بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate studies

Sero-prevalence of Hepatitis B Virus among Prisoners in Khartoum State

الإنتشار المصلي لفيروس إلتهاب الكبد (ب)لدي المساجين في ولاية الخرطوم

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

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الآية

بسم الله الرحمن الرحيم

(يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ ۖ وَإِذَا قِيلَ انْشُزُوا فَانْشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

سورة المجادلة : الآية 11

DEDICATION

To my beloved family

 # Who always picked me up on time and

encouraged me to go on every adventure #

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Firstly, thanks to ALMIGHTY ALLAH who gave me the health and power to carry out this research.

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ABSTRACT

Hepatitis B is an infectious disease caused by Hepatitis B Virus (HBV) which affects the liver. The objective of this study was to study the prevalence of HBV among Sudanese prisoners in Khartoum State. The study was conducted during the period between February and May 2017.

A total of ninety (n=90) blood specimens were obtained randomly from female at prison of obstacles. 5 ml blood was collected from each prisoner. Plasma was obtained by centrifugation at 3000 rpm for 5 min. The plasma was examined for the presence of HBsAg using Enzyme Linked Immuno-Sorbent Assay (ELISA).

Data was collected by a structured questionnaire, which include demographic data such as age, marital status, education level, peivous HBV vaccination, family history of HBV infection, previous surgery or jaundice or needle stick and sharing of some article such as razor and nail clip.

The results showed that out of 90 blood samples investigated, 6 (6.7 %) were positive for HBsAg. The rest 84(93.3%) were negative.

The study concluded that, HBV infection among female prisoners is intermediate (6.7%). And the level of infection is equal according to marital status.

Further studies with large number of samples and more advanced technique are required to validate the results of the present study.

المستخلص

التهاب الكبد الفيروسي (ب) هو عبارة عن مرض معدي يسببه فيروس التهاب الكبد ب الذي يصيب الكبد وقد يتسبب في سرطان خلايا الكبد, الهدف من الدراسه هو تحديد مدى انتشار فيروس التهاب الكبد ب بين المساجين في ولاية الخرطوم في الفترة بين فبراير الى مايو 2017.

جمعت 90 عينه بصوره عشوائيه من النساء بسجن التائبات فى ولاية الخرطوم. جمعت 5 مل من الدم من كل مريض ثم فصل منها المصل. كل العينات خضعت للفحص بحثا عن المستضد السطحي لفيروس الكبد ب باستخدام الإلايزا.

جمعت المعلومات بواسطه استبيان شخصى مع كل سجين ,ويحتوى الاستبيان على معلومات عن العمر , الحاله الاجتماعيه , المستوى التعليمى , التطعيم ضد فيروس التهاب الكبد الوبائى ب , تاريخ الاسره مع الاصابه بالمرض , التعرض المسبق لعمليه جراحيه او الاصابه المسبقه باليرقان , وما اذا تعرض للجرح باله حديديه وايضا مدى مشاركتهم لبعض الاشياء كالامواس او ضفاره الاظافرز

أظهرت النتائج أنه من مجموع 90 عينة فحصت فقط 6(6.7%) عينة أظهرت نتائج إيجابية بينما 84 (93.4%) عينة أظهرت نتائج سلبية.

خلصت الدراسة إلى أن عدوى فيروس إلتهاب الكبد (ب) بين السجينات فى ولاية الخرطوم موجود بنسبة متوسطة(6.7%). وأن نسبة الاصابة متساوية مقارنة بالحاله الاجتماعيه للسيدات.

يوصى بدراسات إضافيه بعدد أكبر من العينات وتقنيات متقدمة للتحقق من نتائج هذه الدراسة.

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CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVE

1.1. Introduction

Hepatitis B virus (HBV) infection and its related complications remain a p rimary public health threat globally. More than 240 million people were c hronically HBV infected with 600,000 deaths per year related to HBV (Sc hweitzer *et al.,* 2015*;* Roberts *et al.,* 2016).

HBV is a silent killer disease of the liver with many carriers not realizing t hat they are infected with the virus (Samuel *et al.,* 2004).

Most of the prisoners originate from marginal socio-economic rural areas and are subject to extreme poverty, pervasive social health problems, limit ed educational opportunities, and illegal behaviors, such as drug injection and unsafe sexual activities (Javadi *et al.,* 2006).

Prisons are incubators for infectious diseases, yet are readily accessible for screening and intervention (**Bick, 2007**). They provide a high-yield opportunity for early disease detection, intervention, and treatment, which would benefit not only prisoners and prison employees, but also family members and the general population due to the high turnover of prisoners (McIntyre *et al.,* 2009).

About 9.25 million people are held in prisons worldwide, with 30 million inmates moving from prison to the community and/or back again each year (Walmsley, 2007; UNAIDS, 2006).

Prisons are typically overcrowded, offer limited access to health care, and harbor high rates of airborne and blood-borne diseases (Niveau, 2006).

The prevalence of HBV infection in prisoners in Lebanon, Hungary, Spain , Indonesia, and Croatia was reported about 2.4%, 1.5%, 2.6%, 5.8%, and 11.3%t, respectively (Burek *et al.*, 2010; Treso *et al.*, 2012).

HBV infection is distributed throughout the world, and it is estimated that around 2000 million people worldwide have been in contact with this path ogenic agent. Despite successful vaccination programs and effective antivi ral therapies, there are over 350 million carriers of HBV surface antigen (HBsAg). Some 150 million of them have active infection and a high risk o f progression to cirrhosis or hepatocellular carcinoma (HCC). HBV-infect ed individuals have a 30-fold higher risk of developing HCC than the rem ainder of the population, and it is estimated that 53% of liver cancers worl dwide are associated with HBV (Lupberger and Hildt, 2007).

HBV infection is one of the major causes of death related to cirrhosis and liver cancer (Ott *et al.*, 2012).

Of note, the epidemiology in Africa is characterized by a much higher HB sAg prevalence in rural than in urban areas (Komas, 2010).

