chronic phases, Hepatitis B virus infection does not results to symptoms; however, cirrhosis and liver cancer may eventually develop (Chang, 2017). Cirrhosis or liver cancer occurs in about 25% of those with chronic disease (Strayer, 2007). Hepatitis B virus infection cannot be spread through touching hands, sharing eating utensils, kissing, hugging, coughing, sneezing, or breastfeeding, despite its high rate of infectivity. The infection might be identified 30 to 60 days after exposure.. Blood tests for components of the virus and antibodies to the virus are frequently used to confirm the diagnosis. Management of Hepatitis B virus is often times prevented by vaccination, by testing blood before transfusion, the use of condoms to prevent infection during sexual intercourse, liver transplantation is sometimes used for cirrhosis (WHO, 2017).

## 1.1 AIM AND OBJECTIVE

To know the prevalence rate of Hepatitis B surface antigen viral marker among patients at LAG Clinic with fever illness.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 OVERVIEW OF HEPATITIS

Hepatitis is a liver infection that causes inflammation (Beasley, 2009).Viruses are the most common aetiology of Hepatitis however, autoimmune Hepatitis and Hepatitis that occurs as a secondary result of medications toxins, and alcohol have also been reported (Brunetto, 2010). Hepadnaviruses are cytotropic to liver cells; however hepadnaviral DNA can also be discovered in the kidney, pancreas, and mononuclear cells in modest levels. Extrahepatic illness is not associated to infection at these sites (Barker *et al*., 1975; Halpern *et al*., 1983; Marion, 1988; Korba *et al*., 1988). There are 5 main Hepatitis viruses, referred to as Hepatitis A, B, C, D and E. These 5 types are of public health significance due to high health associated morbidity and mortality greatest(Sonneveld *et al*., 2011). Hepatitis A is caused by an infection with the Hepatitis A virus (HAV). This type of Hepatitis is most commonly transmitted by consuming food or water contaminated with feces from a person infected with Hepatitis A(Robinson *et al*., 1976). The hepatitis B virus (HBV) and the hepatitis C virus (HCV) are spread by direct contactwith infectious body fluids, such as blood, vaginal secretions, or semen, containing the Hepatitis B virus (HBV). Use of non-sterile injections among drug users; practicing unprotected sex and sharing of contaminated razors are among other factors thatpredisposes to HBV and HCV infections(Liaw and Chu, 2009). The Hepatitis D virus causes a serious liver condition known as hepatitis D (delta virus).HDV is contracted through direct contact with infected blood. Hepatitis D is a rare type of hepatitis that only occurs when Hepatitis B infection is present. Hepatitis D cannot multiply unless Hepatitis B is present. The Hepatitis E virus causes a waterborne disease known as hepatitis E. (HEV). Hepatitis E is primarily found in areas with poor sanitation and is transmitted by ingesting feces-contaminated water.Hepatitis B and C are the most frequent causes of liver cirrhosis and cancer, affecting hundreds of millions of individuals worldwide. Ingestion of contaminated food or water is the most common cause of hepatitis A and E. (Sonneveld *et al*., 2011). Hepatitis B, C, and D are most commonly transmitted through parenteral contact with infected body fluids (Kao, 2002). Acute viral hepatitis infection can cause a variety of symptoms, including jaundice (yellowing of the skin

and eyes), dark urine, excessive exhaustion, nausea, vomiting, and stomach discomfort (Dane et al., 1970).

### 2.2. HEPATITIS B CLASSIFICATION AND STRUCTURE

Hepatitis B virus is classified in the genus Orthohepadnavirus, The genus is classified as part of the Hepadnaviridae family, which contains four other genera, Avihepadnavirus, Herpetohepadnavirus, Metahepadnavirus and Parahepadnavirus. This family of viruses is the only member of the viral order Blubervirales(Mitchell *et al*., 2011).HBV virions are double-shelled particles with three identical envelope glycoproteins on the outer lipoprotein envelope (or surface antigens)(Dane *et al*., 1970; Ganem, 1991). The viral nucleocapsid, or core, is found within the envelope and contains the viral genome, 3.2 kb of relaxed-circular, partly duplex DNA, and a polymerase that is responsible for viral DNA synthesis in infected cells (Robinson *et al*., 1976). DNA sequencing of various HBV isolates each with a distinct geographic distribution (Kao, 2002). In addition to virions, HBV-infected cells produce two subviral lipoprotein particles: 20-nm spheres and filamentous forms of similar width (Robinson et al., 1976).

### 2.3 EPIDEMIOLOGY OF HEPATITIS B VIRUS INFECTION

30% of the world's population, serological evidence of current or prior HBV infection can be discovered (Hatzakis et al., 2013; WHO, 2014). In 2010, HBV infection was responsible for over half of all liver cancer deaths, with global mortality attributable to liver cancer increasing by 62% and that linked to cirrhosis increasing by 29% between 1990 and 2010.(Lozano *et al*., 2012). HBV is spread by coming into touch with infected blood or sperm. There are three major forms of transmission in use. HBV is usually transferred perinatally from infected mothers to newborns in areas with high endemicity. Sexual transmission is the most common mode of infection in low-endemic locations. People with a large number of sexual partners, males who have sex with women, and people with a history of other sexually transmitted infections are at a higher risk of infection. Unsafe injections, blood transfusions, and dialysis are the third most common sources of infection. Despite the fact that blood product screening has reduced transfusion-associated HBV infection, infection from this source is still common in developing nations. Nosocomial infection through contaminated medical, surgical, or dental devices, needle-stick injuries, and organs donated by HBsAg-positive or HBV-DNA-positive donors are all

probable sources of HBV. Household or intimate non-sexual contacts, as well as living in cramped quarters, are all potential dangers. Acute HBV infection has a different prognosis depending on your age. A persistent infection affects approximately 95 percent of infants, 20–30 percent of children (ages 1–5), and less than 5% of adults (Beasley, 2009). The introduction of universal HBV immunization in infants has resulted in a significant reduction in prevalence in several regions of the world. Vaccine coverage, on the other hand, varies greatly, ranging from 90% in the western Pacific and the Americas to 56% in Southeast Asia (Mitchell et al., 2011). As a result, HBV infection prevalence varies greatly over the world. China, Southeast Asia, most of Africa, most Pacific Islands, portions of the Middle East, and the Amazon basin are among the 45 percent of HBV-infected persons living in highly endemic areas (those with a prevalence of 8% or above) (Te and Jesen, 2010). The majority of infections in these locations happen during children or infancy. Some highly endemic nations, like as China, now have overall prevalence of 7–8% and are expected to fall into the intermediate prevalence group in the near future as a result of universal neonatal vaccination. Infected persons live in regions with intermediate prevalence (2–7%), such as south-central and southwest Asia, eastern and southern Europe, Russia, and Central and South America, accounting for roughly 43% of HBV-infected people. There are a variety of transmission patterns in these locations, including newborn, childhood, and adult transmission. The remaining 12% of infected people live in low-endemic countries (prevalence of less than 2%), including as North America, Western Europe, Australia, and Japan. The majority of diseases in these areas are transmitted to adolescents and adults by sexual or parenteral means. In high-income countries, immigration has a significant impact on prevalence. According to a 2012 meta-analysis (Rossi *et al*., 2012), HBV infection was found in 7.2 percent of migrants and refugees, with previous immunity in 39.7 percent. Immigrants are thought to be responsible for 95 percent of newly diagnosed cases of chronic HBV infection in the United States (Mitchell *et al*., 2011).

