Sero-diagnosis of Hepatitis B Virus Surface Antigen among Pregnant Women in Khartoum State

التشخيص المصلي للمستضد السطحي لفيروس إلتهاب الكبد الوبائي لدى النساء الحوامل في ولاية الخرطوم

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

By:

Saif Eleslam Ibrahim Hassan Saad
B.Sc.in Medical Laboratory Science, ShargElNil College, 2000

Supervisor:

Prof. Humodi Ahmed Saeed

2016
الآية

بسم الله الرحمن الرحيم

قال تعالى:

وَمِنَ النَّاسِ والدُّوَابِ والأنْعَامِ مُخْتَلِفُونَ أَلْوَانَهُ كَذَٰلِكَ لَكُنَّ فَإِنَّمَا يَخْشَى اللَّهُ مِن عِبَادِهِ عَلَمَاءٌ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ

صدق الله العظيم

سورة فاطر: الآية (28)
DEDICATION

To my mother, father and brothers
ACKNOWLEDGMENT

All praise is due to ALMIGHTY ALLAH.

I have insufficient words to express my gratitude to my supervisor Prof. Humodi Ahmed Saeed for his priceless inspiration and encouragement from the very beginning has been wonderful experiences. His comments and suggestions encourage me to think critically.

I am grateful to my brother Ammar for his assistance, my teacher and colleagues in the Department of Microbiology, Sudan University of Science and Technology for help and fruitful discussion.

Finally, I would like to thanks my family for their warmth love and confidence.
**ABSTRACT**

Hepatitis B virus (HBV) is a leading cause of liver disease worldwide. There is much data exist concerning HBV during pregnancy in many African countries, but no published data are available in Sudan. The objectives of this study were to investigate HBV among as well as possible risk factors among pregnant women.

Blood specimens were collected from pregnant women attended Omdurman Maternity Hospital in Khartoum State. Sera were separated by centrifugation. Participated women were interviewed by pre-structured questionnaire. Enzyme Linked Immunosorbent Assay (ELISA) technique was use to detect of Hepatitis B Virus surface antigen (HBVsAg).

Ninety (n=90) pregnant women were participated in this study. The age of the participants ranged from 15 to 45 years old. The mean age was 24 years. Most 56 (62.2%) of the participants fall in age ranged from 26 to 35 (%). The majority 53 (58.9 %) of the participants were from Khartoum City, the rest 37 (30.1%) were distributed as follows: Khartoum North 20 (21.8 %), Omdurman 9 (9.8%) and out of Khartoum State 8 (9.5%). Of the investigated women, 59 (65.6%) of the were not vaccinated while 31 (34.4%) vaccinated. 77 (85.6%) were house wife and only 12 (14.4%) employees. Study on detection of HBVsAg revealed that 5 (5.6%) were positive, and the rest 85 (94.4%) were negative.

It is concluded that high percentage of pregnant women were infected with HBV.
Significant ($P< 0.05$) relation was noticed between HBV infected and dental manipulation as well as blood transfusion. The study indicated insignificant ($p<0.05$) association between HBV infection and risk factors, including residence, age occupation and gestational age. Further studies with large number of participants are required to validate the result of this study.
الخلاصة
فيروس التهاب الكبد الوبائي ب من مسببات أمراض الكبد ويُعتبر من أهم المشاكل الصحية في كل من الدول المتقدمة والتأرجح. تتوفُّر بيانات كثيرة بخصوص الالتهاب الوبائي أثناء فترة الحمل في معظم الدول الأفريقية ولكن البيانات المتوفَّرة في السودان قليلة بخصوص البحث لدراسة التهاب الكبد الوبائي ب بين النساء الحوامل بالإضافة للعوامل.

تم جمع عينات الدم من النساء الحوامل من مستشفى الولادة بأمدرمان ولاية الخرطوم، ثم إجراء استبيان للنساء المبحوثات. تم جمع عينات الدم لفضل الأمهات بواسطة جهاز الطرد المركزي (ELISA). تم Pruط لدراسة المستضد السطحي لالتهاب الكبد الوبائي ب.

تراوح أعمار المبحوثات ما بين 10-45 عام المتوسط 24 عام. معظم المبحوثات 56 (62.2%) في عمر يتراوح ما بين 26-35 سنة، الأقلية 53 (58.9%) من ولاية الخرطوم والباقي من الخرطوم بحري 20 (21.8%)، 9 (9.8%)، 8 (9.5%) على التوالي.

59 (65.6%) من المبحوثات لم ياخذن الفاكسينات و 31 (34.4%) منهن أخذن الفاكسينات، 77 (85.6%) من المبحوثات ربات بيوت وفقط 12 (14.4%) عاملات.

أظهرت الدراسة أن ما بين (90) من الحوامل فقط 5 (5.6%) أظهرن نتيجة موجبة HBVsAg الباقية 85 أظهرن نتيجة سالبة (94.4%).

