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 (WHO 2017). Hepatitis B virus is a member of the hepadna virus family, it is a 42, nm enveloped virion with icosahedral nucleocapsidcore containing partially double strand circular DNA genome (Levinson,2014).The chronic infections increases risk of death from childhood hepatic failure, cirrhosis of the liver and liver cancer (Shepard *et al*., 2006). 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, 2002).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.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, 2001). HCWs might include physicians, nurses, doctors, 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). 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. While performing their duties, healthcare workers (HCWs) are frequently exposed to dangerous infectious agents. The risk oftransmission 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, 2002), 2.5% of HIV cases, 40% of both and HCV cases worldwide are the result of occupational exposure among health care workers (Hunchew*etal*.,2014). Adherence to standard precautions, awareness about post exposure prophylaxis is poor in developing countries amongHCWs and documentation of exposures is suboptimal (WHO, 2002).Needle stick injuries are one of the most efficient modes of HBV transmission, most transmission in the healthcare setting probably occurs in the absence ofa 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, 2003). The initial diagnosis of hepatitis can be made on the basis of the clinical symptoms and the presence of liver enzymes in the blood. However, the serology of

HBV infection describes the course and the nature of the disease. Acute and chronic HBV infections can be distinguished by the presence of HBsAg and HBeAg in the serum and the pattern of antibodies to the individual HBV antigens. HBsAg and HBeAg are secreted into the blood during viral replication. The detection of HBeAg is the best correlate to the presence of infectious virus. A chronic infection can be distinguished by the continued finding of HBeAg, HBsAg, or both, and a lack of detectable antibody to these antigens(Murray et al., 2013).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 combinationcompared with HBIG alone in preventing prenatal transmission is presumed to apply to the occupational health setting(CDC 2006).

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 causessignificant morbidity and mortality rate(WHO 2002).

the observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary to transmit the disease(Jha AK *etal.,* 2012).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.

The prevalence of HBsAg among health care workers in Aldueim hospital was 8.7% (Abuelgasim, 2013) , and in Khartoum hospital is 4.4% (Abdalwahab and Nafi ,2014) .The aim of this studywas to determine the prevalence of HBV among health care workers inAldamer locality.

1.3 Objectives

1.3.1General objective

To detect Hepatitis B Virus Infection among Health Care Workers in Aldamer locality River Nile State

1.3.2 Specific objectives

1. To detect hepatitisB surface antigen (HBsAg), among health workers in Aldamer localityusing Enzyme Linked Immune Sorbent Assay technique.

2. To correlate the possible association between hepatitis B virus and selected risk factors(vaccine , accidental injury , blood transfusion , Renal dialysis and surgical operation).

CHAPTER TWO

LITERATURE REVIEW

2.1HBV properties

The hepadnaviruses got their name because they cause hepatitisand they have DNAgenomes.They are known as hepatitis B viruses (HBVs) and are classified in the familyHepadnaviridae. Some members infect mammals and some infect birds; examples include woodchuck HBV and heron HBV. The best known hepadnavirusis 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 hepadnavirusfamily, it is a 42, nm enveloped virion with icosahedralnucleocapsidcore containing partially double strand circular DNAgenome (Levinson,2014).