### 2.3.1 Chronic Hepatitis B Infection in Children

Childhood chronic Hepatitis B has certain distinct characteristics that are mostly determined by the age of original HBV infection and the method of transmission (Shneider*et al*., 2006; Chang, 2007). HBV can be transferred horizontally or perinatally from mother to child. Perinatal infection of newborns by highly infectious HBeAg positive mothers is frequent in Asia, whereas

infection is transferred horizontally by HBsAg positive family members and playmates in the United States, where the number of HBeAg positive moms is significantly lower. Prior to widespread vaccination, perinatal HBV transmission was estimated to account for 40–50% of HBsAg carriers in Taiwan, with horizontal transmission early in life accounting for the remaining instances (Chang, 2007; Chu and Liaw, 2007). Clinical evidence suggests that the natural course of chronic Hepatitis B in children differs depending on whether the infection was acquired perinatally or postnatally.

### 2.3.2 Chronic Hepatitis B Infection in Adult

Adult patients with HBeAg positive chronic Hepatitis are more likely to be men and present in their third or fourth decade of life. Adult patients with HBeAg positive chronic Hepatitis have high levels of HBV-DNA, which can reach 2 billion IU/ml (1010 copies/ml), as well as varied elevations in ALT and histopathological activity. Although the duration of typical HBeAg positive chronic Hepatitis varies and can lead to cirrhosis, approximately 65 percent of patients eventually undergo seroconversion from HBeAg to anti-HBe, which is linked to decreased HBVDNA replication, biochemical remission, and a lower risk of disease progression (Hsu *et al*., 2002; Easl, 2003). In order to achieve regression of fibrosis and hepatic inflammation, a study found that in addition to HBeAg seroconversion, persistent illness remission defined as normal ALT and HBV-DNA levels fewer than 104 copies/ml is necessary (Hui*et al*., 2007). In people with increased ALT, HBeAg seroconversion occurs at a rate of 10–15 % each year (Fattovich*et al*., 2003). According to a recent longitudinal study, up to 90% of Caucasian persons with chronic Hepatitis B clear HBeAg after ten years, with an incidence rate of 18 per 100 person years (Fattovich*et al*., 2009). Older age, higher ALT levels, HBV genotypes B (versus C) and A (vs D), and ethnicity other than Asian are all linked to greater rates of spontaneous HBeAg seroconversion (Fattovich, 2003; Kao and Chen, 2006; Lok and MaMahon, 2007).

## 2.4 PATHOGENESIS OF HEPATITIS B VIRUS INFECTION

### 2.4.1 Immunopathogenesis of Hepatitis B Virus

HBV is a noncytopathic encapsulated virus that belongs to the Hepadnaviridae family and has a largely double-stranded circular form DNA genome (Hsu et al., 2002). Only humans and chimpanzees are infected by this virus, which produces covalently closed circular DNA (cccDNA) in hepatocytes, which serves as a stable template for viral replication and is essential for viral survival.(Tseng and Kao, 2013).More than 250 million people worldwide have chronic HBV infection, putting them at a significant risk of developing end-stage liver disease and liver cancer (Liaw and Chu, 2009). Adults infected with HBV normally have self-limited and transitory hepatitis, with viral clearance and the development of protective antibodies in 95 percent of cases. However, the majority of neonates who contract HBV during pregnancy develop a persistent infection. (Liaw and Chu, 2009).Based on the virus-host interactions, the natural course of chronic HBV infection can be divided into four chronological periods (Liaw and Chu, 2009; Tseng and Kao, 2013). The immune-tolerant phase is defined by active HBV replication, HBV e antigen (HBeAg) positivity, and a normal-to-low blood alanine aminotransferase (ALT) level. The second stage is the immunological clearance phase, which is characterized by elevated blood ALT levels and a decrease in serum HBV DNA load in HBeAg-positive patients. With remission of liver disease, patients lose HBeAg and gain antibody against HBeAg (anti-HBe) in the low-replication or residual phase, also known as the inactive carrier state. During follow-up, however, between 20%–30% of inactive carriers may experience a viral relapse and enter the reactivation phase (HBeAg-negative Hepatitis), which is now recognized as a variant of the immune clearance phase (Liaw and Chu, 2009; Tseng and Kao, 2013). Despite the fact that HBV has been around for over 50 years, its immunopathogenesis is still unknown. Immune responses are a major contributor to the liver damage caused by HBV infection, and immune-related liver damage is triggered by active viral replication (Liaw and Chu, 2009; Tseng et al., 2012). HBV-specific cytotoxic T lymphocytes (CTLs) may cause virus-infected hepatocytes to die at first. CTLs, on the other hand, are unable to completely eradicate the virus, so they recruit HBV-nonspecific inflammatory cells such as bystander T cells, natural killer (NK) cells, and neutrophils, which in turn produce CHB immunopathology (Chisari and Ferrari, 1995; Reherman, 2013). However, why the virus is seldom cleared by repetitive immune responses, and why immune-tolerant individuals do not acquire liver damage despite vigorous

viral multiplication throughout the immune-tolerant phase, is unknown. The involvement of the HBV-specific immune response, on the other hand, is more obvious in acute Hepatitis B. According to this review of the evidence, the immune response controls AHB but fails to combat CHB. Comparing the discrepancies will help researchers better understand CHB disease progression and identify treatment options (Hatzakis et al.,2013).

### 2.4.2 Chronic Hepatitis B Virus Infection Phase

Individuals infected perinatally (90%) or throughout childhood (20–30%), when the immune system is assumed to be immature, have a larger chance of developing chronic HBV infection than immunocompetent people infected during adulthood (1%) (Reherman, 2013). The stages of chronic HBV infection mentioned here apply to those who were infected as children. Based on the virus–host interaction, the natural course of chronic HBV infection can be split into four phases: immunological tolerance, immune clearance, low or non-replication, and reactivation (Easl, 2003; Lok and MaMahon, 2007).

### 2.4.3 Pathogenesis of Chronic Hepatitis B Virus Infection

During acute Hepatitis B, HBsAg vanishes within six months by definition. Longer HBsAg persistence is thought to be a sign of chronic HBV infection. Infection of newborns (from an HBV-infected mother) or infants usually results in a persistent infection because an adequate immune response takes years or decades to develop for unknown reasons (McMahin*et al*., 1985). Even if the immune deficiency is minimal, such as in hemodialysis patients, infection causes persistence in immunocompromised patients (Chisari and Ferrari, 1995; Reherman, 2013). Immune resistance may evolve after a long anergic phase, leading to the selection of escape mutants. HBeAg loses its immunomodulatory role and becomes a worthless side product as soon as cellular immunological responses to HBcAg develop. HBeAg-negative variants with increased HBcAg expression and viral replication frequently take over and partially compensate for the loss of HBV-infected cells that have been killed. Variants with mutant HBcAg and HBsAg T cell epitopes may be chosen, and non-essential preS domain epitopes may be eliminated. In many chronic carriers, immunological control will decrease HBsAg to undetectable levels. Inflammation, increasing fibrosis of the liver, and possibly hepatocellular cancer ensue from the coexistence of cytotoxic immune responses with continuous robust HBV DNA replication (Tseng and Kao, 2013).