وجدت علاقة معنوية (0.05>P) ما بين الإصابة وعقمات الأسنان ونقل الدم. وهناك أرتياح غير معنوي ما بين التحصين، السكن، العمر، والعمر الحملي.

خلصت الدراسة أن نسبة عالية من التهاب الكبد الوبائي وجدته لذلك لا بد من وجود برنامج تعليمي صحي للنساء الحوامل. وأوصى بإجراء مزيد من الدراسات مع عدد كبير من العينات للتحقق من صحة نتائج هذه الدراسة.

VI
TABLE OF CONTENTS

Dedication ......................................................................................................................... II
Acknowledgment ................................................................................................................ III
Abstract ............................................................................................................................... IV
الخلاصة .............................................................................................................................. VI
Table of Contents ............................................................................................................... VII
List of Tables ....................................................................................................................... X
List of Figures ..................................................................................................................... XI

CHAPTER ONE
INTRODUCTION AND OBJECTIVES
1.1 Introduction .................................................................................................................. 1
1.2 Rationale ....................................................................................................................... 2
1.3 Objectives ..................................................................................................................... 3
  1.3.1 General objective .................................................................................................... 3
  1.3.2 Specific objectives ................................................................................................. 3

CHAPTER TWO
LITERATURE REVIEW
2.1 Hepatitis B virus (HBV) .............................................................................................. 4
  2.1.1 Morphology and structure ................................................................................... 4
  2.1.2 Genome ............................................................................................................... 4
2.2 Replication .................................................................................................................... 5
2.3 Serotypes and genotypes ............................................................................................ 6
2.4 Occult hepatitis B ....................................................................................................... 7
2.5 Mutants

2.6 Physical properties

2.7 High risk groups

2.8 Epidemiology

2.9 Pathogenesis

2.10 Clinical features

2.11 Laboratory diagnosis

2.11.1 Clinical chemistry tests

2.11.2 Serological tests

2.11.3 Polymerase chain reaction (PCR) test

2.11.4 Detection of HBV in tissue specimen

2.12 Treatment

2.13 Prevention and vaccination

2.13.1 Current vaccines

2.13.2 Combined prophylaxis

2.13.3 Active immunization

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

3.2 Study area

3.3 Study period

3.4 Study population

3.5 Study variable

3.6 Sample size
LIST OF TABLES

Table 1. Frequency of risk factors of Hepatitis B virus infection among study population
...........................................................................................................................................25

Table 2. Statistical relation of sero-positive HBs Ag using ELISA technique and its risk factors among pregnant women................................................................................................26
List of Figures

Figure 1: Frequency of the HBsAg result among pregnant women........................................28
CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1 Introduction

Hepatitis means inflammation of the liver. It is most common caused by one of the several viruses, such as hepatitis A virus, hepatitis B virus, hepatitis C virus and other viruses, Toxins, bacterial infections, certain drug, and heavy alcohol use can also cause hepatitis (Jawetezet al., 2007).

Hepatitis B virus (HBV) is a leading to cause of liver disease. Approximately one-third of living persons have been infected with HBV at one point in their lives, and 350 million of them have become chronic HBV patients or carriers. More than 1 million people die each year due to HBV-related diseases, such as chronic active hepatitis, cirrhosis, and hepatocellular carcinoma (Ozeret al., 2011).

Transmission of the HBV can occur through of several ways, by the intravenous rote e.g. by transfusion of infected blood or blood product, or by contaminated needles used by drug user (around 30%), tattooists or acupuncturists. By sharing objects that may have a very tiny amount of blood on them, such as a tooth brush, razor, or tools used for manicures. By close personal contact, such as during sexual intercourse, particularly in men having sex with men (homosexual), also heterosexual exposure and at the same time bisexuality (Kumar and Clark., 2009).

Vertical transmission from mother to child in uterus (intrauterine), during parturition or soon after birth (prenatal) which constitute the major one in this category, and from breast
feeding (Kumar and Clark, 2009). Horizontal transmissions occur particularly in children through minor abrasion or close contact with other children, and HBV can survive on household articles, e.g. toys, toothbrush, for prolonged period so transmission may be possible. HBV is present in high titer in blood and exudates (for example from skin lesions) of acutely and chronically infected persons (Kumar and Clark, 2009).

Moderate viral titer are found in semen, vaginal, secretions and saliva, other body fluids that don’t contain blood or serous fluids, such as feces or urine cannot as sources of HBV (Lunget al., 2008). It cannot be spread through sneezing, coughing, hugging or same risk for a hepatitis B infection but some groups are at high risk because their an occupation or life choices (Jawetezet al., 2007).

Sudan is classified as endemic area of hepatitis B surface antigen (HBs Ag), seroprevalence ranging from 6.8% in central Sudan to high 26%4 in southern Sudan (Mudawiet al., 2008).

