

بسم الله الرحمن الرحيم

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seroprevalence of Hepatitis B Virus among Cleaning Workers in Khartoum State Hospitals

الإنتشار المصلي لفيروس إتهاب الكبد الوبائي (ب) لدى عمال النظافة في مستشفيات ولاية
الخرطوم

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الآية

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قال تعالى:

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5).

صدق الله العظيم

سورة العلق - الآيات (1-5)

DEDECATION

I dedicate this research to

My father, mother, brother and sisters

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Firstly, thanks to ALMIGHTY ALLAH for giving me strength to carry out this research.

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ABSTRACT

The objectives of this study were to determine the prevalence of Hepatitis B virus and the possible risk factors among hospital cleaning workers, at Khartoum and Omdurman hospitals.

Ninety (90) subjects were randomly enrolled during the period from February to April 2017. The subjects were males and females, their age ranging from 18 to 70 years. Socio demographic data were collected by structured questionnaire.

5 ml of blood were collected from each worker; Plasma was obtained by centrifugation at 3000 rpm for 5 min.

Hepatitis B Surface Antigen (HBsAg) was detected in plasma using Enzyme Linked Immunosorbent Assay (ELISA). The results showed that out of 90 blood samples investigated, 5 (5.5%) were positive; (3.3%) in female workers and (2.2%) in male workers, and the rest 85 (94.4%) were negative. (3.3%) was detected in Omdurman teaching hospital, the remaining (2.2%) in Khartoum Oncology Hospital and Antalia Medical Center. The study concluded that, hospital cleaning worker is at risk of getting HBV infection and mostly by accidental needle stick injury.

Further studies with large sample size and more advanced techniques are required to validate the result of the present study.

المستخلص

هدفت هذه الدراسة بصورة أساسية إلى تحديد مدى إنتشار الإصابة بالتهاب الكبد الفيروسي "ب" و تحديد عوامل الخطر المحتملة الممهدة للإصابة بالفيروس وسط عمال النظافة ببعض مستشفيات ولاية الخرطوم؛ في الفترة من فبراير و حتى أبريل 2017.

شملت الدراسة بصورة عشوائية 90 من عمال النظافة بالمستشفيات؛ ذكور و إناث؛ تتراوح أعمارهم بين 18-70. جمعت البيانات الإجتماعية بواسطة إستبيان منظم.

تم جمع (5) مل من الدم من العاملين و فصل البلازما عن طريق الطرد المركزي بسرعة 3000 لفة في الدقيقة لمدة 5 دقائق

تم الكشف عن وجود المستضد السطحي لفيروس الكبد "ب" و الذي يعتبر المؤشر الرئيسي للإصابة بالتهاب الكبد الفيروسي "ب" باستخدام تقنية الإليزا أو المقايسة الامتصاصية المناعية للإنزيم المرتبط ؛ و كانت النتيجة إيجابية في 5 (5.5%) من المتطوعين للدراسة، أعلى في الإناث بنسبة (3.3%) من الذكور (2.2%) و كانت النتيجة سلبية في 85 (94.4%) . سجل مستشفى امدرمان التعليمي(3.3%) بينما باقي النسبة كانت في مستشفى الخرطوم للأورام (مستشفى الذرة) و مركز أنطاليا الطبي (2.2%).

خلصت الدراسة إلي أن عمال النظافة بالمستشفيات في خطر للإصابة بالتهاب الكبد الوبائي في الغالب عن طريق الإصابة العرضية بالإبر الملوثة.

مزيد من الدراسات في حجم أكبر من العينات و بتقنيات متطورة، مطلوبة للتحقق من صحة الدراسة الحالية.

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CHAPTER ONE

INTRODUCTION AND OBJECTIVES

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1.1. Introduction

Blood borne infectious disease constitutes a major health burden for the developing countries. Health-care workers are at risk of exposure to occupational infection with subsequent risk of contracting disease, disability and even death. Doctors, nurses, laboratory technologist, and clinical waste handlers are continuously at risk of acquiring blood-borne infections such as human immunodeficiency virus (HIV)/AIDS and hepatitis (Sagoe *et al.*, 2001). Worldwide sharp injuries among health care workers resulted in 16000 hepatitis C virus, 66000 hepatitis B viruses and 1000 human immunodeficiency virus infection (Pruss-Ustun *et al.*, 2005).

Worldwide hepatitis B virus infection (HBV) is estimated to have infected > 2 billion people and causes 0.5 million deaths annually (WHO, 2013). Sudan has a high prevalence rate of blood borne infectious disease (Mudawi, 2008). There are tremendous regional variations and the prevalence of hepatitis B surface antigen (HBsAg) positive patients by country varies from low endemicity levels (<2%) to high levels(>8%) (Schweitzer, 2015). Hepatitis B virus is the most common transmitted blood borne virus in the health care setting. Transmission generally occurs from patient to patient or from patients to health-care personnel via contaminated instruments or accidental needle-stick or sharps injuries. The virus can be transmitted directly through body fluids to mucous membranes, cutaneous scratches, abrasions, burns or other lesions. Indirect transmission can occur from surfaces contaminated with blood or body fluids to mucous membrane. HBV has shown to be survive in dried blood on surfaces at room temperature for at least a week (Alter, 2003). Contaminated needles and syringes represent a particular threat and may be scavenged from waste areas and dump sites and be reused, WHO estimated that, in 2000, injections with contaminated syringes caused 21 million hepatitis B virus infections (WHO, 2004). It was observed that the knowledge of health care workers is

not adequate to prevent them from blood-borne disease, in Sudan; the rate of hepatitis B virus infection among Sudanese healthcare workers is high where the majority of health workers in Elsha'ab Hospital, Khartoum State were not vaccinated (Baraka *et al.*, 2014). The knowledge, attitude, and practice of healthcare workers about HBV infection in central Sudan were poor (Bakry *et al.*, 2011).

