Sero-prevalence of Hepatitis B Virus among Pregnant Women in Red Sea State

 معدل الانتشار المصلي لفيروس التهاب الكبد (ب) لدى الحوامل في ولاية البحر الأحمر

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

BY

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2017
الآية

بسم الله الرحمن الرحيم

يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات والله بما تعملون خبير.

صدق الله العظيم

سورة المجادلة: الآية رقم (11)
DEDICATION

To my Father,

Mother,

and

Friends
ACKNOWLEDGEMENT

First of all thanks to ALMIGTYALLAH for all of his graces. I would like to thank all those who helped me in achieving this work.

Thanks are not enough to explore my thankfulness to my supervisor Prof. Humodi Ahmed Saeed for his continuous supervision and guidance.

I would like to thank Dr. Nasr Mohamed Nasr and Dr. Mohamed Abd-Alla Musa for their help and support. Thank to my colleagues for their collaboration and help.

Great thanks to the patients whom accept participation in this study.
ABSTRACT

Hepatitis B Virus (HBV) occurs worldwide with more than 2 billion people being infected at some time in their lives. Transmission of this virus from carrier mothers to babies can occur during perinatal period. This study was conducted to determine prevalence of HBV infection among pregnant women in Rea Sea State. The study was carried out during the period from April to July 2017.

A total of ninety one (n=91) pregnant women were enrolled in this study. Sociodemographic data including name, age, gestational stage, history of abortion and blood transfusion were collected by structured questionnaire. Blood specimen was collected from each pregnant women. Plasma separated by centrifugation at 3000 rpm for 5 minutes. The plasma were analyzed for the presence of HBsAg using a commercially available enzyme -linked immune sorbent assay “ELISA”. The results showed that out of 91 women enrolled, only 3(3.3%) were positive, the positive cases, 2 in their third trimester, and one in second trimester, two of these positives exposed to blood transfusion in at least one time in their life. While the rest 88(96.7%) were negative.

The study concluded that the seroprevalence of Hepatitis B virus among pregnant women in Red Sea State was low. Pregnant women in third trimester had the highest frequency of infection followed by second one.

Further studies with large sample size and advanced technique are required to validate the results of the study.
المستخلص

ينتشر التهاب الكبد الوبائي (ب) في جميع أنحاء العالم، حيث يصاب به أكثر من 2 مليار شخص في فترة زمنية من حياتهم. الانتقال من الأمهات الحوامل إلى الأطفال يمكن أن يحدث خلال فترة ما قبل وال сразу في الولادة مباشرة. هذه دراسة مقطعيه أجريت لتحديد مدى انتشار فيروس التهاب الكبد الوبائي (ب) بين النساء الحوامل في مستشفى الولاية التعليمي، البحر الأحمر. أجريت هذه الدراسة في الفترة من أبريل إلى يوليو 2017.

شملت الدراسة 91 من النساء الحوامل وتم استخدام الاستبيان لجمع المعلومات الديمغرافية. جمعت عينات الدم من كل مشاركه وتم الحصول على المصل بواسطة جهاز الطرد المركزي عند 3000 دوره في الدقيقة لمدة 5 دقائق. كل العينات خضعت للفحص وذلك للكشف عن المستضد السطحي لفيروس الكبد الوبائي بواسطة تقنيه الأنزيم المناعي المرتبط (الأليزا).

أظهرت النتائج أن من أصل 91 عينة تم فحصها (3.3%) كانت إيجابية للمستضد السطحي لفيروس التهاب الكبد الوبائي (ب)، ومن بين الثلاث نساء الموجبات، اثنين في الطور الثالث من الحمل، وواحد في الـ40-44 من الحمل. وان اثنين منهن تعرضن لعملية نقل دم في مرحلة من حياتهم. و 88(96.7%) كانت سالبه للمستضد السطحي لفيروس التهاب الكبد الوبائي (ب).