1.2 Rationale

Hepatitis B virus is a major cause of liver disease worldwide and a potential cause of substantial morbidity and mortality in the future. The complexity and uncertainty related to the geographical distribution of HBV infection and chronic hepatitis B virus, determination of it associated with risk factors and evaluation of cofactors that accelerate its progression, under-score the difficulties in global prevention and control of HBV.
Study of the hepatitis among pregnant women group is very important to protect transmission of disease to children. Therefore, this study was aimed to study the hepatitis B surface antigen among pregnant women in Khartoum State and to identify possible risk factors associated with hepatitis B infection in them.

1.3 Objectives

1.3.1 General objective

To study the Hepatitis B virus among pregnant women attending Omdurman Maternity Hospital in Khartoum State.

1.3.2 Specific objectives

a. To detect the Hepatitis B surface antigen (HBs Ag) in pregnant women.

b. To compare between HBs Ag and risk factor associated with HBV infection.
CHAPTER TWO
LITERATURE REVIEW

2.1 Hepatitis B virus (HBV)

2.1.1 Morphology and structure

Hepatitis B virus is a member of the hepadnavirus family (Levinson, 2006). The virus particles, virions consist of an outer lipid envelope and icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity. The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells (Sonnabend et al., 2010). The virus is one of the smallest enveloped animal viruses with a virion diameter of 42nm and pleomorphic form exist including filamentous and spherical bodies lacking a core, these particles are not infectious and are composed of the lipid and proteins that form a part of the surface of the virion, which is called the surface antigen (HBs Ag) (Mandelet et al., 2005).

2.1.2 Genome

Hepatitis B virus is a small DNA virus and belong to a group of hepatotropic DNA viruses (hepadnaviruses). The virus consists of nucleocapsid and an outer envelope composed mainly of three antigens (HBs Ag that play a central role in the diagnosis of HBV infection). The nucleocapsid contains HBc Ag, a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins (Seto et al., 2011).
The genome consist of partially double stranded DNA molecules of about 3200 base pairs in length with known sequence as well as genetic organization, the presurface1(pre-s1),pre-surface2(pre-s2) and the surface genes (S) code for the various HBs Ag. The protein encoded by pre core (pre-c), code gene (c) undergoes post transitional modification to yield hepatitis B envelope antigen (HBe Ag), which is a sero-marker for high viral replications the core gene codes for HBc Ag, the major structural protein nucleocapsid. Finally the X gene code the hepatitis B xAg. HBx Ag has been shown to be a potent trans activator for cellular and viral gene (Baumert et al., 2007).

The viral DNA polymerase transcriptase is encoded by the polymerase gene(p) and is a central important for the viral replication. Different from all of mammalian DNA viruses, hepadnaviruses replicate via reverse transcription of a RNA intermediate, the pregenomic RNA, which is strategy central to the RNA retroviruses (Baumert et al., 2007).

2.2 Replication

The life cycle of the HBV is complex. Hepatitis B is one of the few known nonretroviralviruses which used reverse transcription as a part of its replication process. The virus gain entry in to the cell by binding to an unknown receptor on the surface of the hepatocytes and enter it by endocytosis. Because virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transformed to the cell nucleus by host protein called chaperones. The partially double stranded viral DNA is then made fully double stranded and transform in covalently closed circular DNA (cccDNA) that serves as template, for transcription of four viral mRNAs. The largest mRNA, (which is larger
than the viral genome), is used to make the new copies of the genome the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and recycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where the virion p protein synthesized DNA via its reverse transcriptase activity (Levinson, 2006).

2.3 Serotypes and genotypes

For vaccine purposes HBV has one serotype based on HBs Ag, however, for epidemiological purpose there are four subtype of HBs Ag based on a group specific Ag (a) was marked and to sets of mutually exclusive based on these antigenic epitopes which are presented on its envelope proteins, and in to 8 genotype (A-H) according to overall nucleotide sequence variation of the genome.

The genotype has a distinct geographical distribution and is used in tracing the evaluation and transmission of the virus. Differences between genotype affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination. Genotypes differ by at least 8% of their sequences and were first reported in 1988 when six were initially described (A-F). Tow further types have been described (G&H) (Baumert et al., 2007).
2.4 Occult hepatitis B

Occult hepatitis B is defined by the presence of hepatitis B virus DNA in serum or liver in the absence of HBs Ag. Serum HBV level is usually less than 104 copies/ml. Although occult HBV infection has been identified in patient with chronic liver disease two decades ago. Occult HBV infection has been found in patient with hepatocellular carcinoma (HCC), past HBV infection, or chronic hepatitis C virus, and individual without hepatitis B serological markers, the frequency of the diagnosis depends on the relative sensitivity of HBV DNA assay and the prevalence of HBV infection in the population. Collectively around 30% to 35% of HBs Ag negative. Subject with chronic hepatitis with or without HCC have positive serum HBV DNA (range from 5% to 55%) (Mumtaz et al, 2011).

The clinical significance of occult HBV infection remains unclear. Occult infection represents a potential transmission source of HBV via blood transfusion or organ transplantation. Furthermore some studies suggested that occult might affect responsiveness of chronic hepatitis C to interferon therapy and disease progress (Jinlin et al., 2005).

