Sudan University of Science and Technology

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Frequency of Hepatitis B Virus among Health Care Workers in McNimir Hospital, Shendi Locality

معدل الإنتشار لفيروس إلتهاب الكبد الوبائي (النوع ب) وسط العاملين في مجال الرعاية الصحية بمستشفى المك نمر محلية شندي

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Microbiology)

By:

Elsied Altaher Babker Elsied

B.Sc. in Medical laboratory Science, Shendi University, (2017)

Supervisor:

Dr. Wafaa Mohammed Abdalla

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الآية

قال تعالى:

(وَوَصَّيْنَا الْإِنْسَانَ بِوَالِدَيْهِ إِحْسَانًا حَمَلَتْهُ أُمُّهُ كُرْهًا وَوَضَعَتْهُ كُرْهًا وَحَمْلُهُ وَفِصَالُهُ ثَلَاثُونَ شَهْرًا حَتَّى إِذَا بَلَغَ أَشُدَّهُ وَبَلَغَ أَرْبَعِينَ سَنَةً قَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَصْلِحْ لِي فِي ذُرِّيَّتِي إِنِّي تُبْتُ إِلَيْكَ وَإِنِّي مِنَ الْمُسْلِمِين)

صدق الله العظيم

سورة الاحقاف (١٥)

Dedication

To who taught me how to be available member in the community...

My father

To who give me the love and security...

My Mother

To dears brothers and sisters

To my dear Teachers

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First of all I thank **ALMIGHTY ALLAH** for giving me the strength and patience to do this study. Then I would like to thank my supervisor **Dr. Wafaa Mohammed Abdalla** who helped and supported me patiently to complete this work.

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Abstract

Hepatitis B infection is one of the world's major infectious diseases and health care workers (HCWs) have a high risk of occupational exposure to HBV. This descriptive cross-sectional study was conducted during March to October 2019, to detect the frequency of HBV serologically among health care workers in McNimir Hospital. One hundred (n=100) blood specimens were collected from each eligible participants and structured questionnaire was used to collect both demographic and clinical data from them. Enzyme Linked Immunosorbent Assay (ELISA) technique was used to detect Hepatitis B surface antigen (HBsAg).

A total of one hundred health care workers (HCWs) in McNimir Hospital were included in this study, in which 38 (38%) were males and 62 (62%) were females, mostly were at age between 20-30 years (77%) and mostly were physicians (25%) and Lab. technologists (22%).

Among HCWS there were 2 (2%) positive for HBs Ag. There was 2 (2%) females were positive for HBs Ag and all males were negative. There was insignificant association between gender and positivity of HBs Ag.

According to age groups, there was one (1%) between 20-30 years and one (1%) in age range from 51 to 60 years were positive for HBs Ag and there was irrelevant association between age groups and HBs Ag positivity.

There was one (1%) HCW had accidental needle stick and one (1%) didn't expose to any of the possible risk factors were HBs Ag positive and there was meaningless association between sero-positivity of HBsAg and accidental needle stick injury, hemodialysis, previous surgical operation and blood transfusion.

There was 1(1%) nurse and 1 (1%) cleaning staffs were positive for HBsAg while all other HCWs were negative and there was no significant between occupation and HBs Ag positivity.

In conclusion the frequency of HBV infection among HCWs was low.

المستخلص

عدوى التهاب الكبد (ب) هي واحدة من الأمراض المعدية الرئيسية في العالم ، ويواجه العاملون في مجال الرعاية الصحية مخاطر عالية من التعرض المهني لمرض التهاب الكبد (ب). تم إجراء دراسة وصفية مستعرضة خلال الفترة من مارس إلى أكتوبر ٢٠١٩ ، للكشف عن معدل الانتشار المصلي لفيروس التهاب الكبد الوبائي مصلياُ بين عاملي الرعاية الصحية بمستشفى الملك نمر. تم جمع مائة (ن = ١٠٠) عينة دم من كل المشاركين المؤهلين ،وجمعت المعلومات بواسطة استبيان منظم لجمع البيانات الديموغرافية والسريرية. تم استخدام تقنية الامتزاز المناعي المرتبط بالانزيم للكشف عن المستضد السطحي لالتهاب الكبد(ب).

تم تضمين مجموعة مائة من العاملين ف مجال الرعاية الصحة في مستشفى الملك نمر، والتي كان ٣٨ (٣٨ ٪) من الذكور و٦٢ (٦٢ ٪) من الإناث، تتراوح اعمار معظمهم بين (٢٠-٣٠) (٧٧) وكان معظمهم من الأطباء (٢٥) وتقنيو المختبر (٢٢).

بين العاملين في مجال الرعية الصحية كان هناك ٢ (٢%) إيجابية لفيروس التهاب الكبد الوبائي (ب). كان هناك ٢(٢%) من الاناث إيجابيات لفيروس التهاب الكبد الوبائي (ب) وكانت جميع الذكور سلبية. كان هنالك ارتباط ضئيل بين الجنس وإيجابية التهاب الكبد الوبائي.

وفقا للفئات العمرية، كان هناك واحد (١%) بين ٢٠-٣٠ عاما وواحد (١%) في الفئة العمرية من ٥١-٦٠ عاما كان إيجابيا لفيروس التهاب الكبد الوبائي (ب) وكان هنالك ارتباط غير زي صلة بين الفئات العمرية وإيجابية التهاب الكبد الوبائي.

كان هناك واحد (١%) من العاملين في مجال الرعاية الصحية لديه إصابة عصا ابرة عرضية، وواحد (١%) لم يتعرض لأي من عوامل الخطر المحتملة للإصابة بفيروس التهاب الكبد الوبائي (ب)، وكان هناك ارتباط لا معني له بين الإيجابية المصلية واصابة عصا الابرة العرضية، غسيل الكلى، عملية جراحية سابقة ونقل دم.

كانت هناك ممرضة واحدة (١%) وموظفة تنظيف واحدة (١%) إيجابية لفيروس التهاب الكبد الوبائي(ب) بينما كانت جميع سلالات الرعاية الصحية الأخرى سلبية ولم يكن هناك فرق كبير بين الوظيفة وإيجابية التهاب الكبد الوبائي (ب).

خلصت الدراسة الي ان معدل الإصابة بفيروس التهاب الكبد (ب) بين العاملين في مجال الرعاية الصحية منخفضًا.

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List of abbreviations

3TC	Lamivudine
Ab	Antibody
ALF	Acute liver failure
ccc DNA	covalently closed circular DNA
CHB	chronic Hepatitis B virus
CTL	cytotoxic T-lymphocyte
DNA	Deoxyribonucleic acid
ETV	Entecavir
HBc	Hepatitis B core antigen
HBeAg	Hepatitis B viral protein
HBIG	Hepatitis B immunoglobulin
HBsAg	hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCWs	Health care workers
HDV	Hepatitis delta virus
HIV	Human immunodeficiency virus
IFN	Interferon
mRNA	Messenger RNA
MTCT	mother-to-child transmission
NAs	Nucleotide analogues
NSIs	Needle sticks or other sharp device injuries
OBI	Occult HBV infection
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
TAF	Tenofoviralafenamidefumarate
TDF	Tenofovirdisoproxilfumarate
WHO	World health organization

CHAPTER I

INTRODUCTION

CHAPTER I

1. INTRODUCTION

1.1. Introduction

Half a century ago, hepatitis B virus (HBV) is one of the world's most severe infectious illnesses (Yang *et al*., 2017).

HBV is a major global public health problem and worldwide estimates that more than 2 billion people have been infected with HBV and 248 million of these people are chronically infected. About 15% to 25% of persons with chronic HBV infection die from cirrhosis or liver cancer (Nelson *et al*., 2016).

Health care workers (HCWs) are defined as all paid and unpaid persons working in health-care settings who have the potential for exposure to patients and/or to infectious materials, including body substances, contaminated medical supplies and equipment, contaminated environmental surfaces, or contaminated air. They are at a high risk of exposure to blood and body fluid (CDC, 2011).

They have an up to four-fold incidence of this infection in the general population and the main risk factor to acquire HBV infection for HCWs is direct contact with infectious material, especially HBV-infected blood or body fluid (David *et al*., 2018).

Approximately three million HCWs are exposed to percutaneous blood – borne viruses each year and it is estimated that 66000 HBV are acquired annually (Kermode *et al*., 2005).

Occupational exposure to HBV is a well-recognized risk for HCWs and it is dependent on the frequency of percutaneous and permucosal exposure to blood or body fluids containing blood, which commonly occurs due to needle sticks or other sharp device injuries (NSIs) (Hisham *et al*., 2013).