خلصت الدراسة إلى أن معدل انتشار عدوي فيروستهاب الكبد الوبائي (ب) بين النساء الحوامل في ولاية البحر الأحمر كانت منخفضة وكانت النساء الحوامل في الفصل الثاني من الحامل أغلبهم تم التكاثر من الحوامل في الفصل الثالث، وهي الأقل للحمل. يجب إجراء المزيد من الدراسات بعدد أكبر من العينات وتقنيات متقدمة للتحقق من نتائج هذه الدراسة.
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LIST OF ABBREVIATIONS

CC DNA: Covalently closed circular DNA

HBV: Hepatitis B virus

HCC: Hepatocellular carcinoma

HBsAg: Hepatitis B surface antigen

HBsAb: Hepatitis B surface antibody

MTCT: Mother to child transmission
CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hepatitis B is one of the major and common infectious disease of the liver worldwide, caused by a small enveloped DNA virus. This occurs worldwide and constitutes a serious public health problem. Globally, more than 2 billion people have been infected with HBV at some time in their lives. Of these, about 350 million people remain infected chronically and become carriers of the virus, and 1.5 million deaths occurs from HBV related liver diseases, including end stage cirrhosis and hepatocellular carcinoma (HCC) each year (Lavanchy, 2004; Wong et al., 2003). Approximately 65 million of all chronically infected individuals live in Africa (Karmvis and Kew, 2005).

The risk factors for hepatitis B infection are known to be linked to body fluids especially those with high concentration of the virus like blood, semen and vaginal secretions (Parry et al., 2009). Traditional practices that expose people to hepatitis B infection like scarification, ear and nose piercing as well as tattoos have led to higher prevalence in certain zones but not necessarily in pregnancy (Dwivedi et al., 2011). The risk factors for hepatitis B infection during pregnancy vary among communities depending on the cultural practices and some traditional beliefs. In one study carried out in a Nigerian obstetric population the major risk factors identified were higher mean parity, higher number of sexual partners since sexual debut, polygamy and previous sexually transmitted infection (obi et al., 2006). In addition to determining prevalence, risk factors for HBV infection need to be identified in each setting in order to potentially design targeted preventive measures.

Mother-to-child transmission (MTCT) of HBV is responsible for more than one third of chronic HBV infections globally (Nelson et al., 2014). Indeed, prenatal transmission seems to be predominant in high-prevalence areas such as sub-Saharan African countries (Anna and lok., 2002). Children born to mothers
positive for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) have a 70-90% likelihood of prenatal acquisition of HBV infection, and up to 90% of prenatal infections evolve towards chronicity compared to nearly 5% of adult infections (Mc Mahon et al., 1985). Prevention of prenatal transmission of HBV is therefore crucial to tackle the burden of the disease in high endemic sub-Saharan African areas. Effective strategies for reducing the incidence of chronic infections include maternal screening combined with post-exposure prophylaxis consisting of HBV vaccination immediately after delivery in all children born to HBsAg positive mothers, ideally with immunoglobulin prophylaxis (Lee et al., 2006).

Hepatitis B virus (HBV) infection may go undetected. Unawareness of an ongoing infection delays the diagnosis of HBV-related liver disease and favors the spread of the virus (Vazquez et al., 2003). In a study to assess awareness of HBV infection among patients in a clinic in Italy, it was noticed that up to 40.3% of the participants were not aware of their infection (Ippolito et al., 2010).

1.2. Rationale and objectives

1.2.1. Rationale

Hepatitis B virus (HBV) is a major blood-borne and sexually transmitted infectious agent, and represents a serious global public health problem. HBV is approximately 100 times more contagious than human immunodeficiency virus (HIV) and is found in diverse populations and subpopulations (WHO, 2016; Mastet et al., 2005). Globally, 350 million people are chronic carriers. Of these, one million of them are expected to suffer serious illness and death from cirrhosis and hepatocellular carcinoma (HCC) (WHO, 2013). Africa has the second largest number of chronic carriers after Asia, and is considered a region of high endemicity (≥8%) (WHO, 2013). Although it is difficult to assess the exact burden of HBV in Africa, between 56% and 98% of the adult population show evidence of past exposure to HBV infection, and the seroprevalence of
hepatitis B surface antigen (HBsAg) has been estimated to range from 6% to 20% (Kiire, 2000). Studies among pregnant women have shown moderate endemicity, with the prevalence of HBsAg positivity ranging from 3% to 6.1% (Tegegne et al., 2014).