### 2.4.4 Acute Hepatitis B Virus Infection Phase

A moderate, asymptomatic, and sub-clinical sickness affects about two-thirds of individuals with acute HBV infection, which generally goes unnoticed (McMahin*et al*., 1985). A third of individuals with acute HBV infection have Clinical Hepatitis symptoms and signs, which can range from mild constitutional symptoms like fatigue and nausea to more severe symptoms like jaundice and, in rare cases, acute liver failure. Acute Hepatitis B has a clinical incubation time of 2-3 months, however it can vary from 1-6 months after exposure, with the length of the incubation period corresponding to some extent with the level of virus exposure (Barker *et al*., 1972). A short prodromal period of constitutional symptoms such as fever, lethargy, anorexia, nausea, and body aches follows the incubation period. Serum ALT levels rise during this phase, and high levels of HBsAg and HBV DNA are detected. The preicteric phase can continue anywhere from a few days to a week and is followed by jaundice or dark urine. Hepatitis B's icteric phase lasts for a variable amount of time, generally 1-2 weeks, during which virus levels fall. Jaundice goes away after convalescence, although constitutional symptoms can remain for weeks or even months. HBsAg is eliminated, followed by the removal of detectable HBV DNA from serum, during this phase. Acute liver failure affects about 1% of people who have acute Hepatitis B and jaundice (Berk and Popper, 1978). Fever, abdominal discomfort, vomiting, and jaundice are common symptoms of fulminant Hepatitis, which are often followed by disorientation, confusion, and coma. As liver failure progresses, HBsAg and HBVDNA levels drop fast, and some patients are HBsAg-negative by the time hepatic coma sets in. Acute liver failure caused by Hepatitis B requires careful therapy and monitoring, and patients should be transferred to a tertiary medical hospital that offers liver transplantation as soon as possible (Hootnagle*et al*., 1995).

### 2.4.5 Pathogenesis of Acute Hepatitis B Virus Infection

HBV infection is highly replicative for several weeks or months before immune recognition occurs, which takes a long time (McMahin*et al*., 1985). A strong cellular immune response prevents viral replication and removes the majority of HBV-positive hepatocytes, resulting in severe Hepatitis. If the infectious dosage is low (generally less than 1000 ID50), the immune response would begin before many hepatocytes are infected, and the symptoms are modest

enough that they are often overlooked (Barker and Murray, 1972). In the late acute phase, the appearance of neutralizing anti-HBs antibodies precludes the infection of new hepatocytes (Liaw and Chu, 2009).

### 2.4.6 Occult or Lateral Hepatitis B Virus Infection Phase

Seronegative occult or latent HBV infections are another type of atypical HBV infection. This diverse group includes HBsAg-negative patients who are either seronegative for all HBV indicators or positive for anti-HBc and/or anti-HBs antibodies (Liang *et al*., 1990; Paterlini*et al*., 1990; Liang *et al*., 1991). By polymerase chain reaction, many of these individuals had HBV DNA in their livers, serums, or both. Some of these patients have underlying liver disease, which could indicate that they are suffering from hepatocellular injury as a result of their HBV infection. Long-term persistence of viral genomes in the serum and/or liver of animals with biochemical and serologic evidence of viral clearance and recovery from infection has been reported in animal models (Liang *et al*., 1991; Yotsuyanagi*et al*., 1998). HBV infection was transmitted to recipients after liver transplantation if the donors had anti-HBc markers (Dickson *et al*., 1997). Furthermore, resurgence of HBV infection has been recorded in individuals with serologic signs of recovery who are on immunosuppression or chemotherapy (Lok*et al.*, 1991; Blanpain*et al*., 1998; Hui*et al*., 2006; Loomba*et al*., 2008). These findings, combined with the immunologic research mentioned above, give persuasive evidence that HBV infection may not be totally eradicated. Low-level viral replication is likely regulated by an active immune response in patients with serologic evidence of recovery. It's been suggested that HBV mutations are to blame for these occult infections. Although mutations have been found in various parts of the viral genome (Blum *et al*., 1991; Kato *et al*., 1996; Yamamoto *et al*., 1994), there is no conclusive evidence that these mutants are harmful.

### 2.4.7 Symptoms of Hepatitis B Virus Infection

Hepatitis B symptoms and signs range from minor to severe. They normally emerge one to four months after being infected with the virus, but they might appear as soon as two weeks following infection (Pan and Lee, 2013). Abdominal discomfort, dark urine, fever, joint pain, loss of appetite, nausea and vomiting, weakness and exhaustion, yellowing of the skin and whites of the eyes are some of the indications and symptoms of Hepatitis B (jaundice) (Yotsuyanagi*et al*.,

1998). Loss of appetite, joint and muscle discomfort, low-grade fever, and potential stomach ache are all signs of an acute infection. Although the majority of people do not have symptoms, they might appear anywhere between 60 and 150 days after infection, with the average being 90 days or 3 months. Infected patients has more serious symptoms such nausea, vomiting, jaundice (yellowing of the eyes and skin), or a swollen stomach, which should prompt them to seek medical attention (Wen *et al*., 2013).

### 2.4.8 Prevention of Hepatitis B Virus Infection in Humans

HBV infection can be avoided by avoiding contact with infected people and generating immunity in those who have not been exposed. Screening blood donors for HBsAg and implementing universal measures in health-care settings resulted in a considerable reduction in transmission (Krugmann et al., 1979). The addition of HBV DNA testing to screening protocols lowers the risk of transfusion-associated illness even more, although implementation is impeded by the additional expense (Busch, 2004). The most important steps in preventing transmission and decreasing the incidence of transfusion-associated disease are counseling infected people to prevent transmission, screening and vaccination of at-risk adults, and universal vaccination of neonates. Since 1981, there has been a safe and effective vaccine against HBV infection. The majority of vaccines on the market are manufactured from recombinant DNA that solely expresses HBsAg. In addition to the monovalent vaccine, a combination vaccine that also protects against the Hepatitis A virus is available, as well as a multivalent vaccine that protects against diphtheria, tetanus, pertussis, and Haemophilus influenza type B. Immunization against HBV infection had been included in normal children vaccination schedules in 180 countries by the end of 2011 (WHO, 2014), Because the probability of progression from acute to chronic HBV infection is 90% when infection occurs in babies, preventing perinatal transmission of HBV is critical. Despite the use of HBV immunoglobulin and HBV vaccination for passive–active immunoprophylaxis, kids born to women with high HBV DNA titers (>107 copies per mL) still face a significant risk of infection (Wen *et al*., 2013). Antiviral medication for moms with high viraemia may minimize the risk of perinatal transmission even more. The introduction of the HBV vaccine resulted in a decrease in the frequency of not only HBV infection but also hepatocellular cancer during the third trimester of pregnancy (Pan and Lee, 2013). In Taiwan, the proportion of children carrying the HBsAg virus has fallen from 10% in 1984 to 5% in 2009 (Ni

and Chang, 2012). The incidence of hepatocellular carcinoma in children and adolescents fell by 70% as a result of this decline (Chang *et al*., 2009). Between 1990 and 2006, the incidence of acute HBV infection in the United States fell by 81 % (Wasley*et al*., 2006). Overall HBsAg carrier rates fell from 38 % to 27 %, but this decrease was primarily in children and adolescents (Wasley*et al*., 2010). The relatively stable carrier rate in adults has been attributed to immigration of chronically infected people from endemic areas (Mitchell *et al*., 2008).