Knowledge and awareness regarding proper waste management remain low in the absence of training for hospital staff, moreover hospital sanitary workers and scavengers, operate without the provision of safety equipment or immunization, unsegregated waste is illegally recycled, leading to further safety risks, overall hospital waste management in developing countries faces several challenges (Mustafa *et al.*, 2017). Building cleaners are an important group of workers who experience diverse occupational hazards resulting in health problems, the potential for infectious disease was identified among cleaners in medical laboratories and was associated with exposure to broken glass and uncapped needle in the trash (Charles *et al.*, 2009). The risk that cleaners may be exposed to depend on the tasks they perform and also the premises they work in. Most of the time, the employers of cleaning personnel have difficulties in controlling the environment in which the cleaners work although they are responsible for their health and safety (Emmanuella, 2009).

1.2. Objectives

1.2.1. General objectives

To determine the prevalence of hepatitis B virus, among hospital cleaning workers in Khartoum State hospitals.

1.2.2. Specific objectives

1. To investigate the prevalence of hepatitis B surface antigen (HBsAg) in a representative population of cleaning workers in Khartoum hospitals using ELISA.
2. To identify the risk factors aid in transmission of hepatitis B virus to hospitals cleaning workers.
3. To evaluate the knowledge and attitude of the cleaning workers in dealing with hazardous material and protective procedures they follow.

1.3. Rationale

Most of the studies conducted in healthcare workers were focusing on the medical staff such as doctors, nurses and laboratory technician. Cleaning workers are important group, dealing with serious hazard. In most developing countries like Sudan, the cleaning workers often lack formal training, tools and information. Usually, this category of workers is poorly educated and employed as ad-hoc staff with very little formal requirements. As consequence they mostly do not understand the health implications of what they do.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Hepatitis Viruses

Many viruses cause hepatitis. Of these, five medically important viruses are commonly described as “hepatitis viruses” because their main site of infection is the liver. These five are hepatitis A virus (HAV); hepatitis B virus (HBV); hepatitis C virus (HCV); hepatitis D virus (HDV) and hepatitis E virus (HEV) (Levinson, 2008).

2.2. Hepatitis B VIRUS

2.2.1. Historical background

Hepatitis B virus (HBV) is a DNA virus and was first identified in the 1960s. According to the International Committee on Taxonomy of Viruses (ICTV), this virus belongs to the genus *Orthohepadnavirus* of the *Hepadnaviridae* family and along with the *Spumaretrovirinae* subfamily of the *Retroviridae* family, represent the only other animal virus with a DNA genome known to replicate by the reverse transcription of a viral RNA intermediate (Norder *et al.*, 2004; Seeger *et al.*, 2007). Is a blood-borne virus and roughly 75-200 times more infectious than human immune deficiency virus” HIV” (Bowyer and Sim, 2000). Hepatitis B virus was the first human hepatitis virus from which the protein and genome were identified and characterized. Before discovery of the hepatitis viruses, two types of hepatitis transmission were identified based on epidemiological observations. Type A was considered to be predominantly transmitted by fecal-oral route, where type B was transmitted parenterally. In 1996, Blumberg, in a research for polymorphic serum proteins, discovered a previously unknown antigen in the blood of an Australian Aborigine (Australia antigen). Four years later it was recognized that the appearance of this antigen was related to type B hepatitis (Mao *et al.*, 2004). In 1973, the viral nature of the particles discovered by Dane was confirmed by the detection of an endogenous DNA polymerase activity within their core (Schiefke *et al.*, 2004), this

enzyme allowed Shen *et al.*, (2004) to detect and characterize the HBV genome as small, circular, partially double-stranded DNA molecule.

2.2.2. HBV genome

The genome contains four genes (four open reading frames) that encode five proteins, namely, the S gene encodes the surface antigen, the C gene encodes the core antigen and the e antigen, the p gene encodes the polymerase, and the X gene encodes the X protein. The X protein is an activator of viral RNA transcription (Levinson, 2008).

2.2.3. HBV the virion

The virion of hepatitis B (Dane particle) is a spherical lipid-containing structure of approximately 42 to 47nm. The virion consists of a viral envelope, nucleocapsid and a single copy of the partially double-stranded DNA genome. The nucleocapsid is comprised of 120 dimers of core protein and is covered by a capsid membrane embedded with 3 viral envelope proteins, the large(L), middle(M) and small(S) surface proteins (Seeger *et al.*, 2007). The partially double-stranded DNA genome consists of a minus-strand, which spans the full genome, and a plus-strand of DNA spanning roughly two thirds of the genome. Upon infection of the liver cells, the genome is converted to covalently closed circular DNA (ccDNA) of which the plus strand is used for the transcription of viral protein (Bowyer and Sim, 2000; Seeger *et al.*, 2007). Because viral replication takes place via an RNA intermediate and uses reverse transcriptase, an enzyme which lacks proof-reading and is known to have a high error rate, the nucleotide exchange rate is 10⁴ fold higher than that of typical DNA genomes and estimated to be between 0.1 and 0.7 per annum(Zhu *et al.*, 2010).