Studies have reported a lack of awareness of HBV among HCWs; consequently, proper precautions (e.g., use of disposable gloves) against blood-borne infections are lacking in these workers and this observation is consistent with other studies demonstrating that untrained individuals are more likely to be exposed to HBV infection and preventive vaccination against hepatitis B for hospital staff is standard in many countries, but is still not implemented in many resources-poor settings. Therefore WHO recommends monitoring immune responses to the vaccine in addition to compulsory vaccination of HCWs (Mueller *et al.*, 2015).

HBV-infected HCWs also pose a potential risk for patients as there is documented risk of HBV transmission to patients from treating doctors or medicals vaccination is effective in protecting 90-95% adults (Vishal *et al*., 2015).

1.2. Rationale

Hepatitis B is a well documented occupational risk for health professionals, including a laboratory and nursing staff (Elmukashfi *et al*., 2012).

The prevalence of disease is associated with a proper understanding of the mode of transmission of the disease.

Moreover, little is known about the situation and prevalence of the disease in River Nile State, especially among health workers whom may expose to infection. Furthermore, the proper understanding of the prevalence in study area may help in setting further control programs.

So, this study was conducted to detect the frequency of HBV among health care workers in McNimir Hospital.

1.3. Objectives

1.3.1. General objective

To detect the frequency of HBV serologically among health-care workers in McNimir Hospital, Shendi Locality.

1.3.2. Specific objectives

To detect hepatitis B surface antigen (HBsAg) among health-care workers in McNimir Hospital using enzyme linked immunosorbent assay (ELISA), to determine the frequency of HBsAg among health care workers, to associate between hepatitis B infection and gender, age groups and occupation of HCWs and to determine the possible risk factors (accidental needle stick injury, previous surgical operation, hemodialysis and blood transfusion) associated with HBsAg.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

2. LITERATURE REVIEW

2.1. HBV

2.1.1. Classification

HBV belongs to the genus *Orthohepadnavirus* of the family *Hepadnaviridae* and the virion is spherical with a diameter of 42 nm (Faseeha, 2015).

It is the best known hepadnavirusis that infects humans which is commonly referred to HBV and it is a major importance as an agent of disease and death (Levinson, 2014).

Related viruses have been found in woodchucks, ground squirrels, and ducks, suggesting a long evolutionary history of this virus family (Shuping *et al*., 2013).

2.1.2. Genome

The partially double-stranded HBV genome is encased within the core particle, which is wrapped by an envelope consisting of host-derived lipids containing dispersed viral envelope proteins (Shuping *et al*., 2013).

The nucleocapsid encloses the viral genome consisting of two linear strands of DNA held in a circular configuration. One of the strands (the plus strand) is incomplete, so that the DNA appears partially double stranded and partially single stranded. Associated with the *plus* strand is a viral DNA polymerase, which has both DNA-dependent DNA polymerase and RNA-dependent reverse transcriptase functions. Although it is a DNA virus, it encodes a reverse transcriptase and replicates through an RNA intermediate and this polymerase can repair the gap in the plus strand and render the genome fully double stranded (Kumar, 2016).

2.1.3. HBV genotypes

At least 10 HBV genotypes (A to J) with distinct geographic distributions and several HBV mutants (Lin and Kao, 2015).

All the single HBV-genotype infection has the propensity to develop liver diseases but in an HBV endemic area, co-infection or super infection with multiple HBV genotype variants are often observed (Datta *et al*., 2014a).

They differ in more than 7.5% of their nucleotide sequences and which are further subdivided into sub-genotypes with a nucleotide divergence greater than 4%. While genotypes A to H have long been accepted as individual genotypes, three new genotypes (I and J) were proposed more recently (Velkov *et al.,* 2018).

Genotype A is prevalent in Brazil, USA, Canada, Northwest Europe, South Asia, Central African countries, Tunisia and Benin and Genotype B is common in Japan, Taiwan, Philippines, Hong Kong, China, Vietnam, Thailand, Indonesia and United States of America (Mahmood *et al*., 2016).

Genotype C occurs in Australia, Polynesia, Melanesia, Micronesia, Indonesia, China, Hong Kong, Vietnam, Thailand, Japan, Korea, Taiwan, India, Solomon Islands, Brazil and USA. Genotype D is predominant in Mediterranean region, Spain, Albania, Czech Republic, Russia, Turkey, Middle East, Iran, Afghanistan, South Asia, Solomon Islands, Tunisia, Polynesia, Melanesia, Micronesia, Brazil and USA (Mahmood *et al*., 2016).

Genotype E almost exclusively occurs in African people and its presence is more commonly associated with the development of chronic HBV (CHB) infection. Moreover, an epidemiological link has been found between the distribution of HBV genotype E infection and African countries with high incidences of hepatocellular carcinoma (Malagnino *et al*., 2018).

Genotype G (HBV-G) is an aberrant genotype with little sequence divergence, suggesting a recent origin. HBV-G is strongly associated with certain risk groups such as intravenous drug users (IDUs) and men who have sex with men (MSM), but hardly with geography. The origin and epidemiology of HBV-G remain unresolved, is also present in certain risk groups in Europe (Cornelissen *et al.*, 2016).

Genotypes B, C and I are associated with a more frequent vertical transmission from mother to child, a higher transmission rate during sexual contact or injecting drug use has been reported for genotypes A, D and G (Velkov *et al.*, 2018).

Acute genotype A and D infection results in higher chronicity rates than B and C (Lin and Kao, 2011).

2.1.4. Replication

Hepatocytes (liver cells) are the host cells for having the body and in the laboratory, primary cell cultures of human hepatocytes support replication, but unfortunately none of the established cell lines derived from liver tumors can be infected by HBV visions. Some cell lines, however, can be infected using HBV DNA (a procedure known as transfect ion) (Carter and Saunders, 2007*).*

HBV enters hepatocytes via an as yet unknown receptor. Following uncoating and disassembly of the core particle, the viral DNA is delivered to the nucleus and converted into

covalently closed circular (ccc) DNA. In the nucleus the cccDNA serves as a template for viral RNA transcription (Shuping *et al*., 2013).

The cccDNA is complicated with cellular proteins such as histones and other regulatory proteins (Pei*et al*., 2014).

The cccDNA serves as template for transcription of four viral mRNAs and the largest mRNA, (which is larger than the viral genome), that is uses to make the new copies of the genome, the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and recycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where the virion protein synthesized DNA via its reverse transcriptase activity (Levinson, 2014).

2.1.5. HBV transmission

The HBV can survive outside the body for at least 7 days, during this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine and the incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days(WHO, 2018).

There are three important modes of transmission; parenteral transmission, perinatal transmission and sexual transmission (Kumar, 2016).

It is transmitted through percutaneous, mucosal, or non-intact skin exposure to infectious blood or body fluids. HBV is concentrated most highly in blood, and percutaneous exposure is an efficient mode of transmission. Semen and vaginal secretions are infectious, and also can be detected in saliva, tears, and bile. Cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid are also considered potentially infectious. Urine, feces, vomits, nasopharyngeal washings, sputum, and sweat are not efficient vehicles of transmission unless they contain blood because they contain low quantities of infectious HBV. HBsAg found in breast milk is also unlikely to lead to transmission, and hence HBV infection is not a contraindication to breastfeeding (Schillie *et al*., 2018).

2.1.6. Risk groups for HBV

2.1.6.1. Health care workers

The risk of acquisition of this infection in an unvaccinated individual after a single exposure is estimated 32–67% when blood is positive for both hepatitis B surface antigen (HBsAg) and

envelope antigen (HBeAg) and 6% when HBeAg is negative. The World Health Organizationrevealed that in 2000, 66,000 HBV infections among HCWs could have happened o wing to their occupational exposure (Ganczak *et al.*, 2019).

Health care worker are most exposed to HBV due to number of blood exposures sustained during medical procedures, the risk of transmission at each exposure and the prevalence of HBV in general population, particularly in hospitalized patients, lack of training in infection control, and not using protective equipment contribute to contracting HBV at hospital setting (Ganczak *et al.*, 2019).

2.1.6.2. Sexual (heterosexual and homosexual) exposure

Among persons with case reports of HBV infection with information about sexual exposure, 26.4% reported having two or more sexual partners, 3.3% reported sexual contact with an HBV infected person, and 11.8% of males reported having had sex with another male. As many as 10%–40% of adults seeking treatment in clinics have evidence of current or past HBV infection. Among adults with acute HBV infection, 39% were screened or sought care for sexual transmitted infection (STI) prior to becoming infected with HBV (Mahmood *et al*., 2016).

