

Sudan University of Science and Technology
College of Graduate Studies

Seroprevalence of Hepatitis B virus among Pregnant Women
Attending Omdurman Maternity Hospital Clinic
الانتشار المصلي لالتهاب الكبد الفيروسي(ب) لدى الحوامل في عيادة مستشفى الولادة
امدرمان

A thesis submitted in partial fulfillment for the Requirement of M.Sc
Degree in Medical Laboratory Science (Microbiology)

By

Ahd Gamer Edeen Hamied

B.S.c, Medical Laboratory Science (Microbiology) 2013
Faculty of Medicine and Health Sciences (University of Kordofan)

Supervisor

Prof. Yousif Fadlalla Hamed elnil

September 2015

Dedication

For my Dears:

Mother

Father

Brothers

And sisters

Acknowledgments

First of all thanks for **ALMIGHTY ALLAH** for every things, and for giving me chance and power to start and complete this study.

Thanks' to my supervisor: **Prof.YousifFadlalla** for taking my hand to the right way through their instruction and suggestion that help me to complete my project with remarkable flexibility.

I wish to thank **Suhair Ramadan Rehan** Sudan University of Sciences and Technology for technical help in the laboratory. And **WegdanAwadElkareem** (BSc) that help me in collection of sample.

Finally I acknowledge after Allah my **family**, who stimulating me to continue this work and provide me with encouragement and support that make this study possible.

Abstract

Pregnant women are a specific target group to Hepatitis B Virus (HBV) due to the risk of vertical transmission of the infection, which occurs usually in the prenatal period and is the major cause of HBV transmission. The aim of the study was to determine Sero-prevalence of Hepatitis B virus among pregnant women attended Omdurman Maternity Hospital clinic. A total of ninety subjects (n=90) were enrolled in this study. A local antiseptic (70% ethanol) was used to clean the skin. Venous blood (3mLs) were taken from each participant by standard procedures and put in plain containers to clot then centrifuged at 1500 rpm for 5 minutes and serum was obtained and kept at -20°C until used. The serum samples were examined by ELISA to detect HBsAg and HBcAb. One out of 90 (1.1%) pregnant women was found HBsAg positive and 6 out of 90 (6.7%) pregnant women were showed HBcAb positive. All women who gave a positive result they were from urban area and there was no case from the rural. Out of total 6 positive result for HBcAb 3 (50%) of them between the ages 26-34 years. In addition to the total number of abortion 35 only 2 (5.7%) gave a positive result for HBcAb and no abortion in HBsAg positive case. The prevalence of HBV infection among pregnant women attending Omdurman Maternity clinics did not exceed the estimated nationwide prevalence. However, that not means to ignore this low prevalence and giving special attention to the prevention of outbreak of hepatitis B.

ملخص الدراسة

النساء الحوامل مجموعة مستهدفة لالتهاب الكبد الفيروسي (ب) فخطر الانتقال العمودي الذي يحدث عادة قبل فترة الولادة وهو من الاسباب الاساسية للانتقال. قد استهدفت هذه الدراسة معرفة مدى الانتشار المصلي لالتهاب الكبد الفيروسي (ب) لدى النساء الحوامل الموجودين في عيادة مستشفى الولادة امدرمان، وقد شملت الدراسة (90) حامل بعد التعقيم الموضعي بكحول الايثانول ذو تركيز 70% لتعقيم الجلد اخذت (3مل) من الدم من كل مشارك ومن ثم وضعت في حاويات خاوية ليتجلط ثم وضع في جهاز الطرد المركزي (1500) لفة في الدقيقة لمدة 5 دقائق ومن ثم المصل المتحصل عليه حفظ لغاية الاستخدام في درجة حرارة -20 تحت الصفر. ثم فحصت العينات لاختبار الانتجين السطحي والصد البني لالتهاب الكبد الوبائي (ب) بواسطة اختبار الـ روز المناعي الانزيمي. واحد من اصل 90 حامل اعطت نتيجة ايجابية لفحص الانتجين السطحي لفيروس التهاب الكبد الوبائي ب (1.1%). وكذلك 6 من اصل 90 اعطوا نتيجة ايجابية لفحص الصد البني لفيروس التهاب الكبد الوبائي ب (6.6%) باستخدام الـ روز المناعي الانزيمي. كل النساء اللاتي اعطوا نتيجة موجبة في هذه الدراسة كانوا من المدينة ولم تكن هناك حالات من القرى. من مجموع 6 نتائج موجبة لصد البني لالتهاب الكبد الوبائي (ب) 3 (50%) منهم كانت اصابتهم متراوح عمر 26-الى 34 سنة. بالاضافة ان من المجموع الكلي لحالات الاجهاض 35 حالة اثنين فقط (5.7%) اعطوا نتيجة ايجابية للصد بي لفيروس التهاب الكبد الوبائي ولا توجد حالة اجهاض بالنسبة للانتجين السطحي لفيروس. من خلال هذه النتيجة نستنتج ان انتشار التهاب الكبد الوبائي ب لدى حوامل عيادات مستشفى امدرمان لا يتجاوز الحد الاعلى لانتشاره علي الصعيد الدولي لكن ذلك لايعني تجاهل هذه النسبة البسيطة واعطاء اهتمام لمنع انتشار التهاب الكبد الوبائي ب.