2.2.4. Protein composition of HBV particles

I. Surface proteins (HBs proteins):

The pre-S/S gene encodes the three transmembrane glycoproteins of the viral membrane:

- L-protein which provides a ligand for the viral receptor on hepatocytes.
- The M protein represents a form that is larger than HBsAg but smaller than the L-protein, its exact function is unknown as it does not appear to have a prominent function in virion assembly.
- The shortest, S-protein or S-antigen (HBsAg) contain the major antigenic determinants that led to discovery of HBV and is the basis of diagnostic tests for active infections and vaccines against HBV infection (Seeger *et al.*, 2007)

II. HBe protein

The pre-c protein, or e-antigen (HBeAg) as it is serologically termed, is a shorter excreted soluble protein whose exact function is unknown although it is thought to be associated with the regulation of the host immune response in HBV infection (Seeger *et al.*, 2007). Despite close similarities at the sequence level, HBcAg and HBeAg differ in solubility, assembly properties, function, infection kinetics and antigenic specificity (Watts *et al.*, 2010).

III. HBc protein

The pre-C/C or core mRNAs encodes the core and pre-core proteins. Core protein is a cytoplasmic, basic phosphoprotein whose antigenicity has been exploited from early days for the detection and monitoring of ongoing or resolved infections (Seeger *et al.*, 2007)

IV. HBx

The smallest gene, found only in animal hepadnaviruses, is the gene encoding the hepatitis B x antigen, HBxAg or X protein. This protein predominantly occurs as a soluble cytoplasmic protein but has also been associated with the cytoskeleton and in the nucleus (Seeger *et al.*, 2007).

2.2.5. Replication and infective cycle

As with all viruses, the life cycle of HBV and its relatives in the animal kingdom can be divided into several steps: attachment of the virus to the host cells, penetration into the cell, release of the viral genome, expression of viral gene products, replication of the viral genome, finally assembly of virions, and release of the virus (Zhu *et al.*, 2004). HBV have a unique pathway allowing the entry of newly synthesized viral DNA from the cytosol into the nucleus (Zhang, 2016), in addition HBV replicate through an RNA intermediate produces and release antigenic decay particles (Murray *et al.*, 2002).

2.2.6. Mode of Transmission

Transmission of hepatitis B virus; result from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include sexual contact, blood transfusion and transmission with other blood products and vertical transmission from mother to child (perinatal transmission) during child birth (Fairley and Read, 2012); (Buddeberg *et al.*, 2008). There is also other way of transmissions; parental drug abusers develop hepatitis from using shared unsterile equipments, the mortality may be very high in this group (Papatheodoridis *et al.*, 2002). Hospital staff and health care workers; have a higher carrier rate than the general population they get the infection through contact with blood parentally from; pricking or through skin abrasions (Sherlock and Dooley, 2002). The hepatitis B virus can survive outside the body for at least 7days. During this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The incubation period of the HBV is 75 days on average, but can vary from 30 to 180 days (WHO, 2015)

2.2.7. 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. The pathogenesis of hepatitis B is probably the result of this cell-mediated immune injury, because HBV itself does not

cause a cytopathic effect (Levinson, 2008). The chronic carrier state is attributed to a persistent infection of the hepatocyte, which result in the prolonged presence of HBV and HBsAg in the blood. The main determinant of whether a person clears the infection or became a carrier; is the adequacy of the cytotoxic T-cell response, HBV-DNA exists primarily as an episome in the cytoplasm of persistently infected cells; a small number of the copies of HBV-DNA are integrated into cell DNA. A high rate of hepatocellular carcinoma occurs in chronic carriers, the HBV genome has no oncogene, and hepatocellular carcinoma appears to be the result of persistent cellular regeneration that attempts to replace the dead hepatocytes . Alternatively, malignant transformation could be the result of insertional mutagenesis, which could occur when the HBV genome integrates into the hepatocyte DNA (Levinson, 2008)

2.3. Epidemiology of HBV

Over two billion people worldwide have evidence of previous or current hepatitis B virus infection while more than 350 million are chronic carriers of HBV, of whom 25% are at risk of serious illness and eventually death from cirrhosis or hepatocellular carcinoma (WHO, 2013). Three quarters of the world population live in areas with high levels of infection; risk factors for infection include blood transfusion, sexual intercourse, intravenous drug abuse, vertical and horizontal transmission of the virus (Hou et al, 2005). Africa on the whole considered having a high HBV endemicity (Kramvis, 2009). Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5-10% of the adult population is chronically infected , in the Middle East and the Indian subcontinent, an estimated 2-5% of the general population is chronically infected, while in western Europe and north America is less than 1% (WHO, 2016). Sudan is classified among countries with a high hepatitis B surface Antigen (HBsAg) endemicity of more than 8% , exposure to HBV infection ranges from 47% to 78% with a hepatitis B surface antigen seroprevalence ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan (Mudawi *et al.*, 2007).

The prevalence of HBV infection in health care workers and for all those who work at hospital facilities in different sectors is high; this group is at high risk of getting the infection due to occupational contact with blood and body fluids, depending upon the prevalence in the general population.