Preventing mother-to-child transmission of HBV is therefore fundamental for reducing the burden of the disease in sub-Saharan Africa, where it is endemic. An effective strategy for reducing the incidence of chronic infections is maternal screening combined with post-exposure prophylaxis consisting of HBV vaccination immediately after delivery in all infants born to HBsAg-positive mothers, jointly with immunoglobulin prophylaxis (Lee et al., 2006). Moreover, universal immunization of infants against HBV is performed as part of the National Expanded Program on Immunization starting at six weeks. Hence, data regarding HBV in pregnant women are fundamental for health planners and caregivers in order to support evidence-based interventions.

1.3. Objectives

1.3.1. General objective

To study hepatitis B surface antigen among pregnant women in Red Sea State.

1.3.2. Specific objectives

1. To detect Hepatitis B virus among pregnant women.

2. To determine the frequency of hepatitis B surface antigen (HBsAg) among enrolled pregnant women in Red Sea State.

3. To identify potential risk factors associated with the infection.
Chapter Two

Literature Review

2.1. Hepatitis B Virus

Serendipity led to the identification of the Australia antigen, which we now know as hepatitis B surface antigen (HBsAg). An immunodiffusion precipitin line between the HBsAg present in the serum of an Australian Aborigine and the antibody to HBsAg in a patient with hemophilia who had received multiple transfusions provided the first clue. The subsequent development of acute hepatitis in a laboratory technician provided the essential link to the clinical illness. For these achievements, Dr. Baruch Blumberg received the Nobel Prize in Physiology or Medicine in 1976. (Purcell, 1993; Alter, 2005).

2.1.1. Classification

The hepatitis B virus is classified as the type species of the Orthohepadnavirus, which contains three other species: the Ground squirrel hepatitis virus, Woodchuck hepatitis virus, and the Woolly monkey hepatitis B virus. The genus is classified as part of the Hepadnaviridae family, which contains two other genera, the Avihepadnavirus and a second which has yet to be assigned. This family of viruses have not been assigned to a viral order (Mason, 2008). Viruses similar to hepatitis B have been found in all apes.

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins. These serotypes are based on a common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r). The viral strains have also been divided into ten genotypes (A–J) and forty subgenotypes according to overall nucleotide sequence variation of the genome (Hundie et al., 2016). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.
(Karmvis et al., 2005). The serotypes and genotypes do not necessarily correspond.

Genotype D has 10 subgenotypes(Ghosh et al., 2013; Hundie et al., 2016).

2.1.2. Morphology

2.1.2.1. Structure

Hepatitis B virus is a member of the Hepadnavirus family. The virus particle, called Dane particle(WHO, 2015) (virion), consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses. The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen (HBsAg), and is produced in excess during the life cycle of the virus (Locarnini, 2004).
**HBV Structure & Antigens**

![Dane Particle Diagram](image)

- **HBsAg** = surface (coat) protein (4 phenotypes: adw, adr, ayw and ayr)
- **HBcAg** = inner core protein (a single serotype)
- **HBeAg** = secreted protein; function unknown

**Fig:1** structure of hepatitis B virus

### 2.1.2.2. Components

It consists of:

- HBsAg
- HBcAg (HBeAg is a splice variant)
- Hepatitis B virus DNA polymerase
- HBx. The function of this protein is not yet well known (Guo *et al.*, 2009) but evidence suggests it plays a part in the activation of the viral transcription process (Benhenda *et al.*, 2013).

Hepatitis D virus requires HBV envelope particles to become virulent (Chai *et al.*, 2008).

### 2.1.3. Genome

#### 2.1.3.1. Size

Hepatitis B is a noncytopathic enveloped virus with circular double strand DNA genome that cause acute and chronic necroinflammatory liver disease and hepatocellular carcinoma. The genome is unusual because the DNA is not fully
double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full length strand) and 1700–2800 nucleotides long (for the short length strand) (Zoulim and Mason, 2007).