## 2.5 LABORATORY DIAGNOSIS OF HEPATITIS B VIRUS INFECTION

The first viral markers detected in serum are HBV DNA, followed by HBsAg and HBeAg (Krugmann*et al*., 1979). HBsAg can be identified as soon as 1-2 weeks after exposure or as late as 11-12 weeks, and its persistence is a sign of chronicity. HBeAg is associated with high levels of HBV replication and infectivity (Liang and Gheny, 2002). Serum alanine and aspartate aminotransferase (ALT, AST) levels continue to rise a few weeks after viral signs show, and jaundice may develop. HBsAg and HBV DNA normally persist in the serum for the duration of Clinical symptoms and are eliminated with recovery, although HBeAg is frequently cleared early, during the peak of Clinical illness. During acute Hepatitis B, antibodies to HBV proteins appear in a variety of ways. Antibody to HBcAg (anti-HBc) appears just before clinical disease, with the initial antibody primarily being of the immunoglobulin M (IgM) class, which then decreases in titer as IgG anti-HBc levels rise.Antibody to HBeAg (anti-HBe) occurs soon after HBeAg clearance, frequently at the height of Clinical disease. As a result, the emergence of anti-HBe and the disappearance of HBeAg is a good serological marker during acute Hepatitis B, indicating the start of recovery. Antibody against HBsAg develops late in the course of an infection, usually during recovery or convalescence after HBsAg has been cleared. Anti-HBs, the antibody linked with HBV immunity, continue after recovery. However, 10% to 15% of individuals who recover from Hepatitis B do not acquire detectable anti-HBs and instead have anti-HBc as a marker of previous infection. As a result, anti-HBc testing is the most reliable method of determining prior HBV infection, whereas anti-HBs testing is used to determine immunity and vaccine response (Tabor *et al*., 1981). Patients with chronic Hepatitis B have a similar pattern of serological markers at first, with HBV DNA, HBsAg, HBeAg, and anti-HBc showing up. However, viral replication continues in these people, and HBsAg, HBeAg, and HBV DNA are still detected in their blood, typically at high titers. Chronic Hepatitis B can have

a wide range of outcomes. Most people are HBsAg-positive for years, if not their whole lives, and have some kind of chronic liver injury (chronic Hepatitis), which can develop to cirrhosis and fibrosis. People who have been infected with HBV for a long time are at a higher risk of developing HCC. The presence of IgM anti-HBc in serum, especially in a patient with HBsAg and signs, symptoms, or laboratory characteristics of acute Hepatitis, is a reliable indicator of acute Hepatitis B. However, in certain cases, HBsAg is swiftly removed from the blood, and IgM anti-HBc is the only marker detectable when a patient has Hepatitis. Anti-HBc (total) and anti-HBs testing aren't helpful in diagnosis, and HBeAg and anti-HBe testing should be held for people who test positive for HBsAg. The presence of HBsAg in the absence of IgM anti-HBc suggests chronic Hepatitis B, however this diagnosis is normally made when HBsAg has been present for at least 6 months (Krugmann*et al*., 1979; Perrillo*et al*., 1983). HBVDNA testing can also be useful in determining the level of viral replication and possibly in determining prognosis and the need for antiviral therapy. HBV DNA level assays have vastly improved throughout the years (Pawlotsky*et al*., 2008). The current TaqMan real-time polymerase chain reaction–based test has a detection limit of 5-10 HBV DNA copies/mL and can reliably assess a wide range of values. HBV DNA can be discovered early in the course of infection, before other serological markers such as HBsAg or anti-HBc, thanks to this level of sensitivity. As a result, testing for HBV DNA has become a standard method for diagnosing and treating HBV infection. HBV DNA testing is now widely utilized in blood product screening (nucleic acid testing) and monitoring of HBV patients during treatment (Kuhns and Busch, 2006; Loomba and liang, 2007).

## 2.6 TREATMENT STRATEGIES FOR DIFFERENT PATIENT

### 2.6.1 Patients with Liver Failure Related with HBV Infection

Liver failure is a life-threatening condition with a significant fatality rate in the near term (Fontana *et al*., 2015). It can happen after an acute HBV infection or after a reactivation of a chronic HBV infection. Patients with HBsAg or HBV DNA positive liver failure (acute, sub-acute, or acute on chronic) should start therapy as soon as possible. The favorable benefits were largely shown in patients with a MELD (Model End Stage Liver Diseases)score of 20–30, whereas the death rate in patients with a (MELD) score of more than 30 is >90% even with rapid

antiviral treatment, indicating that urgent liver transplantation should be considered in these patients (Yuen, 2015).

### 2.6.2 HBV and HIV Co-infected Patients

Concurrent HBV/HIV infection increased the incidence of all-cause and liver-related death (Konopniek*i et al*., 2015). Higher HBV DNA levels, lower rates of HBeAg loss, and a faster development to cirrhosis are all associated with HIV infection (Thio, 2009). Regardless of CD4 cell level, all patients living with HIV should start antiretroviral medication (ART) right away, according to current guidelines (Gunthard*et al*., 2017). NAs such as LAM, emtricitabine (FTC), TDF, and TAF are effective against both HIV and HBV. As a result, for individuals with HBV/HIV co-infection, the ART backbone should consist of TDF or TAF plus LAM or FTC (Terrault*et al*., 2018). Because of the danger of HIV resistance, these medicines should not be administered as a single agent for HBV treatment in HBV-/ HIV-Y co-infected patients. In the initial months after initiating ART, HBV/HIV-co-infected patients with liver cirrhosis and a low CD4 cell count must be closely monitored for immune reconstitution syndrome and eventual hepatic decompensation.

### 2.6.3 HBV and HCV Co-Infected Patients

Cirrhosis and HCC are more likely to occur in CHB patients who also have HCV infection than in those who only have HBV or HCV infection (Zampino*et al*., 2015; Pol *et al*., 2017). The therapy of HBV/HCV co-infection should be tailored to the patient's HBV and HCV viral loads, ALT levels, and assessment of liver fibrosis or cirrhosis. In co-infected patients with positive HCV-RNA, anti-HCV therapy is recommended. In the IFN-era, using IFN-plus-ribavirin to treat co-infected patients resulted in HCV eradication and HBV suppression. IFN-free and ribavirin-free DAA treatment has been the standard of care for HCV infection since the introduction of direct-acting antivirals (DAA). However, in patients with HCV/HBV co-infection, there is a risk of HBV reactivation during DAA therapy, and life-threatening outcomes have been recorded in some cases (Holmes *et al*., 2017). Co-infected patients should be continuously followed during anti-HCV therapy with DAA by evaluating HBV viral load and ALT levels (Sagnelli*et al*., 2017). HBV antiviral medication should be given concurrently with HCV DAA therapy in HBV/HCV-co-infected individuals who meet the usual criteria for HBV treatment.

### 2.6.4 Chronic HBV Infection during Pregnancy

The effects and safety profile of various antiviral medicines should be considered when creating a treatment strategy for women with CHB who are of reproductive age (Zhou and Terrault, 2017). LdT and TDF are pregnancy category B medicines that are recommended for use in pregnant women with CHB, but ADV and ETV are pregnancy category C treatments that are not suggested for use during pregnancy. Despite the fact that LAM is a pregnancy category C drug, it can be used in pregnant women based on the safety data gathered from its usage in HIV-positive pregnant women. Previous research has shown that LAM (Xu*et al*., 2009), LdT (Han *et al*., 2011; Pan *et al*., 2012), and TDF (Lin *et al*., 2018) are all effective at reducing perinatal HBV transmission. TDF, on the other hand, is preferable in pregnant women with persistent HBV infection because it has a superior resistance profile and greater safety data.

### 2.6.5 Infected Children with HBV

Every year, around 2 million new HBV infections in children under the age of 5 occur (WHO, 2017). At 6–12 months after birth, exposed infants should be tested for HBsAg (Indolfi*et al*., 2018). Most children with chronic HBV infection are in the immune-tolerant phase, which is defined by a high viral load and normal ALT levels, and they do not respond well to existing antiviral medications. The course of HBV-related liver disease in children is often mild, and the majority of children do not fulfill the usual treatment criteria. As a result, the start of antiviral treatment should be approached with prudence. Children with CHB who meet the criteria for antiviral treatment should be treated (Terrault*et al*., 2018). For CHB children with advanced liver disease and cirrhosis, antiviral medication should be started as away (Sarin*et al*., 2016). For children with CHB, antiviral medications such as conventional IFN-α (1 year old), LAM (2 years old), ETV (2 years old), and TDF (12 years old) have been licensed (Terrault*et al*., 2018).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 STUDY SITE

This study was carried out at a LAG Clinic at Yaba.