2.1.1 Genome

Hepatitis B virus is a small DNA virus and belong to a group of microscopy: the infectious visions and the sub viral particles. The infectious virus particles are the so-called Dane particles (Dane *et al.,* 1970), have a spherical, doubleshelledstructure of 42-44 nm containing a single copy of the viral DNA genome, covalently linked to the terminal protein of the virus (Sonabend*etal.,* 2010).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 (Glebeandurban, 2010). 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 secretary pathways through the ER and Golgi. The surface proteins are named the preS1 (or large), the preS2 (or middle) and the S (orsmall), which correspond to the HBsAg. As with nearly all enveloped viruses, theHBV particle also contains proteins of host origin (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 togenotype, that in concert with the core protein (HBcAg) forms the nucleocapsids(Nassal *et al.,*2008).The viral polymerase is covalently bound tothe negative strand by a phosphotyrosine bond. At the 5' end of the positive strand ashort 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 (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 livertumors can be infected by HBV virions. Some cell lines, however, can be infected using HBV DNA (a procedure known as transfect ion) (CarterandSaunders, 2007*)*.The life cycle of the HBV is complex. Hepatitis B is one of the few knownnonretroviralviruses 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 thehepatocytes and enter it by endocytosis (Sonnbend*etal.*,2010). 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 returnedto the nucleus and recycled to produce even more copies (Mandel *etal* .,2005). The long mRNA is thentransported back to the cytoplasm where the virion p protein synthesized DNA via itreverse transcriptase activity (Levinson,2014). Hepatocytes (liver cells) are the host cells for HBVin the body. In the laboratory, primary cell culturesof human hepatocytes support replication, but unfortunatelynone of the established cell lines derived fromliver tumors can be infected by HBV virions. Somecell lines, however, can be infected using HBV DNA(a procedure known as transfection), (*Carter*and Saunders,2007).

2.3HBV Transmission

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

2.3.1Risk groups for hepatitis B in developedCountries

Intravenous drug abusers, homosexual men, sexual contacts of antigen-positive persons, residents in long-stay homes for

mentally handicappedPeople, renal dialysis patients, recipients of multiple blood products (e.g. haemophiliacs), surgeons, dentists and morticians, and infants of infectious HBsAgpositive mothers, (Bannister*etal.*, 2006).

2.4HBV 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(Kramvis*etal.*,2005). Genotype H was recently identified in central America (Arauz-RuizP*etal.*,2002), is well known that HBV genotypes have distinctgeographical distributions. but the two genotypes distribute unevenly in China. We studied 1096 Chinese chronic HBVcarriers 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 ispredominant (55.0%).Genotypes A and D are also found in other areas of China. However, thegenotypes E-H have not been reported in China. Recently, genotype C/D hybrid was identified in Tibet (Cui*etal.*,2002) and genotype B wasfound recombinated with preC/C region of genotype C in China (Luo,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. Itwas reported that HBeAg carrier status tends to be longer and the prevalence of HBeAg appears higher in patients with genotype Cthan with genotype B (Orito, 2001). HBV carriers with genotype B have lower histological

activity scores and genotype C is more prevalence in patients with cirrhosis (*Kaoetal.*,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*etal.*,2000). However, whether patients with genotype B differfrom those with genotype C in development of hepatocellular carcinoma remains controversial. The response of different HBVgenotypes to interferon-Alfa treatment is of increasing interest because the benefit of interferon-Alpha or its pegylated form in combination with other antiviral agents is being explored in the treatment of chronic hepatitis B. In a homogeneous group ofprospectively followed patients from Europe, a recent study demonstrates that genotype A responds better than other HBVgenotypes to standard interferon therapy and represents an independent predictor of a therapeutic success, with a greater impact thanother pre-treatment characteristics, such as HBV DNA or ALT levels (Hou,2000).

2.5 Epidemiology

There are around 350 million chronic carriers of the hepatitisB virus worldwide.

Sudan is classified among the countries with high hepatitis B virus seroprevalence.Exposure tothe virus varied from 47%–78%, with a hepatitis B surface antigen prevalence ranging from 6.8%in central Sudan to 26% in southern Sudan (Mudawi, 2008).

The incidence of acute disease andprevalence of carriage varies considerably from country tocountry. In parts of south-east Asia, 10–20% of the population may be carriers, whereas most countries in Europeand North America have carriage rates below 2%.Where carriage rates are high, acute infection occurs mainly in infants and young children, mostly

via intrapartumand horizontal transmission within households. Skindisease and biting arthropods may facilitate the transfer of body fluids from person to person.In low-prevalence countries most infections are sporadicand arise in adults through needlestick injuries,shared syringes, bites and scratches, or by sexual contact (Chamberlain 2009).