2.5 Mutants

Mutation occurs in the various reading frame of the HBV genome. These mutants can emerge in patient with chronic HBV infection (escape mutant) or can be acquired by infection. HBs Ag mutant are produced by alterations in the 'a' determinants of the HBs Ag proteins with usually a substitution of glycine for arginine at position 145. This result
in changes in the antibody binding domain and the usual tests or HBs Ag may be affected. A mutation in the pre core region when a guanosine (G) to adenosine (A) change creates a stop codon (TAG), prevent the production of HBe Ag, but the synthesis of HBc Ag is unaffected. To detect infectivity, HBV-DNA must always be measured as no HBe Ag will be present. DNA polymerase mutant occur, particularly after lamivudine therapy (Kumaret et al., 2009).

2.6 Physical properties

Unlike enveloped viruses, which are usually unstable and weak in horrible condition, HBV is a very strong virus tolerating extreme condition. The stability of HBs Ag does not always coincide that of the infectious agent. However, both are stable at -20°C for over 20 years and stable to repeated freezing and thawing. The virus also is stable at 37°C for 60 minutes and remains viable after been dried and stored at 25°C for at least one week. HBV (but not HBs Ag) is sensitive to higher temperature 100°C for 1 min, or to longer incubation period (60 or 10 hours) depending on amount of virus present in the sample. HBs Ag is stable at ph 2.4 for 8 up to 6 hrs, but HBV infectivity is lost, sodium hypochlorite 0.5% e.g. (1: 10 chloride bleach ) destroys antigenicity within 3 min at lost protein concentration, but undiluted serum specimen require higher core (5%). HBs Ag is not destroyed by ultraviolet irradiation of plasma or other blood products and viral infectivity may also resist such treatment (Ozeret et al., 2011).
2.7 High risk groups

The health care workers (HCWs) and medical students are at risk of infection with HBV through occupational exposure to blood and infection body fluids and HBV is also the most easily transmitted blood borne pathogens (Amini et al., 2005).

The following groups represent the high risk group (Amini et al., 2005):

a. Infants from mother who are infected at the time of delivery.
b. Partners or individuals living in close household contact with an infected person.
c. Individual with multiple sex partner (past or present).
d. Individual who has been diagnosed with a sexually transmitted disease.
e. Illicit drug user (injection, inhaling, snorting, popping pills).
f. Men who have sex with men.
g. Individual who receive a blood transfusion.
h. Individual who get tattoos or body piercing.
i. Individual who travel to countries where HBV is common.
j. Individual with early kidney disease or undergoing kidney dialysis.
k. Individual who use blood products for medical conditions (i.e. hemophilia).

2.8 Epidemiology

HBV is worldwide health care problem, approximately 350 million people are lifelong carrier, and most of them are in Asia, in USA, an estimated 200,000 new cases occur
annually. It has an effective vaccine which reduces the incidence rate in same area like Taiwan, future problem with HBV is merging mutant strain which may abort the eradication of the virus (Mandelet et al., 2005).

The USA have a lower carrier rate (0.5-2%), but it rises to 10-20% in some parts of Africa, the middle, and the east (Kumar and Clark, 2004). The prevalence of HBV infection varies markedly throughout regions of the world.

Hepatitis B is highly endemic in developing regions with large population such as south East Asia, China, Sub-Saharan Africa and the Amazon Basin, where at least 8% of the population HBV was chronic carrier. In these areas, 70-95% of the population shows post or present serological evidence of HBV infection. Most infections occur during infancy or childhood. Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the rates of chronic liver disease and liver cancer in adult are very high (Jinlinet et al., 2005).

The endemicity of HBV is low in most developed area, such as North America, Northern and Western Europe and Australia (Jinlinet et al., 2005). In these regions, HBV infects 5-70% of the population, and only 0.5-2% of the population are chronic carriers. In these areas, most HBV infections occur in adolescents and young adults (Jinlinet et al., 2005).

**2.9 Pathogenesis**

Among all viral hepatitis, the immune-pathogenesis of hepatitis B has been studied most extensively. The existence of inactive hepatitis B carriers with normal liver histology
and function suggest that the virus is not directly cytopathic. The fact that patients with defects in cellular immune competence are more likely to remain chronically infected rather than to clear the virus is cited to support the role of cellular immune responses in the pathogenesis of hepatitis B related liver injury.

The model that has the most experimental support involves cytolytic T cells sensitized specifically to recognize host and hepatitis B viral antigens on the liver cell surface (Ganem et al., 2004).

Laboratory observations suggest that nucleocapsid proteins (HBc Ag and possibly HBe Ag), present on the cell membrane minute quantities are the viral target antigens that invite cytolytic T cells to destroy HBV (Ganem and Alfred, 2004).

Although the precise mechanism of liver injury in HBV infection remain elusive, studies of nucleocapsid proteins have shed light on the profound immunologic tolerance to HBV of babies born to mothers with highly replicative (HBe Ag positive), chronic HBV infection. In HBe Ag, expressing transgenic mice, In utero exposure to HBs Ag, which is sufficiently small tolerance to both nucleocapsid proteins (Ganem and Alfred, 2004).