World Health Organization estimates that the prevalence of hepatitis B vir us infection in Africa is on average more than 10% (Bwogi *et al*., 2009).

1.2. Rationale

HBV is one of the most important Viruses that cause liver disease and can progress to liver cirrhosis and HCC. HBV accounts for penalty of death pe r year therefore the early diagnosis can help in treatment and even limitati on of its transmission.

HBV studies correlate the prevalence of the disease with certain high risk groups; the prisoners are more susceptible for HBV infection due to their poverty, life style and drug abuse.

1.3. Objective

1.3.1. General objective

To study the prevalence of HBV among Sudanese prisoners at Altaebat prison.

1.3.2. Specific objectives

1. To detect HBsAg Virus among prisoner by ELISA.

2. To determine the prevalence of HBV among Sudanese prisoners at Altaebat pri son.

3. To correlate the presence of HBV with marital status, age, family history, HBV vaccination and previous jaundice or surgery.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Background

Hepatitis B is a potentially life-threatening liver infection caused by the HBV. It is a major global health problem. It can cause chronic infection and puts at high risk of death from cirrhosis and liver cancer (WHO, 2017).

Blumberg won the Nobel Prize in Medicine for his discovery of the hepatitis B virus. He and his colleagues discovered the virus in 1967, developed the blood test that is used to detect the virus and invented the first hepatitis B vaccine in 1969 (Mason, 2008).

Early identification of infected persons with the help of blood tests can break the o ngoing transmission and lead to necessary treatment with antiviral medication (Ng uyen *et al.*, 2007). Household with the infected person and sexual partners that mi ght have become infected. To avoid transmission there are a few measures that HB V positive individuals can take, for example they should notify sexual partners and the people they share their household with to test themselves for HBV and inform them of the need for vaccination. An HBV-infected person can delay and/or preve nt liver disease by limiting their alcohol consumption and by regularly seeking dis ease monitoring (Weinbaum *et al.*, 2009).

Despite availability of a vaccine and antiviral treatment, HBV infection is still a m ajor

Health problem causing considerable morbidity and mortality (Chaiba *et al.*, 2012; Harkisoen *et al.*, 2012)

2.2. General characteristics of HBV

HBV is a DNA virus .This virus belongs to the genus Orthohepadnavirus of the Hepadnaviridae family and, along with the Spumaretrovirinae subfamily of the Retroviridae family, represents 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).

The Dane particle of HBV 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 (Seeger *et al*., 2007).

There are different types of hepatitis B antigens encoded by the HBV genome, Hepatitis B Surface antigen (HBsAg). This protein is the prime constituent of all hepatitis B particle forms and appears to be manufactured by the virus in high quantities. It also contains a highly antigenic epitope which may be responsible for triggering immune response (Yen, 2002).

The second antigen is Hepatitis B Core Antigen (HBcAg) which is the only HBV antigen that cannot be detected directly by blood test, this antigen can only be isolated by analyzing an infected hepatocyte. Also Hepatitis B e Antigen (HBeAg) which is named due to its early appearance during an acute HBV infection. Thought to be located in the core structure of the virus molecule, this antigen can be detected by blood test. If found it's usually indicative of complete virus particles in circulation (Strauss, 2002).

The last one is, HBV *X* Antigen encodes a 16.5-kd protein (HBxAg) with multiple functions, including signal transduction, transcriptional activation, DNA repair,

and inhibition of protein degradation (Zhang *et al.,* 2001).

HBV virus currently encompasses eight genotypes with several genotypes co mprising multiple sub genotypes (Mason *et al*., 2012).

2.3. Replication of HBV

HBV replication is protein-primed reverse transcription, related to, but mechanistically distinct from, retroviral replication (Seeger and Mason, 2000). Replication of HBV can broadly be divided into three phases, infectious vi rions contain in their inner icosahedral core the genome as a partially doub le-stranded, circular; the second phase is upon infection, the relaxed circul ar DNA(RC-DNA) is converted, inside the host cell nucleus, into a plasmi d-like covalently closed circular DNA (cccDNA); and in the last phase fro m the cccDNA, several genomic and sub genomic RNAs are transcribed b y cellular RNA polymerase II; of these, the pregenomic RNA (pgRNA) is selectively packaged into progeny capsids and is reverse transcribed by th e co-packaged P protein into new RC-DNA genomes. Matured RC-DNA containing but not immature RNA containing nucleocapsids can be used f or intracellular cccDNA amplification, or be enveloped and released from the cell as progeny virions (Juergen Beck and Michael Nassal, 2007).

2.4. Viral hepatitis

Hepatitis is a Latin word which means inflammation of liver (Lu SN *et al.* , 2003).

Hepatitis is a general term for inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of Jaundice is a characteristic feature of liver disease, a correct diagnosis can only be made by testing patient's sera for the presence of specific antiviral antigens and antibodies (Hollinger and Liang, 2001).

Viral hepatitis is often a silent disease whose symptoms and signs become evident only after the disease has caused severe liver damage. The sympto ms of hepatitis can take decades to manifest, so many people who are infe cted with hepatitis are unaware that they have the disease and therefore do not seek treatment (Iom, 2010).

2.4.1. Acute viral Hepatitis B.

Acute Hepatitis infections have a one month (4-6 weeks) to as long as 6 months incubation period after transmission as the virus spreads with in the liver. In approximately 65% of acute infections the infection and re solution is clinically silent. Symptoms that are clinically recognized in the remaining cases include decreased appetite, nausea and vomiting, fatigue and abdominal pain as well as jaundice in the more severe cases. These sy mptoms most often result from increased production of pro-inflammatory cytokines such as INF-γ or TNF-α (Seeger *et al*., 2007).

The first serological marker to become detectable during infection is the H BsAg, which usually becomes detectable at 8-12 weeks post-infection, ass uming one month incubation. This marker typically precedes an elevation of serum ALT levels and symptoms of hepatitis by 2 to 6 weeks and remai ns detectable throughout the symptomatic phase. After the onset of jaun dice, HBsAg titers gradually decrease and usually and become undet ectable after 2 to 6 months. Shortly thereafter antibodies against S-antige n (Anti-HBs) become detectable in the serum and may remain detectable i ndefinitely (Dienstag, 2010).