LIST OF CONTENTS

Dedication.....	I
Acknowledgment.....	II
Abstract: English	III
Abstract: Arabic.....	IV
List ofContents	V
List of Tables.....	VIII
List of Figures	VIII

CHAPTER INTRODUCTION

1.1 Background.....	1
1.2 Rationale.....	3
1.3 Objective.....	4
1.3.1 General objective.....	4
1.3.2 Specific objective.....	4

CHAPTER TWOLITERATURE REVIEW

2. Literature review.....	5
2.1 Discovery of HBV.....	5
2.2 Classification of HBV.....	6
2.3 Morphology of HBV.....	7
2.3.1 Structure.....	7
2.3.2 Component.....	7
2.4 Evolution of HBV.....	8
2.5 Genome.....	8
2.5.2 The size.....	8
2.5.2 Encoding.....	8
2.5.3 Genotype.....	9
2.6 Life cycle.....	10
2.7 Antigencity.....	12
2.8 Stability.....	13

2.9 Endemicity.....	13
2.10 Incidence/Epidemiology.....	14
2.11 HBV among Sudanese pregnant women.....	15
2.12 Transmissions of HBV.....	15
2.13 Spectrum of liver disease after HBV infection.....	16
2.14 Sequelae of HBV on pregnancy.....	19
2.15 Laboratory diagnosis of HBV.....	20
2.15.1 Collection, and storage the sample.....	21
2.15.2 Diagnosis.....	21
2.15.2.1 Large scale screening for HBV infection.....	21
2.15.2.2 Small scale screening for HBV infection.....	24
2.16 Preventions.....	24
2.17 Treatments.....	25
CHAPTER TREEMATERIAL AND METHODS	
3. Materials and methods.....	27
3.1 Study Design.....	27
3.2 Study population	27
3.3 Study Area.....	27
3.4 Sampling.....	27
3.5 Selection Criteria.....	27
3.5.1 Inclusion criteria.....	27
3.5.2 Exclusion criteria.....	27
3.6 Ethical consideration.....	27
3.7 Data collection.....	28
3.7.1 Interview with questionnaire.....	28
3.7.2 Blood sample collection.....	28
3.7.3.1 Detection of HBsAg by sandwich ELISA.....	28
3.7.3.2 Detection of HBcAb by competitive ELISA.....	30
3.8 Statistical analysis.....	32

CHAPTER FOUR RESULT

4.1. Detection of HBsAg among pregnant women.....	33
4.2. Detection of HBcAb among pregnant women.....	34
4.3. Relation between HBsAg and HBcAb among pregnant.....	34

CHAPTER FIVE DISCUSSION

5. Discussion.....	37
5.1 Conclusion.....	39
5.2 Recommendations.....	39

REFERENCES

Appendix

FIGURES

2.1 Structure of Hepatitis B Virus (HBV).....	7
2.2 Outline of HBV reproduction life cycle.....	11
2.3 World Distribution of HBV infection.....	14
4.1 Show the Percentage of HBsAg among pregnant women.....	33
4.2 show the percentage of HBcAb among pregnant women.....	34
4.3 Show the percentage of both HBsAg and HBcAb.....	35

TABLES

2.1 Interpretation of HB panel tests result interpretation.....	23
4.1 Show the Percentage of HBsAg among pregnant women.....	33
4.2 show the percentage of HBcAb among pregnant women.....	34
4.3 Show the percentage of both HBsAg and HBcAb.....	35
4.4 Distribution of HBsAg and HBcAb according to risk factors.....	36
4.5 Distribution of HBsAg and HBcAg according to the age.....	36

Abbreviations

ACOG American College of Obstetricians and Gynecologists

ALT Alanine aminotransverase

AST Aspartate aminotransverase

cccDNA Covalently Closed Circular

CDC Center Disease Control

ELISA Enzyme linked Immune Sorbent Assay

HBV Hepatitis B Virus

HCC Hepato Cellular Carcinoma

Hib Haemophilusinflunzae b

HRP Horse Radish peroxidase

MHC Major Histocompatibility Complex

ROIPAC Recommendations of the Immunization Practices Advisory Committee

SPSS Statistical package for Social Science

TMB TetramethyleBenzidine

CHAPTER ONE

CHAPTER ONE

INTRODUCTION

1.1 Background

Hepatitis B, one of the major and common infectious diseases of the liver worldwide is 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 occur from HBV related liver diseases, including end stage cirrhosis and hepatocellular carcinoma (HCC) each year (Lavanchy, 2004; Wang *et al.*, 2003).

With regard to Hepatitis B Virus (HBV), pregnant women are a specific target group due to the risk of vertical transmission of the infection, which occurs usually in the prenatal period and is the major cause of HBV transmission, particularly in countries of the world where HBV has intermediate to high prevalence. Acute HBV infection during pregnancy is usually not severe and is not associated with increased mortality or teratogenicity (Sookoian, 2006). Thus, infection during gestation should not prompt consideration of termination of the pregnancy. However, there have been reports of an increased incidence of low birth weight and prematurity in infants born to mothers with acute HBV infection (Jonas, 2009). Furthermore, acute HBV occurring early in the pregnancy has been associated with a 10 percent prenatal transmission rate (Jonas, 2009). Transmission rates significantly increase if acute infection occurs at or near the time of delivery, with rates reported as high as 60 percent (Sookoian, 2006).