Those most at risk include intravenous drug abusers, homosexualmen, residents and staff of institutions for thementally handicapped, surgeons, dentists, laboratoryworkers, morticians, renal dialysis patients and recipientsof unscreened blood and blood products (Bannister *et al*., 2006). Hepatitis B is highly endemic indeveloping regions with large population such as South East Asia, China, sub-Saharan Africa and the Amazon Basin, where at least8% of the population are HBV chronic carrier. In these areas, 70–95% of the population shows past or present serological evidenceof HBV infection. Most infections occur during infancy or childhood. Since most infections in children are asymptomatic, there islittle 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 also found in other body fluids,including urine, bile, saliva, semen, breast milk, and vaginalsecretions. It is not found in feces, however. Membranecontact 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 withmultiple partners. It can be readily spread from mother toneonate at the time of vaginal delivery—a common modeof 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 duringplacement of tattoos and ear-piercing. Crowded environments such as institutions for the mentally handicapped, (Frederick and Southwick, 2007).

2.6Pathogenesis and immunity

After entering the blood, the virus infects hepatocytes, and viral antigens aredisplayed on the surface of the cells. Cytotoxic T cells mediate an immune attackagainst the viral antigens, and inflammation and necrosis occur. Immune attackagainst viral antigens on infected hepatocytes is mediated by cytotoxic T cells. Thepathogenesis of hepatitis B is probably the result of this cell-mediated immuneinjury, because HBV itself does not cause a cytopathic effect. Antigen–antibodycomplexes cause some of the early symptoms (e.g., arthralgias, arthritis, andurticaria) and some of the complications in chronic hepatitis (e.g.,glomerulonephritis, cryoglobulinemia, and vasculitis), (Levension,2014). Fully differentiated hepatocytes are the primary cell type infected byHBV. The primary cause of hepatic cell destruction appears to bethe cell-mediated immune response, which results in inflammationand necrosis. The cells involved are cytotoxic T cells, which reactspecifically with the fragments of nucleocapsid proteins (HBcAg and(HBeAg), expressed on the surface of infected hepatocytes. Thisresponse also contributes to control of the infection by eliminatingvirus-producing cells. Enhanced natural killer cell activity, as well asproduction of interferon-γ also contributes to limiting the extent ofinfection. Anti-HBsAg antibody, which is the neutralizing antibody,does not appear until well into the convalescence period, when itmay aid in clearing any remaining circulating free virus (Harvey *etal*., 2007).

2.7Clinical Presentation

2.7.1Acute Infection

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 marker (Cornellissn*et al*.,2012).

• 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 toseveral months after the disappearance of HBsAg. Anti-HBs may persist for life, conferring immunity; this is thebasis for current vaccination strategies using noninfectious. HBsAgHBeAg, HBV-DNA, and DNA polymerase appear inserum soon after HBsAg, and all signify active viralreplication. Persistence of HBeAg is an important indicatorof continued viral replication, infectivity, andprobable progression to chronic hepatitis. The appearanceof anti-HBe antibodies implies that an acute infectionhas peaked and is on the wane(Cornellissn*et al*.,2012).

IgM anti-HBc becomes detectable in serum shortlybefore the onset of symptoms, concurrent with elevationof serum aminotransferase levels (indicative ofhepatocytedestruction). Over a period of months, the IgM anti-HBc antibody is replaced by IgG anti-HBc. As in the caseof anti-HAV, there is no specific assay for IgG anti-HBc,but its presence is inferred from decline of IgM anti-HBcin the face of rising levels of total anti-HB (Kumar*et al*.,2013)Initial infection with hepatitis B virus (HBV) maybe asymptomatic in up to 50 per cent of adults and90 per cent of children,When symptomsoccur, they may include anorexia, vague abdominalpain, nausea, vomiting and jaundice,Fevermay be absent or mild (Heymann,2008). Extrahepaticmanifestations such as arthralgia , arthritis,macularrashes,thrombocytopenia,orpapularacrodermatitis(Gianotti-Crosti syndrome) can occur early in thecourse of the illness and may precede jaundice, Acute HBV infection cannotbe distinguished from other forms of acute viralhepatitis on the basis of clinical signs

andsymptoms or nonspecific laboratory findings (American Academy of Pediatrics, 2012).