### 3.2 STUDY POPULATION

This study was carried out among 92 febrile patients consisting of 32 males and 60 females in LAG Clinic, Yaba.

### 3.3 MATERIALS AND EQUIPMENTS USED

Rapid labs HBsAg ELISA kit, serviette, absorbent paper, disposable gloves, Thermo fisher scientific calibrated automatic microwell, Thermo fisher scientific calibrated automatic micropipette, timer, graduated cylinders, calibrated micropipettes with disposable tips, incubator, sterile syringes, Ethylene Diamine Tetra Acetic Acid (EDTA) bottles, plain bottles, centrifuge, tourniquet, cotton wool, methylated spirit and needle disposal unit.

### 3.4 REAGENTS USED

Freshly distilled or deionized water and sodium hypochlorite solution for decontamination.

### 3.5 SAMPLE COLLECTION

5mls of blood samples were collected in EDTA bottles by the Clinic phlebotomist. The blood sample was mixed, held at a room temperature for 30 minutes and centrifuged at 3000rpm for 5minutes. Using a sterile Pasteur pipette, the serum was aseptically separatedinto a clean plain bottle labeled with the patient's delineated data. The serum was stored at 2-8ºc for HBsAg screening using the ELISA kit.

### 3.6 HBsAg DETECTION BY ENZYME LINKED IMMUNOSORBENT ASSAY

The reagents and serum samples was allowed to attain room temperature before use. The wash buffer was prepared by diluting the concentrated wash buffer 1:25, with 1000ml of distilled water. The test was carried out for each sample as follows; using a micropipette calibrated at

100µl, positive control, negative control, and specimen were added to their respective wells apart from the blank.

Separate disposal tips were used for each of the samples, positive control and negative control to avoid decontamination.50µl of the conjugate was added to each well except from the blank well, the conjugate contains the antibody which aids the antigen in the microwell plate to bind. The microwell plate is the mixed by swirling on the work bench for 30 seconds. The microwell plate was then covered with a plate sealer and incubated at 30ºc ± 2ºc for 60 minutes ± 2 minutes. At the end of the incubation, the plate sealer was removed and each well was washed 5 times with 350µl of working buffer per well using the calibrated automated plate washer. After the final washing cycle, the microwell plate was blotted onto a clean absorbent paper to remove any residual wash fluid after washing. 50µl of substrate A and B were added to all the wells including the blank well, the contents in the microwell plate was mixed by swirling for 30 seconds then covered with a plate sealer and then incubated at 37ºc ± 2ºc for 10 minutes ± 1minute avoiding light. The enzymatic reaction between the substrate solutions and the conjugate produces a blue color in positive control and HBsAg positive sample wells. The plate sealer was removed and then 50µl of stop solution was added to each well and was mixed gently. Intensive yellow color develops in the positive control and HBsAg positive sample wells. The plate was then read at 450/630-700nm within 30 minutes.

Table 3.1: The Distribution of Samples into the Microplates Well

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 BLANK | 2 8 | 3 16 | 4 24 | 5 32 | 6 40 | 7 48 | 8 56 | 9 64 | 10 72 | 11 80 | 12 88 |
| B 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 | 81 | 89 |
| C 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 | 82 | 90 |
| D 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 83 | 91 |
| E 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 | 84 | 92 |
| F 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 85 | NC |
| G 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 86 | NC |
| H 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 87 | PC |

Key; NC- negative control, PC- positive control

# CHAPTER FOUR

## 4.0 RESULTS

During the study period, a total of 92 patients were screened for HBsAg using ELISA technique. There were 32 males (34.8%) and 60 females (67.4%). Of the 92 patients tested, 2 male patients were positive for HBs Ag giving a prevalence of 6.3%, within the age limit of 21-30 years and 41-50 years. None of the female patients were positive for Hepatitis B surface antigen.The age and sex distribution of HBSAg among febrile patients is represented in table 4.1. The incidence of HBSAg with sex among febrile patients is represented in table 4.2. The microplate well showing the positive and negative result is represented in figure 4.1.

Table 4.1: Age and Sex Distribution of HBSAg among Febrile Patients

| Age group | Number of patients screened (%) | Sex distribution | | Number of positive patients (%) | Number of negative patients (%) |
|---|---|---|---|---|---|
| | | Male (%) | Female (%) | | |
| 0-10 | 11 (10.87) | 6 (54.56) | 5 (45.45) | 0 (0.00) | 11 (100.00) |
| 11-20 | 28 (30.43) | 11 (39.29) | 17 (50.71) | 0 (0.00) | 28 (100.00) |
| 21-30 | 30 (32.61) | 11(36.76) | 19 (63.33) | 1 (3.33) | 29 (96.66) |
| 31- 40 | 9 (9.78) | 2 (22.22) | 7 (77.78) | 0 (0.00) | 9 (100.00) |
| 41- 50 | 5 (5.43) | 1 (20.00) | 4 (80.00) | 1 (20.00) | 4 (80.00) |
| 51- 60 | 6 (6.52) | 1 (16.67) | 5 (83.33) | 0 (0.00) | 6 (0.00) |
| 61- 70 | 3 (3.13) | 0 (0.00) | 3 (100.00) | 0 (0.00) | 3 (100.00) |

Table 4.2: The Incidence of HBSAg with Sex among Febrile Patients

| Sex | Positive (%) | Negative (%) | Total (%) |
|---|---|---|---|
| Male | 2 (6.25) | 30 (93.75) | 32 (34.78) |
| Female | 0 (0.00) | 60 (100.00) | 60 (65.22) |
| Total | 2 (2.17) | 90 (97.83) | 92 (100.00) |

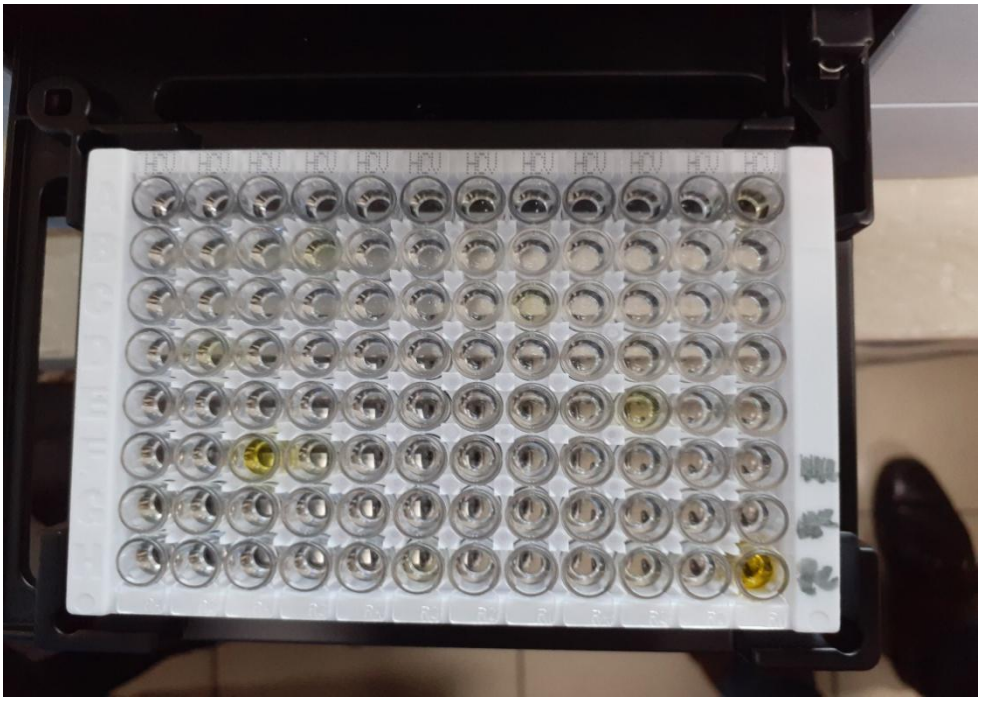

Figure 4.1: A microwell plate showing positive (yellow wells) and negative (colorless well) result.

