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**Detection of Hepatitis B Surface Antigen among Health Care
Workers in Atabra locality River Nile State**

الكشف عن فيروس التهاب الكبد الوبائي (النوع ب) بين العاملين في مجال الرعاية الصحية في محلية
عطبرة ولاية نهر النيل

**A dissertation submitted in partial fulfillment for the requirements of M.Sc
degree in Medical Laboratory Science (Microbiology)**

By

Einas Mohammed Ahmed Eltayeb Mohammed

(B.Sc in Medical laboratory Science, Shendi University, 2013)

Supervisor

Dr. Leila Mohammed Ahmed

PHD in Microbiology

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الآية

قال تعالى:

﴿ يَا أَيُّهَا النَّاسُ اتَّقُوا رَبَّكُمُ الَّذِي خَلَقَكُمْ مِنْ نَفْسٍ وَاحِدَةٍ وَخَلَقَ مِنْهَا زَوْجَهَا وَبَثَّ مِنْهُمَا رِجَالًا كَثِيرًا وَنِسَاءً وَاتَّقُوا اللَّهَ الَّذِي تَسَاءَلُونَ بِهِ وَالْأَرْحَامَ إِنَّ اللَّهَ كَانَ عَلَيْكُمْ رَقِيبًا ﴾

صدق الله العظيم

سورة النساء الآية (1)



DEDECATION

TO

MY Mother

**A strong and gentle soul who taught me to trust in Allah ,believe in
hard work and that so much could be done with little**

My Father

**For earing an honest living for us and for supporting and
encouraging me to believe in my self**

My sisters

Whom support and love me

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Thanks to the **ALMIGHTY ALLAH** for giving me the strength and patience to complete this work successfully.

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ABSTRACT

Hepatitis B infection is one of the world's major infectious diseases. Health care workers (HCWs) have a high risk of occupational exposure to many blood- borne viruses including HIV, Hepatitis B and Hepatitis C viruses. This cross-sectional study was conducted during period from March to June 2018., to determine the sero-prevalence of Hepatitis B virus (HBsAg) and to determine the possible association between Hepatitis B virus and selected risk factors among health care workers in Atbara Teaching Hospital and selected health centers. Blood samples were obtained from eligible participants, sera separated by centrifugation and structured questionnaire was used to collect both demographic and clinical data. Enzyme Linked Immunosorbent Assay (ELISA) technique was used to detect Hepatitis B surface antigen (HBsAg). Ninety two (n=92) HCWs participated in this study, the males were 47/92 (51.1%) and females 45/92 (48.9%), 23/92 (25%) were vaccinated and 69/92 (75%) were not vaccinated. The sero-positivity was higher in female than male, and the infection according to occupational practice was higher among laboratory technologist 3/(3.3%) followed by cleaning staff 3/(3.3%) and Nurses 2/(2.2%). Three of the vaccinated participants were infected and the disease was more frequent in those who were exposed to an accidental injury during their work (4 out of 8 infected participants).

ملخص البحث:

عدوي الكبد الوبائي هي احد الأمراض المعدية الرئيسية في العالم. ويتعرض العاملون في مجال الرعاية الصحية لخطر كبير من التعرض المهني للإصابة من الفيروسات المنقولة عن طريق الدم فيما ذلك فيروس نقص المناعة المكتسبة، فيروس الكبد (ج) وفيروس الكبد الوبائي(ب). هذه الدراسة مقطعية أجريت في الفترة من شهر مارس إلى يونيو 2018م لتحديد الانتشار المصلي لفيروس التهاب الكبد الوبائي ولتحديد احتمالية الارتباط بين فيروس التهاب الكبد الوبائي و عوامل الخطر المحددة للعاملين بالرعاية الصحية الأولية بمستشفى عطبرة التعليمي والمراكز الصحية المختارة. جمعت عينات الدم من المتطوعين المستهدفين وفصلت بجهاز الطرد المركزي وجمعت معلومات بواسطة استبيان منتظم لجمع البيانات الديموغرافية والسريية معاً، استخدمت تقنية اليزا للكشف عن مستضد الكبد السطحي. اثنان وتسعون من العاملين في مجال الرعاية الصحية شاركوا في هذه الدراسة . وكان عدد الذكور 47 والإناث 45 ،مجموعهم 92. 23 منهم تم تطعيمهم و69 لم يتم تطعيمهم . وكان من المستغرب أن 3 من المشاركين الذين تم تطعيمهم كانوا مصابين . كانت ايجابية المصل اعلي في الإناث من الذكور. وجدت زيادة العدوى وفقا للممارسة المهنية 3من بين اختصاصي المختبرات الطبية ،3 من موظفي التنظيف و 2 من الممرضين .كما كان المرض أكثر انتشارا في أولئك الذين تعرضوا للوخز بالإبر أثناء فترة العمل وكان عدد المصابين 4.

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LIST OF ABBREVIATIONS

Abbreviations	Full name
ALT	Alanine Transaminase
CDC	Center for Disease Control
CCCDNA	Covalently closed circular Deoxyribo Nucleic Acid
C.O	Cut-Off
DNA	Deoxyribo-Nucleic Acid
ELISA	Enzyme Linked Immuno sorbent assay
EIA	Enzyme Immune Assay
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B envelope antigen
HBIG	Hepatitis B immunoglobulin
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	HepatoCellular Carcinoma
HCV	Hepatitis C virus
HCW	Health Care Workers
HDV	Hepatitis Delta Virus
HRP	HorseRadish Peroxidase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NC	Negative Control
OD	Optical Density
PCR	Polymerase Chain Reaction
RNA	RiboNucleic Acid
SPSS	Statistical Package for the Social Sciences
TMB	TetraMethylBenzidine
WHO	World Health Organization