2.4.2. Chronic viral Hepatitis B

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 levels of HBV DNA and both HBsAg and HBeAg positivity (Dienstag, 2010).

This phase ends when the immune system matures (in younger carriers) or recovers and begins to control and clear the virus. The end of the immune clearance (or immune active) phase is often marked by HBeAg seroconversion when HBeAg levels become undetectable and Anti-HBe antibodies appear. This is considered a good clinical sign and marks the beginning of an inactive carrier state because high HBeAg levels are indicative of high viral replication and infectivity, whereas high Anti-HBe levels indicate a low level of viral replication with low to moderate infectivity (Bowyer *et al*., 2011; Dienstag 2010).

2.5. Pathogenesis of HBV

The Hepatitis B Virus is a blood-borne virus and roughly 75 – 200 times more infectious than HIV (Bowyer *et al*., 2011).

The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days. The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic (WHO, 2017).

Hepatitis B Virus primarily interferes with the functions of the liver by replicating in liver cells. The virions bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV-preS-

specific receptors are expressed primarily on hepatocytes; however, viral DNA and proteins have also been detected in extra hepatic sites suggesting that cellular receptors for HBV may also exist on extra hepatic cells. During HBV infection the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, in particular virus-specific cytotoxic T lymphocytes (CTLs) contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines which are then used to purge HBV from viable hepatocytes (Lannacone *et al.*, 2007).

2.6. Signs and symptoms

Acute infection with Hepatitis B Virus is associated with acute viral hepatitis – an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result (Terruault and Samael. 2005).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma . Symptoms outside of the liver are present in 1–10% of HBV-infected people and include serum-sickness–like syndrome, acute necrotizing vacuities (polyarteritis nodosa), membranous glomerulonephritis, and papular acrodermatitis of childhood The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice. The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after the onset of jaundice (Lang, 2009).

2.7. Transmission

HBV is highly contagious and is transmitted by percutaneous and permuc osal exposure to infected blood and other body fluids (i.e. semen and vagi nal fluid). The highest concentrations of the virus occur in blood and wou nd secretions Moderate concentrations of HBV are found in semen and va ginal fluid, and lower concentrations occur in saliva. HBV is not spread b y air, food, or water. Other common modes of transmission include mothe r-to-infant, child-to-child, unsafe injection practices and blood transfusion s, and sexual contact, Perinatal transmission from HBsAg-positive mother s to their newborn infants (vertical) or transmission from one child to anot her (horizontal) is a major source of HBV infections in many countries wh ere chronic HBV infection is highly endemic, also the spread of HBV fro m child to child usually happens in household settings but may also occur in child daycare centers and schools (WHO, 2001).

In prisoners significant risk factors were intravenous drug abuse (Kazi *et a l.*, 2010; Fayyaz *et al.*, 2006).

2.8. High risk group

High risks group includes people with multiple sex partners, previously in fected with STD, homosexual men, people who have a sexual partner with hepatitis, people who are addicted to injection drugs or who have partners who use them, people who share a household with someone chronically in fected with hepatitis, Health care workers, Dialysis patients, patients HCV and HIV infections and patient who is taking immunosuppressive or cytot oxic therapy (Dienstag, 2008).

2.9. Epidemiology

HBV infection affects over 350 million people worldwide and over one million die annually of HBV-related chronic liver disease (Jinlin *et al*., 2005).

According to the estimates of the global burden of disease study, deaths due to vir al hepatitis increased by 63% from 0.89 million to 1.45 million between 1990 and 2013, and Africa is one of the regions with highest mortality rate (Stanaway *et al.*, 2013).

The prevalence of HBV infection varies according to the geographic region, and is categorized as high (≥ 8%), intermediate (2%-7%), or low (< 2%) endemicity (Fitr ia *et al.*, 2015; Gunardi, *et al.*, 2014).

2.10. Laboratory Diagnosis

The specimen of choice for the diagnosis of HBV infection is blood. Serol ogical tests for viral antigens and antibodies are typically used for diagnos tic 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 tes ting involves automated enzyme immunoassays that depend on colorimetr ic measurement, care should be taken to avoid hemolysis of the sample be cause it may interfere with the ability of the assay to accurately detect thes e markers (Mel Krajden *et al.*, 2005).

The diagnosis of HBV infection and its associated disease is based on a co nstellation of clinical, biochemical, histological, and serologic findings. A number of viral antigens and their respective antibodies can be detected in serum after infection with HBV, and proper interpretation of the results is

essential for the correct diagnosis of the various clinical forms of HBV inf ection (T. Jake Liang, 2010).

serological assays for the detection of HBV antigens (HBsAg and HBeAg) and antibodies (anti-HBs, anti-HBc and anti-HBe) should be performed t o assess the phase of chronic hepatitis B. HBV DNA can be monitored in serum by means of DNA hybridization with signal amplification, to assess disease activity and candidacy for antiviral therapy and to determine respo nse to treatment. Liver biopsy is essential to confirm the diagnosis, to iden tify any intercurrent disease affecting the liver, to stage the fibrosis and to grade the necroinflammation (Raymond and Graham, 2004).

HBV DNA testing can also be helpful in the assessment of level of viral re plication and possibly helpful in assessing prognosis and need for antiviral therapy (Pawlotsky *et al.*, 2008).

PCR tests have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment (Zoulim, 2006).

2.11. Treatment

There is no specific treatment for acute hepatitis B. Therefore, care is aimed at maintaining comfort and adequate nutritional balance, including replacement of fluids lost from vomiting and diarrhoea while chronic hepatitis B infection can be treated with drugs, including oral antiviral agents. Treatment can slow the progression of cirrhosis, reduce incidence of liver cancer and improve long term survival. WHO recommends the use of oral treatments - tenofovir or entecavir, because these are the most potent drugs to suppress hepatitis B virus. They rarely lead to drug resistance as compared with other drugs, are simple to

take (1 pill a day), and have few side effects so require only limited monitoring. In most people, however, the treatment does not cure hepatitis B infection, but only suppresses the replication of the virus. Therefore, most people who start hepatitis B treatment must continue it for life (WHO, 2017).