2.4. Clinical presentation

2.4.1. Acute HBV infection:

The incubation period ranges from 2 to 20 weeks (average 8-12 weeks). The onset of is usually insidious beginning with non-specific prodromal constitutional and gastrointestinal symptoms including; malaise, anorexia, vomiting and flu-like symptoms of pharyngitis, cough, coryza, photophobia, headache and myalgias. Prodromal symptoms abate or disappear with onset of jaundice, although anorexia, malaise and weakness may persist. These events can be related to circulating immune complexes (Livezey *et al.*, 2002). The usual clinical attack diagnosed in the adult tends to be more severe than for hepatitis A or C, however, the overall picture is similar. The self-limited, benign icteric disease usually lasts less than 16 weeks, jaundice rarely exceeds 4 weeks. Occasionally, a prolonged benign course is marked by increased serum transaminase value for more than 16 weeks, relapses are rare. Choestatic hepatitis with prolonged deep jaundice and pruritus is unusual (Craxi and Cooksley, 2003). Physical examination reveals mild tender hepatomegaly in over 70% of cases. Mild splenomegaly and posterior cervical lymphadenopathy is found in 15-20% of Cases (D. Valla, 2003). The first serological marker to become detectable during infection is the HBsAg at 8-12 weeks post-infection, after the onset of jaundice HBsAg titres gradually decrease and usually become undetectable after 2 to 6 months; shortly thereafter antibodies against S-antigen (Anti-HBs) become detectable in the serum and may remain detectable indefinitely. HBcAg is not normally found in the serum as it is either intracellular in hepatocyte or sequestered within the virion, class switch in the immunoglobulins from IgM to IgG occurs around 6 months post infection (Dienstag, 2010). A third serological marker,

HBeAg, is readily detectable either concurrently or shortly after S-antigen. This marker associated with high level of viral replication, become undetectable shortly after the characteristic peak in serum ALT activity (Dienstag, 2010).

The most severe cases of acute infection lead to complete liver failure and are termed fulminant hepatitis; this form of hepatitis is distinguished from others by a 100-fold increase in serum transaminase levels (ALT) in contrast to the 10-fold increase found in non-fulminant cases (Seeger *et al.*, 2007). Of those acutely infected, 5-10% of adults, 90% of neonates and 25-30% of children will develop a persistent or chronic infection (Bowyer and Sim, 2000)

2.4.2. Chronic HBV infection:

Chronic hepatitis B, or the persistence of HBsAg and HBV disease for more than 6 months, is host and virus dependant and presents in several distinct phases based on differing levels of viral replication and intensity of the immune response . Carriers experience an initial immune tolerant phase characterized by near normal levels of ALT, high level of HBV DNA and both HBsAg and HBeAg positivity (Dienstag, 2010). This phase ends when the immune system matures (in younger carriers) or recover, and begin to clear the virus; this often marked by HBeAg seroconversion when HBeAg become undetectable and Anti-HBe antibodies appear. This is a good sign because HBeAg high level is indication of high viral replication and infectivity (Bowyer *et al.*, 2011). In some cases, patients may fail to undergo seroconversion and remain in the immune active phase which is associated with an increase in ALT and high but variable HBV DNA titers. During this phase the virus cause severe liver damage whiles the host immune system unable to control the infection (Seeger *et al.*, 2007). This eventually contributes to liver cirrhosis and hepatocellular carcinoma (Kramvis, 2009).

2.5. Laboratory diagnosis

Serological tests for viral antigens and antibodies are typically used for diagnostic screening and can be performed on either serum or plasma. Both HBV antigens and antibody are stable at room temperature for days, at 4°C for months, and frozen at -20°C to -70° C for many years. Because modern testing involves automated enzyme immunoassay that depend on colourimetric or chemiluminescence signal measurement, care should be taken to avoid hemolysis of the sample because it may interfere with the ability of the assay to accurately detect these markers. A number of nucleic acid-based tests, which have been the subject of recent reviews, are available to directly detect HBV-DNA in serum or plasma (Pawlotsky, 2002).

2.5.1. Clinical chemistry tests:

Clinical chemistry investigations are helpful in differentiating hepatocellular jaundice due to viral hepatitis from hemolytic jaundice and obstructive jaundice (Cheesbrough, 2000).

- a. Serum aminotransferase enzyme activities (ALT-AST) are increased in the pre icteric stage.
- b. Urobilinogen can be found in urine, and when there is jaundice.
- c. Serum bilirubin levels are increased and bilirubin is present in the urine. Many viral hepatitis infections, however an icteric (without jaundice) but aminotransferase activity increased. Measurement of serum albumin can provide information on the severity of the hepatitis.

2.5.2. Serological tests:

The diagnosis is made on the basis of the blood sample which used to demonstrate antibodies against hepatitis or hepatitis components in the patient's blood (El Mishad, 2007).

- The most important laboratory test for detection of early HBV infection is the immunoassay for surface antigen (HBsAg), which appear during the incubation

period and is detectable in most patients during the prodrome and acute disease; if it is level remain high for more than 6 months, this means the patient became a chronic carrier of HBV (Levinson, 2008).

- Hepatitis B surface antibody (HBsAb) usually appears 4 weeks after the AG disappear. The presence of this Antibody means that the infection is at the end of its active stage and the patient cannot pass the infection to others (El Meshad, 2007).
- Hepatitis B envelope antigen (HBeAg); testing this antigen determine how contagious the patient is, and can be used to monitor the effectiveness of treatment (El Meshad, 2007).
- Hepatitis B core antigen (HBcAg) appears about one month after an active HBV infection. It can be found in people who had infection in the past and those with long-term (chronic) HBV. Anti-HBc suggests an acute and continuing viral replication. IgM anti-HBc may be the only indicator of recent infection in a period when HBs Ag has disappeared and anti-HBsAg is not detectable in serum (Kumar and Clark, 2009).