2.7.2Chronic Infection

While the majority of individuals infected withHBV are able to clear the virus, some individualsfail to mount an adequate immune response,leading to chronic infection (Conlyand Johnston,2007). The exactmechanisms by which chronic liver injury occurs inHBV infection are not known (Kozieland*Siddiqui*,2010). HepatitisBvirusinfection becomes chronic in approximately90 per cent of infants infected at birth (American Academy of Pediatrics,2012) If chronic infection is established, the spectrumof illness ranges from the healthy carrier state to allof the sequelae of chronic hepatitis, including mildto moderate fibrosis, compensated cirrhosis, hepaticdecompensation and hepatocellular carcinoma(HCC), The single most important riskfactor for HCC is cirrhosis (Pungpapong*et al*., 2007). Individuals who areimmunosuppressed or have an underlying chronicillness are at increased risk of developing chronicinfection (Heymann, 2008;American Academy of Pediatrics,2012). Factors that may influence thenatural history of chronic infection include gender,race, alcohol use, and co-infection with hepatitis A,hepatitis C or hepatitis D viruses or humanimmunodeficiency virus (HIV) (American Academy of Pediatrics,2012). Antiviraltherapy can modify the natural history of chronic HBVinfection (YimandLok,2006).Superinfection or co-infection is not uncommon in patients with chronic HBV infection. Acutehepatitis delta virus (HDV) may be acquired as co-infection simultaneously with HBV or as asuperinfection in a patient who is already a carrierof HBV (Sherman,2007). Infection with HDV in

HBVinfected individuals is associated with more severe and/or progressive liver disease than is HBVmonoinfection.The natural course following acute hepatitis C virus(HCV) superinfection has not been well studied.The long-term prognosis following acute HCVsuperinfection is worse than that following HDVsuperinfection (Liaw,2004).

2.8 HBV antigen

All three coat protein of HBV contain HBsAg , Which is highly immunogenic and induce anti-HBs antibody (humoral immunity). Structural viral proteins induce specific T-lymphocyte capable of eliminate HBV infected cells. HBsAg is heterogeneous antigenically , with acommon antigen designated a , and two pairs of mutually exclusive antigens , d , y , w (including several sub determinants) and r , resulting in 4 major sub types , adw ,adr ,ayr, (Hollinger and Liang, 2001).

2.9. Laboratory Diagnosis

2.9.1Serologic and Virologic Markers

The two most important serologic tests for the diagnosis of early hepatitis B are thetests for HBsAgand for IgM antibody to the core antigen. Both appear in theserum early in the disease (Levinson,2014).After a person is infected with HBV, the first virologicmarker detectable in serum within 1–12 weeks, usuallybetween 8 and 12 weeks, is HBsAg(Dan *et al*,2010)Both acutely and chronically infected individuals have HBsantigenaemia. The diagnosis of acute disease is confirmedby demonstrating IgM anti-HBc in the serum. This appears2 weeks after HBsAg, and disappears a fewmonths after uncomplicated infection. IgG anti HBc persistsprobably lifelong, and is a marker of previous infection.The stage of evolution of antigenaemia and

antibodyproduction is determined by EIA tests. Viral persistencecan be confirmed by PCR-based detection of HBV DNAin serum. Detection of HBe is still used as a marker of enhancedinfectivity and risk of chronic liver disease. (Bannister*etal.,*2006).