Chapter One

Introduction

Rationale

Objectives

Chapter one

INTRODUCTION

1.1. Introduction

Hepatitis B viral infection is a major global health problem with predilection for the liver and is known to commonly lead to chronic infection after acute infection. The chronic infections increases risk of death from childhood hepatic failure, cirrhosis of the liver and liver cancer (Shepard *et al.*, 2006; Mustaphas *et al.*, 2007). The earliest recognition of the public health importance of hepatitis B virus infection is thought to have occurred when it appeared as an adverse event associated with a vaccination campaign (WHO, 2011). More than 300 million people have chronic liver infections globally and about 600,000 people die annually from acute or chronic complications of hepatitis B infection. The highest prevalence of hepatitis B infection is in sub-Saharan Africa and East Asia, majority of the people in these regions become infected during childhood and between 5–10% of the adult population is chronically infected (WHO, 2009). Approximately three million health care workers (HCW) are exposed to percutaneous blood – borne viruses each year. It is estimated that 66000 hepatitis B virus (HBV) are acquired annually (Kermode *et al.*, 2005). The infections are important risk factors for hepatocellular carcinoma and other liver related morbidity (Omer *et al.*, 2001). The HBV carrier rate varies widely from 0.01% to 20% in different geographical regions of the world. The HCW including clinicians, nurses, laboratory technicians, other hospital technicians, administration and cleaning staff are exposed to an increased risk of occupational infection with HBV (Tarantola *et al.*, 2006). 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. HCWs might include physicians, nurses, nursing assistants, therapists, technicians, emergency medical service personnel, dental personnel,

pharmacists, laboratory personnel, autopsy personnel, students and trainees, contractual staff not employed by the healthcare facility, and persons (e.g. clerical, dietary, housekeeping, laundry, security, maintenance, administrative, billing and volunteers) not directly involved in patient care but potentially exposed to infectious agents that can be transmitted to and from HCWs and patients (CDC, 2011). While performing their duties, healthcare workers (HCWs) are frequently exposed to dangerous infectious agents. The risk of transmission of vaccine-preventable infections, both from patients to HCWs and from personnel to patients, other HCWs, and visitors is substantial (Almuneef *et al.*, 2006). Health care workers are at a high risk of exposure to blood and body fluids. Needle stick injuries, cuts and splashes are common occupational accidents exposing health care providers to different blood borne pathogens. Transmission of hepatitis B virus, human immune deficiency virus (HIV), and hepatitis C virus (HCV) has been related to injuries and frequency of exposure. According to world health organization (WHO), 2.5% of HIV cases, 40% of both and HCV cases worldwide are the result of occupational exposure among health care workers (CDC, 1999). Adherence to standard precautions, awareness about post exposure prophylaxis is poor in developing countries among HCWs and documentation of exposures is suboptimal (WHO, 2002). Healthcare workers have been historically recognized as being at increased risk of HBV infection, effective vaccines are available to prevent HBV infection and universal immunization programs are now advocated, needle stick injuries are one of the most efficient modes of HBV transmission, most transmission in the healthcare setting probably occurs in the absence of a documented percutaneous injury, there is evidence from a Cochrane Library systematic review to support occupational health guidelines that all healthcare workers should be offered HBV vaccination and that the vaccine is safe (Jefferson *et al.*, 2003). Healthcare workers who have not been immunized, HBIG and HBV vaccine are recommended after a significant exposure. Although the effectiveness of HBIG and HBV vaccine has not been evaluated in the occupational health setting, the

increased efficacy of this combination compared with HBIG alone in preventing prenatal transmission is presumed to apply to the occupational health setting (Beasley *et al.*, 1983).

1.2. Rationale

Health care workers have a high risk of occupational exposure to many blood borne viruses, hepatitis B virus is a major health problem and causes significant morbidity and mortality rate. the observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary to transmit the disease. 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 represent a source of infection. Furthermore, the proper understanding of the prevalence in study area may help in setting further control programs. Hepatitis B exposure was assessed in 311 health care workers in Uganda highly endemic country .60.1 % of health care workers have evidence of hepatitis B infection ,with 8.7 % being chronic carrier and one (0.3%) acutely infected ,needle stick injuries reported by 77% of HCW.

In Tanzania ,the prevalence of acute or chronic HBV infection in HCW was found to be 8.8%

The evidence of HBsAg among health care workers in wad madani hospital was 22% .The aim of this study was to determine the prevalence of HBsAg among health care workers in Atbara locality.

1.3 Objectives

1.3.1 General objective

To detect hepatitis B surface antigen (HBsAg) among healthcare workers in Atbara locality.

1.3.2 Specific objectives

1. To detect hepatitis B surface antigen (HBsAg) among health workers in Atbara locality by using ELISA technique.
2. To correlate the possible association between hepatitis B virus and selected risk factors.

Chapter Tow

Literature review

CHAPTER TWO

LITERATURE REVIEW

2.1 HBV properties

The hepadna viruses got their name because they cause hepatitis and they have DNA genomes. They are known as hepatitis B viruses (HBVs) and are classified in the family Hepadnaviridae. Some members infect mammals and some infect birds; examples include woodchuck HBV and heron HBV. The best known hepadnavirus that which infects humans; it is commonly referred to as HBV, and is of major importance as an agent of disease and death. Duck HBV, on the other hand, is non-pathogenic in its natural host (Carter and Saunders, 2007). Hepatitis B virus is a member of the hepadnavirus family, it is a 42, nm enveloped virion with icosahedral nucleocapsid core containing partially double strand circular DNA genome (Levinson,2014).

2.1.1 Genome

Hepatitis B virus is a small DNA virus and belong to a group of hepatotropic DNA viruses (hepadnaviruses). The virus consists of nucleocapsid and an outer envelope composed mainly of three antigens (HBs Ag that play a central role in the diagnosis of HBV infection). The nucleocapsid contains HBc Ag, a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins (Setout *al.*, 2011). The genome is made up of two strands of DNA, one of which is incomplete; hence the DNA is partly single stranded and partly double-stranded. A short sequence is triple-stranded as a result of a complementary sequence at the 5' ends, and this results in the DNA having a circular conformation, the genome is very small, with a length of about 3.2 kb (p). At the 5' end of each of the DNA strands there is a covalently linked molecule: a capped RNA on the short strand and a protein (P) on the long strand. (Carter and Saunders 2007). Three types of viral particles can be visualized in the infectious serum by electron microscopy: the infectious virions and the sub viral particles. The infectious virus particles are the so-called Dane particles (Dane *et al.*, 1970), have a spherical, double shelled structure of 42-44 nm containing a

single copy of the viral DNA genome, covalently linked to the terminal protein of the virus. A hallmark of HBV infection is the presence of two additional types of particles, the spheres and the filaments, which are exclusively composed of hepatitis B surface proteins and host-derived lipids (Glebe *et al.*, 2007). Since they do not contain viral nucleic acids, the sub viral particles are non-infectious. The spherical structures measure around 22 nm in diameter, while the filaments are of similar width, but of variable lengths. The viral membrane contains three viral surface proteins and is acquired by the virus during budding into the endoplasmic reticulum, whereas the viral particles are transported via the secretory pathways through the ER and Golgi. The surface proteins are named the preS1 (or large), the preS2 (or middle) and the S (or small), which correspond to the HBsAg. As with nearly all enveloped viruses, the HBV particle also contains proteins of host origin (Glebe, 2007; Glebe and Urban, 2010). The HBV genome consists of a partially double-stranded relaxed circular DNA of approximately 3200 nucleotides in length, varying slightly from genotype to genotype, that in concert with the core protein (HBcAg) forms the nucleocapsids (Nassal *et al.*, 2008). Within the Dane particle the negative strand of the viral DNA is present in full-length, carrying the complete genetic information. In contrast, the positive strand spans only $\sim 2/3$ of the genome in length, whilst its 3' end is variable in size (Summers *et al.*, 1988). The viral polymerase is covalently bound to the negative strand by a phosphotyrosine bond. At the 5' end of the positive strand a short RNA oligomer originating from the pre-genomic RNA residually remains bound covalently after the viral DNA synthesis. The negative strand also contains small redundancy of 8-9 nucleotides in length on both the 5' end and the 3' end, named the R region. These redundant structures are essential for viral replication (Seeger, 1986; Nassal, 2008).