# CHAPTER FIVE

## 5.0 DISCUSSION

Hepatitis virus infection is an infection of the liver that is transmitted through sexual contact, blood borne exposure, transmission from mother to child during delivery, sharing of object that pierce the skin, child to child and house hold contact(Gunthard*et al*., 2017). Several authors have reported the prevalence of Hepatitis B antigen in Nigeria. In a study by Wasley*et al*., the authors reported HBV prevalence to be 8.2% in Yola, Adamawa State, Northeastern Nigeria(Wasley*et al*., 2006). In a similar study by Mbaawuaga*et al*.,aprevalence of 11.0% was reported in Makurdi, Benue State, North-central Nigeria (Mbaawuaga*et al*., 2008). In a similar study on the prevalence of HBsAg among patients in Abeokuta, Southwest Nigeria,Okonko*et al.*, showed the prevalence of HBsAg among132 patientsto be 6.6% (Okonko*et al.*, 2010). Compared to other studies, findings from this study showed a significantly low prevalence of 2.17%. Thissuggeststhat observed prevalence is significantly affected by size of population being investigated. The overall prevalence rate of HBsAg was 2.17% among the study population was noted to be among the age groups 21-30 (3.3%) and 41-50 (20.0%).In accordance with the World Health Organization (WHO) classification of assessing severity of HBV infection in endemic countries, the rate observed in this study is regarded as moderate seroprevalence level of HBV infection (WHO, 2010). WHO in 2010, defines low prevalence to be <2%, moderate prevalence as 2–8%, and high prevalence as >8% HBsAg positivity. The observed prevalence can be attributed to the small number of patient recruited for this study.

## 5.1 CONCLUSION AND RECOMMENDATION

This study shows a low prevalence of Hepatitis B virus among patients in LAG Clinic Lagos, Southwest, Nigeria. It is therefore recommended thatemphasis be placed on sensitization and routine testing of HBV by various stakeholders in the health sector. Furthermore, screening of HBV should also be included in the list of infectious diseases to be tested for between intending couples, pregnant women, and in athletes involved in contact sport. The federal ministry of health should make effort towards inclusion of HBV vaccine in the national vaccine program for children as this will help guarantee a HBV-free future.

## REFERENCES


Barker, L. F. and Murray, R. (1972).Relationship of virus dose to incubation time of Clinical Hepatitis and time of appearance of Hepatitis–associated antigen.*Journal of medical science*,**263**:27-33.

Barker, L. F., Maynard, J. and Purcell R. (1975). Hepatitis B virus infection in chimpanzees: titration of subtypes. *Journal of Infectious Disease*,**132**: 451-8.

Barker, L. F., Shulman, N. R., Murray, R., Hirschman, R.J., Ratner, F., Diefenbach, W.C. and Geller, H.M (1996)."Transmission of serum Hepatitis.1970". *Journal of the American Medical Association*. **276** (10): 841–844.

Berk, P.D. and Popper, H. (1978) Fulminant hepatic failure.*Journal of the American Medical Association*.**69**:349-400.

Blanpain, C., Knoop, C., Delforge, M.L., Antoine, M., Peny, M.O., Liesnard, C., *et al* (1998). Reactivation of Hepatitis B after transplantation in patients with preexisting anti-Hepatitis B surface antigen antibodies: report on three cases and review of the literature. Transplantation,**66**:883-886.

Blum, H.E., Galun, E., Liang, T.J., von, Weizsacker, F. and Wands, J.R.(1991). Naturally occurring missense mutation in the polymerase gene terminating Hepatitis B virus replication. *Journal of virology*,**65**:1836-1842.

Brunetto, M.R. (2010) A new role for an old marker, HBsAg.*Journal of Hepatology*,**52**:475–477

Busch, M.P. (2004) Should HBV DNA NAT replace HBsAg and/or anti-HBc screening of blood donors *Journal ofClinical Biology*, **11:** 26–32.

Chang, M.H. (2017) Hepatitis B virus infection.*Seminar of Medicine*; **12**:160–167.

Chang, M.H., You, S.L. and Chen, C.J. (2009) Taiwan Hepatoma Study Group. Decreased incidence of hepatocellular carcinoma in Hepatitis B vaccinees: a 20-year follow-up study. *Journal of cancer*,**101:** 1348–55.

Chisari, and Ferrari, C. (1995) Hepatitis B virus immunopathogenesis.*Annual Review of Immunology*, **13**:29–60.

Chu, and Liaw, Y.F. (2007) Chronic Hepatitis B virus infection acquired in childhood: special emphasis on prognostic and therapeutic implication of delayed HBeAg seroconversion. *Journal Viral Hepatitis*,**14**:147–152.

Dane, D.S., Cameron, C.H. and Briggs, M. (1970) Virus-like particles in serum of patients with Australia-antigen-associated Hepatitis.*Lancet*,**1**:695–698

Dickson, R.C., Everhart, J.E., Lake, J.R., Wei, Y., Seaberg, E.C., Wiesner, R.H., *et al* (1997) Transmission of Hepatitis B by transplantation of livers from donors positive for antibody to Hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database.*Gastroenterology*,**113**: 1668-1674.

EASL International Consensus Conference on Hepatitis B (2003).Consensus statement.*Journal of Hepatology*,**38**: 533-540.

Fattovich, G. (2003) Natural history and prognosis of Hepatitis B. *Seminar Liver Disease*, **23**: 47–68.

Fattovich, G., Olivari, N., Pasino, M., D'Onofrio, M., Martone, E. and Donato, F. Long-term outcome of chronic hepatits B in Caucasian patients: mortality after 25 years. Gut 2009 in press.

Fontana, R.J., Ellerbe, C., Durkalski, V.E., Rangnekar, A., Reddy, R.K., Stravitz, T., *et al* (2015) Two-year outcomes in initial survivors with acute liver failure: results from a prospective, multicenter study. **35**(2):370–380

Galibert, F., Mandart, E., Fitoussi, F., Tiollais, P. and Charnay, P. (1978) Nucleotide sequence of the Hepatitis B virus genome (subtype ayw) cloned in E. coli. *Nature*,**281**:646–650

Ganem, D. (1991) Assembly of hepadnaviral virions and subviral particles. *Microbiology Immunology*;**168**: 61-83.

Gunthard, H.F., Aberg, J.A., Eron, J.J., Hoy, J.F., Telenti, A., Benson, C.A., *et al* (2017) Antiretroviral treatment of adult HIV infection: *recommendations of the International Antiviral Society-USA Panel*, 312(4):410–425

Halpern, M.S., England, J.M., Deery, D.T., Petcu, D.J., Mason, W.S. and Molnar-Kimber, K.L. (1983). Viral nucleic acid synthesis and antigen accumulation in pancreas and kidney of Peking ducks infected with duck Hepatitis B virus. *Academy ScienceU S A*, **80**:4865-9.

Han, G.R., Cao, M.K., Zhao, W., Jiang, H.X., Wang, C.M., Bai, S.F., *et al* (2011) A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for

the prevention of perinatal transmission of Hepatitis B virus infection. *Journal of Hepatitis*,**55**:1215–1221

Hatzakis, A., Van.,Damme, P. and Alcorn, K.(2013) The state of Hepatitis B and C in the Mediterranean and Balkan countries: report from a Beasley RP. Rocks along the road to the control of HBV and HCC.*Ann Epidemiology*, **19:** 231–34.