Four different stages have been identified in the viral life cycle of hepatitis B: The first stage is immune tolerance. The duration of this stage for healthy adults is approximately 2-4 weeks and represent the incubation period. For newborn, the duration of this period is often decades. Acute viral replication is known to continue despite little or no elevation in the amino transfer’s levels and no symptoms of illness (Ganjiet al., 2011).
In the second stage, an inflammatory reaction with a cytopathic effect occurs. HBe Ag can be identified in the sera, and a decline of the level hepatitis B virus (HBV) DNA is seen. The duration of this stage for patient with acute infection is approximately 3-4 weeks (symptomatic period). For patient with chronic infection, 10 years or more may elapse before cirrhosis develops (Ganjiet al., 2011). In third stage, the host can target the infected hepatocytes and the HBV viral replication no longer occurs, and HBeAb can be detected (Ganjiet al., 2011).

The HBV DNA levels are lower or undetectable, and amino transferase levels are within the references range- in this stage, an integration of the viral genome into the host's hepatocytes genome takes place. HBs Ag still present (Ganjiet al., 2011).

In the fourth stage, the virus cannot be detected and antibodies to viral antigens have been produced. Different factors have been postulated to influence the evolution of these stages, including age, sex, immunosuppressant, and co-infection with other viruses (Ganjiet al., 2011).

Recent experiments suggest that some of the inflammatory by product, notably interferon g (INF g) and TNFα can have antiviral effects that do not involve killing of the target cells. When cytotoxic T lymphocytes are transferred to mice that bear replicating HBV, viral DNA and RNA throughout the liver rapidly disappear, even from viable, uninjured hepatocytes, an effect that can be blocked by the administration of Abs to TNFα and IFNg. Such non cytocal antiviral effects may be important for viral clearance in natural infection. In fact, cytokine release triggered by unrelated hepatic infection in HBV.
Transgenic mice can have the same effect (Ganjiet al., 2011). This phenomenon may explain the suppression and occasional clearance of chronic HBV infection in patient with superimposed acute hepatitis caused by unrelated viruses (Ganjiet al., 2011).

2.10 Clinical features

The prodromal symptoms of viral hepatitis are systemic and quite variable. Constitutional symptoms of anorexia, nausea and vomiting, fatigue, malaise, arthralgias, headache, photophobia, pharyngitis, cough, and coryza may precede the onset of jaundice by 1 to 2 weeks (Kumar and Clark., 2009).

Symptoms resulting from acute hepatitis B infection among adult are common, with jaundice occurring approximately 12 weeks after initial infection (Kumar and Clark., 2009). Chronic active hepatitis follows hepatitis B infection in 3% of cases. Many people with a chronic hepatitis B remain symptom free for up to 30 years, but others experience ongoing symptoms similar to those of acute hepatitis B. Chronic hepatitis B is a serious disease that can result in long-term health problems (Kumar and Clark., 2009). Rarely, a few spider angiomas appear during the icteric phase and disappear during convalescence (Kumar and Clark., 2009).

During the recovery phase, constitutional symptoms disappear, but usually some liver enlargement and abnormalities in liver biochemical test are still evident (Ganem and Alferd., 2004).
Immune complex-mediated tissue damage appear to play a path genetic role in the extrahepatic manifestation of acute hepatitis B, the occasional prodromal serumsickness-like syndrome observed in acute hepatitis B appears to be related to the deposition in tissue blood vessel walls of HBs Ag-Anti-HBs circulating immune complexes, lending to activation of the complement system and depressed serum complement levels. In patient with chronic hepatitis B, other types of immune complex disease may be seen. Glomerulonephritis with the nephritic syndrome is occasionally observed; HBs Ag; immunoglobulins, and C3 deposition has been found in the glomerular basement membrane. While polyarteritis nodosadovelopsin considerably less than 1% of patient with chronic HBV infection, 20-30% of patient with polyarteritis nodoso have HBs Ag in serum. In these patient, the affected small and medium size arterioles have been shown to contain HBs Ag, immunoglobulin, and complement components. Another extra hepatic manifestation of viral hepatitis, essential mixed cryoglobulinemia (EMC), was reported initially to be associated with hepatitis B (Kumaret et al., 2009, Ganem and Alferd, 2004) and Jinlin et al., 2005).

The disorder is characterized clinically by arthritis coetaneous vasculitis (palpable Purpura), and occasionally with Glomerulonephritis and serologically by the presence of circulating cyroprecipitable immune complexes of more than one immunoglobulin class (Ganem and Alferd., 2004).
2.11 Laboratory diagnosis

2.11.1 Clinical chemistry tests

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

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

2.11.2 Serological tests

Hepatitis B is diagnosed from the results of specific HBV blood test (serology) that reflect the various component of the HBV. The diagnosis is made on the basis of the blood sample which used to demonstrate antibodies against hepatitis B, or hepatitis B components in the patient blood (El Mishad, 2007).