Enzyme linked immuonsorbant assay (ELISA): the procedure involves attaching antibodies or antigens to a solid surface and combining (immunosorbent) the coated surface with the test material, an enzymes system then linked to the complex, the remain g enzymes are washed away, and the extend of enzyme activity is measured. This gives an indication that antigens or antibodies are present in the test material (Pommerville, 2004).

2.5.3. Polymerase chain reaction:

Polymerase chain reaction (PCR) relies on the specificity of base pairing between short synthetic oligonucleotide probes and complementary sequences in a complex mixture of nucleic acids to prime DNA synthesis using a thermostable DNA polymerase. Multiple cycles of primer annealing, extension, and thermal denaturation are carried out in an

automated process, resulting in a massive amplification (2^n -fold increase after n cycles of amplification) of the target sequence located between the two primers (Alan J, 2005).

2.5.4. Detection of HBV in tissue specimen:

Because of its ability to generate high viremia and antigenaemia, HBV infection is usually easy to diagnose with the aid of the serum sample. Liver biopsies are necessary to examine the degree of the inflammation, necrosis and fibrosis, and repeated biopsies are required to follow the progression of the disease or the success of antiviral therapy. Because of the risk associated with the biopsy, it should be done only if the clinical, biochemical and virological data suggested severe disease. Biopsy may be stained for HBs Ag, pre-s1 antigen, HBc Ag or HBV-DNA (Topley and Wilsons, 2007).

2.6. Treatment

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontaneously. Early antiviral treatment may be required in less than 1% of people, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer, treatment lasts from six months to year, depending on medication and genotype ((Hollinger and Lau, 2006).

Although none of the available drugs can clear the infection; they can stop the virus from replicating. There are seven medications licensed for the treatment of hepatitis B infection, include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude), and the two immune system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys). The world health organization recommended a combination of tenofovir and entecavir as first-line agents (WHO, 2015).

2.7. Prevention and vaccination

Prevention depends on avoiding risk factors. These include not sharing needles and having safe sex, standard safety precautions in laboratories and hospitals must be enforced strictly to avoid accidental needle punctures and contact with infected body fluids (Lung and Fung, 2008).

2.7.1. Current vaccines:

Hepatitis B vaccine can prevent hepatitis B and its consequence, including liver cancer and cirrhosis. The currently used vaccine consists of small envelope(s) protein and middle; envelope (m) protein assembled into 22 nm particles. S vaccines are produced by processing of HBs Ag purified from plasma of HBV carrier and from yeast cells, expressing recombinant vaccine prepared by expressing in carbohydrate (CHO) (Mumtaz *et al.*, 2011). It cannot cause infection; the vaccine is usually given as 3 or 4 shots over a 6-month period (CDC, 2016).

Infants should get their first dose of hepatitis vaccine at birth and will usually complete the series at 6 months of age. All children and adolescents younger than 19 years of age who have not yet get the vaccine; should also be vaccinated. The vaccine is also recommended for those who are at risk of getting the infection such as; people who have household contact with someone infected, health care and public safety worker, travelers to regions with increased rate of HBV infection and anyone who wants to be protected (CDC, 2016).

2.7.2. Combined prophylaxis:

It should be given to staff with accidental needle stick injury; all newborn babies of HBs Ag-positive mothers; regular sexual partner of HBs Ag-positive patients, who have been found to be HBV-negative. For adult a dose of 500 IU of specific hepatitis B immunoglobulin (HBIG) (200 IU to new born) is given, and the vaccine intramuscular (I.M) is given at another site (Timbury, 1997).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a descriptive cross-sectional study conducted to detect Hepatitis B virus infection among cleaning workers in Khartoum State hospitals.

3.1.2. Study area

Hospital cleaning workers in Omdurman Teaching Hospital, Alshaab Teaching Hospital, Khartoum Oncology Hospital, Bahry Teaching Hospital and Antalia Medical Centers were sampled. The practical part of this study was carried out in the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology.

3.1.3. Study duration

The study was conducted during the period from February to May 2017.

3.1.4. Study population

Cleaning workers in Khartoum State hospitals.

3.2. Sample size and sampling technique

A total of ninety (n=90) hospital cleaning workers were selected randomly to participate in this study.

3.3. Ethical consideration

A verbal consent was taken from the study recruits; before proceeding with the study and collecting blood samples and after explaining the study and its goal.

3.4. Data collection

Data collected from the participants through structured questionnaires.

3.5. Collection of blood specimens

The blood specimens were collected from subjects after their consent. The venipuncture technique were used for collecting; the suitable vein was located, then the skin was cleaned by alcohol pad, sterile syringe (5ml) was used to collect about 5ml of blood, then the blood was dispensed in a sterile EDTA container with ethylenediaminetetraacetic acid anticoagulant.

3.6. Laboratory work

3.6.1. Preparation of plasma

Blood specimens were centrifuged at 3000 rpm for 5-10 minutes to obtain plasma. Then obtained plasma was preserved at -20°C until the analysis time.