Holmes, J.A., Yu, M.L. and Chung, R.T. (2017) Hepatitis B reactivation during or after direct acting antiviral therapy – implication for susceptible individuals.

Hou, J., Karayiannis, P., Waters, J., Luo, K., Liang, C. and Thomas, H.C.(1995) A unique insertion in the S gene of surface antigen–negative Hepatitis B virus Chinese carriers. *HEPATOLOGY*, **21** :273-278.

Hsu, Y.S., Chien, R.N., Yeh, C.T., Sheen, I.S., Chiou, H.Y., Chu, C.M., *et al*.(2002) Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic Hepatitis B, **35**:1522–1527.

Hui, C.K., Cheung, W.W., Zhang, H.Y., Au, W.Y., Yueng, Y.H. and Leung, A.Y.(2006) . Kinetics and risk of de novo Hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy.*Gastroenterology*, **131**: 59-68.

Hui, C.K., Leung, N., Shek, T.W.H, Yao, H., Lee, W.K. and Lai, J.Y. (2007) Sustained disease remission after spontaneous HBeAg seroconversion is associated with reduction in fibrosis progression in chronic Hepatitis B Chinese patients. *Hepatology*,**46**:690–698.

Indolfi, G.E.P., Dusheiko, G., Siberry, G., Chang, M.H., Thorne, C., *et al* (2018) Hepatitis B virus infection in children and adolescents. *Lancet Gastroenterology Hepatology*, **23***: 567-589*

Kao, and Chen, D.S. (2006) HBV genotypes: epidemiology and implications regarding natural history.*Current Hepatology*, **5**:5–13

Kao, J.H. (2002) Hepatitis B viral genotypes: Clinical relevance and molecular characteristics. *Journal of Gastroenterology Hepatology*, **17**: 643- 50.

Kato, J., Hasegawa, K., Torii, N., Yamauchi, K. and Hayashi, N. (1996). A molecular analysis of viral persistence in surface antigen-negative chronic Hepatitis B. *HEPATOLOGY*, **23**:389-395.

Konopnicki, D., Mocroft, A., de Wit, S., Antunes, F., Ledergerber, B. and Katlama, C. (2005) Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. AIDS **19**:593–601

Krugman, S., Overby, L.R., Mushahwar, I.K., Ling, C.M., Frosner, G.G. and Deinhardt, F. (1979) Viral Hepatitis, type B. Studies on natural history and prevention re-examined. *New England Journal of Medicine*, **300**: 101-106.

Kuhns, M.C. and Busch, M. (2006) New strategies for blood donor screening for Hepatitis B virus: nucleic acid testing versus immunoassay methods, **10**:77-91.

Liang, T.J. (2009). "Hepatitis B: the virus and disease". *Hepatology*, **49** : S13–21.

Liang, T.J. and Ghany, M. (2002).Hepatitis B e antigen–the dangerous endgame of Hepatitis B. *New England Journal of Medicine*,**347**:208-210.

Liang, T.J., Baruch, Y., Ben-Porath, E., Enat, R., Bassan, L. and Brown, N.V. (1991) Hepatitis B virus infection in patients with idiopathic liver disease.*HEPATOLOGY*, **13**:1044-1051.

Liang, T.J., Blum, H.E. and Wands, J.R. (1990) Characterization and biological properties of a Hepatitis B isolated from a patient without HBV serologic markers. *HEPATOLOGY*, **12**:204-212.

Liaw, and Chu, C.M. (2009) Hepatitis B virus infection.*Lancet*, **373:** 582–92.

Lin, Y., Liu, Y., Ding, G., Touqui, L., Wang, W., Xu, N., *et al* (2018) Efficacy of tenofovir in preventing perinatal transmission of HBV infection in pregnant women with high viral loads. **8**:15514

Lok, A.S., Liang, R.H., Chiu, E.K., Wong, K.L., Chan, T.K. and Todd, D. (1991) Reactivation of Hepatitis B virus replication in patients receiving cytotoxic therapy, Report of a prospective study. Gastroenterology,**100:** 182-188.

Loomba, R, and Liang, T.J. (2007) Treatment of chronic Hepatitis B;**12**: H33-H41.

Loomba, R., Rowley, A., Wesley, R., Liang, T.J., Hoofnagle, J.H., Pucino, F., *et al*.(2008) Systematic review: the effect of preventive lamivudine on Hepatitis B reactivation during chemotherapy. *Ann International Medicine*,**148**:519-528.

Lozano, R., Naghavi, M. and Foreman, K. (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010, **380:** 2095–128.

Marion, P.L. (1988) Use of animal models to study Hepatitis B viruses.*The Medical Virology*, **35**:43-75.

Mbaawuaga, E.M., Enenebeaku, M.N.O., Okopi, J.A. and Damen, J.G. (2008) Hepatitis B Virus (HBV) Infection among Pregnant Women I Makurdi, Nigeria. *African Journal of Biomedical Research*,**11**, 155-159.

McMahon, B.J., Alward, W.L., Hall, D.B., Heyward, W.L., Bender, T.R. and Francis, D.P. (1985). Acute Hepatitis B virus infection: relation of age to the Clinical expression of disease and subsequent development of the carrier state. *Journal of Infectious Diseases*,**151**: 599-603.

Mitchell, T., Armstrong, G.L., Hu, D.J., Wasley, A. and Painter, J.A, (2011). The increasing burden of imported chronic Hepatitis B—United States, 1974–2008; **6:** e27717.

Okonko, I.O., Soleye, F.A., Amusan, T.A., Udeze, A.O., Alli, J.A., Ojezele, M.O., Nwanze, J.C. and Fadeyi, A (2010) *Seroprevalence of Hbsag among Patients in Abeokuta, South Western Nigeria*, **10**:40-44

Pan, and Lee, H.M. (2013) Antiviral therapy for chronic Hepatitis B in pregnancy.*Seminar of Liver Diseases*,**33:** 138–46

Pan, C.Q., Han, G.R., Jiang, H.X., Zhao, W., Cao, M.K., Wang, C.M., *et al* (2012) Telbivudine prevents vertical transmission from HBeAg-positive women with chronic Hepatitis B. *Clinical Gastroenterology Hepatology*,**10**:520–526

Pawlotsky, J.M., Dusheiko, G., Hatzakis, A., Lau, D., Lau, G., Liang, T.J., *et al*.(2008) Virologic monitoring of Hepatitis B virus therapy in Clinical trials and practice: recommendations for a standardized approach. *Gastroenterology*,**134**:405-415.

Perrillo, R.P., Chau, K.H., Overby, L.R. and Decker, R.H. (1983) Anti-Hepatitis B core immunoglobulin M in the serologic evaluation of Hepatitis B virus infection and simultaneous infection with type B, delta agent, and non-A, non-B viruses. *Gastroenterology*, **85**:163-167.

Pol, S., Haour, G., Fontaine, H., Dorival, C., Petrov-Sanchez, V. Bourliere, M. *et al* (2017) The negative impact of HBV/HCV co-infection on cirrhosis and its consequences. **46**:1054–1060

Rehermann, B. (2013) Pathogenesis of chronic viral Hepatitis: differential roles of T cells and NK cells. *Medical Journal*; **19**:859–68

Robinson, W.S. and Lutwick, L.I. (1976) The virus of Hepatitis, type B. *New England Journal of Medicine*,**295**: 1168-75.

Rossi, C., Shrier, I. and Marshall, L. (2012) Seroprevalence of chronic Hepatitis B virus infection and prior immunity in immigrants and refugees: a systematic review and meta-analysis; **7:** e44611.