On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer (Hollinger and Lau, 2006).

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. As from 2008, there are seven medications licensed for treatment of Hepatitis B infection in the United States antiviral drugs Lamivudine, Adefovir, Tenofovir, Telbivudine, Entecavir, and the two immune system modulators interferon Alpha-2a and PEGylated Interferon Alpha-2a (Pegasys). The use of interferon, which requires injections daily or thrice weekly, has been substituted by long-acting PEGylated interferon, which is injected only once weekly (Albert and Caporaso, 2011).

2.12. Prevention

2.12.1. Passive immunoprophylaxis

This is done either with standard Immunoglobulin, containing modest levels of anti-HBs or hepatitis B immunoglobulin (HBIG), containing high-titer anti- HBs (Weinbaum *et al.,* 2009).

2.12.2. Active immunization

These includes either, Purified, noninfectious 22-nm spherical forms of HBsAg derived from the plasma of healthy HBsAg carriers or Plasma-derived vaccine, supplanted by a genetically engineered vaccine derived from recombinant yeast, consisting of HBsAg particles that are nonglycosylated but are otherwise indistinguishable from natural HbsAg (Weinbaum *et al.,* 2009).

2.11.3. Pre-exposure prophylaxis

It is indicated for health workers exposed to blood; hemodialysis patients and staff; residents and staff of custodial institutions for the developmenta lly handicapped; injection drug users; inmates of long-term correctional fa cilities; persons with multiple sexual partners; persons such as hemophilia cs who require long-term, high-volume therapy with blood derivatives; ho usehold and sexual contacts of HBsAg carriers; persons living in or travell ing extensively in endemic areas; unvaccinated children under the age of 1 8; unvaccinated children who are immigrants from endemic countries (We inbaum *et al.*, 2009).

2.13. Previous studies

There are many studies which indicate a high prevalence of HBV infection among prisoners, 13% - 47% of prisoners in the USA had HBV infection by (Anonymous, 2004). In other study that conducted by (Van et *al.,* 1993) on Amsterdam, which found that 68% of addict prisoners are infected with HBV infection.

The prevalence of HBV infection in prisoners in Lebanon, Hungary, Spain , Indonesia, and Croatia was reported about 2.4%, 1.5%, 2.6%, 5.8%, and 11.3%t, respectively (Burek *et al.,* 2010; Treso *et al.,* 2012).

The prevalence of any HBV serological marker was 17.9% with Infection rates varied from 14% (prison for females) to 23.5% (prison for males) thi s was conducted in a Brazilian study(Alcione *et al*., 2010).

25.2% of prisoners had antigen or core or surface antibodies to HBV by (Solomo n et al., 2004) on Maryland.

The sero-prevalence of HBV (8.1%) infections among the inmates of a pri son in Bologna by (Sabbatani *et al.,* 2004*).*

In Pakistan the prevalence of HBV among prisoners is 5.9% the study was

conducted by (Abdul *et al.,* 2009).

When the subjects were examined according to the presence of hepatitis B , HBsAg positivity was detected in 2.6% among Prisoners in Turkey (Der ya *et al.,* 2016).

High infection prevalence among inmates represents 20.2 % of hepatitis B virus a mong Males in Rhode Island Prisons by (Grace et al., 2004).

Another study on India detected that HBsAg among prisoners was 2.72% and found that, slightly higher percentage of HBsAg was found among ma les i.e., 3.19% than females i.e., 1.16% (Shweta *et al.,* 2015).

Of the 970 participants, 264 (27.2%) were positive for any of HBV serolo gical markers, and 706 (72.8%) individuals were serologically negative fo r all HBV markers in prisoners related to injection drug users in Iran (Dan eshmand *et al.,* 2013).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE
MATERIAL AND METHOD

3.1. Study design

3.1.1. Type of study

This was a descriptive cross sectional study conducted to determine seroprevelance of HBV among Sudanese prisoners.

3.1.2. Study area

prison on Khartoum State.

3.1.3. Study duration

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

3.1.4. Study population

Sudanese female prisoners on Altaebat prison at Khartoum State.

3.2. Sample size and sampling technique

A total of ninety (n=90) prisoners were selected randomly in this study.

3.3. Ethical consideration

Approval to conduct this study was obtained from College Ethical Committee, Sudan University of Science and Technology.

3.4. Data collection

3.4.1. Questionnaire

A structured questionnaire was used for collection of both qualitative and quantitative data. Which include demographic information such as age, marital status, education level, pervious HBV vaccine, family history of HBV infection, previous surgery or jaundice, previous needle stick, and sharing of some article such razor and nail clip.

3.5. Laboratory methods

3.5.1. Blood specimens

The blood specimens were collected from each prisoner after his consented. The venipuncture were use for collection, the suitable vein was located, using sterile syringe (5 ml) to collect the blood after cleaning the skin area with alcohol pads

and the blood will be dispensed in a sterile EDTA container.

3.5.2. Preparation of specimens

Blood specimens were centrifuged at 3000 for 5 minutes to obtain plasma, and then the obtained plasma was preserved at -20°c until the serological analysis.

3.5.3. Analysis of specimens

The specimens were analyzed for the presence of HBsAg by a commercially available enzyme linked immunosorbent assay “ELISA” kit (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, and BT41 IQS United Kingdom). The assays were performed following the instruction of the manufacturer. Positive and negative controls were included in each assay. According to the information included in the kit’s insert, with specifIty 99.94 %.

3.5.4. Principle of the assay

The test is an enzyme immunoassay based on a "sandwich principle polystyrene micro titer strip wells have been coated with monoclonal anti-HBs (antibodies to HBsAg). Patient’s serum or plasma sample is added to the microwells. During incubation the specific immune complex is formed in case of presence of HBsAg in the sample is captured on the solid phase. After washing to remove sample serum protein, second antibody conjugated to the enzyme HRP and directed against a different epitope of HBsAg is added to the well. 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 solution a solution containing TMB and urea peroxide are added to the wells. In the presence of the antibody antigen antibody HRP sandwich immune complex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction using the stop solution. The color 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 colorless.