Mother-to-infant transmission represents a basic factor in maintaining chronic HBV infection and usually depends on the degree of maternal infectivity, especially in the prenatal period (Wang *et al.*, 2003). It has long been recognized that prevention of prenatal transmission is a high priority in the attempt to decrease the global burden of chronic HBV (Jonas, 2009). Pregnant women constitute a special subgroup which is

subject to routine HBsAg screening because of the risk of vertical transmission. At the same time, pregnancy can modify the course of hepatitis B infection (Lin *et al.*, 2006; Soderstrom *et al.*, 2003).

1.2. Rationale

Viral hepatitis during pregnancy is associated with high risk of maternal complications and has become a leading cause of fetal death. Hepatitis B virus (HBV) infection still has a relatively high incidence and prevalence worldwide. The most important mode of HBV transmission globally is perinatal, from the mother to her newborn baby. If babies were not vaccinated at birth, many of these develop lifelong HBV infections, and many develop liver failure or liver cancer later in life (Lavanchy, 2004). If a pregnant woman is an HBV carrier and is also HBeAg-positive, her newborn baby has 90% likelihood to be infected and become a carrier. Of these children, 25% will die later from chronic liver disease or liver cancer. 10-20% of women seropositive for HBsAg transmit the virus to their neonates in the absence of immunoprophylaxis (Hollinger and liang , 2001).

Although infection with Hepatitis B Virus (HBV) remains a global public health problem, little is known about epidemiology in Sudan concerning pregnant. That due to improper Unitarian managing screening program to all state for both the mother and fetus, That can lead to serious outcome in the coming years (will increase the incidence of disease) in our population. If routine free screening is adopted that will enhance prevention of prenatal transmission which is an important component of global efforts to reduce the burden of chronic HBV since vertical transmission is responsible for approximately one-half of chronic infection worldwide.

1.3. Objective:

1.3.1. General objective:

To determine the sero-prevalence hepatitis B virus among pregnant women attending antenatal clinic (Omdurman Maternity Hospital)

1.3.2. Specific objective:

- 1.To determine the frequency of hepatitis B surface antigen (HBsAg) By sandwich ELISA among pregnant women attending Omdurman maternal hospital Khartoum, Sudan..
- 2.To detect total anti HBc Ag in pregnant women by using competitive ELISA.
- 3.To identify potential risk factors associated with the infection including age, accommodation, history of jaundice, history of blood transfusion and previous miscarriage.

CHAPTER TWO

CHAPTER TWO

2. Literature Review

2.1 Discovery of the Hepatitis B Virus

In brief, the story of hepatitis B started with a search for inherited differences and ended with the discovery of a new virus. As a medical anthropologist in the early 1950's, Dr. Blumberg was interested in the genetics of disease susceptibility. He wondered whether inherited traits could make different groups of people more or less susceptible to the same disease.(Hepatitis B Foundation,2011)

Dr. Blumberg and his team traveled the globe to collect blood samples from native populations in remote parts of the world. They planned to look for genetic differences, and then study whether these differences were associated with a disease. However, since they did not have the technology to analyze these blood samples at the genetic level, a new indirect method had to be developed; they turned their attention to hemophiliac patients.Dr. Blumberg reasoned that hemophiliacs who had received multiple blood transfusions would have been exposed to blood serum proteins that they themselves had not inherited, but had been inherited by their donors. As a result of this exposure, the immune systems of the hemophiliac patient would produce "antibodies" against the foreign blood serum proteins, or "antigens", from the donors. Since antibodies are programmed to lock onto specific antigens, Dr. Blumberg decided to use antibodies from hemophiliac patients to test the blood samples collected around the world.(Hepatitis B Foundation,2011)

Using this new lab technique for matching antibodies with antigens, an unusual match was identified between an antibody from a New York hemophiliac and an antigen found in the blood sample of an Australian aborigine, which they called the "Australia antigen".A series of research and clinical observations led to confirmation that the

"Australia antigen" caused hepatitis B and thus, this was how the hepatitis B virus was discovered in 1967.(Hepatitis B Foundation,2011).

. 2.2. 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 *et al.*, 2008). Viruses similar to hepatitis B have been found in all apes (*orangutan, gibbons, gorillas and chimpanzees*), in Old World monkeys and in a New World woolly monkeys suggesting an ancient origin for this virus in primates,(Dupinay *et al.*, 2013).

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins, and into eight genotypes (A–H) according to overall nucleotide sequence variation of the genome. 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 (Kramvis *et al.* , 2005; Magnus and Norder 1995).

Unclassified species

A number of as yet unclassified Hepatitis B like species has been isolated from bats. (Drexler *et al.*,2013)

2.3 Morphology

2.3.1. Structure (Fig 2.1)

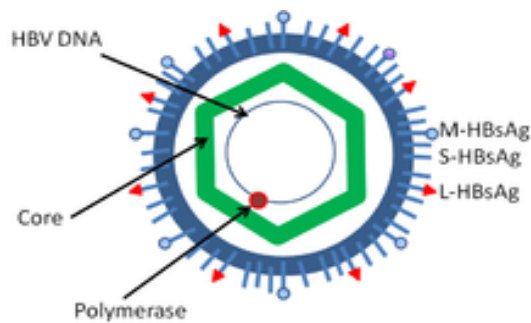


Fig 2.1: The structure of hepatitis B virus

Hepatitis B virus is a member of the *Hepadnavirus* family (Zuckerman, 1996). The virus particle, (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. (Locarnini, 2004) 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. (Howard, 1986).