Sagnelli, E., Sagnelli, C., Macera, M., Pisaturo, M. and Coppola, N. (2017) An update on the treatment options for HBV/HCV co-infection. *Journal of Pharmacotherapy*,**18**:1691–1702

Sarin, S.K., Kumar, M., Lau, G.K., Abbas, Z., Chan, H.L., Chen, C.J., *et al* (2016) Asian-Pacific Clinical practice guidelines on the management of Hepatitis B: a 2015 update. *Hepatology*,**10**:1–98

Shneider, B.L., Gonza`lez-Peralta, R. and Roberts, E.A. (2006) Controversies inthe management of pediatric liver disease: Hepatitis B, C and NAFLD; Summary of a Single Topic Conference.*Hepatology*,**44**:1344–1354.

Sonneveld, M.J., Zoutendijk, R. and Janssen, H.L (2011) Hepatitis B surface antigen monitoring and management of chronic Hepatitis B. *Journal of Viral Hepatitis*,**18**:449–457

Tabor, E., Hoofnagle, J.H., Barker, L.F., Pineda-Tamondong, G., Nath, N., Smallwood, L.A., *et al*. (1981) Antibody to Hepatitis B core antigen in blood donors with a history of Hepatitis. Transfusion,**21**:366-371.

Terrault, N.A., Lok, A.S.F., McMahon, B.J., Chang, K.M., Hwang, J.P., Jonas, M.M., *et al* (2018) Update on prevention, diagnosis, and treatment of chronic Hepatitis B: AASLD 2018 Hepatitis B guidance. *Hepatology*, **67**: 1560–1599

Thio, C.L. (2009) Hepatitis B and human immunodeficiency virus co-infection.*Hepatology*, **49**:138–S145

Tseng, T.C. and Kao, J.H. (2013) Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic Hepatitis B: new trick of old dog. *Journal ofGastroenterology*, **48**:13–21.

Tseng, T.C., Liu, C.J. and Chen, C.L. (2012) Serum Hepatitis B virus-DNA levels correlate with long-term adverse outcomes in spontaneous Hepatitis B e antigen seroconverters. *Journal of Infectious Disease*; **205**:54–63.

Valenzuela. P., Gray, P., Quiroga, M., Zaldivar, J., Goodman, H.M. and Rutter, W.J (1979) Nucleotide sequence of the gene coding for the major protein of Hepatitis B virus surface antigen. *Nature*,**280**:815–819

Wasley, A. Kruszon-Moran, D. and Kuhnert, W. (2006) The prevalence of Hepatitis B virus infection in the United States in the era of vaccination. *Journal of Infectious Disease*, **202:** 192–201.

Weinbauer, M.G., Fuks, D. and Peduzzi, P. (1993) Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea.*Applied and EnvironmentalMicrobiology*, **59**: 4074–4082.

Wen, W.H., Chang, M.H. and Zhao, L.L. (2013) Mother-to-infant transmission of Hepatitis B virus infection: significance of maternal viral load and strategies for intervention. *Journal of Hepatology*,**59:** 24–30.

WHO (2017) Global Hepatitis report, 2017. [2017; cited 2019 ]

WHO (2017) Global Hepatitis report, 2017. World Health Organization, Geneva

WHO. Hepatitis B. Fact Sheet N°204. http://www.who.int/mediacentre/factsheets/fs204/en/ (2014).

Xu, W.M., Cui, Y. T., Wang, L., Yang, H., Liang, Z.Q., Li, X.M., *et al* (2009) Lamivudine in late pregnancy to prevent perinatal transmission of Hepatitis B virus infection: a multicentre, randomized, double-blind, placebo-controlled study. *Journal of Viral Hepatitis,***16**:94–103

Yamamoto, K., Horikita, M., Tsuda, F., Itoh, K., Akahane, Y., Yotsumoto, S., *et al*. (1994). Naturally occurring escape mutants of Hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to Hepatitis B surface antigen. *Journal of Virology*,**68**:2671-2676.

Yotsuyanagi, H., Yasuda, K., Iino, S., Moriya, K., Shintani, Y., Fujie, H., *et al*. (1998). Persistent viremia after recovery from self-limited acute Hepatitis B. *HEPATOLOGY*, **27**:1377-1382.

Zampino, R., Pisaturo, M.A., Cirillo, G., Marrone, A., Macera, M., Rinaldi, L., *et al* (2015) Hepatocellular carcinoma in chronic HBV-HCV co-infection is correlated to fibrosis and disease duration. *Hepatology*,**14**:75–82

Zhou, K. and Terrault, N. (2017) Management of Hepatitis B in special populations.*Best Practice Clinical Gastroenterology*, **31**:311–320.

## APPENDIX 1

**Elisa Kit**

**Microwell plate**

White microwell plate.

1 plate (96 wells). Each well is coated with Anti-HBsAg.

**Negative control**

Normal serum non-reactive for HBsAg.

Colorless fluid.preservative; 0.1% proclin 300.

**Positive control**

Inactivated serum containing HBsAg and negative for HCV, HIV -1 and -2; preservative:0.1% proclin 300.

**HBsAg conjugate**

Anti- HBsAg bound to peroxidase; preservative:0.1% proclin 300

**Concentrated wash buffer**

Tris-HCL buffer containing 0.1% Tween 20; preservative: 0.1% proclin 300

**Substrate A**

Citrate-phosphate buffer containing hydrogen peroxide; preservative 0.1% proclin 300

**Substrate B**

Buffer containing tetramethylbenzidine (TMB); preservative 0.1% proclin 300

**Stop solution**

0.5 sulfuric acid

**Plate sealers**

To cover the microwell plates during incubation to prevent contamination or evaporation of the wells.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.047 | 0.219 | 0.145 | 0.164 | 0.141 | 0.119 | 0.169 | 0.097 | 0.098 | 0.149 | 0.164 | 0.176 |
| 0.215 | 0.199 | 0.130 | 0.150 | 0.176 | 0.156 | 0.137 | 0.112 | 0.120 | 0.123 | 0.137 | 0.217 |
| 0.214 | 0.235 | 0.166 | 0.117 | 0.152 | 0.164 | 0.147 | 0.201 | 0.124 | 0.196 | 0.123 | 0.221 |
| 0.150 | 0.194 | 0.165 | 0.095 | 0.163 | 2.974 | 0.137 | 0.183 | 0.100 | 0.159 | 0.133 | 0.141 |
| 0.203 | 0.152 | 0.147 | 0.127 | 0.179 | 0.092 | 0.150 | 0.107 | 0.148 | 0.140 | 0.128 | 0.162 |
| 0.234 | 0.216 | 0.135 | 3.379 | 0.159 | 0.170 | 0.190 | 0.120 | 0.162 | 0.159 | 0.097 | 0.216 |
| 0.196 | 0.163 | 0.101 | 0.079 | 0.189 | 0.214 | 0.198 | 0.211 | 0.171 | 0.238 | 0.195 | 0.270 |
| 0.185 | 0.156 | 0.098 | 0.179 | 0.193 | 0.197 | 0.225 | 0.174 | 0.197 | 0.254 | 0.205 | 2.390 |

## APPENDIX II

Blank = 0.047

$NC_1$ = 0.216

$NC_2$ = 0.270

PC = 2.390

Mean absorbance for negative control = 0.243

NC×: mean absorbance of negative control – blank absorbance= 0.243 -0.047= 0.196

Cut Off value = NC× + 0.070 = 0.196 + 0.070= 0.266

Interpretation of results

Non reactive: specimens with absorbance less than the cut off value are non-reactive for HBsAg and may be considered negative.

Reactive; specimens with absorbance greater than or equal to the cut off value are considered initially reactive for HBsAg. The specimen should be retested in duplicate before final interpretation.