2.1.6.3. Hemodialysis patients

Since the initiation of HBV vaccination and additional infection control precautions for HBV in dialysis centers, the incidence of HBV infection among hemodialysis patients has declined approximately 95%. Since 1995, the annual incidence has been stable and HBsAg sero-prevalence has remained at 1%.Receipt of dialysis was reported in <1% of acute HBV surveillance cases with information reported to CDC (Schillie*et al*., 2018).

2.1.6.4. Travelers to countries where HBV is endemic

Short term travelers to countries in which HBV infection is of high or intermediate endemicity typically are at risk for infection only through exposure to blood in medical or disaster relief activities, receipt of medical care that involves parenteral exposures, sexual activity, or drug use. Monthly incidence of 25–420 per 100,000 travelers has been reported among long term travelers to countries where the disease is endemic (Schillie *et al.*, 2018).

2.1.7. Epidemiology

According to the WHO and the Centers for Disease Control and Prevention (CDC), 257 million people are living with HBV. Moreover, 20,900 acute hepatitis B cases were reported in 2016.

Hepatitis B is highly prevalent in the African, Western Pacific, Eastern Mediterranean, South-East Asia, and European regions, respectively (Al-Sadeq *et al*., 2019).

According to a recent systematic review and meta-analysis estimating the worldwide prevalence of chronic HBV infection, in 2010, about 248 million individuals were hepatitis B surface antigen (HBs Ag) positive. More than 780,000 people die every year due to hepatitis B related complications including cirrhosis and hepatocellular carcinoma and the prevalence of HBV chronic infection is particularly high in Africa and the prevalence reaching up to 22 % in South Sudan. In Cameroon, seroprevalence of HBs Ag has been reported to be 10.1 % in a general population of blood donors, 10.2 % among pregnant women and 23.7 % among HIV-infected patients (Tatsilong *et al.*, 2016).

Sudan is classified among the African countries with high HBV endemicity. The reported prevalence of HBV chronic infection, characterized by the detectable level of HBV surface antigen (HBsAg), varied from region to region and ranged between 5 and 7% in the general population and 26% in hospital outpatients. The prevalence of adults having been in contact with HBV and identified by the presence of anti-core antibodies (anti-HBc) was high, ranging between 47.5 and 67%. The introduction of vaccination and the screening of blood and blood products during the past 8 years is expected to reduce the rate of HBV infection and the carrier pool (Mahgoub *et al*., 2011).

2.1.8. Pathogenesis

After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. The pathogenesis of hepatitis B is probably is the result of this cell-mediated immune injury, because HBV itself does not cause a cytopathic effect. Antigen–antibody complexes cause some of the early symptoms (e.g., arthralgias, arthritis, and urticaria) and some of the complications in chronic hepatitis (e.g., glomerulonephritis, cryoglobulinemia, and vasculitis) (Livenson, 2014).

2.1.9. Clinical presentations

2.1.9.1. Acute infection

Acute liver failure (ALF), earlier known as fulminant hepatitis, is a rare but dramatic clinical syn drome characterized by the sudden loss of hepatocytes, resulting in an individual with no preexis ting liver disease (Chen *et al*., 2018).

After exposure to the virus, there is a long, asymptomatic incubation period, which may be followed by acute disease lasting many weeks to months. The natural course of acute disease can be tracked using serum markers (Kumar *et al*., 2013).

During the acute infection, hepatitis B does not appear to induce an intra-hepatic innate immune response and instead, it acts as a 'stealth' virus early in the infection (Spearman *et al*., 2013).

Hepatitis B surface antigen (HBsAg) appears before the onset of symptoms, peaks during overt disease, and then declines to undetectable levels in 3 to 6 months (Kumar *et al*., 2013).

Anti-HBs antibody does not rise until the acute diseases over and usually is not detectable for a few weeks to several months after the disappearance of HBsAg. It may persist for life, conferring immunity; this is the basis for current vaccination strategies using non-infectious. HBsAg, HBeAg, HBV-DNA, and DNA polymerase appear in serum soon after HBsAg, and all signify active viral replication. Persistence of HBeAg is an important indicator of continued viral replication, infectivity, and probable progression to chronic hepatitis. The appearance of anti-HBe antibodies implies that an acute infection has peaked and is on the wane (Kumar *et al*., 2013).

2.1.9.2. Chronic infection

Chronic HBV is defined as persistence of serum HBsAg for more than 6 months (Yim *et al*., 2019).

Chronicity occurs rarely when the HBV infection is contracted in adulthood, but is common in neonates and young children (Spearman *et al*., 2013).

It is estimated that there are 240 million people who are chronically infected with HBV globally 15%–40% of infected patients will develop serious liver disease, resulting in up to 1.2 million deaths per year. HBV infection is the tenth leading cause of death worldwide (Burns and Alexander, 2014).

Chronically infected patients are unable to sustain an immune response to HBV and may experience intermittent episodes of hepatocyte destruction in an attempt to clear virally infected hepatocytes, in what can be termed 'flares' (Niederau, 2016).

The complication rate of chronic hepatitis B is associated with the degree of viral replication, inflammation, and fibrosis. The risk for cirrhosis is also increased in the presence of fibrosis, long disease duration, male gender, co-comorbidities like alcohol consumption, diabetes mellitus type 2, obesity, and co-infection in particular with HDV or HIV (Niederau, 2016).

Age is also an important host factor determining the risk of chronicity. Following acute exposure to HBV, 90% of neonates born to HBeAg-positive mothers, 20 - 50% of infants and children under the age of 5 years, and <5% of adults will develop chronic hepatitis B infection. Viral variants may also influence the course and outcome of the disease. In addition, and only rarely and in the setting of profound immune suppression, the virus can be directly cytopathic (Spearman *et al*., 2013).

Most chronic HBV-infected patients have low viral replication with a lack of HBV e antigen (HBeAg) and absence of significant findings from liver biopsy. Generally, these subjects have good outcome and low risk of developing cirrhosis or liver cancer. They are called inactive carriers. On the other hand, there are patients who develop more severe histological lesions and have high HBV DNA levels. These subjects have high risk of developing HBV complications after years or decades of chronic infection (Galizzi *et al.*, 2010).

Other presentation of chronic HBV infection is the immune-tolerant phase, which is common among newborns of HBV female carriers. This situation is usually detected among children and teenagers. There is high HBV replication, HBeAg persistence, and high infectivity potential. Liver biopsy shows mild histological activity, and biochemical analysis shows aminotranferase levels under the reference range (Galizzi*et al.*, 2010).

2.1.9.3. HBV and hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and about 350 million people globally are chronically infected with HBV. Chronic HBV infection accounts for at least 50% cases of HCC worldwide (Xie, 2017).

Risks of HCC among HBV-infected patients vary by several factors, the major one being serum HBV-DNA levels. Although there is no discrete cutoff level, having greater than Log_{10} 5/mL viral copies confers a 2.5- to threefold greater risk over an 8- to 10-year follow-up period than does having a lower viral load. The cumulative incidence of HCC increases with serum HBV-DNA levels. A recent hospital-based cohort study further validated the HCC risk, showing it started to increase when the HBV-DNA level was higher than 2000 IU/ml. In addition to HBV-DNA levels, the clinical significance of quantitative hepatitis B surface antigen (HBsAg) has become increasingly recognized (Omata *et al*., 2017).

One particularly important intermediate aspect of a decades-long chronic HBV infection includes the development of HBV-associated cirrhosis prior to HCC development and it is generally

accepted that the majority, potentially as much as 70–90%, of all HCC occurs in the context of decompensated cirrhosis and a strong relationship exists between chronic HBV infection and cirrhosis (Lamontagne, 2016).

There are three reported mechanisms by which HBV promotes carcinogenesis: HBV proteins are involved in many signaling pathways in hepatocytes, thereby affecting the expression and functions of specific genes and contributing to live disorders and most of these changes are associated with HCC, integration of HBV DNA into the host genome alters the function of endogenous genes or induces chromosomal instability, and Inflammation-mediated alteration of specific signaling pathways contributes to tumorigenesis. Chronic inflammation plays a vital role in the development of HCC and repeated cycles of inflammation-induced apoptosis and hepatocyte regeneration increase the risk of hepatocarcinogenesis (Xu *et al*., 2014).

2.1.9.4. Occult hepatitis

Occult HBV infection (OBI) was first described in the late 1970s (Kwak and Kim, 2014).

It is characterized by negative HBsAg and detectable HBV-DNA in the liver and/or serum, with or without anti-HBc (Elbedewy *et al*., 2015).

Occult HBV infection is associated with a risk of HBV transmission through blood transfusion, hemodialysis, and liver transplantation (Kim and Kim, 2015).

It may indirectly favor HCC development and it has been shown that the persistence of very low viral replicative activity during OBI may induce mild liver necro-inflammation continuing for life, and substantial clinical evidence indicates that OBI can accelerate the progression of liver disease towards cirrhosis that is considered the most important risk factor for HCC development (Pollicino and Saitta, 2014).