2.2. Replication:

Hepatocytes (liver cells) are the host cells for having the body. 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 virions. Some cell lines, however, can be infected using HBV DNA (a procedure known as transfection) (Carter and Saunders, 2007). The life cycle of the HBV is complex. Hepatitis B is one of the few known non retroviral viruses which used reverse transcription as a part of its replication process. The virus gain entry in to the cell by binding to an unknown receptor on the surface of the hepatocytes and enter it by endocytosis. Because virus multiplies via RNA made by host enzyme, the viral genomic DNA has to be transformed to the cell nucleus by host protein called chaperones. The partially double stranded viral DNA is then made fully double stranded and transform in covalently closed circular DNA (cccDNA) that serves as template, for transcription of four viral mRNAs. The largest mRNA, (which is larger than the viral genome), is used 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 p protein synthesized DNA via its reverse transcriptase activity (Levinson, 2014). Hepatocytes (liver cells) are the host cells for HBV in the body. 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 virions. Some cell lines, however, can be infected using HBV DNA(a procedure known as transfection), (Carter and Saunders, 2007).

2.3 HBV Transmission

The three main modes of transmission are via blood, during sexual intercourse, and perinatally from mother to newborn (Levenson, 2014).

2.3.1 Risk groups for hepatitis B in developed Countries

Intravenous drug abusers, homosexual men, sexual contacts of antigen-positive persons, residents in long-stay homes for mentally handicapped People, renal dialysis patients, recipients of multiple blood products (e.g. haemophiliacs), surgeons, dentists and morticians, and infants of infectious HBsAg positive mothers, (Bannister *et al.*, 2006).

2.4 HBV Genotype and Its Clinical Significance

Based on an intergroup divergence of 8% or more of the complete genomes, HBV can be classified in to 7 genotypes, i.e. A-G (Okamoto *et al.*,1988; Norder *et al.*,1992). Genotype H was recently identified in central America (Arauz-Ruiz *et al.*,2002), is well known that HBV genotypes have distinct geographical distributions. The geographical distributions of HBV genotypes are summarized in Table 2. The prevalent HBV strains in China are genotype B and C (Zhu *et al.*,1999). but the two genotypes distribute unevenly in China. We studied 1096 Chinese chronic HBV carriers from 9 provinces in Mainland China. Four major genotypes A, B, C and D were found and their prevalence were 1.2%,41%, 52.5% and 4.3%, respectively. In northern China, genotype C is predominant(85.1%), while in southern China, genotype B is predominant (55.0%).Genotypes A and D are also found in other areas of China. However, the genotypes E-H have not been reported in China. Recently, genotype C/D hybrid was identified in Tibet (Cui *et al.*,2002) and genotype B was found recombined with pre C/C region of genotype C in China (Luo *et al.*, 2004). Accumulated data suggest the importance of genotype, subgroup and recombination that may influence the biological characteristics of virus and clinical outcome of HBV infection. Several studies reported a correlation of HBV genotypes with HBeAg clearance, liver damage, and the response to IFN treatment. It was reported that HBeAg carrier status tends to be longer and the prevalence of HBeAg appears higher in patients with genotype C than with genotype B (Orito *et al.*, 2001). HBV carriers with genotype B have lower histological activity scores and genotype C is more prevalence in patients with

cirrhosis (*Kao et al.*, 2000). Furthermore, a retrospective study showed that HBV genotype B is associated with a higher rate of IFN-induced HBeAg clearance compared with genotype C (*Kao et al.*, 2000). However, whether patients with genotype B differ from those with genotype C in development of hepatocellular carcinoma remains controversial. The response of different HBV genotypes to interferon-Alfa treatment is of increasing interest because the benefit of interferon-Alfa or its pegylated form in combination with other antiviral agents is being explored in the treatment of chronic hepatitis B. In a homogeneous group of prospectively followed patients from Europe, a recent study demonstrates that genotype A responds better than other HBV genotypes to standard interferon therapy and represents an independent predictor of a therapeutic success, with a greater impact than other pre-treatment characteristics, such as HBV DNA or ALT levels (*Houet et al.*, 2000).

2.5 Epidemiology

There are around 350 million chronic carriers of the hepatitis B virus worldwide. The incidence of acute disease and prevalence of carriage varies considerably from country to country. In parts of south-east Asia, 10–20% of the population may be carriers, whereas most countries in Europe and North America have carriage rates below 2%. Where carriage rates are high, acute infection occurs mainly in infants and young children, mostly via intra partum and horizontal transmission within households. Skin disease and biting arthropods may facilitate the transfer of body fluids from person to person. In low-prevalence countries most infections are sporadic and arise in adults through needle stick injuries, shared syringes, bites and scratches, or by sexual contact.

Those most at risk include intravenous drug abusers, homosexual men, residents and staff of institutions for the mentally handicapped, surgeons, dentists, laboratory workers, morticians, renal dialysis patients and recipients of unscreened blood and blood products (*Bannister et al.*, 2006). Hepatitis B is highly endemic in developing regions with large population such as South East

Asia, China, sub-Saharan Africa and the Amazon Basin, where at least 8% of the population are HBV chronic carrier. In these areas, 70–95% of the population shows past or present serological evidence of HBV infection. Most infections occur during infancy or childhood. Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the rates of chronic liver disease and liver cancer in adults are high (alter,2003)Hepatitis B virus is spread from person to person primarily by blood and blood products. Blood transfusion remains a major mode of transmission in the United States; however, screening of donors has reduced the risk to 1 in 63,000 transfusions. Screening tests fail to exclude small percentage of donors who have infectious viral particles in their blood despite being negative for HBsAg. Hepatitis B virus is also found in other body fluids, including urine, bile, saliva, semen, breast milk, and vaginal secretions. It is not found in feces, however. Membrane contact with any of these body fluids can result in transmission. The virus can be spread to sexual partners, and its prevalent in homosexual men and heterosexuals with multiple partners. It can be readily spread from mother to neonate at the time of vaginal delivery—a common mode of transmission in developing countries. Intravenous drug abusers have a high incidence of hepatitis B. Reuse of needles has also led to transmission of the virus during placement of tattoos and ear-piercing. Crowded environments such as institutions for the mentally handicapped, (Frederick and Southwick, 2007).