All patients with chronic infections have the viral component called HBs Ag. When both HBs Ag and HBe Ag are present, the infectiousness of the disease is at is highest and in
the long run, those patient are at increased risk of developing complications (El Mishad, 2007).

Hepatitis B surface antigen is the earliest indicator of hepatitis B infection. This Antigen may be present before symptoms of HBV infection are present. If this Ag level remains high for more than 6 months, this means the patient became a chronic carrier of HBV (El Mishad, 2007).

Hepatitis B surface Antibody (HBs Ab) usually appear about 4 weeks after hepatitis B surface Ag disappear. The presence of this antibody means that the infection is at the end of its active stage and the patient cannot pass the virus to others. Occasionally, your test may shows that you have both the HBs Ab and Ag antibodies indicating the presence of mutant strain of HBV. In this case, you are still contagious (El Mishad, 2007).

Hepatitis B envelope Ag (HBe Ag) is an HBV protein that is present during an active HBV infection. This is test determines how contagious the patient is testing for this antigen can also be used to monitor the effectiveness of treatment for HBV (El Mishad, 2007).

The hepatitis B virus DNA tests measure how much genetic material is present. A high level of HBV DNA means that the virus is multiplying in your body and you are very contagious. If you have chronic HBV, an elevated viral DNA level means you are an increased risk for liver damage and may want to consider treatment with antiviral medicine. Testing for HBV DNA is also used to monitor the effectiveness of treatment
for chronic HBV infection. HBV DNA testing is more sensitive test than HBe Ag for detecting HBV in the blood (Pommerville., 2004).

Other HBV tests are not done as often hepatitis B core Ab(HBcAb) is an antibody to the hepatitis B core antigen that appears about one month after an active HBV infection. It can be found in people who had an infection in the past and those with long-term (chronic) HBV. Some blood banks test for this antibody when screening donated blood for hepatitis B (Chempeand Harvey.,2008).

Anti-HBc is the first antibody appear and have a high titers of IgM, anti –HBcsuggest an acute and continuing viral replication. It persists for many months. IgManti-HBc may be the only serological indicator of recent HBV infection in a period when HBs Ag has disappear and anti –HBs is not detectable in the serum. Anti-HBe appears after the anti-HBc and is appearance relates to a decreased infectivity(Kumarand Clark., 2009).

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

It is based on the use of DNA fragment called the gene probe (39). Gene probe is relatively small, single stranded DNA segment that can hunt for complementary fragment of DNA (Mumtazet et al., 2011).

To use a gene probe effectively, it is valuable to increase the DNA to be searched. The polymerase chain reaction (PCR) accomplishes this task (Pommerville., 2004).

2.11.4 Detection of HBV in tissue specimen

Because of its ability to generate high viremia and antigenaemia, HBV infection is usually easy to diagnose with the aid of serum sample. Liver biopsies are necessary to examine the degree of the inflammation, necrosis and fibrosis, and repeated biopsies are required to follow the progression of the disease or the success of antiviral therapy. Because of the risk associated with biopsy, it should be done only if the clinical, biochemical and virological data suggested severe disease. Biopsy may be stained for HBs Ag, pre-s1 antigen, HBe Ag or HBV DNA (Topley and Wilsns., 2011).

2.12 Treatment

Patient experiencing the acute symptoms of the hepatitis should receive symptomatic treatment. The most common complications from an acute infection are dehydration and/or electrolyte imbalance from vomiting. This can corrected with fluid administration antiemetic may be employed (Kumar and Clark., 2009). Treatment use for HBV infection are recombinant subcutaneous interferon Alfa (10Mu thrice weekly), oral
lamivudine (100mg once a day), oral adefovir (10mg once a day) and oral enticavir (0.5g once a day) has recently been approved. The main goal of treatment of chronic hepatitis B is to suppress HBV replication and to induce remission of the liver disease before development of cirrhosis and hepatocellular carcinoma (Souza et al., 2004).

2.13 Prevention and vaccination

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

2.13.1 Current vaccines

The currently used European HBV vaccines consist of the small envelope(s) protein and middle pre-s2 envelope (m) protein assembled into 22 nm particles.

Determinant and several subtype determinants. S vaccines are produced by processing of HBs Ag purified from plasma of HBV carrier and from yeast cells (Saccharomyces cerevisiae) expressing recombinant vaccine prepared by expression in CHO cells (Mumtaz et al., 2011).