OBI may be related mainly to mutations in the HBV genome, although the underlying mechanism of it remains to be clarified. Mutations especially within the immunodominant "α" determinant of S protein are "hot spots" that could contribute to the occurrence of OBI *via* affecting antigenicity and immunogenicity of HBsAg or replication and secretion of virion (Zhu *et al*., 2016).

It is a clinical class of HBV infection and can appear in two forms: seropositive OBI and seronegative OBI. In seropositive OBI, serum HBV DNA is detectable and both anti-HBc/anti-HBs IgGs are positive or only anti-HBcIgG is positive, while in seronegative OBI, only HBV

DNA is detectable in serum/or liver tissue, but anti-HBcIgG/anti-HBs IgGs are negative in serum (Makvandi, 2016).

The frequency of the diagnosis depends on the relative sensitivity of both HBsAg and HBV DNA assays. It also depends on the prevalence of HBV infection in the population (Allain, 2004).

Occult HBV in blood donors has a wide range of potential origins within the natural history of the infection and it may originate from recovered infections with anti-HBs and persistent, low-level, viral replication, escape mutants undetected by the HBsAg assays or healthy chronic carriage and the last situation is mostly found with anti-HBc only (Allain, 2004).

2.1.10. Immunomechanism against HBV

Unlike many other viruses, HBV infection is characterized by a delayed kinetics of viral replicati on and further uniqueness of HBV is its inability to trigger a classic innate immune response.

Data *in-vitro* and *in-vivo* has shown the absence of activation of type I Interferon (IFN) genes during the logarithmic phase of HBV expansion and the absence of pro-inflammatory cytokines in the serum of patients in the early phases of acute infection. So the virus is causes of inability to activate a classical innate immune response and whether HBV actively suppress innate immunity or only evade its recognition (Hong and Bertoletti, 2017).

It is able to trigger adaptive immune response, which usually prompts the death of infected hepatocytes leading to hepatic injury and damage. The intention of which is to remove virus infected cells. In this immune response, both CD4 T cells (T helper cells) and CD8 T (cytotoxic T-lymphocyte (CTL) cells are activated CD4 T cells are robust producers of cytokines and are required for the efficient development of CTLs and B cells, which produce anti-HBV antibody to reduce the levels of circulating virus studies of HBV infected chimpanzees, suggest that CD4 T cells have no direct effect on viral clearance and liver disease (Lu, 2011).

CD8 T cells clear HBV-infected hepatocytes through cytolytic and non-cytolytic mechanisms, reducing the levels of circulating virus, whereas B-cell antibody production neutralizes free viral particles and can prevent reinfection. This antiviral immune response is induced in adults after acute HBV infection and leads to HBV control. In contrast, chronic HBV patients fail to mount such an efficient antiviral response (Tan *et al*., 2015).

Broadly reactive CD4 T cells are predominantly detectable during acute infection, whereas their numbers decline during chronic infection. Both, CD4 and CD8 T cell responses are deterministic

of whether an acute infection is resolved, or whether it progresses to chronic infection (Prieto and Dorner, 2017).

Depletion of CD4 T cells at the peak of HBV infection in chimpanzees does not affect the rate of viral clearance or the extent of liver damage, thereby supporting this hypothesis. However, CD4 T cells may be necessary to instruct and maintain anti-HBV CTLs and the specific CTL response plays a significant role in viral clearance and the pathogenesis of liver damage (Lu, 2011).

In acute HBV infection, initial damage to the liver corresponds kinetically with the entry of HBV-specific CTLs into the liver. Furthermore, depletion of these cells at the peak of viremia delays the onset of liver damage and viral clearance in chimpanzees (Lu,2011).

The association of CTLs with liver injury is also observed in patients with acute viral hepatitis who successfully clear HBV and in patients with chronic HBV infection, CTLs seem to be suppressed, although low levels of CTLs exist in the infected liver. Reactivation of the killing mediated by CTLs usually leads to the clearance of HBV in patients with chronic infection (Lu, 2011).

2.1.11. Laboratory diagnosis

2.1.11.1. Serologic and virologic markers

Serological markers for HBV infection consist of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc IgM and IgG and the identification of serological markers allows to; identify patients with HBV infection; to elucidate the natural course of chronic hepatitis B (CHB); to assess the clinical phases of infection; and to monitor antiviral therapy (Song and Kim, 2016).

Viral persistence and be confirmed by PCR-based detection of HBV DNA in serum and detection of HBe is still used as a marker of enhanced infectivity and risk of chronic liver disease (Bannister *et al.*, 2006).

2.1.11.1.1. Viral capsid surface antigen and the antibody directed against the surface antigen (anti-HBs)

Hepatitis B surface antigen (HBsAg) is the expressed protein on the surface of the virus and one of the early viral markers for HBV active or acute infection. HBsAg level in the serum is associated with cccDNA levels inside host hepatic cells, defining a clinical relevance of this marker (Al-Sadeq *et al*., 2019).

Several studies have reported the association between transcription activity of cccDNA in the liver and serum HBsAg levels and differences in the serum HBsAg levels during the different

phases of infection indicate the distribution of cccDNA during the respective phases of the disease. The serum HBsAg titers are higher in patients with HBeAg-positive CHB than in HBeAg-negative CHB and monitoring of quantitative HBsAg levels predicts treatment response to interferon and disease progression in HBeAg-negative CHB patients with normal serum alanine aminotransferase levels (Song and Kim, 2016).

Anti-HBs is known as a neutralizing antibody, and confer long-term immunity and in patients with acquired immunity through vaccination, anti-HBs is the only serological marker detected in serum. In the past HBV infection, it is present in concurrence with anti-HBc IgG (Song and Kim, 2016).

Occasionally, the simultaneous appearance of HBsAg and anti-HBs has been reported in patients with HBsAg positive and in most cases, anti-HBs antibodies are unable to neutralize the circulating viruses, thus these patients are regarded as carriers of HBV(Song and Kim, 2016).

2.1.11.1.2. Antibody directed against the core antigen (anti-HBc)

Hepatitis B core antigen is detected in infected hepatocytes, butis not released into serum; however, Immunoglobulin M antibody directed against HBcAg (anti-HBc) is usually the earliest anti-hepatitis B antibody detected in the infected patient (Frederick and Southwick, 2007).

The IgM anti-HBc is usually interpreted as a marker for early acute disease; however, in some patients, anti-HBc IgM levels can persist for up to 2 years after acute infection, and in patients with chronic active hepatitis, IgM antibody levels can rise during periods of exacerbation. An anti-HBc IgM titer is particularly helpful for screening blood donors, because this antibody is usually present during the window between HBsAg disappearance and anti-HBs appearance.

The Immunoglobulin G antibodies directed against the core antigen develop in the later phases of acute disease and usually persist for life (Frederick and Southwick, 2007).

2.1.11.1.3. Secreted core antigen (HBeAg) and its antibody (anti-HBe)

Secreted core antigen (HBeAg) marker indicates viral replication and risk of transmission of infection, and seroconversion of HBeAg to anti-HBe is associated with remission of liver disease. However, some anti-HBe reactive subjects continue to have active viral replication and hepatic disease caused by mutations in the pre-core and core region in the HBV genome, which reduces the production of HBeAg (Villar *et al*., 2015).

2.1.11.1.4. Polymerase chain reaction (PCR) test

PCR is a simple, yet elegant, enzymatic assay, which allows for the amplification of a specific DNA fragment from a complex pool of DNA (Garibyan and Avashia, 2013).

PCR can be performed using source DNA from a variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes. Only trace amounts of DNA are needed for PCR to generate enough copies to be analyzed using conventional laboratory methods. For this reason, PCR is a sensitive assay (Garibyan and Avashia, 2013).

However, quantitation of HBV DNA in the serum provides an alternative to cccDNA detection, through less invasive method. According to the recommendations of the Taormina Group, detection of very low levels of HBV DNA should be done with highly sensitive PCR using primers specific for highly conserved sequences (genotype independent) of different HBV genomic regions. It has been observed that sensitivity of the HBV DNA detection by PCR may vary across different genetic regions of the HBV genome (Datta *et al*., 2014b).

Most PCR based methods of HBV DNA detection for clinical purposes have a sensitivity of 50-200 IU/mL with dynamic range of 4-5 $\log_{10}$ IU/m (Allain, 2004). In comparison, real-time PCR based assays have higher sensitivity (5-10 IU/mL) with a wider dynamic range 8-9 $\log_{10}$ IU/Ml (Datta *et al*., 2014b).