2.6 Pathogenesis and immunity

After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens, and inflammation and necrosis occur. Immune attack against viral antigens on infected hepatocytes is mediated by cytotoxic T cells. The pathogenesis of hepatitis B is probably 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), (Levenson,2014). Fully differentiated hepatocytes are the primary cell type infected by HBV. The primary cause of hepatic cell destruction appears to be the cell-mediated immune response, which results in inflammation and necrosis. The cells involved are cytotoxic T cells, which react specifically with the fragments of nucleocapsid proteins (HBcAg and HBeAg), expressed on the surface of infected hepatocytes. This response also contributes to control of the infection by eliminating virus-producing cells. Enhanced natural killer cell activity, as well as production of interferon- γ also contributes to limiting the extent of infection. Anti-HBsAg antibody, which is the neutralizing antibody, does not appear until well into the convalescence period, when it may aid in clearing any remaining circulating free virus (Harvey., Cornelissen. and Fisher, 2007).

2.7 Clinical Presentation/Natural History

2.7.1 Acute Infection

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

- HBsAg appears before the onset of symptoms, peaks during overt disease, and then declines to undetectable levels in 3 to 6 months.
- 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. Anti-HBs may persist for life, conferring immunity; this is the basis for current vaccination strategies using noninfectious. HBsAgHBeAg, 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.

IgM anti-HBc becomes detectable in serum shortly before the onset of symptoms, concurrent with elevation of serum aminotransferase levels (indicative of hepatocytolysis). Over a period of months, the IgM anti-HBc antibody is replaced by IgG anti-HBc. As in the case of anti-HAV, there is no specific assay for IgG anti-HBc, but its presence is inferred from decline of IgM anti-HBc in the face of rising levels of total anti-HB (Kumar *et al.*, 2013). Initial infection with hepatitis B virus (HBV) may be asymptomatic in up to 50 per cent of adults and 90 per cent of children. When symptoms occur, they may include anorexia, vague abdominal pain, nausea, vomiting and jaundice. Fever may be absent or mild (Heymann, 2008). Extra hepatic manifestations such as arthralgias, arthritis, macular rashes, thrombocytopenia or papular acrodermatitis (Gianotti-Crosti syndrome) can occur early in the course of the illness and may precede jaundice. Acute HBV infection cannot be distinguished from other forms of acute viral hepatitis on the basis of clinical signs and symptoms or nonspecific laboratory findings (American Academy of Pediatrics, 2012).

2.7.2 Chronic Infection

While the majority of individuals infected with HBV are able to clear the virus, some individuals fail to mount an adequate immune response, leading to chronic infection (Conly and Johnston, 2007). The exact mechanisms by which chronic liver injury occurs in HBV infection are not known (Koziel and Siddiqui, 2010). Hepatitis B virus infection becomes chronic in approximately 90 per cent of infants infected at birth (American Academy of Pediatrics, 2012). If chronic infection is established, the spectrum of illness ranges from the healthy carrier state to all of the sequelae of chronic hepatitis, including mild to moderate fibrosis, compensated cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC). The single most important risk factor for HCC is cirrhosis (Pungpapong and Poterucha, 2007). Individuals who are immunosuppressed or have an underlying chronic illness are at increased risk of developing chronic infection (Heymann, 2008; American

Academy of Pediatrics, 2012). Factors that may influence the natural history of chronic infection include gender, race, alcohol use, and co-infection with hepatitis A, hepatitis C or hepatitis D viruses or human immunodeficiency virus (HIV) (American Academy of Pediatrics, 2012). Antiviral therapy can modify the natural history of chronic HBV infection (Yimand, 2006). Super infection or co-infection is not uncommon in patients with chronic HBV infection. Acute hepatitis delta virus (HDV) may be acquired as co-infection simultaneously with HBV or as a super infection in a patient who is already a carrier of HBV (Sherman *et al.*, 2007). Infection with HDV in HBV infected individuals is associated with more severe and/or progressive liver disease than is HBV mono infection. The natural course following acute hepatitis C virus (HCV) super infection has not been well studied. The long-term prognosis following acute HCV super infection is worse than that following HDV super infection (Liaw *et al.*, 2004). Individuals co-infected with the parasite *Schistosoma* (Schistosomiasis) are more likely to have more severe hepatitis B manifestations and become chronic carriers of HBV (Plourde, 2008).

2.8 HBV and Hepatocellular Carcinoma :

Epidemiologic studies have demonstrated that there is a consistent and specific causal association between HBV infection and HCC (Beasley *et al.* 1981; Chen *et al.* 1996). In patients with persistent HBV infection, the risk of HCC was 100 times higher than in non-infected individuals (Beasley *et al.* 1981). The global distribution of hepatocellular carcinoma correlates with the geographic prevalence of chronic carriers of HBV, who number 400 million worldwide. The highest rates are in Southeast Asia and sub-Saharan Africa, with the HCC incidence >50/100,000 populations (Bosch *et al.*, 1999). Virological factors in the pathogenesis of hepatocellular carcinoma have recently been defined. Both retrospective and prospective studies strongly supported the relation between positive HBeAg and the risk of HCC (Lin *et al.*, 1991). A prospective study in Taiwan (Yang *et al.*, 2000) showed that relative risk of HCC among men who were positive for both HBsAg and HBeAg were much higher than that among

men who were positive for HBsAg alone . HBV DNA was identified as the most important predictor of the development of hepatocellular carcinoma in HBsAg-positive patients with different clinical conditions, therefore, efforts at eradicating or reducing the viral load may reduce the risk for HCC. Additionally, HBV genotype might play a role in the development of HCC. The data from Taiwan showed that genotype C is associated with more severe liver disease including cirrhosis and hepatocellular carcinoma (HCC), whereas genotype B is associated with the development of HCC in young non cirrhotic patients (Ishikawa *et al.*, 2001; Ikeda *et al.*, 2003; Ohata *et al.*, 2004).