2.1.4. Life cycle

The life cycle of hepatitis B virus is complex. Hepatitis B is one of a few known non-retroviral viruses which use reverse transcription as a part of its replication process.

Attachment

The virus gains entry into the cell by binding to a receptor on the surface of the cell and enters it by clathrin-dependent endocytosis. The cell surface receptor has been identified as the Sodium/Bile acid co transporting peptide SLC10A1 (also named NTCP).

Penetration

The virus membrane then fuses with the host cell's membrane releasing the DNA and core proteins into the cytoplasm.

Uncoating

Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus. It is thought the capsid is transported on the microtubules to the nuclear pore. The core proteins dissociate from the partially double stranded viral DNA is then made fully double stranded and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs.
Replication

The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase.

Assembly

These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and re-cycled to produce even more copies.

Release

The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity (Benhenda et al., 2013).

![Fig:2HBV replication cycle](image-url)
2.2. Epidemiology

It is estimated that approximately 2 billion people worldwide have evidence of past or present infection with hepatitis B virus (HBV), and 248 million individuals are chronic carriers (Ott et al., 2012; Schweitzer et al., 2013).

2.3. HBV among pregnant women in Sudan

Sudan is a highly endemic area for HBV with high seroprevalence (Mudawi, 2008; Mahgoub et al., 2011). Exposure to the virus varied from 47%-78%, with the hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in southern Sudan. Hepatitis B virus was the comments cause of chronic liver disease and hepatocellular carcinoma and was the second comments cause of acute liver failure in the Sudan. Viral hepatitis during pregnancy is associated with high risk of maternal complication, has a high rate of vertical transmission causing fetal and neonatal hepatitis and it has been reported as a leading cause of maternal mortality in Sudan.

2.4. Transmission:

HBV is spread by blood, body fluids, and close personal contact. In developing countries the main routes of transmission are:

a. Neonatal with an HBV carrier mother infecting her infant, usually during birth or soon after birth following close contact,

b. Transfer of HBV via open wounds, bites, cuts and grazes,

c. Sexual transmission,

d. Transfusion of infected blood or blood products when donor blood is not screened

e. Needle sticks injury and other sharps injuries,

f. Contamination of eye,

g. reuse of HBV contaminated needles, syringes, lancets, razors, and instruments including those used in tattooing, ear piercing, acupuncture, and tribal ceremonies
h. Possibly sharing cups and spoons.

Up to 20% become chronic carriers, particularly those infected between 1–5 years. Worldwide, there are estimated to be 450 million chronic carriers of HBV. Hepatitis B has an incubation period of 2–6 months (Cheesbrough, 2006; GE and Dongliag, 2009).

2.5. Pathogenicity

HBV disease is a Nermo-inflammatory liver disease with variable severity, with hepatocytes representing the primary target cells for HBV (Chisari et al., 2009; Hovart and Tegtmeier, 2011). Infection leads either to acute disease that resolve or chronic one lasting for years. Persistent infection by HBV is often associated with chronic liver disease that can lead to development of cirrhosis and hepatocellular carcinoma. It is suggested that HBV that is not directly cytopathic for the infected hepatocyte (Chisari et al., 2009). Rather, viral clearance and disease pathogenesis are largely mediated by adaptive immune response in HBV infection. It is widely believed that cytotoxic T-lymphocytes are the cells that clearer viral infections by killing HBV-infected cells. This is likely to occur in adult patient, unlike in neonates, where neonatal tolerance to HBV is responsible for viral persistence following mother-infant transmission. This leading to high rates of chronic infections leading to liver cirrhosis and HCC (Chisari et al., 2009). Multifactorial mechanisms contribute to development of HCC is chronic HBV infection. Most tumors in HBV-associated HCC result from random integration of HBV DNA in host cell DNA resulting in down-regulation of cellular growth control mechanisms. Also, certain HBV proteins may directly participate in HCC development. For example, HBV x gene product trans activates cellular genes that control cellular growth (Chisari et al., 2009).