3.6.2. Analysis of plasma

Plasma was analyzed for the presence of “HBsAg” by a commercially available enzyme-linked immunosorbent assay “HBsAg ELISA” kit (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, AntrimBT41 1QS United Kingdom). The assays were performed following the instructions of the manufacturer. Positive and negative controls were included in the assay. According to the information included in the kit’ s insert, the immunoassay used has specificity 99.94%.

3.6.3. Principle of the assay

The test is an enzyme-immunoassay based on a “sandwich” principle. Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs (antibody to HBsAg). Patient’s plasma sample is added to the microwells. During incubation, the specific immune-complex formed in case of presence of HBsAg in the sample, is captured on the

solid phase. After washing to remove sample serum proteins, second antibody conjugated to the enzyme HRP “horse raddish peroxidase” and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRP conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound HRP conjugate, chromogen solutions containing TMB and urea peroxidase are added to the wells. In the presence of the antibody-antigen-antibody HRP sandwich immune-complex, the colourless chromogens are hydrolyzed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction using the stop solution. The colour intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colourless.

3.6.4. Procedure

All reagents and specimens were settled to reach room temperature; 20µl of specimen diluents was added to each well except the blank, then 100µl of positive control, negative control and specimen were added to their respective wells. The plate was covered with plate cover and incubated for 60 minutes at 37°C. At the end of incubation period, 50µl of HRP-conjugate was added to each well except the blank; the plate covered again and incubated for 30 minutes at 37°C. By the end of incubation period each well was washed 5 times with diluted wash buffer. Finally 50µl of chromogen A and chromogen B solutions were added to each well including blank, then the plate was incubated at 37°C for 15 minutes and stop solution added at the end.

3.6.5. Quality control and calculation of the result

Reagents, standard and control were checked for storage, stability and preparation before starting work. Each microplate was considered separately when the results was calculated and interrelated; the results were calculated by relating each specimen absorbance (A) to the cut off (c.o.) of the plate.

Calculation of cut off value (C.O.) = $NC \times 2.1$ (NC is mean of the three negative controls).

3.6.6. Data analysis

Processing and analysis of data were carried out by means of the statistical package for the social sciences (SPSS). A descriptive statistic frequency was used to assess the risk; cross tabulation (chi-square) was used to compare the variable with positive result.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

4.1. Results

During February to April 2017 a total of ninety (90) blood specimens were collected from hospital cleaning workers at Khartoum State hospitals to determine the prevalence of hepatitis B virus using ELISA (enzyme linked immunosorbent assay). These hospitals include; Omdurman Teaching Hospital 44 samples (51.1%), Alshaab Teaching Hospital 8 samples (8.8%), Antalia Medical Center 10 samples (11%), Bahry Teaching Hospital 2 samples (2.2%) and Khartoum Oncology Hospital “Alzara” 26 samples (28.8%), as shows table (1). 58 (64.4%) were females and 32 (35.5%) were males as shows table (2) with a mean age (38.9) years. Of the ninety samples (90), 5 samples (5.5%) were positive for the presence of hepatitis B surface antigen (HBsAg), 2 (2.2%) were males and 3 (3.3%) were females as shows figure (1), distributed among different age group as shows figure (3).

The five (5) positive samples distributed as 3 samples (3.3%) from Omdurman teaching hospital, one sample from Khartoum oncology hospital (1.1%) and one sample from Antalia medical center (1.1%) as shows figure (2).

The results revealed no significant difference ($P>0.05$) between prevalence of HBsAg and the possible profounder predisposing to infection including; accidental needle stick injury, history of blood transfusion, family history of hepatitis infection and history of surgery, as shows table (3).

The participants were also questioned about the education level, marital status, and behavior of wearing protective clothes, HBV vaccination and job training; as these considered confounding factors table (4).

Table 1. Distribution of participants according to hospitals

Hospital	Frequency	Percent %
Omdurman Teaching Hospital	44	48.9
Alshaab Teaching Hospital	8	8.9
Khartoum Oncology Hospital	26	28.9
Antalia Medical Center	10	11.1
Bahry Teaching Hospital	2	2.2
Total	90	100.0