2.9 Occult Hepatitis B

Occult hepatitis B is defined by the presence of HBV DNA in serum or liver in the absence of HBsAg (Hou *et al.*, 2001; Hu *et al.*, 2002). Serum HBV level is usually less than 10⁴ copies/ml. Although occult HBV infection has been identified in patients with chronic liver disease two decades ago (Brechot *et al.*, 1985), its precise prevalence remains to be defined. Occult HBV infection has been found in patients with HCC, past HBV infection, or chronic hepatitis C, and individuals without HBV serological markers. The frequency of the diagnosis depends on the relative sensitivity of HBV DNA assays and the prevalence of HBV infection in the population. Collectively, around 30% to 35% of HBsAg-negative subjects with chronic hepatitis with or without HCC have positive serum HBV DNA (range from 5% to 55%). The prevalence of HBV DNA is higher in anti-HBc positive, but anti-HBs-negative patients, ranging from 7% to 60% in populations highly exposed to HBV. HBV DNA is much less frequently identified in HBsAg-negative patients with acute, and particularly fulminant hepatitis at around 10% and 7% in serum and liver samples (Brechot *et al.*, 2001). Viral DNA persistence is not, however, restricted to patients with liver disease and may be observed in subjects with normal liver parameters, including blood and/or organ donors. Overall, occult HBV infection is seen in 7%-13% of anti-HBc-positive and/or anti-HBs-positive subjects, and in 0% to 17% of blood donors. The clinical significance of occult HBV infection

remains unclear. Occult HBV infection represents a potential transmission source of HBV via blood transfusion or organ transplantation. In addition, occult HBV infection has been associated with cryptogenic chronic hepatitis and hepatocellular carcinoma. Furthermore, some studies suggested that occult hepatitis B might affect responsiveness of chronic hepatitis C to interferon therapy and disease progress (Brechot *et al.*, 2001).

2.10 Laboratory Diagnosis

2.10.1 Serologic and Virologic Markers

The two most important serologic tests for the diagnosis of early hepatitis B are the tests for HBsAg and for IgM antibody to the core antigen. Both appear in the serum early in the disease (Levenson, 2014). After a person is infected with HBV, the first virologic marker detectable in serum within 1–12 weeks, usually between 8 and 12 weeks, is HBsAg (Dan and Fauci, 2010). Both acutely and chronically infected individuals have HBs antigenaemia. The diagnosis of acute disease is confirmed by demonstrating IgM anti-HBc in the serum. This appears 2 weeks after HBsAg, and disappears a few months after uncomplicated infection. IgG anti-HBc persists probably lifelong, and is a marker of previous infection. The stage of evolution of antigenaemia and antibody production is determined by EIA tests. Viral persistence can be confirmed by PCR-based detection of HBV DNA in serum. Detection of HBe is still used as a marker of enhanced infectivity and risk of chronic liver disease. (Bannister *et al.*, 2006).

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

The HBsAg test was the first available for detecting hepatitis B. HBsAg appears in serum within 1 to 10 weeks after exposure; its disappearance within 4 to 6 months indicates recovery. The persistence of HBsAg beyond 6 months indicates chronic disease. The disappearance of HBsAg may be preceded by the appearance of anti-HBs, and during this period, patients may develop a serum sickness-like illness. In a large percentage of patients, anti-HBs does not rise to detectable levels for several weeks to months after the disappearance of HBsAg.

During this window HBsAg and anti-HBs are both negative, and if these two tests alone are used for screening blood donors, a small percentage of infected donors may be missed. To prevent this occurrence, blood banks also test for IgM antibody directed against HBcAg. Anti-HBs rises slowly over 6 to 12 months and usually persists for life, providing protection against re-infection.

2.10.1.2 Antibody directed against the core antigen (anti-HBc)

HBcAg is detected in infected hepatocytes, but is not released into serum; however, IgM antibody directed against HBcAg (anti-HBc) is usually the earliest anti-hepatitis B antibody detected in the infected patient. The IgM anti-HBc is usually interpreted as a marker for early acute disease; however, in some patients, anti-HBcIgM 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-HBcIgM 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 IgG antibodies directed against the core antigen develop in the later phases of acute disease and usually persist for life.

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

Naked DNA strands and associated proteins make up HBeAg. The presence of HBeAg in serum indicates active viral replication, and it persists in patients with chronic disease, its presence correlating with infectivity. As the patient with acute hepatitis B recovers, HBeAg disappears, and anti-HBe appears. Seroconversion from HBeAg to anti-HBe usually corresponds with the disappearance of hepatitis B virus DNA from the serum.

2.10.3 Polymerase chain reaction (PCR) test

It is based on the use of DNA fragment called the gene probe (39). Gene probe is relatively small, single stranded DNA segment that can hunt for complementary fragment of DNA (Mumtaz *et al*, 2011). To use a gene probe effectively, it is valuable to increase the DNA to be searched. The polymerase chain reaction (PCR) accomplishes this task (Pommerville, 2004).

2.10.3.1 Hepatitis B viral DNA (HBV-DNA)

Quantization 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. In patients with fulminant hepatitis, assays for HBV-DNA has been positive in the absence of other positive markers for HBV(Frederick and Southwick, 2007).

2.11 Treatment

No antiviral therapy is typically used in acute hepatitis B. For chronic hepatitis B, entecavir (Baraclude) or tenofovir (Viread) are the drugs of choice. They are nucleoside analogues that inhibit the reverse transcriptase of HBV. Interferon in the form of peginterferon alfa-2a (Pegasys) is also used. Other nucleoside analogues such as lamivudine (Epivir-HBV), adefovir (Hepsera), and telbivudine (Tyzeka) are used less frequently. A combination of tenofovir and emtricitabine (Emtriva) is also used (Levenson, 2014).

2.11.1 Drugs active against HBV

Lamivudine, 100 mg daily, orally (also used for HIV), adefovir dipivoxil, 10 mg daily, orally, tenofovir (used for HBV/HIV co-infected patients), alternative: interferon alpha, 5–10 MIU three times weekly, subcutaneously for 6 months. If an antiviral drug effective against HBV is also being used to treat HIV co-infection, the HIV-treatment dose should be given (this is often higher than the dose for HB) (Bannister *et al.*, 2006).

2.12 Prevention

Prevention involves the use of either the vaccine or hyperimmune globulin or both (Levenson, 2014).