2.3.2. Components

It consists of:

HBsAg

HBcAg

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).

2.4. Evolution

The early evolution of the Hepatitis B, like that of all viruses, is difficult to establish. The divergence of *orthohepadnavirus* and *avihepadnavirus* occurred ~125,000 years ago. Both the *Avihepadnavirus* and *Orthohepadna viruses* began to diversify about 25,000 years ago. (Van Hemert *et al.*, 2011). The branching at this time lead to the emergence of the *Orthohepadna* genotypes A–H. Human strains have a most recent common ancestor dating back to 7,000 to 10,000 years ago. A second estimate of the origin of this virus suggests a most recent common ancestor of the human strains evolved ~1500 years ago. (Zhou and Holmes , 2007) The most recent common ancestor of the avian strains was placed at 6000 years ago.

2.5. Genome

2.5.1. Size

The genome of HBV is made of circular DNA, but it 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), (Kay and Zoulim ,2007).

2.5.2. Encoding

The negative-sense, (non-coding), is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand by viral

polymerase and removal of a protein molecule from the (-) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (-) sense strand and the ends are rejoined.

There are four known genes encoded by the genome called C, P, S, and X. The core protein is coded for by gene C (HBcAg). HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg) (Beck and Nassal, 2007). The function of the protein coded for by gene X is not fully understood (Bouchard and Schneider, 2004), but some evidence suggests that it may function as a transcriptional transactivator.

2.5.3. Genotypes

Genotypes differ by at least 8% of the sequence and have distinct geographical distributions and this has been associated with anthropological history. Within genotypes subtypes have been described. There are eight known genotypes labeled A through H. (Kramvis *et al.*, 2005) A possible new "I" genotype has been described (Olinger *et al.*, 2008). But acceptance of this notation is not universal (Kurbanov *et al.*, 2008).

Two further genotypes have since been recognized. (Hernández *et al.*, 2014). The current listing now runs A through to J. Several subtypes are also recognized. There are at least 24 subtypes. Different genotypes may respond to treatment in different ways. (Palumbo, 2007; Mahtab *et al.*, 2008).

Individual genotypes

Type F which diverges from the other genomes by 14% is the most divergent type known. Type A is prevalent in Europe, Africa and South-east Asia, including the Philippines. Type B and C are predominant in Asia; type D is common in the Mediterranean area, the Middle East and India; type E is localized in sub-Saharan Africa; type F (or H) is restricted to Central and South America. Type G has been found in

France and Germany. Genotypes A, D and F are predominant in Brazil and all genotypes occur in the United States with frequencies dependent on ethnicity.

2.6. 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 cotransporting peptide .

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. (Beck and Nassal ,2007; Bruss ,2007).

Release

The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity (Fig.2.2).