2.13.2 Combined prophylaxis

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

12.13.3 Active immunization

This is with a recombinant yeast vaccine produced by insertion of a plasmid containing the gene of HBs Ag in to yeast. Dosage regimen. Three injections (at 0,1and 6 months) are given in to deltoid muscle ; this gives short-term protection in over 90% of patient – people who are over 50years of age or clinically ill and for immunocompromised (including those with HIV infection and AIDs) have a poor antibody response; more frequent and larger doses are required. Antibody levels should be measured at 7-9 months after the initial dose in all at-risk groups. There are few side-effects from vaccine, soreness at the site of injection may occurs, with very occasionally a fever, rash or a flu–like illness (Kumar and Clark., 2009).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study design

3.1.1 Type of study
This is a descriptive cross sectional study.

3.2.1 Study area
The study was carried out in Omdoreman Maternity Hospital Khartoum, Sudan. Practical part was of this study done in Disease preventive & screening Centre Laboratory in UAE.

3.3 Study period
The study was carried out from October 2015 to December 2015.

3.4 Study population
Pregnant women attending Omdurman Maternity Hospital in Khartoum state.

3.5 Study variable
Variable like age, gender, residence, martial state, tattooing, dental treatment, smoking, pregnancy, education and vaccination were considered.

3.6 Sample size
A total of 90 samples whom agree to participate were enrolled pregnant women.

3.7 Sample technique
It was convenient non probability random sample (quota).

3.8 Data collection
Data were collected using interviewing questionnaire covered general information which include Age, , martial state, tattooing, dental treatment, smoking, education and vaccine (Appendix 1).
3.9 Collection of blood specimens

Under sterile condition, 5ml of venous blood sample was withdrawn from each one using vacationer tubes. The serum was separated by centrifugation at 5000rpm for five minutes. Serum was store at -20°C until used.

3.10 Detection of HBV

Sera samples were tested for the presence of HBs Ag using Enzyme LinkImmuno-Sorbent AssayELISA (Bio-Rad) (Monolisa HBs Ag ULTRA) (appendix 2) the procedure was done according to the instructions of the manufacturer.

3.10.1 ELISA technique

3.10.1.1 Principles

An enzyme-immunoassay based on a sandwich principle was applied in this study. Microtiter well had been coated with monoclonal anti-HBs (antibody to HBs Ag), which constitute the solid-phase antibody. The test sample was incubated in each well; when HBs Ag present in the sample it bound to the solid-phase antibody. Subsequently guinea-pig anti-HBs, which have been labeled with the enzyme horseradish peroxidase (HRP), was added with positive reaction this labeled antibody becomes bound to any solid-phase antibody HBs Ag complex previously formed. Incubation with substrate produced a blue color in the test-well, which turns yellow when the reaction was stopped with sulphuric acid. When the sample did not contain HBs Ag, the labeled antibody cannot be bound specifically and only a low background color developed.

3.10.1.2 Assay procedure
The reagent and sera were allowed to reach room temperature. 100 uL of each control (Non-Reactive Control, and Reactive Control) and serum were added to the appropriate microtiter Plate well, and then 50ul of HRP-conjugate was transferred to each well except blank and mixed well and incubated for 60 minutes at 37°C. The Microtiter Plate was washed 4 times and soaked for second 20-30seconds with diluted wash buffer. 100 uL of substrate was added into each and incubated for 30 minutes at 37°C. This distribution can be visually controlled: There is a clear different of coloration between empty well and well with the pink substrate solution. This distribution can also be controlled automatically riding at 490 nm (optional).

100 uL stop solution was added this distribution can be visually controlled. The substrate initially pink become uncolored for the non-reactive sample wells and turn blue to yellow for the positive well (R3).

Reading of the optical densities at 450/620-700 nm and interpretation of the result, were interpreted.

**Reader**

Calculate the mean measured absorbance value for negative control (A1, B1, G1, and D1).

Calculated cut-off: mean R3 + 0.050.

Positive test is <R3+ 0.050.

Negative test >R3+0.050.

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

3.12 Ethical consideration

Each individual invited to participate in the study was informed about the purpose of study before data collection. The study was approval by Ethical committee, College of Medical Laboratory Science, Sudan University of Science and Technology.

CHAPTER FOUR
RESULTS

24
4.1 Results

Ninety pregnant women were participated in this study. All participants fall in age ranged from 15 to 45 years old. The mean age was 24 years; Most of participants (62.2 %) fall in age ranged 26-35 (%). According to geographical residence, the majority 53 (58.9 %) from Khartoum, Bahri 20 (21.8%), Omdurman 9 (9.8%) and out of Khartoum 8 (9.5%).

59 (65.6%) not vaccinated will 31 vaccinated (34.4%). 77 (85.6%) were house wife while 12 (14.4%) employee (Table 1).

The hepatitis B surface antigen were founded in 5 (5.6%) of study population. This rest (85) were negative for Hepatitis B virus while 5 were positive for Hepatitis B virus (Table 2). Most of the infected women were in age (26-35).

Table 2 showed statistical relation of HBsAg sero-diagnosis and its risk factors among pregnant women using ELISA technique.

The results indicated significant relations (P value < 0.05) between HBsAg infection and dental treating and blood transfusion. While it has insignificant association with vaccine, residence, age, occupation and gestational age (Table 2).