2.12.1 Passive Immunoprophylaxis

Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb. It is used to provide immediate, passive protection to individuals known to be exposed to HBsAg-positive blood (e.g., after an accidental needle-stick injury) (Levenson, 2014). Immunoprophylaxis is recommended for all infants born to

HBsAg positive mothers. Current dosing recommendations are 0.13ml/kg HBIG immediately after delivery or within 12 hours after birth in combination with recombinant vaccine. The combination results in a higher-than-90% level of protection against perinatal acquisition of HBV (Stevens., Taylor. And Tong. 1987). Between 3.7% to 9.9% of infants still acquire HBV infection perinatally from HBV infection mothers, despite immunoprophylaxis. Failure of passive and active immunoprophylaxis in this setting may be the result of in utero transmission of HBV infection, prenatal transmission related to a high inoculum, and/or the presence of surface gene escape mutants. To study the interruptive effect of HBIG before delivery in attempt to prevent intrauterine transmission of HBV, a large-scale, random-control study was conducted in China (Zhu *et al.*, 2003). In this study, nine hundred and eighty HBsAg carrier pregnant women were randomly divided into HBIG group and control group. Each subject in the HBIG group received 200 IU or 400 IU of HBIG intramuscularly at 3, 2 and 1 months before delivery, in addition to newborns receiving HBIG intramuscularly. By this way, the rate of intrauterine transmission in this group fell to 5.7%, compared to 14.3% in control group. ($P < 0.001$). However, the preventive effect of HBIG administration before delivery needs to be confirmed by more study in the future. Hepatitis B immune globulin remains a central component of prophylaxis in HBV-infected patients undergoing liver transplantation. HBIG monotherapy given at a high dosage can prevent recurrence in 65% to 80% of patients. Because the cost of long-term prophylaxis with high-dose HBIG is extremely high and combination therapy using HBIG with a nucleoside analog is more uniformly effective, the current protocol is combination HBIG with a nucleoside analog after liver transplantation. These combination protocols have reduced the rate of virologic breakthrough to 10% or less (Terrault and Vyas, 2003).

2.12.2 Active Immunization

Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and its subsequent complications. The first-generation hepatitis B vaccine, an inactive plasma-derived vaccine, became available in 1982. Consequently, the second generation of HB vaccine, a DNA recombinant HB vaccine was also available for general use in 1986. Both of the vaccines were proven to be safe and efficacious in preventing HBV infection. (WHO) recommended that hepatitis B vaccination should be included in national immunization system in all countries with a hepatitis B carrier prevalence (HBsAg). By May 2002, 154 countries had routine infant immunization with hepatitis B vaccine (Lavanchy, 2004). The world's first universal vaccination program for HBV infection was launched in 1984 in Taiwan (Ni *et al.* 2001). During the first 2 years of the program, coverage was provided mainly for infants whose mothers were carriers of HBsAg. Vaccination was subsequently extended, first to all newborns and then to unvaccinated preschool-age and elementary school-age children. Since 1991, catch-up vaccinations have been given to children in the first grade. This program reduced the overall HBsAg prevalence rate from 9.8% in 1984 to 1.3% in 1994 among children <15 years of age. The HBV carrier population was further reduced through improved maternal screening (Chen *et al.*, 1996). In 1999, vaccination rates were 80–86% for young children and higher than 90% for older children; the prevalence of HBsAg was reduced to 0.7% for children younger than 15 years of age (Ni *et al.*, 2001). To evaluate the long-term efficacy of hepatitis B (HB) vaccination in newborns, one of the longest HB vaccine follow-up studies in the world was conducted in Shanghai, China (Zhou *et al.*, 2003). Children who were born in 1986 and immunized with hepatitis B vaccine at birth were followed up at least once a year. Serum HBsAg, anti-HBc and anti-HBs were tested. The positive rates of HBsAg in the vaccine group with the period of 16 years were 0.46%-0.97%, the average being 0.61%, which was much lower than those of baseline before vaccination and external control.

Chapter Three

Materials and Methods

The long-term efficacy of newborn vaccination was 85.42%. In countries such as Italy and the United States, the incidence of acute hepatitis B has declined dramatically during the past decade after vaccination program for HBV infection, particularly among persons in younger age group (Da villa, 2000). Universal HB vaccination was proven to be effective in the prevention of HCC in several large cohort studies in Southeast Asia (Chang *et al.*, 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This study was a descriptive cross-sectional study.

3.2. Study area:

This study was conducted in Atabra locality, is on the River Nile in Sudan, about 300 km from Khartoum.

3.3 Study duration

The study was carried out between March to June 2018.

3.4. Study population

Health care workers including (Laboratory technologist, Nurses , Pharmacist and Cleaning staff).

3.5. Sample size

Ninety-two(n=92) health care workers were recruited for this study .

3.6 Ethical consideration

Ethical approval to conduct this study in the region was obtained from the Health Services Director in Atabra locality and verbal consent was obtained from participants before collection of the blood samples.

3.7. Data collection

A structured questionnaire was used to collect demographic and clinical data.

3.8 Collection of blood specimens

Under sterile condition Five ml of venous blood sample was withdrawn from each participant, then waited until sample clotted the serum was separated by centrifugation at 5000 rpm for five minutes, serum was separated into plain vacutainers then stored at -20C° until used.

3.9 Laboratory investigation

HBV surface antigen (HBsAg) was screened by HBsAg (high sensitivity) - ELISA Kit.

3.9.1 ELISA technique:

Method: ELISA (Enzyme linked immune sorbent assay)

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

For screening of blood donors

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 as an aid the diagnosis of liver disease

3.9.1.1. Principle

The test is an enzyme linked immune sorbent assay based on sandwich principle. Polystyrene microtiter wells have been coated with a monoclonal HB antibodies to HBsAg patterns serum or plasma sample is added to the micro wells. During incubation the specific –immune complex formed in the case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins ,Second antibody conjugate to the enzyme HRP and directed against different epitopes of HBsAg is added to the conjugated antibodies will be bound to any ant-HBs-HBsAg complexes previously formed during the first incubation, and The un bound HRP conjugate is then removed by washing after washing to unbound HRP conjugate, chromogen solutions containing TMB and urea peroxidas are added to the wells .in the presence of antibody-antigen , antibody HRP sandwich immune- complex ,the colorless chromogen are hydrolyzed by the bound HRP conjugate to blue colored product .The blue colure turns yellow after stopping the reaction by using the stop solution. The color intensity can be measured and it is proportional to the amount of the antigen captured in the wells and it is amount respectively. wells containing samples negative for HBsAg remain colorless.

3.9.1.2. Assay procedure:

The reagent and samples were allowed to reach room temperature. Numbered of wells including two negative control e.g. (B1, C1) and one blank (e.g. A1) and one blank (e.g. A1, neither samples nor HRP conjugate should be added into the blank wells).