2.6. Laboratory diagnosis

Diagnosis is based on clinical and laboratory findings. It is impossible to differentiate HBV infection on clinical ground alone; so, differentiate diagnosis
should be established on the results of laboratory testing. Both serological and molecular methods are available and used to distinguish between acute and chronic infections (CDC, 2012).

2.6.1. Specimen

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 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 marker (Hovart and Tegtmeier, 2011).

2.6.2. Serology

Several commercial assays are available to detect HBV specific antibodies, which determine the stage of the disease and immunity due to vaccination (Hovart and Tegtmeier, 2011).

2.6.2.1. Hepatitis B markers (Ags and Abs) in blood

2.6.2.1.1. Hepatitis B Ags

a. HBs Ag: is detected during I.P acute or chronic (remain for 6 months or years)

b. HBcAg: is not detectable only in nuclei of liver cells.

c. HBeAg: active hepatitis infection acute or chronic found in presence of HBs Ag. Indicates that the serum contain high of infective HBV and is highly contagious (Hovart and Tegtmeier, 2011).
2.6.2.1.2. Hepatitis B Abs

a. HBs Ab: appears after disappearance of HBs Ag and its presence indicate immunity (protection against infection) remain for 6 months.

b. HBcAb: appear with the onset of clinical disease and remains after recovery. Detected in recent infections (IgM) indicates viral replication.

c. HBeAb: appears as HBeAg disappears and it indicates recovery. Are not detectable after 6 months. There is a period of several weeks when HBs Ag has disappeared but HBs Ab is not yet detectable. This is the window phase at this time, the HBcAb is always positive and is used to make the Diagnosis (Hovart and Tegtmeier, 2011).

2.7. Prevention

Passive immunization with hepatitis B immunoglobulin is recommended for treating persons within 48 hours of exposure to hepatitis B virus along with infants born of mother with hepatitis B virus. Hepatitis B vaccines have recently been prepared from the 22nm spherical surface antigen particles and also synthetically from chemical produced hepatitis B proteins. The synthetically produced vaccine is still in an experimental stage, the best way to prevent hepatitis B is by getting vaccinated (Tenney and Daniel, 2009).

2.8. Hepatitis B Vaccine

The first licensed hepatitis B vaccines were plasma-derived and composed of purified HBsAg; most currently available hepatitis B vaccines are produced by recombinant DNA technology. Hepatitis B vaccines are typically given in a three-dose series, but vaccine formulations employing two- and four-dose schedules have also been licensed in the United States for use in some age groups. Only single-antigen hepatitis B vaccine can be given at birth and to infants younger than 6 weeks of age. Hepatitis B vaccine is given as a series of 3shots over a period of 6 month, the entire is needed for long .Either passive and Active immunization. Single-antigen vaccines can be administered concurrently with other vaccines at any age, and many combination vaccines
containing hepatitis B antigens are licensed in the United States and elsewhere. When used in the appropriate age group and given at the manufacturer's recommended dose, hepatitis B vaccines are considered equivalent in their immunogenicity and effectiveness and can be used interchangeably.

Adults over 40 years of age and immunosuppressed persons are less likely to develop protective concentrations. Because hepatitis B vaccines are highly immunogenic (Tenney and Daniel, 2009).

Hepatitis B vaccines are also highly effective if given as post exposure immunoprophylaxis to prevent prenatal transmission. Hepatitis B vaccine and hepatitis B immune globulin administered within 12–24 hours after birth, followed by completion of a three-dose vaccine series, has been shown to be 89%–98% effective in preventing acute and chronic HBV infection in infants born to women who are positive for both HBsAg and HBeAg. Hepatitis B vaccine without hepatitis B immune globulin is as effective in preventing prenatal infection as vaccine alone in most studies and is used in areas where cost or other considerations make the use of hepatitis B immune globulin impractical. The major determinant of the effectiveness of post exposure immunoprophylaxis for infants of HBsAg-positive mothers is on-time administration of the initial doses of vaccine and hepatitis B immune globulin (Tenney and Daniel, 2009).