Table 1. Frequency of risk factors of Hepatitis B virus infection among study population
<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Result</th>
<th>Count</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion</td>
<td>Yes</td>
<td>9</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>81</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>Dentistry</td>
<td>Yes</td>
<td>9</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>81</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Khartoum</td>
<td>53</td>
<td>58.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bahry</td>
<td>20</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Umdurman</td>
<td>9</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Out of Khartoum</td>
<td>8</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>Housewife</td>
<td>77</td>
<td>85.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee</td>
<td>12</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Vaccine</td>
<td>Yes</td>
<td>31</td>
<td>34.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>59</td>
<td>65.6</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>1st trimester</td>
<td>18</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd trimester</td>
<td>22</td>
<td>24.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd trimester</td>
<td>49</td>
<td>54.4</td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td>15-25</td>
<td>22</td>
<td>24.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26-35</td>
<td>56</td>
<td>62.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36-45</td>
<td>12</td>
<td>13.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Statistical relation of sero-positive HBs Ag using ELIA technique and its risk factors among pregnant women
<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Result</th>
<th>HBsAg Positive</th>
<th>HBsAg negative</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count</td>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>Yes</td>
<td>5</td>
<td>4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Dentistry</td>
<td>Yes</td>
<td>5</td>
<td>4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Khartoum</td>
<td>5</td>
<td>48</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>Bahry</td>
<td>0</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Omdurman</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Out of Khartoum</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>housewife</td>
<td>3</td>
<td>74</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>Employee</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Vaccine</td>
<td>Yes</td>
<td>0</td>
<td>59</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>1st trimester</td>
<td>1</td>
<td>17</td>
<td>0.738</td>
</tr>
<tr>
<td></td>
<td>2nd trimester</td>
<td>2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd trimester</td>
<td>2</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td>15-25</td>
<td>1</td>
<td>21</td>
<td>0.766</td>
</tr>
<tr>
<td></td>
<td>26-35</td>
<td>3</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36-45</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

☐ Spearman’s correlation was used to calculate P value.

☐ P value <0.05 consider significant.
**Fig 1. Frequency of the HBsAg result among pregnant women.**
5.1 Discussion

Hepatitis B is a disease of significant public health impact. Prevention strategies are important for the effective control of this infection, including universal vaccination and the use of immunoglobulin in certain situations. For this purpose, data about prevalence rates are needed, especially in populations at risk for disease transmission, such as pregnant women.

In this study the frequency of HBs Ag among pregnant women was found similar to that reported carried out in Brazil (Souza et al., 2012) relatively low compared to study carried out by (Abou et al., 2009 and Ding Y et al., 2013). The difference of frequency of HBs Ag among the same subject might be owing to diverse of life, influence of risk factor and to the method used to assess the occurrence of hepatitis infection.

Generally, results of this study were considerably lower than that reported at Central Sudan (6.8%) (Mudawi, 2008) and at South Sudan (26.4%), (Abou et al., 2009) reported 6.25% sero-positive among blood donors in Nyala, south Darfur, however Sudan is considered as endemic area of HBV infection, (Elsheik et al., 2007) reported sero-prevalence of 5.6% among antenatal care attendants in central Sudan.

The present study revealed that most candidate had not received the vaccine, even so (Hung and his colleagues reported moderate response to HBV vaccine (51.7%) among street involving youths Aged 15-24 years old (Gilbert et al., 2010).
The present results showed significant statistical relation between HBs Ag and blood transfusion, and dental treatment this result similar to study carried out by Souza et al., 2004).

Agegroup, residence, occupation, gestational age and vaccine did not contribute significantly to increase HBs Ag in this study. Similarly, (Fathimoghaddam et al., 2011) and Khamduanget al.,(2013), reported only age as risk factor affecting HBV infection among Mashhad population.
5.2 Conclusion

1. 5 (5.6 %) of the study population were sero-positive for HBs Ag.

2. The results showed significant association of HBs Ag and dental treating, blood transfusion.

3. The study indicated insignificant ($P<0.05$) association between HBV infection and other factors, including residence, age, occupation and gestational age.

5.3 Recommendations

1. HBV vaccine should be adjusted to cover all pregnant women.

2. Establishment of health education program as well academic education for increased awareness about hepatitis.

3. Hepatitis should be diagnosed as routine investigation among pregnant women.

4. Further studies with large number of participants are required to validate the result of this study.
REFERENCES


17. **Monica C (2000)**. District laboratory practice in tropical countries part 2 Low priced United Kingdom. CambridgeUniversitypress.


Appendices

Questionnaire on Study of Sero-diagnosis of Hepatitis B Surface Antigen among Pregnant Women in Khartoum State

Data collection Sheet

General data
1. Name……………………………………………………………………………

2. Gender
Male ( ) Female ( )

3. Age………………

4. Education level
Primary ( ) secondary ( ) university ( )

5. Blood transfusion
Yes ( ) No ( )

6. Dental treatment
Yes ( ) No ( )

7. Tattooing
Yes ( ) No ( )

8. Vaccine
Yes ( ) No ( )