Then added 20ul of sample diluents to each well except the blank and mixed by topping the plate gently. and added 100ul of positive control and negative control and specimen to their respective wells by using separate disposable tip for each specimen negative control and positive control to avoid contamination. Then added 50 ul HRP conjugate to each well except the blank and mixed tapping the plate gently. And covered the plate with plate cover and incubated for 30 minutes for 37°C at the end of the incubation removed and discard the plate cover. Washed each well 5 times with diluted wash buffer. Each time allowed the micro wells to soaked for 45 second, After the five washing and plotting paper or clean towel, and tap it to remove any remainders. After washing dispense 50ul of chromogen A and 50 ul of chromogen B solutions was added into each well including the blank and mixed by tapping the plate gently. incubated the plate at 37°C for 15 minutes. Stopped the reaction by using a multichannel pipette, added 50 ul stop solution into the each well and mixed gently the absorbance measured at 450 nm. and calculated the cut-off value and evaluated the result and read the absorbance within 5 minutes after the stopping the reaction.

Interpretation of results:

Each micro plate should be considered separately when calculated and interpreting result of the assay ,regardless of the number of the plate concurrently processed the results are calculated by relating each samples optical density (OD) value to the cut-off (C.O.) of the plate .if the cut-off reading spaced on single filter plate reader, results should be calculated by subtracting the blank well OD value from the print report value of samples and

Chapter Four

Results

controls , In case the reading spaced on dual filter plate reader, don't subtract the blank well OD from the print report values of samples and controls.

Cutoff value (C.O.) = *NC*2.1

*NC=the mean absorbance value of two negative controls

Negative result: sample giving an absorbance less than the cut off value are considered negative, which indicate no HBV surface antigen has been detected with this HBsAg ELISA kits.

Positive result: sample giving an absorbance greater than the cut off value are considered initially reactive, which indicate HBV surface antigen has been detected with this HBsAg ELISA kit.

3.10. Statistical analysis

The data analysis was done through Statistical Package for the Social Scinces (SPSS) version 22 and Chi-square test was used to assess the association between various variables.

CHAPTER FOUR

RESULTS

4.1 Results

A total ninety- two health care workers (HCWs) who were considered at occupational risk of contracting HBV infection were enrolled in this study. Forty seven (51.1%) were male and 45/92 (48.9%) were female, the sero-positivity among males was 2 (2.2%) and among females was 6 (6.5%) from the total infected participants 8 (8.7%).

Table 4.1 The distribution of HBsAg positive according to gender

Sex	HBV result		Total
	Positive	Negative	
Male	2	45	47
	2.2%	48.9%	51.1%
Female	6	39	45
	6.5%	42.4%	48.9%
Total	8	84	92
	8.7%	91.3%	100.0%

p-value = 0.122 (p-value > 0.05) Result indicated insignificant

Table 4.2 HBV result and vaccination

HBV results	vaccine		Total
	vaccinated	Non vaccinated	
Positive	3/3.3%	5/5.4%	8/8.7%
Negative	20/21.7%	64/69.6%	84/91.3%
Total	23/25%	69/75	92/100%

p-value = 0.393 insignificant.

Twenty three (25%) of the participants were vaccinated and 69/92 (75%) were not vaccinated (by using questionnaire).

Table 4.3 Frequency of HBV results among marital status

HBV result	Marital status		Total
	Married	Single	
Positive	3/3.3%	5/5.4%	8/8.7%
Negative	44/47.8%	40/43.5%	84/91.3%
Total	47/51.1%	45/48.9%	92/100%

p-value = 0.421 insignificant .

While 47 (51.1%) were married and 45(48.9%) were single .

Table 4.4: Frequency of Hepatitis B virus result among health care workers

Health care workers	HBV positive result	HBV negative result
Lab technologist	3 /37.5%	37/44%
Nurse	2 /25%	31/37%
Pharmacist	0 /0%	3/3.5%
Cleaning staff	3 /37.5%	13/15.5%

p-value = 0.289(p-value > 0.050 result indicated that in significant association between occupation practice.

Table 4.5 Distribution of HBV infection according to accidental injury

HBV Results	Injury		Total
	Yes	No	
Positive	4/4.3%	4/4.3%	8/8.7%
Negative	20/21.7%	64/69.6%	84/91.3%
Total	24/26.1%	68/73.9%	92/100%

p-value 0.107 (p-value >0.05) insignificant.

Twenty four 24 out of 92 were exposed to an accidental injury

Table 4.6 Association of HBsAg results and blood transfusion

HBV results		Blood transfusion		Total
		Yes	No	
Positive		0/0.0%	8/8.7%	8/8.7%
Negative		2/2.2%	82/89.1%	84/91.3%
Total		2/2.2%	90/97.8%	92/100%

p- value = 0.569 (p-value >0.05) insignificant.

Chapter Five

Discussion

Conclusion

Recommendations

Table 4.7: Distribution of HBV infection according to age groups

AGE GROUP	HBV results		Total
	Positive	Negative	
20-40 years	8	70	78
	8.7%	76.1%	84.8%
41-60 years	0	14	14
	.0%	15.2%	15.2%
Total	8	84	92
	8.7%	91.3%	100.0%

p-value = 0.210 (p-value > 0.05) insignificant.

CHAPTER FIVE

5.1 Discussion

In this study the sero-prevalence of HBsAg was assessed for 92 (HCWs) at Atabra locality hospital and selected health centers. Only 8 (8.7%) were positive for HBsAg and this result is similar to those reported from Tanzania where the prevalence of hepatitis B virus among HCWs in tertiary hospital was (7%) (Mueller *et al.* 2015) and the results are similar to those reported from Yemen and Palestine which were (9.9%) and (9.60) respectively (Alhurabiet *al.*, 2004; Jadallah *et al.*, 2005). While, they disagree with other report from White Nile State, Sudan which was (27%) (Abuelgasimet *al.*, 2013). The result is higher than other studies done in Korea which was (2.4 %) (Shin *et al.*, 2006), Morocco which was (1%) (Djeriri *et al.*, 2008), Khartoum which was (4.4%) (Abdalwhaband Nafi, 2014) and Lagos State in Nigeria which was (1.5%) (Abiola *et al.* 2016). The variation between some of the results particularly that carried at River Nile State could be attributed to the difference in the population and the sample size.