2.9. Vaccine safety

The safety of hepatitis B vaccine has been demonstrated in a large, prospective clinical trial and post licensure safety analyses. The hepatitis B vaccine is now one of the most widely used vaccines in the world. In the United States alone, more than 60 million adults and adolescents and more than 40 million infants and children have been vaccinated. The most commonly reported adverse events associated with hepatitis B vaccine are pain at the injection site and temperature greater than 37.7°C. Vaccination of newborns does not increase the number of febrile episodes or sepsis evaluations (Lewis et al., 2001).
2.10. Duration of immunity

Anti-HBs, the only easily measurable correlate of vaccine-induced protection, declines in the years following vaccination, making confirmation of vaccine-induced immunity in persons vaccinated years ago impractical, if not impossible. However, despite declines in anti-HBs to levels that are less than protective, results from multiple long-term follow-up studies indicate that immunized persons are still protected against HBV infection. No clinical cases of hepatitis B have been observed in 10- to 22-year follow-up studies among immunocompetent vaccinated populations, and only rare chronic infections have been documented (Banatvala and Van Damme 2003; Dentiger et al., 2005). Most vaccines in long-term (10 or more years after vaccination) follow-up studies will develop a rapid rise in antibody (anamnestic response) if given an additional (booster) dose of hepatitis B vaccine, despite prebooster anti-HBs concentrations below 10 mIU/ml. This response simulates a response that would occur after exposure to HBV and provides indirect evidence of protective immune memory (Williams, 2003).

2.11. HBV variants

Vaccine failures due to HBV variants with mutations in the small surface protein (S) gene (S mutants) have occurred in perinatally exposed infants who received hepatitis B vaccine or hepatitis B immune globulin appropriately and who have concentrations of anti-HBs that are usually protective (Hus et al., 1999). There has been concern that these HBV variants, which are sometimes resistant to the neutralizing effect of anti-HBs, could threaten the effectiveness of hepatitis B immunization programs and that immunization may accelerate the formation of HBV variants. Despite these concerns, there are several reasons to believe that hepatitis B vaccination will continue to reduce disease burden (Nainan et al., 2002).
2.12. Impact of hepatitis B vaccination

The major objective of hepatitis B immunization is prevention of chronic infection, which prevents sequels such as cirrhosis and hepatocellular carcinoma. Because HBV-related cirrhosis and hepatocellular carcinoma usually occur in adults who were infected with HBV as children, decades must pass before the most significant benefits of HBV vaccination are realized. This lengthy interval creates a challenge in monitoring the impact of hepatitis B immunization programs. In the short term, demonstration of a reduction in the HBV-related disease burden relies on indirect measures such as surveillance for acute (symptomatic) hepatitis B, which represents a small but consistent proportion of new infections, and serial cross-sectional seroprevalence studies in populations targeted for vaccination. In the long term, declines in incidence rates and mortality from HBV-related hepatocellular carcinoma can be detected in countries with well-established cancer surveillance systems and registries (Chan and Juliana, 2008).

2.13. Previous study

Many studies have been published in the last decades addressing various aspects of HBV in Sudan. A recent study in Khartoum State on pregnant women showed an HBsAg carrier rate of 5.6% with a very low prevalence of HCV infection of 0.6%. (Elsheikh et al., 2007) An earlier study in the Gezira state in central Sudan showed that 70% of HBsAg positive women of child bearing age were also HBeAg-positive, an important risk factor for vertical transmission of the infection. Mother to child transmission of HBV infection was studied in Juba, southern Sudan on eighty eight mother and child pairs. In nine HBsAg positive mothers, five of their children were infected (55.5%), where as in seventy-nine HBsAg-negative mothers only nine children were HBsAg-positive (11.4%), again pointing towards infection in early childhood in southern Sudan. It was however difficult to conclude that the infection was
vertical in these cases as the mean age of children studied was 15.5 months (Woodruff et al., 1986).
CHAPTER THREE
MATERIALS and METHODES

3.1. Study design

3.1.1. Types of study

This is a cross-sectional study conducted to detect Hepatitis B virus infection among pregnant women in Red Sea State.

3.1.2. Study area

The study was carried out in Port Sudan Maternity Hospital. The practical part of this study was done 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 April to July 2017

3.1.4. Study population

Pregnant women attending Port Sudan Maternity Hospital.