2.9.2Polymerase chain reaction (PCR) test

It is based on the use of DNA fragment called the gene probe (39). Gene probe isrelatively small, single stranded DNA segment that can hunt for complementary fragmentof DNA.To use a gene probe effectively, it is valuable to increase the DNA to be searched (Pommerville, 2004).

2.9.2.1 Hepatitis B viral DNA (HBV-DNA)

Quantizationof viral DNA in serum is most commonlyused in the assessment of patients with chronicactive hepatitis. In the patient with acute hepatitis,this test provides no significant advantagesover that for HBeAg. Both tests indicate activeviral replication. In patients with fulminanthepatitis, assays for HBV-DNA has been positive in the absence of other positive markers forHBV(Frederick and Southwick,2007).

2.10. 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 arenucleoside analogues that inhibit the reverse transcriptase of HBV. Interferon in theform of peginterferon alfa-2a (Pegasys) is also used. Other nucleoside analoguessuch as lamivudine (Epivir-HBV), adefovir (Hepsera), and telbivudine (Tyzeka) areused less frequently. A combination of tenofovir and emtricitabine (Emtriva) is alsoused (Levinson,2014).

2.10.1Drugs active against HBV

Lamivudine, 100 mg daily, orally (also used for HIV),**a**defovirdipivoxil, 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 beingused to treat HIV co-infection, the HIV-treatment doseshould be given (this is often higher than the dose for HB) (Bannister *et al*., 2006).

2.11. Prevention

Prevention involves the use of either the vaccineorhyperimmune globulinor both (Levinson,2014).

2.11.1Passive Immunoprophylaxis

Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb. It isused to provide immediate, passive protection to individuals known to be exposedto HBsAg-positive blood (e.g., after an accidental needle-stick injury) (Levenson,2014).Immunoprophylaxis is recommended for allinfants born to HBsAg positive mothers. Current dosing recommendations are 0.13ml/kg HBIG immediately after delivery or within12 hours after birth in combination with recombinant vaccine. The combination results in a higher-than-90% level of protectionagainst perinatal acquisition of HBV (Beasley *etal.*,2008). Between 3.7% to 9.9% of infants still acquire HBV infection perinatally from HBVinfectionmothers, despite immunoprophylaxis. Failure of passive and active immunoprophylaxis in this setting may be theresult of in utero transmission of HBV infection, prenatal transmission related to a high inoculums, and/or the presence of surfacegene escape mutants (Beasley *etal.,* 2008). To study the interruptive effect of HBIG before delivery in attempt to prevent intrauterine transmission ofHBV, a large-scale, random-control study was conducted in China (Zhu,2003). 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 livertransplantation. HBIG monotherapy given at a high dosage can prevent recurrence in 65% to 80% of patients. Because the cost oflong-term prophylaxis with high-dose HBIG is extremely high and combination therapy using HBIG with a nucleoside analog ismore uniformly effective, the current protocol is combination HBIG with a nucleoside analog after liver transplantation. Thecombination protocols have reduced the rate of virologic breakthrough to 10% or less (Terraultand Vyas, 2003).

2.11.2Active Immunization

Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and itssubsequent 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 (Krugman,2009). The WHO recommended that hepatitis B vaccination should be included in national immunization system in all countries withahepatitis B carrier (HBsAg). By May 2002, 154 countries hardoutine 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, 2001). During the first 2years of the program, coverage was provided mainly for infants whose mothers were carriers of HBsAg. Vaccination wassubsequently extended, first to all newborns and then to unvaccinated preschool-age and elementary school-age children.