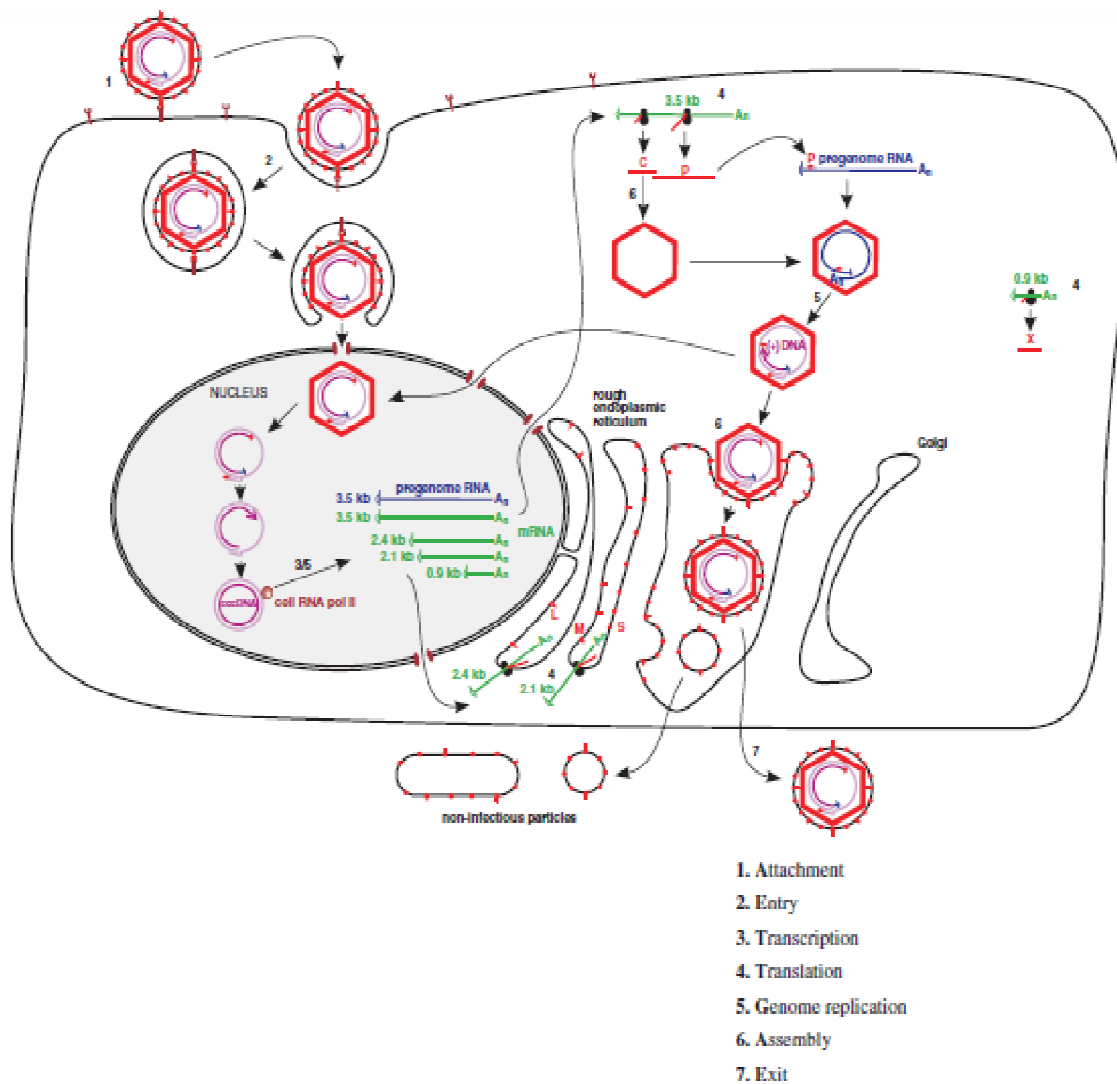


Fig:2.2 Outline of HBV replication cycle. Genome replication involves synthesis of RNA in the nucleus, then copying from RNA to DNA. (John and Venetia, 2007)

2.7. Antigenicity

All three coat proteins of HBV contain HBsAg, which is highly immunogenic and induces anti-HBs (humoral immunity). Structural viral proteins induce specific T-lymphocytes, capable of eliminating HBV-infected cells (cytotoxic T-cells; cellular immunity). HBsAg is heterogeneous antigenically, with a common antigen designated a, and two pairs of mutually exclusive antigens, d and y, and w (including several subdeterminants) and r, resulting in 4 major subtypes: adw, ayw, adr and ayr. The distribution of subtypes varies geographically. Because of the common determinants, protection against one subtype appears to confer protection to the other subtypes, and no difference in clinical features has been related to subtypes (Robinson ,1994). Subtype ayr is rare in the world, but it is commonly found in small populations in Oceania. (Lavanchy, 2004)

The c antigen (HBcAg) is present on the surface of core particles. HBcAg and core particles are not present in the blood in a free form, but are found only as internal components of virus particles (Lavanchy,2004;Robinson ,1994).The core antigen shares its sequences with the e antigen (HBeAg), identified as a soluble antigen, but no crossreactivity between the two proteins is observed. (Robinson ,1995).

Viral oligopeptides of 8-15 amino acids are loaded on host cell MHC-class I molecules and are transported to the surface of the cell. HBV-specific T-lymphocytes can then detect infected cells and destroy them. This cell deletion triggered by inflammation cells may result in acute hepatitis. When the infection is self-limited, immunity results. If HBV is not eliminated, a delicate balance between viral replication and immune-defence prevails which may lead to chronic hepatitis and liver cirrhosis. In chronically infected cells the HBV DNA may integrate into the host cell DNA. As a long term consequence, integration may lead to hepatocellular carcinoma. (Hollinger and liang ,2001).

2.8.Stability

The stability of HBV does not always coincide with that of HBsAg. Exposure to ether, acid (pH 2.4 for at least 6 h), and heat (98°C for 1 min; 60°C for 10 h) does not destroy immunogenicity or antigenicity. However, inactivation may be incomplete under these conditions if the concentration of virus is excessively high. Infectivity is lost after autoclaving at 121°C for 20 min or dry heat treatment at 160°C for 1 h. HBV is inactivated by exposure to sodium hypochlorite (500 mg free chlorine per litre) for 10 min, 2% aqueous glutaraldehyde at room temperature for 5 min, heat treatment at 98°C for 2 min, Sporidicin (Ash Dentsply, York, PA) (pH 7.9), formaldehyde at 18.5 g/l (5% formalin in water), 70% isopropylalcohol, 80% ethyl alcohol at 11°C for 2 min, Wescodyne (a iodophor disinfectant) diluted 1:213, or combined β -propiolactone and UV irradiation. (WHO,1993). HBV retains infectivity when stored at 30°C to 32°C for at least 6 months and when frozen at -15°C for 15 years. HBV present in blood can withstand drying on a surface for at least a week.

2.9. Endemicity

There are no seasonal preferences for primary HBV infections (Robinson ,1994). Hepatitis B is highly endemic in all of Africa, some parts of South America, Alaska, northern Canada and parts of Greenland, Eastern Europe, the eastern Mediterranean area, south-east Asia, China, and the Pacific Islands, except Australia, New Zealand and Japan. In most of these areas, 5 to 15% of the population are chronically infected carriers of HBV, and in some areas may also carry HDV, which may lead to severe liver damage (Fig 2.3). Even in low endemicity countries such as the USA, mortality from HBV was five times that from *Haemophilus influenzae b* (Hib) and ten times that from measles before routine vaccination of children was introduced(Lavanchy, 2004).