3.2. Sample size

A total of ninety one (n=91) pregnant women were enrolled in this study.

3.3. Collection of specimens

Blood specimen were collected from pregnant women after their consent. The vein puncture technique were used for collection the suitable vein was located, then the skin was cleaned by 70% (v/v) ethanol sterile syringe (5 ml) was dispensed in sterile blood container (with anticoagulant).
3.4. Laboratory work

3.4.1. Preparation of specimens

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

3.4.2. Analysis of specimens

The specimens were analyzed for the presence of HBsAg by a commercially available enzyme-linked immunosorbent assay “HBsAg ELISA” kit (Biorex Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT4I IQS United Kingdom).

The assays were performed following the instructions of the manufacturer. Positive and negative controls were included in each assay. According to the information included in the kit’s insert, the immunoassay used has specificity 99.94%.

3.4.3. 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). Patients’ plasma 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 plasma proteins, second antibody conjugated to the enzyme HRP and directed against a different epitopes 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 solution containing TMB and urea peroxidise are added to the wells. In 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 proportion 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.

3.4.4. Procedure

All reagents and specimens were settled to reach room temperature, 20ul of specimen diluent was added to each well except the blank, then 100ul 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, 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.

3.4.5. 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 450nm.

3.4.6. Interpretation of results

Positive more than cut of value.

Negative less than cut of value.
CHAPTER FOUR

RESULTS

4.1. Results

A total of ninety one women were enrolled in this study. The study was conducted during the period from April to July 2017. Out of 91 women investigated only 3(3.3%) were positive, while the rest 88(96.7%) were negative (Figure 1).

The prevalence of HBsAg in pregnant women according to the age groups; there was insignificant statistical association between age group and HBsAg positivity (P value = 0.277). The prevalence of 3.3% was equally distributed as (1.1%) in age group 15-20, 31 – 35 and 36 – 40 respectively (Table 1).

Study of HBsAg sero-positivity in different trimester among the women revealed that, there was no significant statistical differences between samples screened and trimester with HBsAg positivity (P value = 0.534). However, the highest prevalence of 2.2% was found amongst HBsAg positive mothers in their 3rd trimester(Table 2). Distribution of enrolled women according gestational stage (Table 3).

Study of HBsAg Sero-positivity among women in different trimester revealed that, there was significant statistical differences between samples screened and blood transfused with HBsAg positivity (P value = 0.000) (Table 4). Distribution of enrolled women according to history of abortion, blood transfusion, jaundice and vaccination (Table 5).
Table 1. Prevalence of hepatitis B surface antigen (HBsAg) in pregnant women, according to the age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>% of positive</th>
<th>% of negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 - 20</td>
<td>1.1</td>
<td>5.5</td>
</tr>
<tr>
<td>21 - 25</td>
<td>0.0</td>
<td>36.3</td>
</tr>
<tr>
<td>26 - 30</td>
<td>0.0</td>
<td>29.7</td>
</tr>
<tr>
<td>31 - 35</td>
<td>1.1</td>
<td>20.8</td>
</tr>
<tr>
<td>36 - 40</td>
<td>1.1</td>
<td>3.3</td>
</tr>
<tr>
<td>41 - 45</td>
<td>0.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 2. Correlation between trimester and positive HBsAg (n=91).

<table>
<thead>
<tr>
<th>Trimester</th>
<th>% of positive HBsAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0.0</td>
</tr>
<tr>
<td>2nd</td>
<td>2.7</td>
</tr>
<tr>
<td>3rd</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Table 3. Distribution of enrolled women according gestational stage.