Since1991, catch-up vaccinations have been given to children in the first grade. This program reduced the overall HBsAg prevalence ratefrom 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,1996). In 1999, vaccination rates were 80–86% for young children and higher than 90% for olderchildren; 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-termefficacy of hepatitis B (HB) vaccination in newborns, one of the longest HB vaccine follow-up studies in the world was conducted inShanghai, China (Zhou, 2003). Children who were born in 1986 and immunized with hepatitis B vaccine at birth were followed up at leastonce a year. Serum HBsAg, anti-HBc and anti-HBs were tested. The positive rates of HBsAg in the vaccine group with the period of16 years were 0.46%-0.97%, the average being 0.61%, which was much lower than those of baseline before vaccination and externalcontrol. The long-term efficacy of newborn vaccination was 85.42%. In countries such as Italy and the United States, the incidenceof acute hepatitis B has declined dramatically during the past decade after vaccination program for HBV infection, particularlyamong persons in younger age group (Davilla,2000).Universal HB vaccination was proven to be effective in the prevention of HCC in several large cohort studies in SoutheastAsia(Chang *et al.,*1997).

CHAPTER THREE

MATERIAL AND METHODS

3.1. Study design

This study was a descriptive cross-sectional study**.**

3.2. Study area:

This study was conducted in Aldamerlocality, which located in the River Nile in Sudan, it is about 300 km north Khartoum.

3.3Study duration:

The study was carried out during July 2019 to November2019.

3.4. Study population:

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

3.5. Sampling technique:non probability sample

3.6. Inclusion criteria:

Health care worker in hospital of Aldamer town during the study period were the candidates of the study.

3.7. Exclusion criteria:

Health care workers who were not in the study area during data collection and field worker were excluded.

3.8. Sample size:

Ninety-two(n=92) health care workers were recruited for this study, 23 male and 69 female. The objective were divided in to two age group 20-40 years, 41-60 years.

3.9. Ethical consideration:

Ethical approval was obtained from the Research Committee of College Medical Laboratory Science of Sudan University of Science and Technology and also from the Health Services Director in Aldamer Locality and verbal consent was obtained from participants before collection of the blood samples.

3.10. Data collection:

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

3.11. Collection of blood specimens:

Under sterile condition 3 ml of venous blood sample was withdrawn from each participant, the sample were let to clot on bench and serum was separated by centrifugation at 5000rpm for five minutes, serum wascollected into plain containers then stored at -20C° until used.

3.12. Laboratory investigation:

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

3.13.1ELISA 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 plasmait is intended used

for screening of blood donors and for monitoring individuals with a higher than normal risk of contracting hepatitis (Technicians or nursing personnel in renal dialysis units or clinical laboratories as an aid for diagnosis of liver disease).

3.13.1.1. Assay procedure:

The reagent and samples were allowed to reach room temperature. The wells including two negative control (B1, C1) and one blank (A1), (A1, neither samples nor HPR conjugate should be added

into the blank wells) and D1, E1 positive control.

Twenty ul of sample diluents was added to each well except the blank and mixed by toping the plate gently,then 100ul of positive control and negative control and specimen were added to their respective wells by using separate disposable tip for each specimen negative control and positive control to avoid contamination.Then incubated at 37 ˚C for 45 minute.Then 50 ulHRP conjugate was added to each well except the blank and mixed by tapping the plate gently. The plate was then covered and incubated for 30

minutes at 37˚C.After incubation, the cover was removed and the plate content was discard. Each well was washed 5 times with diluted wash buffer.After washing dispense 50ul of chromogen A and 50ul 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 minutesStopped the reaction by using a multichannel pipette, added 50ul 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

3.13.1.2.Interpretation of results:

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

*NC=the mean absorbance value of two negative controls.

Sample giving an absorbance less than the cut off value are considered negative, which indicate no HBVsurface antigen has been detected with this HBsAg ELISA kits and sample that give an absorbance greater than the cut off value are considered initially reactive, which indicate HBVsurface antigen has been detected with this HBsAg ELISA kit.

3.11. Statistical analysis

The data entered checked and analyzed with Statistical Package for the Social Scinces (SPSS)version22and Chi-square test was used to assess the association between various variables.