The incidence of infection in laboratory technologist (3 participants), nurses (2 participants), and cleaning staff (3 participants) could be justified by the frequent contact of those HCWs with sources of infection (e.g., accidental needle stick injuries). Such incidents might occur while giving an injection or after injection, including recapping contaminated needle, and handling infected sharps before and after disposal, contaminated blood during sampling, unsafe sharps waste management, and reuse of injection equipment to administer injection to more than one person. Although majority of the participants in this study were not vaccinated against Hepatitis B virus infection 25% only. Some of the vaccinated participants were infected by HBV and this could be due to poor response to vaccine, the participants were immunosuppressed, or they were vaccinated with non-effective vaccine. In this study only eight were positive for HBsAg which represent (8.7%) and this could be due to understanding of HCWs to the safety protocols that prevent against blood borne infections.

5.2 Conclusions

In conclusion, this study has shown that only 8.7% of the HCWs at Atabra locality were positive for HBsAg. Laboratory technologists were the most affected and only one from those who had blood transfusion was positive. HCWs with frequent injuries had higher prevalence of HBV infection than others

Chapter Six

References

Appendices

5.3. Recommendations

- 1- HCWs should be screened regularly for Hepatitis B virus and other blood-borne infections.
- 2- Further studies should be conducted with larger sample size to confirm these results.
- 3- HCWs should be vaccinated against Hepatitis B virus (HBV) and ensure they are assessed for immunity (post-vaccination management).

Chapter Six

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إقرار بالموافقة

الاسم :

العمر : العنوان :

أوافق أنا بمحض إرادتي في المشاركة في البحث العلمي المتعلق بدراسة الكشف عن فيروس الكبد الوبائي (النوع ب) بين العاملين في مجال الرعاية الصحية الأولية في محلية عطبرة.

ولاية نهر النيل

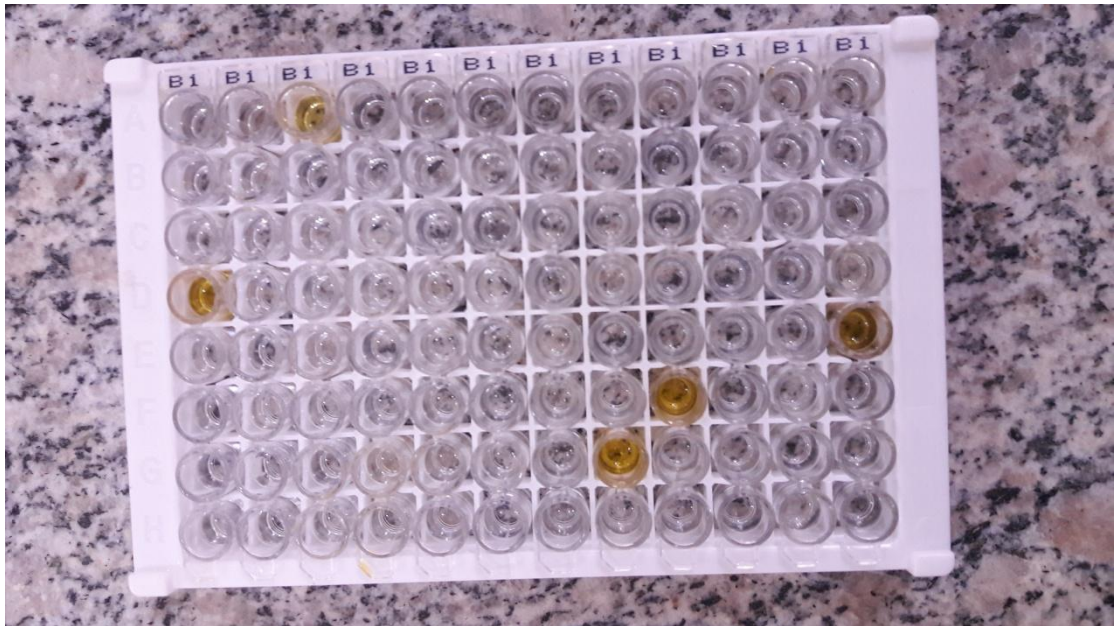
إيناس محمد احمد الطيب محمد

بعد أن شرح لي انه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال كما يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من المراحل

البحث بإشراف :

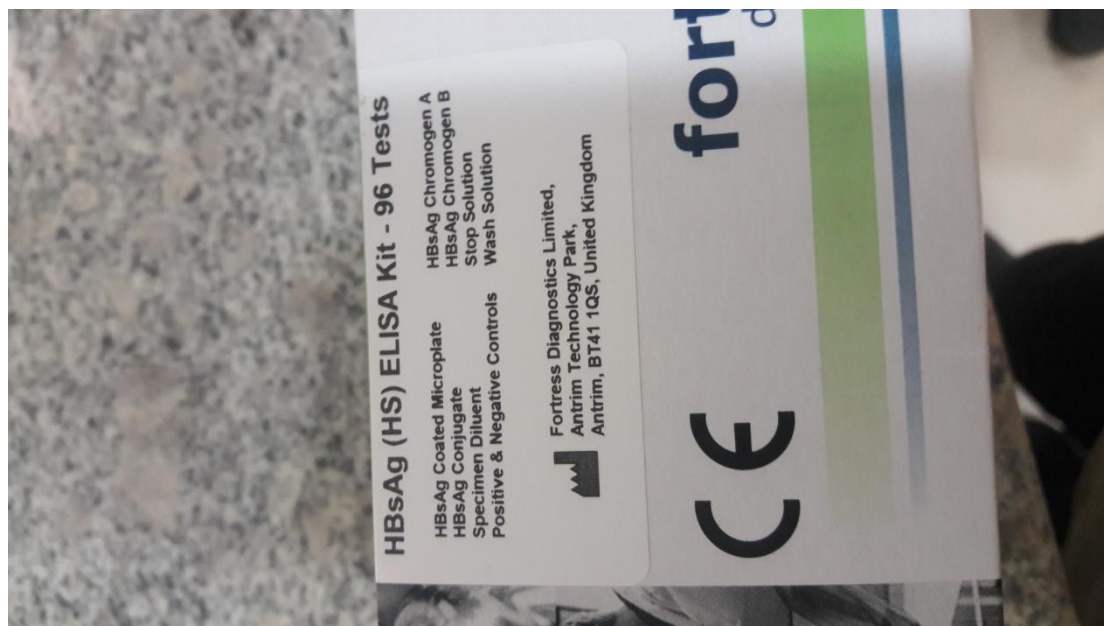
د. ليلى محمد احمد عبد القادر

Appendix (2)



ELISA Results

Appendix (4)



ELISA Kit in study