<table>
<thead>
<tr>
<th>Gestational stage</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st trimester</td>
<td>19</td>
<td>20.9</td>
</tr>
<tr>
<td>2nd trimester</td>
<td>36</td>
<td>39.6</td>
</tr>
<tr>
<td>3rd trimester</td>
<td>36</td>
<td>39.6</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 4. Positive HBs Ag among blood transfused patient

<table>
<thead>
<tr>
<th>Blood transfusion</th>
<th>Positive HBs Ag</th>
<th>HBsAg Negative</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>2</td>
<td>5</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Distribution of enrolled women according to history of abortion, blood transfusion, jaundice and vaccination

<table>
<thead>
<tr>
<th>History</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>12/91</td>
<td>13.2/100</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>7/91</td>
<td>7.7/100</td>
</tr>
<tr>
<td>Jaundice</td>
<td>18/91</td>
<td>19.8/100</td>
</tr>
<tr>
<td>Vaccination</td>
<td>7/91</td>
<td>7.7/100</td>
</tr>
<tr>
<td>Total</td>
<td>44/91</td>
<td>48.4/100</td>
</tr>
</tbody>
</table>

Figure 1: Percentage of positive among study population:
CHAPTER FIVE

DISCUSSION

5.1 Discussion

This study observed a 3.3% prevalence rate of HBsAg among the study participants. This result nearly agrees with the result obtained in Ghana 2.4% by Kfutwah et al.,(2012) and 2.6% reported by Al-Mazrou et al.,(2004) and relatively high compared with study conducted in Saudi Arabia that shows the sero-prevalence of HBsAg was 1.6%. This rate is low, compared to previous studies in Ethiopia 7.8% that reported by Metaferia et al.,(2016) and far less than that reported by Al-Shamahy,(2000).

The differences in the prevalence rate may be due to the geographic variation among regions or due to difference in the detection method used. It is also worth noting that, globally there is an age related decrease in Hepatitis B prevalence, which may be attributed to the introduction of HBV testing in pregnant women combined with immunoglobulin prophylaxis (Kfutwah et al., 2012).

With regards to age, the present study revealed that the age of a pregnant woman is not influence her chances of becoming a HBs Ag carrier  P.value =0.277 and this disagree with result obtained in Ghana ( P< 0.001).

The prevalence of HBsAg within these groups (15-20, 31-35 and 36-40) was 1.1% for each. This represents 3.3% off all positive cases. In similar studies conducted in Nigeria and India, a high seropositivity was found in 18-24 and 21-25 years group respectively. In Ghana the prevalence of HBsAg within age group (46-50) was 100% (1/1). This represents 20% off all positive cases.

Number of weeks of pregnancy (Trimester) in our study also had no statistical significant effect on the chances of been HBs Ag positive, P. value = 0.534, though the highest prevalence was observed in pregnant women in their third trimester (2/36), 2.2%. This is followed by those in their second (1/36) and first trimester (0/19).
Out of the participants who had previously blood transfusion(s), 22.2% were positive for HBsAg. This result faraway with result reported in Nigeria 6.1% by Adegbesan-Omilabu et al., (2015).

5.2. Conclusions

It is clear from this study that Port Sudan in Red Sea State is among the areas of lower Hepatitis B prevalence. To sustain and improve on the current situation routine screening of pregnant women is recommended in all health facilities in the country, to identify HBsAg positive mothers for the administration of the birth dose to children born to these women immediately after birth.

5.3. Recommendation

1. Effective health planning strategies are required to increase the public awareness of infection with viral diseases.
2. Urgent further good quality epidemiological studies are needed.
3. Wide spread education and vaccination programs between the pregnant women is recommended.
4. Further in depth studies using large sample size and advanced techniques such as molecular methods (e.g: real time polymerase chain reaction are highly recommended.
REFERENCES


44. **Tenney F and Daniel J (2009).** Long term monitoring shows hepatitis B virus to endeavor in nucleosides naïve patients is rare though5 years of therapy. *Hematology* 49(5):1503-1514.


Appendix (1)

Questionnaire

Sudan University of Science and Technology
College of Graduate Studies

Seroprevalence of HBsAg among pregnant women in Red Sea State

Number:…………………………… Date:……………………

Name: ………………………………………………………………………

Age:………………

Address:……………………………………………………………………

Job:…………………..

Gestational age:

First trimester ( ) Second trimester ( ) Third trimester ( )

History of abortion: Yes ( ) No ( )

Blood transfusion: Yes ( ) No ( )

Jaundice: Yes ( ) No ( )

Vaccinated: Yes ( ) No ( )

Laboratory finding: Positive ( ) Negative ( )