CHAPTER FOUR

RESULTS

Results

A total ninety- two health care workers (HCWs)who were considered at occupational risk of contracting HBVinfection were enrolled in this study. In this study 23/92 (25%)were male and 69/92 (75%) were female. The mean age ofmales was 30 ± 10 years and females was 30 ± 10years. The working were classify into two age group, 20-40 and 41-60 years. The number of target object that rangebetween 20-40 was 72(78%) while those rang between 41-60 years was 20(22%). Regarding marital status 46(50%) were single and 46(50%) were married.

58(63%) were urban and 34(37%) were rural. Study volunteers were classified according to their occupation to six groups, the nurse 30(33%), doctors 28(31%), laboratory technologist 13(14%), pharmacist 8(9%), cleaning staff (8%) and laboratory assistants 4(4%). From the total workers only one was positive, it was from the cleaning staff. There was no statistical significant between positivity and gender, vaccine,marital status ,injury ,blood transfusion, age group ,renal dialysis ,surgical operation , locality (*p.value* :0.56 , 0.49 , 0.3, 0.35 , 0.85 . 0.4 , 0.9 , 0.68 , 0.19 respectively).

Table 4.1: Frequency of demographic data

Subject		Frequency	Percentage	Total
Gender	Male	23	25%	92 (100%)
	Female	69	75%	
Marital status	Married	46	50%	92 (100%)
	Single	46	50%	
Age group	20-40	72	78%	92(100%)
	41-60	20	22%	
Locality	Urban	58	63%	92 (100%)
	Rural	34	37%	

Table 4.2 The distribution of HBsAg according to demographic data

		HBV	
		Positive	**Negative**
Gender	male	0 /92 (0.0%)	23/92 (25%)
	female	1/92 (1.1%)	68/92 (73.9%)
Marital status	married	1/46 (2.2%)	45/46 (97.8%)
	single	0/46(0%)	46/46(100%)
Age group (years)	20---40	1/72 (1.4%)	71/72 (98.6%)
	41---60	0/22 (0%)	22/22 (100%)
locality	urban	o/58(0%)	58/58(100%)
	rural	1/33(3%)	32/33 (97%)

Table 4.3 Frequency of HBV result among health care worker

Health care workers	HBV	
	Positive	Negative
Laboratory technologist	0/92(0%)	13/92(14%)
Laboratory asistants	0/92(0%)	4/92(4%)
Nurse	0/92(0%)	30/92(33%)
Pharmacist	0/92(0%)	8/92(9%)
Cleaning staff	1/92(1.1%)	7/92(7.9%)
Doctors	0/92(0%)	28/92(31%)

Table 4.4Frequency of HBV result among risk factors

		HBV	
		Positive	**Negative**
Vaccine	vaccinated	0/92(0%)	30/92(33%)
	Non vaccinated	1/92(1.%)	61/92(66%)
Accidental injury	Yes	0/92(0%)	43/92(47%)
	No	1/92(1%)	48/92(52%)
Blood transfusion	Yes	0/92(0%)	3/92(3%)
	No	1/92(1%)	88/92(96%)
Renal dialysis	Yes	0/92(0%)	1/92(1%)
	No	1/92(1%)	90/92(98%)
Surgical operation	Yes	0/92(0%)	13/92(14%)
	No	1/92(1%)	78/92(85%)

CHAPTER FIVE

DISCUSION,CONCLOSIONS, &RECOMMENDATIONS

5.1 Discussion

Hepatitis B virus remains a major problem in developing countries .The HB viruses (HBV) express antigens such as HBsAg (surface antigen) on its surface, provoking both cell-mediated and humoralresponses(Gill and Beeching ,2004).