In endemic areas of Africa and Asia, the epidemiological patterns differ from those seen in North America and Western Europe. In these regions, most infections occur in infants and children as a result of maternal-neonatal transmission or close childhood contact, although percutaneous exposure with contaminated needles or following unsafe injections is always a possibility. The chronic liver disease and HCC associated with HBV infections are among the most important human health problems in high-prevalence regions.

2.11. 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 a hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in southern Sudan. Hepatitis B virus was the commonest cause of chronic liver disease and hepatocellular carcinoma and was the second commonest cause of acute liver failure in the Sudan. Viral hepatitis during pregnancy is associated with high risk of maternal complications, 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. 5.6% of pregnant women that attending in the Omdurman Maternity Hospital were positive for HBVsAg irrespective to their age, parity and socio-demographic characteristics (Hatim, 2008).

2.12. Transmission of HBV

HBV is transmitted through percutaneous or parenteral contact with infected blood, body fluids, and by sexual intercourse. (Ganem and Shneider, 2001) HBV is able to remain on any surface it comes into contact with for about a week, e.g. table-tops, razor blades, blood stains, without losing infectivity.

HBV does not cross the skin or the mucous membrane barrier. Some break in this barrier, which can be minimal and insignificant, is required for transmission. HBV is a large virus

and does not cross the placenta, hence it cannot infect the fetus unless there have been breaks in the maternal-fetal barrier, e.g. via amniocentesis. Still, pregnant women who are infected with HBV can transmit their disease to their babies at birth. If not vaccinated at birth, many of these babies develop lifelong HBV infections, and many develop liver failure or liver cancer later in life.

Sexual intercourse with multiple partners or with persons who have multiple partners can be dangerous. One should not judge by appearance: most infected people look perfectly healthy and have no symptoms of disease, yet may be highly infectious. All persons who are hepatitis B surface antigen (HBsAg) positive are potentially infectious. The many millions of people around the world who become HBV carriers are a constant source of new infections for those who have never contracted the virus. (Robinson ,1995) Hepatitis B is the only sexually transmitted infection for which there is a protective vaccine.

2.13. Spectrum of liver disease after HBV infection

The infecting dose of virus and the age of the person infected are important factors that correlate with the severity of acute or chronic hepatitis B. Only a small proportion of acute HBV infections are recognized clinically. Less than 10% of children and 30-50% of adults with acute HBV infection will have icteric disease. (WHO, 2001). Most cases of acute hepatitis are subclinical, and less than 1% of symptomatic cases are fulminant.

Clinical phases of acute hepatitis B infection

The acute form of the disease often resolves spontaneously after a 4-8 week illness. Most patients recover without significant consequences and without recurrence. Young children rarely develop acute clinical disease, but many of those infected before the age of seven will become chronic carriers. The incubation period varies usually between 45 and 120 days, with an average of 60 to 90 days. The variation is related to the amount of virus in the inoculum, the mode of transmission and host factors (Mahoney and Kane, 1999).

In patients with clinical illness, the onset is usually insidious with tiredness, anorexia, vague abdominal discomfort, nausea and vomiting, sometimes arthralgias and rash, often progressing to jaundice. Fever may be absent or mild (Chisari and Ferrari, 1997). The icteric phase of acute viral hepatitis begins usually within 10 days of the initial symptoms with the appearance of dark urine followed by pale stools and yellowish discoloration of the mucous membranes, conjunctivae, sclerae, and skin. Jaundice becomes apparent clinically when the total bilirubin level exceeds 20 to 40 mg/l. It is accompanied by hepatomegaly and splenomegaly. About 4-12 weeks thereafter, the jaundice disappears and the illness resolves with the development of natural, protective antibodies (anti-HBs), in about 95% of adults.

Clinical features of chronic hepatitis B

Non replicative phase. Markers of viral replication are either absent or below detection level, and the inflammation is diminished. However, if cirrhosis has already developed, it persists indefinitely.

Sustained increases in the concentrations of the liver enzyme aminotransferases together with the presence of HBsAg for >6 months is regarded as indicative of chronic hepatitis. Up to 20% of the chronic persistent hepatitis cases progress to cirrhosis. In cirrhosis, liver cells die and are progressively replaced with fibrotic tissue leading to nodule formation. The internal structure of the liver is deranged leading to the obstruction of blood flow and decrease in liver function. This damage is caused by recurrent immune responses stimulated by the presence of the virus. Because liver inflammation can be totally symptomless, progression of inflammation to cirrhosis can occur without the knowledge of the patient (Viral Hepatitis Prevention Board, 1996).

HBV and hepatocellular carcinoma (HCC)

Persons at increased risk of developing HCC, who contracted hepatitis B in early childhood (Lavanchy, 2004), only about 5% of patients with cirrhosis develop HCC. On the other hand, between 60 and 90% of HCC patients have underlying cirrhosis. Primary liver cancer is the eighth most common cancer in the world. Up to 80% of liver cancers are due to HBV. When HCC presents clinically, the disease is fatal. The median survival frequency of HCC patients is less than 3 months. However, if the cancer is detected early, there is a 85% chance of a cure. Treatment involves surgery, hepatic irradiation, and anticancer drugs (Robinson, 1995).