3.5.5. Procedure

All reagents and specimens were settled to reach room temperature, 20ul of specimen diluents was added to each well except the blank then 100ul of positive control, and negative control. The specimens 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, 50ul of HRP-conjugate was added to each well except the blank; the plate was covered 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 50ul 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 was added.

3.5.6. Quality control and calculation of the results

Reagent, standard and control were checked for storage, stability and preparation before starting work. Each miocroplate 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 × 2.1 (NC is mean of the three negative controls).

The OD value of the blank well must be less than 0.080 at 450nm.

The OD value of the positive control must be more than 0.80 at 450nm.
The OD value of the negative control must be less than 0.1 at 450 nm

3.5.7. Interpretation of results

Positive more than cut of value.

Negative less than cut of value.

3.6. Data analysis

The data that collected from questionnaire and laboratory results were analyzed by SPSS version 16 computerized program.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 90 female prisoners were included in this study; the specimens were collected from prison of Obstacles, data regarding their age, marital status, family history, HBV vaccination, sharing objects, needle stick and previous jaundice, surgery or blood transfusion were gathered by direct interview.

Their age ranges from 15 to 70 years, classified into three age groups as in (figure 1). 51(56.7%) of prisoners lie between the age group of (15-32) years, 5(9.9%) of them are HBV positive and 46(90.1%) were negative. 32 (35.6%) of them lie in the second age group of (33-50) years, 1 (3.2%) was HBV positive and the rest 31(96.8%) were negative. 7(7.7%) in the last age group (51-70) years, all were HBV negative.(Table 1).

Their blood specimens were processed for HBsAg by ELISA 6.7% (6/90) were HBV positive while the other 93.3% (84/90) were HBV negative as seen in (Table 2 & Figure 2).

Of the study population 60 %(54/90) were married, 40% (36/90) were single as seen in (Table 3). The positive results distributed between the two marital status (3/33) and (3/51) in single and married respectively (Figure 3).

Only(2/90) had HBV vaccine the rest, 97.8 % (88/90) had no HBV vaccination, the family history with HBV infection 16.7 % (15/90), 23.3% (21/90) went through at least one surgery, 21.1% (19/90) of the study group get transfused with blood or blood product at least once, 38.9% (35/90) were previously jaundiced and 16.7%(15/90) get injured throw needle stick, with their prevalence to HBV infection, all shown in(Table 4).

Table 1. Frequency of HBV among Prisoners according to their age group

Age group	Total		HBV			
			Negative		Positive	
	NO	%	NO	%	NO	%
15-32 years	51	56.7	46	90.1	5	9.9
33-50 years	32	35.6	31	96.8	1	3.2
51-70 years	7	7.7	7	100	0	0
—	90	100	84	-	6	-

Results	**Samples**	
	No.	**%**

Positive	6	6.7
Negative	84	93.3
Total	90	100

Table 2. Frequency of HBV among prisoners

Table 3. Distribution of prisoners according to the marital status

Marital status	Prisoners	
	No.	%
Married	54	60
Single	36	40

Total	90	00

Table 4. Frequency of HBV in relation to prisoner's situation

Situation	Status	NO.	Positive HBV		Negative HBV	
			Frequency	Percent %	Frequency	Percent %
Marital status	Married	54	3	5.5	51	94.5
	Single	36	3	8.3	33	91.7
HBV Vaccine	YES	2	0	0	2	100

	NO	88	6	6.8	82	93.2
Family history	YES	15	1	6.6	14	93.4
	NO	75	5	6.6	70	93.4
Previous jaundice	YES	35	1	2.8	34	97.2
	NO	55	5	9.0	50	91.0
Previous surgery	YES	21	1	4.7	20	95.3
	NO	69	5	7.2	64	92.8
Needle stick	YES	15	1	6.6	14	93.4
	NO	75	5	6.6	70	93.4
Sharing objects	YES	19	1	5.2	18	94.8
	NO	71	5	7.0	66	93.0
Previous Blood transfusion	YES	6	1	16.6	5	83.4
	NO	84	5	5.9	79	94.1