Table 2. Participants' distribution according to gender

Gender	Frequency	Percent %
males	32	35.6
females	58	64.4
Total	90	100.0

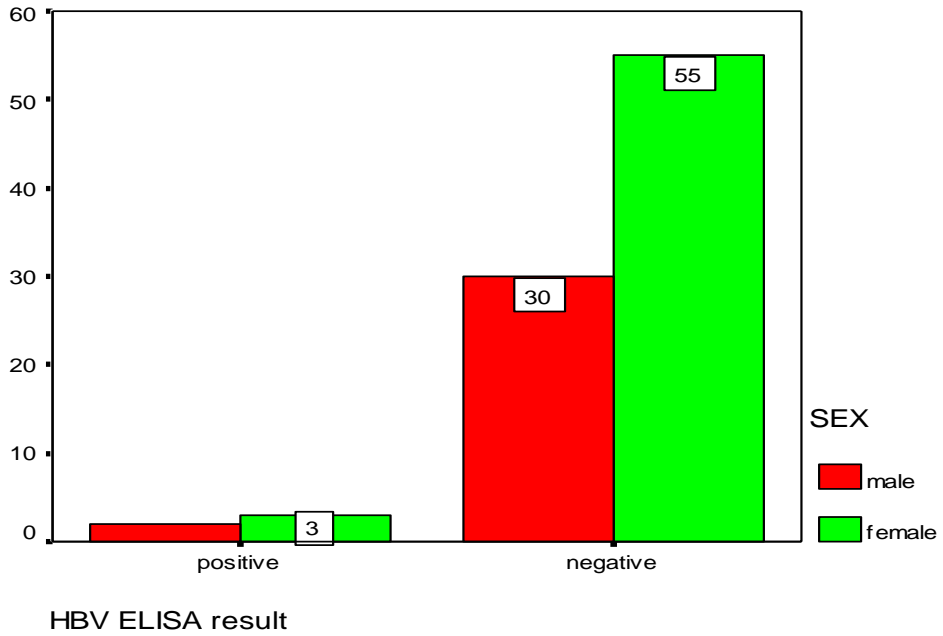


Figure 1. Distribution of positive HBV specimens among sex

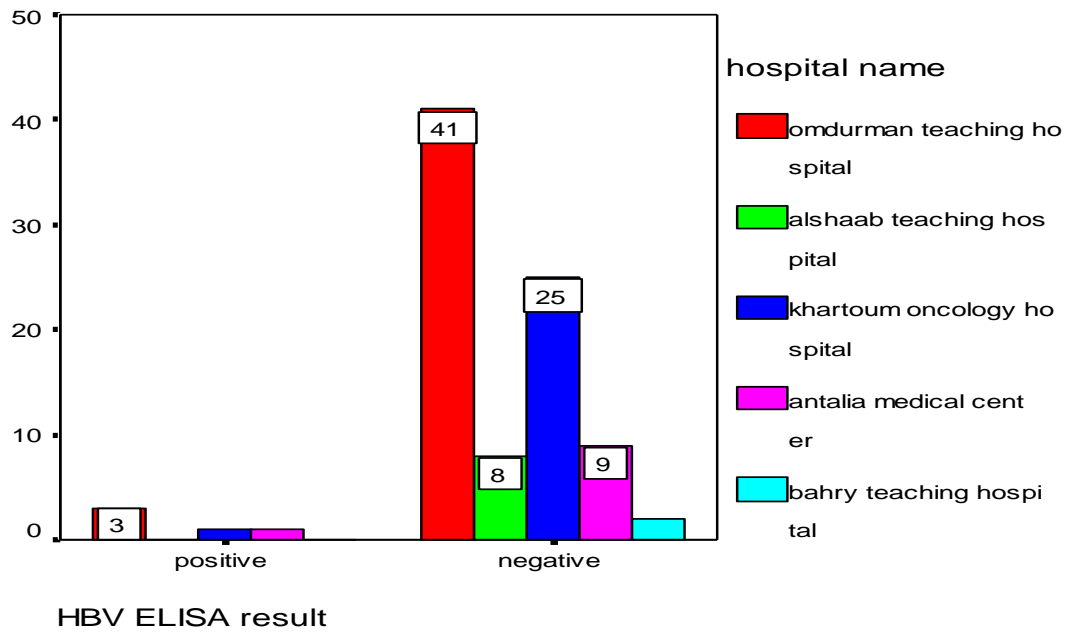


Figure 2. Distribution of HBsAg positive and negative specimens among different hospitals

Table 3. Possible risk factors and profounder predisposing to HBV infection among hospital cleaning workers

Risk factor	No. tested	No. of positive HBsAg	percentage
Needle stick injury	23 (25.5%)	2	2.2%
History of blood transfusion	8 (8.9%)	0	0%
Family history of hepatitis	10 (11.1%)	0	0%
History of surgery	1 (1.1%)	0	0%

Chi-square =0.446

Table 4. Confounding factors affecting hospital cleaning workers

Factors			Percentage
HBV vaccination	YES	39	43.3%
	NO	51	56.7%
Job training	YES	71	78.9%
	NO	19	21.1%
Protective clothes and equipments	YES	87	96.7%
	NO	3	3.3%
Education level	illiterate	50	55.6%
	basic	31	34.4%
	High school	9	10.0%
	university	0	0%