Progression to fulminant hepatitis B

Fulminant hepatitis B is a rare condition that develops in about 1% of cases. It is caused by massive necrosis of liver substance and is usually fatal (Mahoney and Kane, 1999).

Survival in adults is uncommon, prognosis for children are rather better. Remarkably, the few survivors usually recover completely without permanent liver damage and no chronic infection. Genetic heterogeneity of HBV, coinfection or superinfection with other viral hepatitis agents, or host immunological factors, may be associated with the development of fulminant hepatitis B.(Zuckerman, 1996).

Extrahepatic manifestations of hepatitis B:

Extrahepatic manifestations of hepatitis B are seen in 10-20% of patients as

Transient serum sickness-like syndrome

With fever ($<39^{\circ}\text{C}$), skin rash, polyarthritis (acute articular symmetrical inflammation, painful, fusiform swelling of joints of hand and knee, morning stiffness). Symptoms usually precede the onset of jaundice by a few days to 4 weeks and subside after onset of jaundice and may persist throughout the course of the disease. No recurrent or chronic arthritis occurs after recovery (Lavanchy, 2004). Immune complexes (e.g. surface

antigen-antibody) are important in the pathogenesis of other disease syndromes characterized by severe damage of blood vessels (Mahoney and Kane, 1999).

Acute necrotizing vasculitis (polyarteritis nodosa)

With high fever, anemia, leucocytosis, arthralgia, arthritis, renal disease, hypertension, heart disease, gastrointestinal disease, skin manifestations, neurologic disorders. Highly variable disease with mortality rate of 40% within 3 years unless treated. The diagnosis is established by angiography (Robinson, 1995).

Membranous glomerulonephritis

Is present in both adults and children. Remission of nephropathy occurs in 85 to 90% of cases over a period of 9 years and is associated with clearance of HBeAg from serum.

papular acrodermatitis of childhood (Gianotti-Crosti syndrome)

A distinctive disease of childhood. Skin lesions, lentil-sized, flat, erythematous, and papular eruptions localized to the face and extremities, last 15 to 20 days. The disease is accompanied by generalized lymphadenopathy, hepatomegaly, and acute anicteric hepatitis B of ayw subtype. Immune complexes have been found in the sera of all patients with fulminant hepatitis, but are seen only infrequently in non fulminant infections. Perhaps complexes are critical factors only if they are of a particular size or of a certain antigen-to-antibody ratio (Zuckerman, 1996).

2.14. Sequelae of HBV on pregnant

10-20% of women seropositive for HBsAg transmit the virus to their neonates in the absence of immunoprophylaxis. In women who are seropositive for both HBsAg and HBeAg vertical transmission is approximately 90% (ACOG, 2007). In patients with acute hepatitis B vertical transmission occurs in up to 10% of neonates when infection occurs in the first trimester and in 80 -90% of neonates when acute infection occurs in the third

trimester (ACOG, 2007). Chronic infection occurs in about 90% of infected infants, 30% of infected children aged <5 years , and 2%--6% of adults. Among persons with chronic HBV infection, the risk of death from cirrhosis or hepatocellular carcinoma is 15%-25%. HBV infection does not appear to be cause birth defects, but there appears to be a higher incidence of low birth weight among infants born to mothers with acute infection during pregnancy (Shepard, 1998) . However, acute hepatitis did increase the incidence of prematurity (Hieber *et al.*, 1977).

Who to test

Test all pregnant women at the first prenatal visit for hepatitis B surface antigen (HBsAg). More than 90% of women found to be HBsAg-positive on routine screening will be HBV carriers, routine follow-up testing later in pregnancy is not necessary for the purpose of screening. In special situations, such as when the mother is thought to have acute hepatitis, when there has been a history of exposure to hepatitis, or when particularly high-risk behavior such as parenteral drug abuse has occurred during the pregnancy, an additional HBsAg test can be ordered during the third trimester (ROIPAC,1988).

Test all susceptible contacts (including all family members) with hepatitis B panel (HBsAg, antiHBc, antiHBs). Screening and vaccination of susceptible contacts should be done by the family's pediatrician, primary health-care provider, or the physician evaluating the clinical status of the HBsAg-positive pregnant women.

2.15. Laboratory Diagnosis

Diagnosis is based on clinical, laboratory, and epidemiologic findings. HBV infection cannot be differentiated on the basis of clinical symptoms alone, and definitive diagnosis depends on the results of serologic testing (CDC, 2012).

2.15.1. Collection, transport and storage of sample

HBV antigen or antibodies are stable at room temperature for several hour to days, can be stored at -20 °C to – 70°C for many years. Repeat freezing and thawing can lead to degradation of HBV serologic markers .furthermore ,use of hemolyzed sample should be avoided as interferes with detection signals .plasma is separated from blood collected in containers with EDTA or citrate dextrose as the anticoagulant (Horvart and Tegtmeier,2011).

2.15.2.Diagnosis

2.15.2.1.Large-scale screening for HBV infection

Diagnosis of hepatitis is made by:

1.Biochemical assessment of liver function. Initial laboratory evaluation should include: total and direct bilirubin, ALT, AST, alkaline phosphatase, prothrombin time, total protein, albumin, serum globulin, complete blood count, and coagulation studies. (Hollinger and liang ,2001).