2.1.11.1.4.1. Real time PCR

Unlike traditional PCR, real-time PCR, with its increased accuracy, wider linear range, and reproducibility, is widely used for the quantitative detection of HBV DNA. Currently, most HBV DNA quantification reagents use one pair of primers and a single probe for a given HBV genotype test. If HBV genetic variations exist in these primer or probe regions, the actual viral load of HBV will be underestimated by the assay. Mutations in the probe region of the COBAS Amplicor test caused by lamivudine led to the underestimation of the HBV DNA level of a chronic hepatitis patient (Liu *et al*., 2017).

2.1.11.1.5. Hepatitis B viral DNA (HBV-DNA)

Quantitation of viral DNA in serum is most commonly used in the assessment of patients with chronic active hepatitis. In the patient with acute hepatitis, this test provides no significant advantages over that for HBeAg. Both tests indicate active viral replication and in patients with fulminant hepatitis, assays for HBV-DNA have been positive in the absence of other positive markers for HBV (Frederick and Southwick, 2007).

2.1.12. Treatment

Anti-HBV therapy, over the last few years, promised an enhanced effectiveness against HBV (Singh *et al*., 2018).

Nucleostide analogues**(**NAs) that inhibit RT are therefore used to prevent HBV replication, including lamivudine (3TC), entecavir (ETV) and tenofovir (conventionally in the form of tenofovirdisoproxilfumarate (TDF), but more recently available as the prodrug, tenofoviralafenamidefumarate (TAF), with mostly historical use of other agents including telbivudine (LdT) and adefovir (ADV) (Mokaya *et al*., 2018).

Choice of TDF/TAF or ETV is determined by availability, cost, safety profile and barrier to resistance and in Africa, the choice of agent is usually limited to 3TC or TDF (Mokaya *et al*., 2018).

2.1.13. Prevention

Prevention is far simpler than treatment, particularly in the case of HBV, which requires lifelong treatment in most cases. Besides avoiding transmission from infected people via blood supply screening and universal precautions, vaccination is the most important means of reducing the global burden of disease (Rajbhandari and Chung, 2016). Wherever possible, immunization before exposure to HBV is preferred (Joshi, 2001). Efficient HB vaccines have been available since the early 1980s (Jourdain *et al*., 2016).

Vaccination in adults is recommended in high-risk groups at risk for infection by sexual exposure (e.g., men who have sex with men, people with multiple sexual partners, those seeking evaluation and treatment for sexually transmitted disease), or in persons at risk for infection by percutaneous or mucosal exposure to blood (e.g., injection drug users, household contacts of HBsAg+ patients, patients on hemodialysis, institutionalized patients, health-care workers, and public safety workers) (Rajbhandari and Chung, 2016).

Vaccination is also recommended in international travelers to regions with high or intermediate endemicity for HBV infection, persons with chronic liver disease, and with HIV infection.

Vaccination in children is recommended as part of the regular schedule of childhood immunizations. Thirty-five years after the availability of a safe and effective vaccine, universal vaccination of all children is finally available now in 184 of 196 countries in the world (Rajbhandari and Chung, 2016).

Global vaccine coverage with all three doses of vaccine is estimated at 82% (Rajbhandari and Chung, 2016).

2.1.14. Active Immunization

Prevention of HBV infection by immunization is the best way to eliminate HBV-related diseases. The HBV vaccine is the first human vaccine using a viral antigen from infected persons, which is safe and effective. Active immunization by the vaccine yields long-term immunity. Because in endemic areas the major infection route comes from maternal transmission and the outcome of perinatal transmission results in a very high rate (90%) of chronic infection and the best timing of initial HBV immunization, therefore, should be within 24 h after birth, followed by subsequent doses of HBV vaccine during infancy (Chang and Chen, 2015).

Active immunization with three or four doses of HBV vaccine was proved to be immunogenic in more than 90% of infants of non-carrier mothers or HBeAg-negative carrier mothers. In infants of highly infectious (HBeAg seropositive HBsAg carrier) mothers, the prevention efficacy of using HBV vaccines was only ∼75% (Chang and Chen, 2015).

The development of an HBV vaccine using HBsAg protein from HBV carriers as the immunogen to induce anti-HBs, the protective antibody against HBV infection, is a successful pioneer in the history of vaccine development. During the past three decades, it is proved to be safe and successful in protecting people from HBV infection and the related diseases worldwide (Chang and Chen, 2015).

Vaccination strategies against HBV include administration of traditional HBsAg vaccine, human anti-HBV surface antibody (anti-HBs), T cell vaccine, DNA vaccines, apoptotic cells expressing HBV antigens, and viral vectors expressing HBV proteins (Das *et al*., 2019).

Parenteral HBV immunoglobulin is occasionally used to provide instant protection until an effective response in the host immune system occurs and also among individuals who do not form an effective immune response to conventional HBV vaccination (Das *et al*., 2019).

With regard to HBV protection, both monovalent and combined vaccines were found to provide similar sero-protection or vaccine response rates and HBV vaccines are available as a single-antigen formulation and in combination with other vaccines (Das *et al*., 2019).

The single antigen vaccines are recommended for use at birth and the combined vaccines are usually not recommended at birth (‘Pediarix’ for individuals aged 6 weeks–6 years and ‘Twinrix’ for individuals aged ≥18 years) (Das *et al*., 2019).

2.1.15. Passive immunoprophylaxis

There are a number of settings in which post exposure prophylaxis in the form of passive immunization alone or in conjunction with hepatitis B vaccine is either necessary or desirable. Before the advent of vaccine, passive immunization with anti-HBs was the sole option (Joshi, 2001).

Hepatitis B immunoglobulin (HBIG) is a purified solution of human immunoglobulin that could be administered to the mother, newborn, or both and it offers protection against HBV infection when administered to pregnant women who test positive for HBeAg or HBsAg, or both. When HBIG is administered to pregnant women, the antibodies passively diffuse across the placenta to the child to protect against HBV infection. This works best during the last third of pregnancy (Eke *et al.*, 2017).

Approximately 20 years ago, China implemented an HBV immune prophylaxis strategy, which led to a 90% reduction (to 0.96%) in HBsAg carriers <5 years old by 2006. Approximately 90% of infants born to both HBsAg-positive and HBeAg-positive mothers will become HBsAg carriers if no immunoprophylaxis is given. The most effective way to prevent mother-to-child transmission (MTCT) of HBV infection is by immunizing all susceptible individuals with adequate hepatitis B immune globulin (HBIG) and hepatitis B vaccines at birth, especially newborn infants born to HBV-positive mothers (Wang *et al*., 2016).

Standard HBV vaccination with or without HBIG for newborns born to HBeAg-positive mothers should affect the rate of chronic HBV progression. HBV vaccination combined with HBIG for newborns born to HBeAg-positive mothers can reduce the chronic progression of HBV infection acquired prenatally (Zixiong *et al*., 2015).

2.2. Previous studies

A Study among health care workers in Korea reveals that; the overall positive rates of HBsAg and anti-HBs were 2.4% (14/571) and 76.9% (439/571), respectively and a number of HBsAg and anti-HBs co-negative cases were 118 (20.7%). The positive rates of HBsAg among the male and female groups were the same (2.4%); however, the positive rate of anti-HBs was higher in the females (79.5%) than in the males (67.5%) (Shin *et al*., 2006).

In primary hospitals of North-west Ethiopia from the total 268 HCWs and 120 medical waste handlers (MWHs) screened for hepatitis B surface antigen, 7 (2.6%) and 3 (2.5%) were positive respectively and the overall hepatitis B virus infection was 10 (2.6%) (Yizengaw *et al.*, 2018).

In northern Tanzania among the 442 HCWs, the prevalence of chronic hepatitis B virus infection was 5.7% (25/442) (Shao *et al*., 2018).

While in west Africa, Freetown, Sierra Leone a total of 211 HCWs were included with a median age of 39.0 years (range: 18–59). Of the participating HCWs, 172 (81.5%) participants were susceptible (all markers negative), 21(10.0%) were current HBV (HBsAg positive) and nine (4.3%) were considered immune because of past infection (HBsAg negative and anti-HBc positive; anti-HBs positive). Additionally, nine (4.3%) participants displayed immunity to the virus as a result of prior hepatitis B vaccination (only anti-HBs positive) (Qin *et al*., 2018).

In Sudan at different Khartoum hospitals, 90 HCWs were enrolled in a study conducted by Abdalwhab and Nafi, (2014) which found that; 4 (4.4%) were positive for HBsAg.

CHAPTER III

MATERIALS AND METHODS

CHSPTER III

3. MATERIALS AND METHODS

3. Materials and Methods

3.1. Study design

This is descriptive, cross-sectional, hospital based study.

3.2. Study area

This study was conducted in McNimir Hospital, Shendi locality, River Nile State.