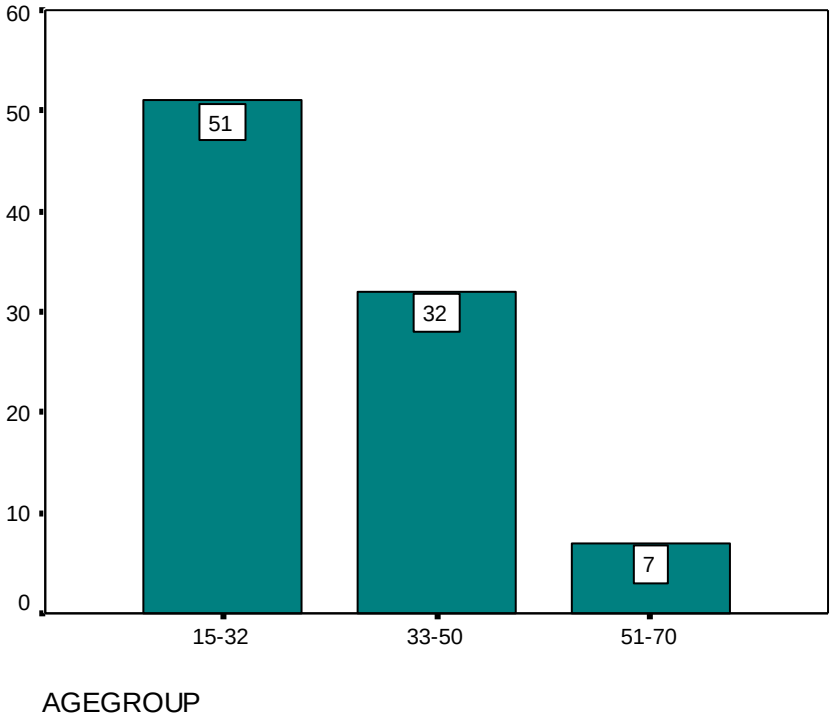

Figure 1. Distribution of the prisoners according to their age groups

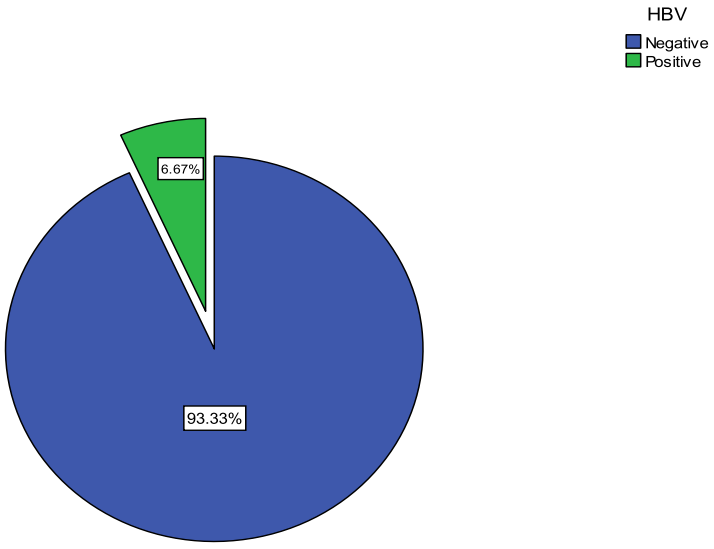

Figure 2. Frequency of HBV among Prisoners

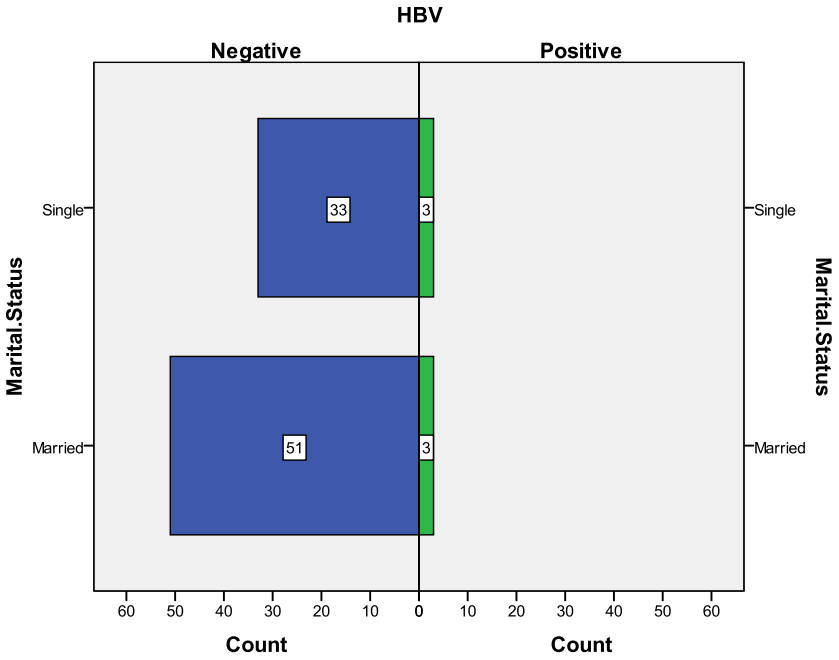

Figure 3. Distribution of positive HBV result between the 2 martial statuses

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE
DISCUSSION

5.1. Discussion

HBV is a serious growing problem in African and Arab countries, and they have great affect on community and future generation's health. So we need great effort to conduct studies that may give guidelines for proper planning to deal with the health problem that related to HBV infection.

The present study aimed at detection of HBV among prisoners in Khartoum State. Out of 90 blood specimens investigated, only 6(6.7%) were positive, the remaining 84(93.3%) were negative.

This result is similar to that obtained in Pakistan by Abdul *et al.*, (2009) w hich showed that the prevalence of HBV among prisoners is 5.9%. But dis agrees with that reported in a Brazilian study by Alcione *et al.*,(2010) whi ch reported that 14% prison of females were positive for HBV. This differ ence may be due to the high endimicity of Brazil with HBV infection.

Their age ranges from 15 to 70 years, classified into three age groups. 51(56.7%) of prisoners lie between the age group of (15-32) years, 5(9.9%) of them are HBV positive and 46(90.1%) were negative. 32 (35.6%) of them lie in the second age group of (33-50) years, 1 (3.2%) was HBV positive and the rest 31(96.8%) were negative. 7(7.7%) in the last age group (51-70) years, all were HBV negative.

The positive results distributed between the two marital status (3/33) and (3/51) in single and married respectively.

5.2. Conclusion

1. The prisoners with poor knowledge about HBV infection and there were poor prevention from the virus; only 2 from total of 90 prisoners were vaccinated.

2. There is moderate exposure to HBV infection in prisoners female.

3. The exposure to HBV infection is equal related to marital status.

4. The highest percentage of HBV infection among the age group (15-32) years.

5.3. Recommendations

1. Screening of the blood for any prisoners before administration to the prison.

2. Extensive vaccination against HBV is recommended.

3. Increase the Educational level about the virus, its transmission and prevention for the prisoners.

4. More health care level for the prisoners.

5. Further studies with large number of samples and more advanced technique are required to validate the results of the present study.

REFERENCES

REFERENCES

1. **Albert A. and Caporaso N. (2011).** HBV therapy guidelines. *Ital J Gastroentrol*. **43**(1): 57-63.
2. **Alcione Cavalheiro Faro Stief, Regina Maria Bringel Martins, Sônia Maria Oliveira, Mauricio Antonio Pompilio, Sonia Maria Fernandes, Paula Guerra Murat, Gina Jonasson Mousquer, Sheila Araújo Teles, Graciele Rodrigues Camolez, Roberta Barbosa Lopes Francisco and Ana Rita Coimbra Motta-Castro. (2010).** Seroprevelance of HBV infection and associated factors among prison animates in State of Brazil. *SciELO Analytics*, **43**(5): 10-15.
3. **Anonymous (2004).** Centres for disease control and prevention. Transmission of hepatitis B virus in correctional facilities. Georgia 1999-2002. *MMWR*, **53**(30): 678-81.
4. **Bick J. A. (2007). Infection control in jails and prisons. *Clin Infect Dis* 45: 1047–1055.**
5. **Burek V, Horvat J, Butorac K, and Mikulic R. (2010).** Viral hepatitis B, C and HIV infection in Croatian prisons. *Epidemiol Infect.* **138**(11):1610–20.
6. **Bwogi J, Braka F, Makumbi I *et al*. (2009).** Hepatitis B infection is highly endemic in Uganda: findings from a national serosurvey. *Afr Health Sci* **9**:98–108.
7. **Chaiba E, Coimbraa BGMM, Galva˜oa FHF, Tatebea ER,Shinzatoa MS,.D'Albuquerquea LAC and Massad E (2012)**. Does anti-hepatitis B virus vaccine make any difference in long-term number of liver transplantation? *Clin Transplant*: **26**: E590-E595.
8. **Daneshmand Dana, Nokhodian Zary, Adibi Peyman, and Ataei Behrooz. (2013).** Infectious Diseases and Tropical Medicine Research Center, *The Scientific **World Journal,*** **10**(4):57-61.

9. **Derya Keten, Mehmet Emin Ova, Hamit Sirri Keten, Alper Keten, Evrim Gulderen, Seray Tumer, Ahmet Caliskan, and Suleyman Kulotu.(2015).** *Adv Biomed Res*,**4**:17.
10. **Di Marco V and Craxi A. (2009).** Chronic hepatitis B: who to treat and which choice of treatment? Expert Rev Anti Infect Ther, vol. 7, pg. 281-291.
11. **Dienstag JL. (2008).** Hepatitis B virus infection. *N Engl J Med*., **359**(14):1486–1500.
12. **Fayyaz M, Qazi MA, Ishaq M, Chaudhary G M, and Bukhari MH. (2002). Frequency of hepatitis B and C seropositivity in prisoners.** *Biomedica*, **9(3):186-90.** .
13. **Grace E. Macalino, David Vlahov, Stephanie Sanford-Colby, Sarju Patel, Keith Sabin, Christopher Salas, Josiah D. and Rich. (2004).** *Am J Public Health*. **94**(7): 1218–1223
14. **Gunardi H Zaimi LF, Soedjatmiko AR, and Muljono DH**. **(2014).** Current prevalence of hepatitis B infection among parturient women in Jakarta, Indonesia. *Acta Med Indones*. **46**(1):3-9.
15. **Harkisoen S, Arends JE, van Erpecum KJ, van den Hoek A, and Hoepelman AI (2012).** Hepatitis B viral load and risk of HBV-related liver disease: from East to West? *Ann Hepatol* **13**(11): 164-171.
16. **Hollinger F. and Lau D. (2006).** HBV pathway to recovery through treatment. *Clini J Gastro* **35**(4): 895-931.
17. **IARC. (2012).** http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.
18. **IOM. (2010).** Hepatitis and Liver Cancer: A National Strategy for Prevention and Control of Hepatitis B and C. Washington, DC: National Academies Press. www.***iom***.edu/viralhepatitis.
19. **Javadi AA, Avijgan M, and Hafizi M. (2006).** Prevalence of HBV and HCV infections and associated risk factors in addict prisoners. *Iran J Public Health.* **35**(4): 33–6.

20. **Juergen Beck and Michael Nassal. (2007).** *World J Gastroenterol*. 13(1): 48–64.

21. **Kazi AM, Shah SA, Jenkins CA, Shepherd BE, and Vermund SH. (2010). Risk factors and prevalence of tuberculosis, human immunodeficiency virus, syphilis, hepatitis B virus, and hepatitis C virus among prisoners in Pakistan. *Int J Infect Dis*. 14S: e60-e66.**

22. **Komas NP, Bai-Sepou S, Manirakiza A, Leal J, Bere A, Le and Faou A. (2010). The prevalence of hepatitis B virus markers in a cohort of students in Bangui, Central African Republic.** *BMC Infectious Disease*, **10:** 226.

23. **Lang T. (2009).** HBV and disease. *Balti J Md*., **49**(5):18-21.

24. **Lannacone M., Sitia G., Ruggeri Z., and Guidotti L. (2007).** HBV pathogen in animal model. *J Hepatol* **46**(4): 45-47.

25. **Lu SN, Chen TM, Lee CM, Wang JH, Tung HD, and Wu JC. (2003). *J Med Virol.* 70(1):74-80.**

26. **Lupberger J. and Hildt E. (2007).** Hepatitis B virus-induced oncogenesis. *World J Gastroenterol*. **13**(1):74-81.

27. **Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, Germanidis G, Lee SS, Flisiak R, Kaita K *et al*.(2008).** Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med.*, **359**(23):2442-2455.

28. **Mason S. (2008).** Hepadna Viridae Index of International Committee on Taxonomy of Viruses http:/ www.ncbi .gov/ICTVDB aindex.

29. **Mason w., Gerlich w., Taylor J., Kann M., Loeb D., Sureau S., Magnius L., and Norder H. (2012).** Family hepadnavirdae. Virus Taxonomy ninth report of the international committee of the taxonomy of viruses.Amestrdam: Elsevier PP. 445-455.

30. **McIntyre, A.F., Studzinski, A., Beidinger, H.A., and Rabins C. (2009).** STD, HIV/AIDS, and hepatitis services in Illinois County jails. *Sex Transm Dis.*, **36**(2): S37–40.
31. **Niveau, G**. **(2006).** Prevention of infectious disease transmission in correctional settings: a review. *Public Health*. 120(1): 33–41.
32. **Norder, H., Courouce´ , A.-M., Coursaget, P., Echevarria, J. M., Lee, S.-D., Mushahwar, I. K., Robertson, B. H., Locarnini, S. and Magnius, L. O. (2004).** Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* **47**(6): 289–309.
33. **Ott JJ, Stevens GA, Groeger J, and Wiersma ST**. **(2012).** Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. *BMC Infect disease* **30**(12):131.
34. **Pawlotsky JM, Dusheiko G, Hatzakis A, Lau D, Lau G, Liang TJ, Locarnini S, Martin P, Richman DD, and Zoulim F. (2008).** *Gastroenterology*. **134**(2):405-15.
35. **Raymond D'Souza, MRCP and Graham R Foster**, PhD FRCP Digestive Disease Research Centre, Queen Mary's School of Medicine and Dentistry, Barts and The London NHS Trust, London, UK. Correspondence to: Professor Graham R Foster, DDRC, Royal London Hospital, Turner Street, London E1 2AD, UK E-mail: ku.ca.lumq@retsof.r.g.
36. **Sabbatani S1, Giuliani R, Fulgaro C, Paolillo P, Baldi E, and Chiodo F. (2004).** HIV Ab, HCV Ab and HBsAg seroprevalence among inmates of the prison of Bologna and the effect of counselling on the compliance of proposed tests. **28**(3):163-8.
37. **Samuel D, Muller R and Alexande G (2010).** Educational Research, National Hepatitis B Virus Programme. *Biotechnology,* **9**(37):6162-6166.

38. **Schweitzer, A., Horn, J., Mikolajczyk, R. T., Krause, G. and Ott, J. J. (2015).** Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet,* **386**(10003): 1546–1555.

39. **Seeger C and Mason WS. (2000).** *Microbiol Mol Biol Rev.* **64**(1):51-68.

40. **Solomon, L., Flynn, C., Muck, K. *et al*. (2004).** *J Urban Health*, **81**(1): 25-37.

41. **Stanaway JD, Flaxman AD, Naghavi M, *et al.* (2013).** The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study. *Lancet*, **388**(16):1081–1088.

42. **Roland Strauss Theodor-Boveri. (2002).** Institute for Biowissenschaften, LS Genetic and Neurobiology, Am Hubland, 97074 Würzburg, Germany.

43. **T. Jake Liang. (2016).** The X files of hepatitis B. *Nature,* **531**(4):313-314.

44. **Terruault N. and Samael D. (2005).** Management of the HBV in the Liver Transplantation Setting. *Euro J.*, **4** (7): 16-32

45. **Treso B, Barcsay E, Tarjan A, Horvath G, Dencs A, Hettmann A, *et al.* (2012).** Prevalence and correlates of HCV, HBV, and HIV infection among prison inmates and staff, Hungary. *J Urban Health*, **89**(1):108–16.

46. **UNAIDS. (2006).** Report on the global AIDS epidemics. UNAIDS 10th Anniversary Special edition. Geneva, Switzerland: UNAIDS, 06-20E.

47. **Van Ameijden EJ, Van den Hoek JA, Mientjes GH, and Coutinbo RA (1993).** A longitudinal study on the incidence and transmission patterns of HIV, HBV and HCV infection among drug users in Amsterdam. *Eur J Epidemiol*, **9**(3): 255-62.

48. **Walmsley R. (2007).** World prison population. London: King's College London.: http://wwwkclacuk/depsta/law/research/icps/downloads/world-prison-pop-seventhpdf.
49. **Weinbaum CM, Mast EE and Ward JW. (2009).** Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *Hepatology*. **49**:35-44.
50. **World Health Organization (2000).** Hepatitis B Fact sheets no 204.
51. **WHO (2017)**. Hepatitis B - World Health Organization.
52. **Zhang, Z., N. Torri, Z. Hu, J. Jacob, and T. J. Liang. (2001).** X-deficient woodchuck hepatitis virus mutants behave like attenuated viruses and induce protective immunity in vivo. *J. Clin. Investig*. **108:**1523-1531.
53. **Yen, T.S. Benedict. (2002).**The Molecular Biology of Hepatitis B Virus. In Hepatitis Viruses, ed. Ou, J.-H. James. Kluwer Academic Publishers, Norwell, Massachusetts, pp 51-79.
54. **Zoulim F. (2006).** New nucleic acid diagnostic test in viral hepatitis http:/ www.intermedpress .com.

APPENDICES

بسم الله الرحمن الرحيم

Sudan University of Science and technology

Faculty of MLS-Microbiology

Questionnaire

Prevalence of HBV among prisoners in Khartoum State

Subject number: _____ _____ :رقم المشارك

Sex: M ( ) F ( ) الجنس: ذكر( ) أنثى( )

Age: _____ _____ :العمر

Head country:_____ _____ :المدينة

Religion: _____ _____ :الديانة

Education:_____ _____ :المستوى التعليمي

Occupation: _____ _____ :العمل

Date of entry: _____ _____ :تاريخ الدخول

Previous HBV vaccine: تاريخ التطعيم ضد فيروس التهاب الكبد الوبائي (ب): نعم ( ) التاريخ:_____

Yes ( ) Date: _____

No ( ) لا ( )

Previous disease(s): الامراض السابقة:

_____ _____

Family history: تاريخ العائلة:

_____ _____

Previous Surgery:	عملية جراحية سابقة
Yes ( ) No ( )	نعم ( ) لا ( )
Hemodialysis:	اجراء عملية غسيل الكلى
Yes ( ) No ( )	نعم ( ) لا ( )
Previous Jaundice:	الاصابة ب اليرقان:
Yes ( ) No ( )	نعم ( ) لا ( )
Other behaviors (Sharing nails clip, razor, and needle stick…):	عادات اخرى (مشاركه ضفارة ، امواس الحلاقة ، اى معدات اخرى ،.....):
_____	_____
_____	_____
I consent voluntarily to participate as a participant in this study.	*أنا أوافق طوعا على المشاركة في هذه الدراسة.*
Name of Participant:	اسم المشارك:
_____	_____
Signature of Participant:	إمضاء المشارك:
_____	_____

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