In this study the sero-prevalence of HBsAg was 1(1%) were positive for HBsAg, the sameresult to those reported in Morocco by Djeririwhich was (1%) (Djeriri2008). This result confirmed by Said in Sudan 2019 which obtained similar result (2%) (Said, 2019).While they disagree with other report from Tanzania where the prevalence of hepatitis B virus among HCWs in tertiary hospital was (7%) (Mueller.2015) and from Yemen and Palestinewhich were (9.9%) and (9.6) respectively (Alhurabi, 2004; Jadallah *et al.*, 2005),White Nile State, Sudan which was (8.7%) (Abuelgasim, 2013).The result is lower than other studies done in Koreawhich was (2.4 %) (Shin *et al.,*2006), Khartoum which was (4.4%)(Abdalwhaband Nafi, 2014) .Seroprevalence was recorded in the females (2.0%) than males (0.0%) students (AbdElrahman*et al.*, 2018), because of the highEndemicity of the disease, different sample size and poor application of prevention and control program.One of ninety two (1.1%) is positive result found in cleaning staff compared with result of Abuelgasim which showed the majority of positive cases in cleaning staff (8.7%) (Abuelgasim , 2013).The positive result was found in females as

the same result found in female in Shendiby Said (Said, 2019). This positive female are located in urban and have history of needle stick injury ,and have note taken blood transfusion or renal dialysis , surgical operation and vaccinatin . The incidence of infection in cleaning staff (1 participants) could be justified by the frequent contact of those HCWs with sources of infection (e.g., accidental needle stick injuries). Although majority of the participants in this study were not vaccinated against Hepatitis B virus infection it showed in this study only one was positive for HBsAg which represent(1.1%) and this could be duto understanding of HCWs to the safety protocols that prevent against blood borne infections.

5.2 Conclusions

It is concluded that the seroprevelance ofHBsAg among HCWs in Aldamerlocality is low compared to other local studies in different states.

5.3. Recommendations

1- HCWs should be screened regularly for Hepatitis B virus and other blood-borne infections.

2- Further studies should be conductedwith larger sample size to support these results.

3- HCWs should be vaccinated against Hepatitis B virus (HBV) and ensure they are assessed for immunity (post-vaccination management).

4- Disposal and deal of waste by scientific and professional way.

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APPENDICES (1)

Sudan University of science and Technology

College of Graduate Studies

Questionnaire

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

Questionnaire on Detection of Hepatitis B Virus among health care workers in Aldamer locality , River Nile State – Sudan.

Data collection Sheet

General data

1. ID. number…………………………………………………………
2. Gender

Male ( )   female( )

3.Age………………………………………………………………..

4.locality ………………………………………………………………..

Urban ( )   rural ( )

5.Marital status

Married( )   single( )

6.Type of occupation…………………………………………………...

7. Vaccine

Yes ( ) No( )

8..Blood transfusion

Yes( ) No( )

9.Surgical operation

Yes ( ) No( )

10.Renal dialysis

Yes( ) No( ).

11.Have you taken a sharp instrument?

Yes( ) No( )

Appendix (2)

ELISA plate

Appendix (3)

ELISA Kit in study

fortress diagnostics

BXE0742A
96 Tests
STORE AT 2-8°C
FOR IN- VITRO DIAGNOSTIC USE ONLY

HBsAg (HS)

High sensitivity - ELISA

Intended Use:

Principle of the Assay:

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	[illegible]
Positive Control	[illegible]
HRP - Conjugate Reagent	[illegible]
HBsAg Sample Diluent	[illegible]
Stock Wash Buffer	[illegible] (Dilute 1 in 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C).
Chromogen Solution A	[illegible] (Ready to use and once open, stable for one month at 2-8°C)
Chromogen Solution B	[illegible] (Ready to use and once open stable for one month at 2-8°C)
Stop Solution	[illegible]
Plastic Sealable Bag	1 Unit
Plate Cover	1 Sheet
Package Inserts	1 Copy

Additional Materials And Instruments Required But Not Provided:

Specimen Collection and Transportation:

1. Sample Collection

2. Transportation and Storage

Special Instructions for Washing Plates:

Storage and Stability:

Precautions and Safety:

Assay Procedure:

Step1 Reagents preparation:

Fortress Diagnostics Limited Unit 2C Antrim Technology Park, Antrim [illegible] (United Kingdom)

ELISA sheet