3.3. Study duration

The study was carried out between March to October 2019.

3.4. Study population

Health care workers including (laboratory technologists, nurses, cleaning staff, physicians, pharmacists and radiologist) were enrolled in this study.

3.4.1. Inclusion criteria

Health care workers, males and females, with different age groups.

3.4.2. Exclusion criteria

Trainees and visitor doctors.

3.5. Ethical considerations

Ethical approval to conduct this study was obtained from the Scientific Research Committee, Collage of Medical Laboratory Science, Sudan University of science and technology and Health Services Director in Shendi Locality. Verbal consent was obtained from participants before collection of the blood specimens after informed about the importance of the study.

3.6. Sample size

The calculation of sample size was done according to below formula, because populations lower than 10,000:

$n=N/1+N\ (d)^2$

n= sample size

N= population

D= degree of precision (0.04)

$n=320/\ (1+320\ (0.04)^2)$

n=211

But one hundred (n=100) health care workers were enrolled in this study due to the high cost.

3.7. Sampling Technique

Non-probability, convenience sampling, was used in this study.

3.8. Data collection

Data were collected through direct interview with each candidate. The interview instrument (Questionnaire) consists of 9 questions**.**

3.9. Collection of blood specimens

Under sterile conditions five ml of venous blood sample was withdraw from each participant, and then waited until sample clotted. Serum was separated by centrifugation at 5000 rpm for five minutes, and collected in plain containers by syringe then stored at -20˚C until analyzed.

3.10. Laboratory test

3.10.1. Enzyme linked immune sorbent assay (ELISA)

It was used to screen HBV surface antigen (HBsAg) which indicates to HBV infection.

3.10.1.1. Procedure

The steps were followed the manufacturing's instructions (For Tress, China) as follow: the reagent and samples were allowed to reach room temperature. Then 20ul of sample diluents was added to each well except the blank and mixed by toping the plate gently and 100ul of positive control and negative control and specimens were added to their respective wells. Then 50ul HRP conjugate was added to each well except the blank and mixed tapping the plate gently and incubated for 30 minutes for 37˚C. By the end of the incubation the plate cover was removed and discarded, washed each well 5 times with diluted wash buffer. Each time allowed the micro wells to soaked for 45 seconds, after the five washing 50ul of chromogen A and 50ul of chromogen B solutions were dispensed into each well including the blank and mixed by tapping the plate gently, the plate was incubated at 37˚C for 15 minutes and the reaction was stopped by adding 50ulof stop solution into the each well and mixed gently. The absorbance was measured at 450 nm and calculated the cut-off value and evaluated the result. The absorbance was read within 5 minutes after the stopping the reaction.

3.10.1.2. Interpretation of result

Negative result: sample gave an absorbance less than the cut-off value (??) are considered negative, which indicates no HBsAg has been detected with this HBsAg ELISA kits.

Positive result: sample gave an absorbance greater than the cut-off value (??) are considered initially reactive, which indicates HBsAg has been detected with this HBsAg ELISA kit.

Borderline results: it were reported as negative results.

3.11. Data analysis

Data were computed and analyzed by using Statistical Package for the Social Sciences (SPSS) software program version 16.0. Frequencies were expressed in form of tables and significant differences were determined using Chi-square test at $p\text{-}value \leq 0.05$.

CHAPTER IV

RESULTS

CHAPTER IV

4. RESULTS

4.1. Results

A total of one hundred health care workers (HCWs) in McNimir Hospital were included in this study, in which 38 (38%) were males and 62 (62%) were females (table (4.1)), mostly were at age between 20-30 years (77%) (table (4.2)) and mostly were physicians (25%) and Lab. technologists (22%) as explained in table 4.3.

Among HCWS there were 2 (2%) positive for HBs Ag as illustrated in table (4.4).

There was 2 (2%) females were positive for HBs Ag and all males were negative. There was insignificant association between gender and positivity of HBs Ag as showed in table (4.5).

According to age groups, there was one (1%) between 20-30 years and one (1%) in age range from 51 to 60 years were positive for HBs Ag and there was irrelevant association between age groups and HBs Ag positivity as explained in table (4.6).

There was one (1%) HCW had accidental needle stick and one (1%) didn't expose to any of the possible risk factors were HBs Ag positive and there was meaningless association between sero-positivity of HBsAg and accidental needle stick injury, hemodialysis, previous surgical operation and blood transfusion as explained in table (4.7).

There was 1(1%) nurse and 1 (1%) cleaning staffs were positive for HBsAg while all other HCWs were negative and there was no significant between occupation and HBs Ag positivity as in table (4.8).

Table (4.1): Distribution of HCWs according to gender

Gender	Frequency	Percentage
Male	38	38%
Female	62	62%
Total	100	100%

Table (4.2): Frequency of age groups among HCWs

Age Groups	Frequency	Percentage
20-30 years	77	77%
31-40 years	9	9%
41-50 years	6	6%
51-60 years	8	8%
Total	100	100%

Table (4.3): Distribution of HCWs according to occupation

Occupation	Frequency	Percentage
Lab. technologists	22	22%
Nurses	19	19%
Cleaning staff	19	19%
Physicians	25	25%
Pharmacists	8	8%
Radiologists	7	7%
Total	100	100%

Table (4.4): The sero-positivity of HBs Ag among HCWs

HBs Ag Results	Frequency	Percentage
Positive	2	2%
Negative	98	98%
Total	100	100%

Table (4.5): The association between positivity of HBs Ag and gender among HCWs

Gender	HBs Ag Results		Total	*P. value*
	Positive	Negative		
Males	0 (0%)	38 (38%)	38 (38%)	0.26
Females	2 (2%)	60 (60%)	62 (62%)	
Total	2 (2%)	98 (98.0%)	100 (100.0%)	

Table (4.6): The association between HBs Ag results and age groups among HCWs

Age Groups	HBs Ag Results		Total	*P. value*
	Positive	Negative		
20-30 years	1 (1%)	76 (76%)	77 (77%)	0.172
31-40 years	0 (0%)	9 (9%)	9 (9%)	
41-50 years	0 (0%)	6 (6%)	6 (6%)	
51-60 years	1 (1%)	7 (7%)	8 (8%)	
Total	2 (2%)	98 (98%)	100 (100%)	

Table (4.7): The association between HBs Ag results and possible risk factors among HCWs

Possible Risk factors	HBs Ag results		Total	*P. value*
	Positive	Negative		
Accidental needle stick	1 (1%)	25 (25%)	26 (26%)	0.929
Previous surgical operation	0 (0%)	9 (9%)	9 (9%)	
Hemodialysis	0 (0%)	5 (5%)	5 (5%)	
Blood transfusion	0 (0%)	6 (6%)	6 (6%)	
No exposure to risk factor	1 (1%)	53 (53%)	54 (54%)	
Total	2 (2%)	98 (98%)	100 (100%)	

Table (4.8): The association between HBs Ag results and occupation among HCWs

Occupation	HBs Ag Results		Total	P. value
	Positive	Negative		
Lab. technologists	0 (0%)	22 (22%)	22 (22%)	0.782
Nurses	1 (1.0%)	18 (18%)	19 (19%)	
Cleaning staff	1 (1.0%)	15 (15%)	16 (16%)	
Physicians	0 (0%)	25 (25%)	25 (25%)	
Pharmacists	0 (0%)	8 (8%)	8 (8%)	
Radiologists	0 (0%)	7 (7%)	7 (7%)	
Total	2 (2%)	98 (98%)	100 (100%)	

CHAPTER V

DISCUSION, CONCLOSIONS AND RECOMMENDATIONS

CHAPTER V

5. DISCUSION, CONCLOSIONS AND RECOMMENDATIONS

5.1. Discussion

In this study100 HCWs was investigated for HBs Ag at McNimir Hospital and only 2 (2%) were positive for HBs Ag, which was similar to those reported from Peshawar, Pakistan by Attaullah *et al*. (2011) (2.18%) and Yizengaw *et al*. (2018) (2.6%) in Primary Hospitals of North-West Ethiopia.

The frequency in this study was higher when compared with the previous study conducted at Tertiary Care Center in India which was 0.4% (Singha *et al.*, 2011).

This result was lower than studies done in: Enugu, South-East, Nigeria by Omotowo *et al*. (2018) which was 14.2%, Northern Tanzania by Shao and his colleagues, (2018) (5.7%) and in Khartoum which was 4.4% (Abdalwhab and Nafi, 2014). The differences might be due to the different levels of exposure to potential risks environment and could be due to different in sample size.

In the present study the frequency was more common in females (2(2%)), which was disagreed with the result conducted in Gondar Hospital, Northwest Ethiopia in which the hepatitis B infection was more frequent among males (13/332), while females were 2/332 (Gebremariam *et al*., 2019).