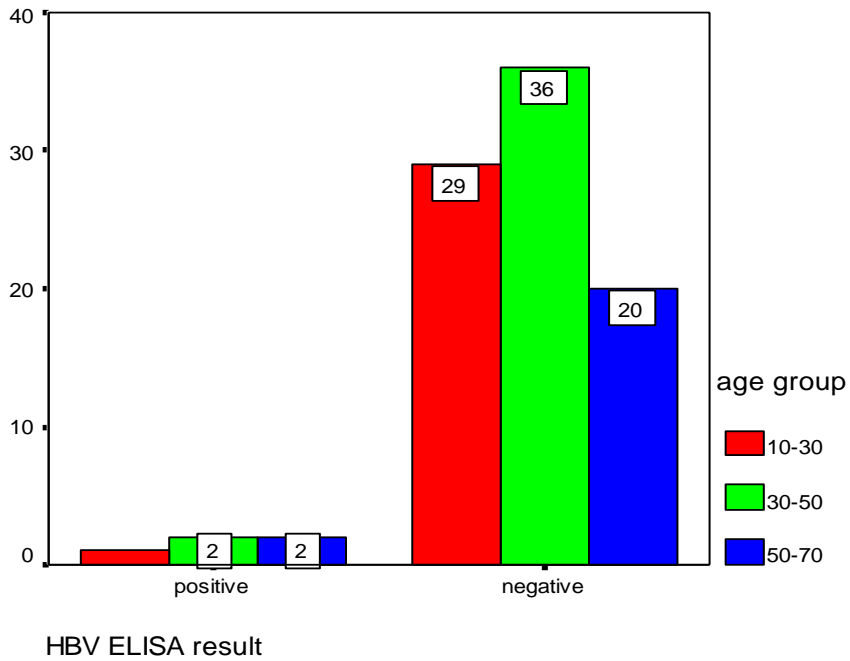


Figure 3. Distribution of positive specimens among age groups

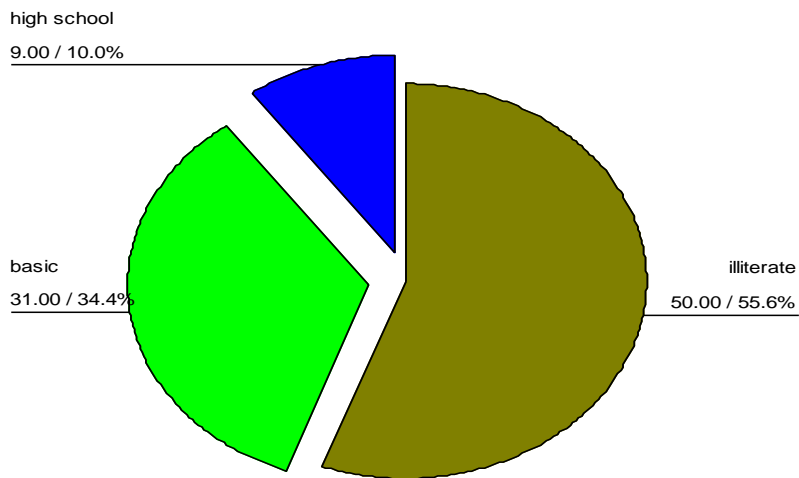


Figure 4. Education level among hospital cleaner

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

In this study, the prevalence of HBV among hospital cleaning workers was (5.5%) which is in line with that reported by Prù s-ù stü n *et al*, (2005) in Ethiopia (6%), and Abdalwhab *et al*, (2014) in Khartoum (4.4%) in Khartoum and Mueller A (2015) in Tanzania hospitals (7%); in which all of these studies were focused in the health care workers in general including doctors, technician, nurses and cleaning staff. Most of the studies don't focus on cleaning staff as the main study population. Data collected by structured questionnaire revealed that (55.6%) of the participant were illiterate, (34.4%) had basic education and only (10%) had reach high school education, this affect on the awareness of the workers about hazardous material they face in the work, around (78.9%) of them said that they had job training at least one time since they started work. Only 39 (43.3%) of the participants had a complete dose of HBV vaccine; which is important especially for the group at risk of getting the infection like health care workers.

87 (96.7) wear a protective clothes and gloves at work; also they knew the behavior of washing hands and do not handle needles and hazardous materials with naked hands, most of them depend on themselves on buying clothes and gloves for protection because the hospital do not provide them with their basic needs. 23(25.5%) of the participant had accidental needle stick injury at work which they reported it as most accident they face at work; while in 2016 a study made by Abdelmoneim kheir found that (46%) of health care workers of various categories of tertiary care hospital in Khartoum had needle stick injury with a mean of 6.1 injuries/year; this refer to that not all hospital staff follow the role of putting the needles and sharp objects in the safety box. By myself I noticed many needles in the ground, although the results obtained in this study shows no significance difference between prevalence of HBsAg and the possible profounding predisposing factors. 3 cases (3.3%) were positive for HBsAg and not vaccinated before against

Hepatitis B virus; this reflect that there are no standard vaccination protocols for HBV implemented in hospitals.

Further in-depth studies, using large sample size at a wide study population of cleaning workers, and employing recent molecular characterization techniques (e.g. Real time – Polymerase chain reaction RT-PCR) are strongly needed to resolve these clinically important viral infections in Sudan.

CONCLUSION

1. The prevalence of Hepatitis B virus among hospital cleaning workers was found to be (5.5%).
2. The obtained results emphasize the importance of screening programs for hepatic viral infection for health care workers and especially for cleaning workers.
3. The results and the data obtained can reflect the general situation of the hospital cleaning workers.

RECOMMENDATIONS

1. Effective health planning program is required to increase the public awareness of infection with viral disease.
2. Standard vaccination protocols and educational program are recommended for the health care workers in general and especially cleaning workers staff.
3. Implementation of quality control and infection control programs is necessary.
4. Accurate identification and management of the hazardous materials and objects.
5. Regular check of cleaning workers protective clothes, equipment and procedure they follow for protection.
6. Regular job training and advisory lectures are required.
7. Regular screening program is necessary for all workers.

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APPENDICES

Appendix
Questionnaire

Prevalence of hepatitis B virus among hospital cleaning workers at Khartoum state
hospitals

- Serial no:
- Age: sex: male female
- Residence:
- Marital status:
Single: married: others:
- Duration of employment:.....
- Educational level:
- Basic high school university others
- General health status:
Recent or previous disease:.....
History of drugs:
- Haemodialysis: Yes No
- History of surgery: Yes No
- Family history of hepatitis:
Yes NO
- Job training or educationally and advisory lectures :
Yes No
If yes; how many time per year:
- Type of protective equipment regularly used:
Gloves masks clothes boots
Others mention:.....

History of blood transfusion:

Yes No

• Vaccination:

Yes No

• Accidental needle stick injury:

Yes No

If yes; how many times:.....

What precaution you took:

• Common accidents at work:

