Sudan University of Science and Technology
College of Graduate studies

Sero-prevalence of Hepatitis B Virus among Prisoners in Khartoum State
الانتشار المصلي لفيروس إلتهاب الكبد (ب) لدى المصابين 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

يا أيها الذين آمنوا إذا قيل لكم تفسحوا في المجالس فأنشروا فإن شرها فانشروا يرفع

اللهم الذين آمنوا منكم الذين أنتموا العالم ذو رحمة و[w]اللهم بما تعملون خير

سورة المجادلة : الآية 11
DEDICATION

*To my beloved family*

# Who always picked me up on time and encouraged me to go on every adventure #
ACKNOWLEDGEMENT

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

My thanks and appreciation to my supervisor, Prof. Humodi Ahmed Saeed, for his constructive guidance and supervision. My thanks extended to Dr. Nasr M. Nasr/ and Dr. Ahmed Intisar for technical help.

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Last, but not least, my thanks to all prisoners who participated in this study, with my best wishes for them to take their freedom as soon as possible.
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, previous 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 الدراسة هو تحديد مدى انتشار فيروس التهاب الكبد ب بين المساجين في ولاية الخرطوم في الفترة بين فبراير إلى مايو 2017.

جمعت 90 عينة بصوره عشوائية من النساء بسجن التنانين في ولاية الخرطوم. جمعت 5 مل من الدم من كل مريض ثم فصل منها المصل. كل العينات خضعت لفحص بحثا عن المستضد السطحي لفيروس الكبد ب باستخدام الإلإيرا.

جمعت المعلومات بواسطة استبيان شخصي مع كل سجين ويحتوي الاستبيان على معلومات عن العمر، الحالة الاجتماعية، المستوى التعليمي، التعرض ضد فيروس التهاب الكبد الوثني ب، تاريخ الامهار، تاريخ التعرض، لمراقبة جراحية أو الاصابه الجراحية بالبرقين، وما اذا تعرض للجرح بالله حديثيه وايضا مدى مشاركتهم لبعض الاشياء كالامموا والضفار الإضافي.

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 التهاب الكبد (ب) بين السجينات في ولاية الخرطوم موجود بنسبة متوسطة(6.7). وأن نسبة الاصابة متساوية مقارنة بالحاله الاجتماعية للسيدات.

يوصي بدراسات إضافية 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 primary public health threat globally. More than 240 million people were chronically HBV infected with 600,000 deaths per year related to HBV (Schweitzer et al., 2015; Roberts et al., 2016).

HBV is a silent killer disease of the liver with many carriers not realizing that 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, limited 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%, 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 pathogenic agent. Despite successful vaccination programs and effective antiviral therapies, there are over 350 million carriers of HBV surface antigen (HBsAg). Some 150 million of them have active infection and a high risk of progression to cirrhosis or hepatocellular carcinoma (HCC). HBV-infected individuals have a 30-fold higher risk of developing HCC than the remainder of the population, and it is estimated that 53% of liver cancers worldwide 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 HBsAg prevalence in rural than in urban areas (Komas, 2010).

World Health Organization estimates that the prevalence of hepatitis B virus 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 per year therefore the early diagnosis can help in treatment and even limitation 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 prison.

3. To correlate the presence of HBV with marital status, age, family history, HBV vaccination and previous jaundice or surgery.
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 ongoing transmission and lead to necessary treatment with antiviral medication (Nguyen et al., 2007). Household with the infected person and sexual partners that might have become infected. To avoid transmission there are a few measures that HBV 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 prevent liver disease by limiting their alcohol consumption and by regularly seeking disease monitoring (Weinbaum et al., 2009).

Despite availability of a vaccine and antiviral treatment, HBV infection is still a major 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 Orthohepadnavir us of the Hepadnaviridae family and, along with the Spumaretrovirin ae subfamily of the Retroviridae family, represents the only other animal v irus with a DNA genome known to replicate by the reverse transcription o f a viral RNA intermediate (Norder et al., 2004).

The Dane particle of HBV is a spherical lipid-containing structure o f approximately 42 to 47nm. The virion consists of a viral envelope, nucleocapsid and a single copy of the partially double-stranded DN A 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 comprising 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 virions contain in their inner icosahedral core the genome as a partially double-stranded, circular; the second phase is upon infection, the relaxed circular DNA (RC-DNA) is converted, inside the host cell nucleus, into a plasmid-like covalently closed circular DNA (cccDNA); and in the last phase from the cccDNA, several genomic and sub genomic RNAs are transcribed by cellular RNA polymerase II; of these, the pregenomic RNA (pgRNA) is selectively packaged into progeny capsids and is reverse transcribed by the co-packaged P protein into new RC-DNA genomes. Matured RC-DNA containing but not immature RNA containing nucleocapsids can be used for 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 symptoms of hepatitis can take decades to manifest, so many people who are infected 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 within the liver. In approximately 65% of acute infections the infection and resolution 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 symptoms 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 HBsAg, which usually becomes detectable at 8-12 weeks post-infection, assuming one month incubation. This marker typically precedes an elevation of serum ALT levels and symptoms of hepatitis by 2 to 6 weeks and remains detectable throughout the symptomatic phase. After the onset of jaundice, HBsAg titers gradually decrease and usually and 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 (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 permucoosal exposure to infected blood and other body fluids (i.e. semen and vaginal fluid). The highest concentrations of the virus occur in blood and wound secretions. Moderate concentrations of HBV are found in semen and vaginal fluid, and lower concentrations occur in saliva. HBV is not spread by air, food, or water. Other common modes of transmission include mother-to-infant, child-to-child, unsafe injection practices and blood transfusions, and sexual contact. Perinatal transmission from HBsAg-positive mothers to their newborn infants (vertical) or transmission from one child to another (horizontal) is a major source of HBV infections in many countries where chronic HBV infection is highly endemic, also the spread of HBV from 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 al., 2010; Fayyaz et al., 2006).

2.8. High risk group

High risks group includes people with multiple sex partners, previously infected 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 infected with hepatitis, Health care workers, Dialysis patients, patients HCV and HIV infections and patient who is taking immunosuppressive or cytotoxic 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 viral 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. 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 immunoassays that depend on colorimetric 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 (Mel Krajden et al., 2005).

The diagnosis of HBV infection and its associated disease is based on a constellation 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 infection (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 to 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 response to treatment. Liver biopsy is essential to confirm the diagnosis, to identify 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 replication 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 developmentally handicapped; injection drug users; inmates of long-term correctional facilities; persons with multiple sexual partners; persons such as hemophiliacs who require long-term, high-volume therapy with blood derivatives; household and sexual contacts of HBsAg carriers; persons living in or travelling extensively in endemic areas; unvaccinated children under the age of 18; unvaccinated children who are immigrants from endemic countries (Weinbaum 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%, 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) this was conducted in a Brazilian study(Alcione et al., 2010).

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

The sero-prevalence of HBV (8.1%) infections among the inmates of a prison 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 (Deruya et al., 2016).

High infection prevalence among inmates represents 20.2% of hepatitis B virus among 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 males 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 serological markers, and 706 (72.8%) individuals were serologically negative for all HBV markers in prisoners related to injection drug users in Iran (Daneshmand 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 seroprevalence 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, previous 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 used 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 specificity 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 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 × 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

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total</th>
<th>HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-32 years</td>
<td>51</td>
<td>56.7</td>
</tr>
<tr>
<td>33-50 years</td>
<td>32</td>
<td>35.6</td>
</tr>
<tr>
<td>51-70 years</td>
<td>7</td>
<td>7.7</td>
</tr>
<tr>
<td>__</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

Results

<table>
<thead>
<tr>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
</tr>
</tbody>
</table>
Table 2. Frequency of HBV among prisoners

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>Negative</td>
<td>84</td>
<td>93.3</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Distribution of prisoners according to the marital status

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Prisoners</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Married</td>
<td>54</td>
</tr>
<tr>
<td>Single</td>
<td>36</td>
</tr>
<tr>
<td>Situation</td>
<td>Status</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
</tr>
<tr>
<td></td>
<td>Single</td>
</tr>
<tr>
<td>HBV Vaccine</td>
<td>YES</td>
</tr>
</tbody>
</table>

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

Total  | 90 | 00
<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>1</th>
<th>6.6</th>
<th>14</th>
<th>93.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>75</td>
<td>5</td>
<td>6.6</td>
<td>70</td>
</tr>
<tr>
<td>Previous jaundice</td>
<td>YES</td>
<td>35</td>
<td>1</td>
<td>2.8</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>55</td>
<td>5</td>
<td>9.0</td>
<td>50</td>
</tr>
<tr>
<td>Previous surgery</td>
<td>YES</td>
<td>21</td>
<td>1</td>
<td>4.7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>69</td>
<td>5</td>
<td>7.2</td>
<td>64</td>
</tr>
<tr>
<td>Needle stick</td>
<td>YES</td>
<td>15</td>
<td>1</td>
<td>6.6</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>75</td>
<td>5</td>
<td>6.6</td>
<td>70</td>
</tr>
<tr>
<td>Sharing objects</td>
<td>YES</td>
<td>19</td>
<td>1</td>
<td>5.2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>71</td>
<td>5</td>
<td>7.0</td>
<td>66</td>
</tr>
<tr>
<td>Previous Blood transfusion</td>
<td>YES</td>
<td>6</td>
<td>1</td>
<td>16.6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>84</td>
<td>5</td>
<td>5.9</td>
<td>79</td>
</tr>
</tbody>
</table>
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
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) which showed that the prevalence of HBV among prisoners is 5.9%. But disagrees with that reported in a Brazilian study by Alcione et al., (2010) which reported that 14% prison of females were positive for HBV. This difference 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


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.


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


APPENDICES
Subject number: __________________________

Sex: M ( )  F ( )

Age: __________________________

Head country: __________________________

Religion: __________________________

Education: __________________________

Occupation: __________________________

Date of entry: __________________________

Previous HBV vaccine:
Yes ( )  Date: __________________________
No ( )

Previous disease(s):
_____________________________________________________

Family history:
_____________________________________________________

Date of entry: __________________________

Previous disease(s):
_____________________________________________________

Family history:
_____________________________________________________
Previous Surgery:
Yes (    ) No (    )

Hemodialysis:
Yes (    ) No (    )

Previous Jaundice:
Yes (    ) No (    )

Other behaviors (Sharing nails clip, razor, and needle stick...):

______________________________
______________________________

I consent voluntarily to participate as a participant in this study.

Name of Participant:

______________________________

Signature of Participant:

______________________________