According to age groups, there was 1 (1%) between 20-30 years and 1 (1%) between 51-60 years, where in other study conducted in Gambella Hospital, South Western Ethiopia there were 7.69% between 21- 25 years of age (Tanga *et al*., 2019).

There were no significant association between sero-positivity of HBsAg and accidental needle stick injury, hemodialysis, previous surgical operation and blood transfusion but in study conducted in a tertiary hospital in Tanzania show that some risk factors (needle stick injuries, blood transfusion, operation and i.m./i.v. drug administration) were found to be significantly associated with chronic hepatitis B infection (HBsAg+) (Mueller *et al*., 2015).

In the current study, there was one (1%) nurse and one (1%) cleaning staff were positive for HBs Ag while all other HCWs were negative, that was slightly mismatched to a study conducted in Khartoum in which 1.1% nurse, 1.1% cleaning staffs and 2.2% laboratory technologist were positive for HBs Ag (Abdalwhab and Nafi, 2014).

The frequency of infection was 1 (1%) nurse and 1 (1%) cleaning staff could be justified by the frequent contact of those HCWs with sources of infection (e.g., accidental needle stick injuries), such frequent may happen during or after injection, including recapping contaminated needles and managing infected sharps before and after disposal, contamination of blood during sampling, unsafe sharps waste management.

5.2. Conclusions

The frequency of hepatitis B virus infection among healthcare workers was low.

5.3. Recommendations

- Further studies with large sample size and different laboratory techniques are recommended.
- Special preventive measures (vaccination), which are fundamental way to protect HCWs against HBV infection, should be mandatory for them.
- Continuous medical education to HCWs should be reinforced to improve their knowledge in order to protect them.

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Appendix (1)

Questionnaire

Sudan University of Science and Technology

Frequency of Hepatitis B Virus among Health Care Workers in McNimir Hospital, Shendi Locality

ID. Number: ……..…

Age: ……

Gender: Male ( ) female ( )

Occupation: ……...

Medical history:

Have you taken a sharp instrument? Yes ( ) No ( )

If the answer is yes, what is the procedure used in the hospital to encroach the accident?

.....

Blood transfusion? Yes ( ) No ( )

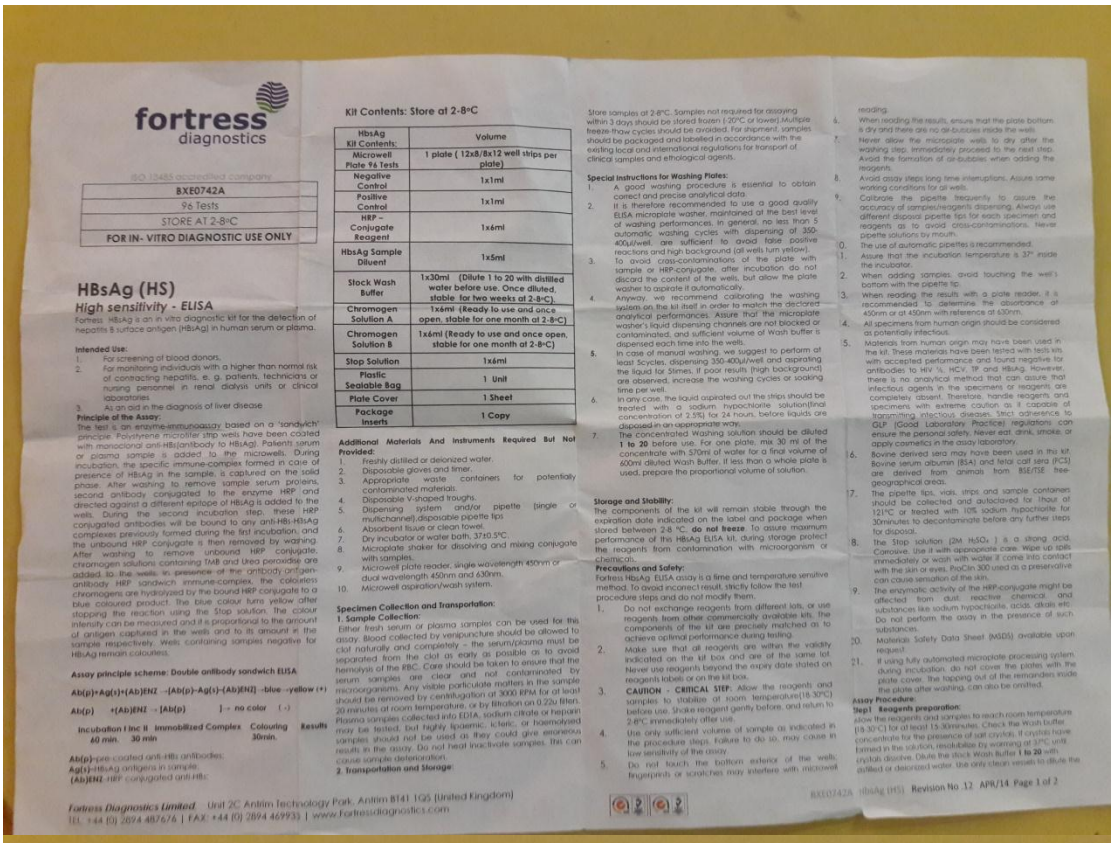
Surgical operation? Yes ( ) No ( )

Hemodialysis? Yes ( ) No ( )

Investigations:

HBsAg: +ve ( ) -ve ( )

Appendix (2)



fortress diagnostics

ISO 13485 accredited company

BXE0742A

96 Tests

STORE AT 2-8°C

FOR IN- VITRO DIAGNOSTIC USE ONLY

HBsAg (HS)

High sensitivity - ELISA

Fortress HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

Intended Use:

1. For screening of blood donors.
2. For monitoring individuals with a higher than normal risk of contracting hepatitis, e. g. patients, technicians or nursing personnel in renal dialysis units or clinical laboratories
3. As an aid in the diagnosis of liver disease

Principle of the Assay:

The test is an enzyme-immunoassay based on a 'sandwich' principle. Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs(antibody to HBsAg). Patients serum or plasma sample is added to the microwells. During incubation, the specific immune-complex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins, second antibody conjugated to the enzyme HRP and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRP conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound HRP conjugate, chromogen solutions containing TMB and Urea peroxide are added. In the wells in presence of the antibody antigen-antibody HRP sandwich immune-complex, the colourless chromogens are hydrolyzed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction using the Stop solution. The colour intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colourless.

Assay principle scheme: Double antibody sandwich ELISA

Ab(p)-Ag(s)-(Ab)ENZ →(Ab(p)-Ag(s)-(Ab)ENZ) -blue →yellow (+)

Ab(p) +(Ab)ENZ → [Ab(p)]→ no color (-)

Incubation I Inc II	Immobilized Complex	Colouring	Results
60 min. 30 min		30min.	

Ab(p)-pre coated anti-HBs antibodies
Ag(s)-HBsAg antigens in sample
(Ab)ENZ-HRP conjugated anti-HBs

Kit Contents: Store at 2-8°C

HbsAg Kit Contents:	Volume
Microwell Plate 96 Tests	1 plate (12x8/8x12 well strips per plate)
Negative Control	1x1ml
Positive Control	1x1ml
HRP – Conjugate Reagent	1x6ml
HbsAg Sample Diluent	1x5ml
Stock Wash Buffer	1x30ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C).
Chromogen Solution A	1x6ml (Ready to use and once open, stable for one month at 2-8°C)
Chromogen Solution B	1x6ml (Ready to use and once open, stable for one month at 2-8°C)
Stop Solution	1x6ml
Plastic Sealable Bag	1 Unit
Plate Cover	1 Sheet
Package Inserts	1 Copy

Additional Materials And Instruments Required But Not Provided:

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel),disposable pipette tips
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microplate shaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

Specimen Collection and Transportation:

1. Sample Collection:

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

2. Transportation and Storage:

Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

Special Instructions for Washing Plates:

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to realize the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispersing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 30 ml of the concentrate with 570ml of water for a final volume of 600ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

Storage and Stability:

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C, **do not freeze**. To assure maximum performance of this HBsAg ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Precautions and Safety:

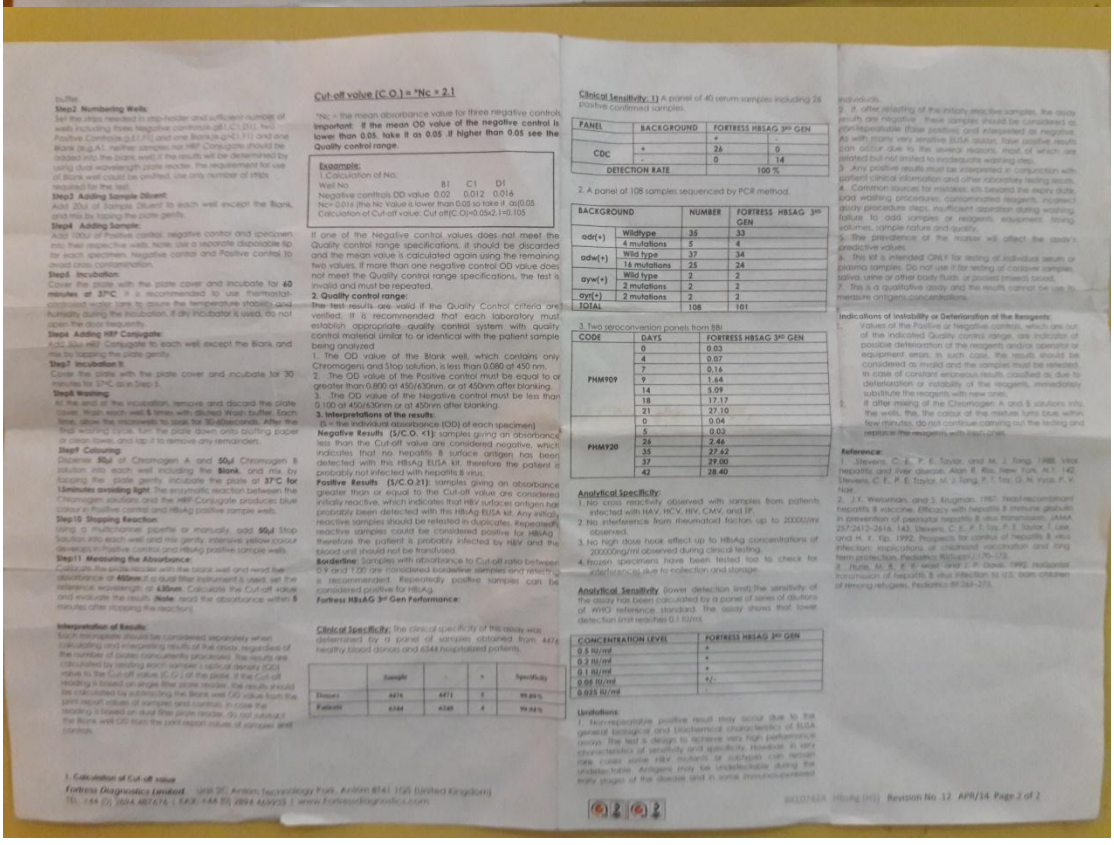
Fortress HbsAg ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits, the components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediatley proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37° inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as it capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in the kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediatley or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

Assay Procedure:

Step1 Reagents preparation:

allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.



Step2 Numbering Wells:

Set the strips needed in strip-holder and sufficient numbers of wells including three Negative controls (B1,C1,D1), two Positive Controls (E1,F1) and one Blanks (G1,H1) and one Blank (A1,A1, neither samples nor HRP Conjugate should be added into the blank well, if the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step3 Adding Sample Diluent:

Add 20ul of Sample Diluent to each well except the Blank, and mix by tapping the plate gently.

Step4 Adding Sample:

Add 100ul of Positive control, negative control and specimen into their respective wells, note: use a separate disposable tip for each specimen, Negative control and Positive control to avoid cross-contamination.

Step5 Incubation:

Cover the plate with the plate cover and incubate for **60 minutes at 37°C**. It is recommended to use thermostat-controlled water bath to assure the temperature stability and humidity during the incubation. If dry INCUbator is used, do not open the door frequently.

Step6 Adding HRP Conjugate:

Add 50ul HRP Conjugate to each well except the Blank and mix by tapping the plate gently.

Step7 Incubation II:

Cover the plate with the plate cover and incubate for 30 minutes at 37°C as in Step 5.

Step8 Washing:

At the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash Buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn the plate down onto soiling paper or clean towel, and tap it to remove any remainders.

Step9 Colouring:

Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue colour in Positive control and HBsAg positive sample wells.

Step10 Stopping Reaction:

Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensive yellow colour develops in Positive control and HBsAg positive sample wells.

Step11 Measuring the Absorbance:

Carefully wipe moisture on the blank well and read the absorbance at **450nm**.If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results (**Note:** read the absorbance within **5** minutes after stopping the reaction).

Interpretation of Results:

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (O.D.) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well O.D. value from the joint report values of samples and controls, in case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the joint report values of samples and controls.

Cut-off value (C.O.) = *Nc × 2.1

*Nc = the mean absorbance value for three negative controls.

Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05 .If higher than 0.05 see the Quality control range.

Example:

1.Calculation of Nc:

Well No:	B1	C1	D1
Negative controls OD value	0.02	0.012	0.016

Nc= 0.016 (the Nc Value is lower than 0.05 so take it as)0.05

Calculation of Cut-off value: Cut off(C.O)=0.05x2.1=0.105

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1): samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit. therefore the patient is probably not infected with hepatitis B virus.

Positive Results (S/C.O.≥1): samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surfaces antigen has probably been detected with this HBsAg ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for HBsAg therefore the patient is probably infected by HBV and the blood unit should not be transfused.

Borderline: Samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

Fortress HBsAG 3rd Gen Performance:

Clinical Specificity: the clinical specificity of this assay was determined by a panel of samples obtained from 4476 healthy blood donors and 4344 hospitalized patients.

Sample	-	+	Specificity
Donors	4476	4471	5	99.89%
Patients	4344	4340	4	99.94%

Clinical Sensitivity: 1) A panel of 40 serum samples including 26 positive confirmed samples.

PANEL	BACKGROUND	FORTRESS HBSAG 3RD GEN	
		+	-
CDC	+	26	0
	-	0	14
DETECTION RATE		100 %	

2. A panel of 108 samples sequenced by PCR method.

BACKGROUND		NUMBER	FORTRESS HBSAG 3RD GEN
adr(+)	Wildtype	35	33
	4 mutations	5	4
adw(+)	Wild type	37	34
	16 mutations	25	24
ayw(+)	Wild type	2	2
	2 mutations	2	2
ayr(+)	2 mutations	2	2
TOTAL		108	101

3. Two seroconvension panels from BBI

CODE	DAYS	FORTRESS HBSAG 3RD GEN
PHM909	0	0.03
	4	0.07
	7	0.16
	9	1.64
	14	5.09
	18	17.17
	21	27.10
PHM920	0	0.04
	5	0.03
	26	2.46
	35	27.62
	37	29.00
	42	28.40

Analytical Specificity:

1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 2000IU/ml observed.
3. No high dose hook effect up to HBsAg concentrations of 2000ng/ml observed during clinical testing.
4. Frozen specimens have been tested too to check for interferences due to collection and storage.

Analytical Sensitivity (lower detection limit):the sensitivity of the assay has been calculated by a panel of series of dilutions of WHO reference standard. The assay shows that lower detection limit reaches 0.1 IU/ml.

CONCENTRATION LEVEL	FORTRESS HBSAG 3RD GEN
0.5 IU/ml	+
0.2 IU/ml	+
0.1 IU/ml	+
0.05 IU/ml	+/-
0.025 IU/ml	-

Limitations:

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristic of ELISA assays. The test is design to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative. These samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing steps.
3. Any positive results must be interpreted in conjunction with patient's clinical information and other laboratory testing results.
4. Common sources for mistaken kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedures steps, inefficient aspiration during washing, failure to add samples or reagents, equipment, wrong volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) bloods.
7. This is a qualitative assay and the results cannot be use to measure antigens concentrations.

Indications of Instability or Deterioration of the Reagents:

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the well, this, the colour of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

Reference:

1. Stevens, C. E., P. E. Taylor, and M. J. Tong. 1985. Viral hepatitis and liver disease. Alan R. Liss, Inc., New York, N.Y. 142.
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2. J.Y. Weissman, and S. Krugman. 1987. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA. 257:2612-2616. 143. Stevens, C. E., P. T. Toy, P. E. Taylor, T. Lee, and H. Y. Yip. 1992. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long term protection. Pediatrics 90(suppl.):170–178.
3. Hulse, M. S., E. Y. Wong, and J. P. Davis. 1982. Horizontal transmission of hepatitis B virus infection to U.S. born children of Hmong refugees. Pediatrics 69:269–273.

1- Calculation of Cut-off Value

Fortress Diagnostics Limited. Unit 2C Antrim Technology Park, Antrim BT41 1QS (United Kingdom)
TEL: +44 (0) 2894 487676 | FAX: +44 (0) 2894 469933 | www.fortressdiagnostics.com