2.Diagnosis is confirmed by demonstration in sera of specific :

Antigens: Three clinical useful antigen systems have been identified for hepatitis B: Hepatitis B surface antigen (**HBsAg**)

Hepatitis Bcore antigen(**HBcAg**)

Hepatitis B e antigen (**HBeAg**)

HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious (Mahoney and Kane, 1999).

Acute hepatitis patients who maintain a constant serum HBsAg concentration, or whose serum HBeAg persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver. The presence of HBeAg is associated with relatively high infectivity and severity of disease.

Antibodies :Three clinical useful antibody systems have been identified for hepatitis B: Antibody to HBsAg (**anti-HBs**) Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity. (Lavanchy,2004).

Antibody (**anti-HBc IgM and anti-HBc IgG**) is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. This test may therefore reliably diagnose acute HBV infection. IgG anti-HBc generally remains detectable for a lifetime (Robinson ,1995).

Antibody to HBeAg (**anti-HBe**) Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity. Anti-HBe replaces HBeAg the resolution of the disease (Mahoney and Kane, 1999) .

3.Tests specific for complete virus particles or DNA and DNA polymerase-containing virions, and for hepatitis Delta antigen (HDAg) and hepatitis Delta virus (HDV) RNA in liver and serum are available only in research laboratories (Hollinger and liang ,2001) .

Figure 2.1: Interpretation of the Hepatitis B Panel Tests Results Interpretation

Tests	Results	Interpretation
HBsAg	negative	susceptible
anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	immune due to natural infection
anti-HBc	positive	
anti-HBs	positive	
HBsAg	negative	immune due to hepatitis B vaccination
anti-HBc	negative	
anti-HBs	positive	
HBsAg	positive	acutely infected
anti-HBc	positive	
IgM anti-HBc	positive	
anti-HBs	negative	
HBsAg	positive	chronically infected
anti-HBc	positive	
IgM anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	four interpretations possible *
anti-HBc	positive	
anti-HBs	negative	

Source: <http://www.cdc.gov/ncidod/diseases/hepatitis/b/Bserology.htm>

2.15.2.2.Small-scale screening for HBV infection

- Immunofluorescence studies
- In situ hybridization
- Immunohistochemistry
- Thin-section electron microscopy

Are used to examine pathological specimens for the presence of HBV-associated antigens or particles, providing information about the relationship between HBV DNA replication and HBV gene expression. Within the hepatocyte, HBsAg localizes in the cytoplasm, and HBcAg is seen in the nucleus and/or the cytoplasm. Detection of complete virions in the liver is uncommon.

-DNA hybridization techniques and RT-PCR assays: have shown that almost all HBsAg/HBeAg-positive patients have detectable HBV DNA in their serum, whereas only about 65% of the HBsAg/anti-HBe-reactive patients are positive. All patients who recover from acute hepatitis B are negative for HBV DNA. On the other hand, some patients infected chronically who have lost their HBsAg remain HBV DNA positive. (Robinson ,1995).

2.16. Prevention

Pregnancy is not a contraindication to vaccination.

For vaccination of adults 20 years of age and older: 1-mL dose by intramuscular injection into the deltoid muscle, at initial visit, then one month and six months after the first dose, for a total of three doses.

Postexposure Prophylaxis for Susceptible Pregnant Women:

After Exposure to Persons Who Have Acute Hepatitis B: When exposure has occurred as a result of sexual contact within 14 days after the most recent sexual contact administer.'

- A course of HBV vaccine into the deltoid as above
- A dose of Hepatitis B immune globulin (HBIG) 0.06 mL/kg IM into the contralateral arm.
- For prophylaxis after percutaneous or mucous membrane injury, a second dose of HBIG should be given 1 month later.(CDC,1990).

Exposure to Persons Who Have Chronic HBV Infection

Active postexposure prophylaxis with hepatitis B vaccine alone is recommended for sex or needle-sharing partners and non-sexual household contacts of persons with chronic HBV infection.

Newborns Born to Hepatitis B Carriers

Newborns born to hepatitis B carriers should receive hepatitis vaccine (HBIG) within 12 hours of birth (CDC, 2006).

2.17. Treatment

The treatment of acute HBV infection is supportive. Patients should be hospitalized if they have coagulopathy, encephalopathy, or severe debilitation (ACOG,2007).Persons with chronic hepatitis B should be referred to health-care professionals with experience in the treatment of hepatitis B for treatment with alpha-interferon or lamivudine. Interferon does not appear to adversely affect the embryo or fetus. However, the data is limited, and the potential benefits of interferon use during pregnancy should clearly outweigh possible hazards (Briggs *et al.*, 2005; Hiratsuka *et al.*, 2000). Initial data do not suggest that Lamivudine is teratogenic (Briggs *et al.*,2005) ,Lamivudine has been used in the latter

half of pregnancy in attempt to prevent perinatal transmission of hepatitis B virus infection with mixed success (Van Zonneveld *et al.*,2003;Kazim *et al.*,2002).

CHAPTER THREE

CHAPTER TREE

Materials and methods

3.1. Study design

This study was descriptive, cross-sectional and hospital based study.

3.2. Study population

Pregnant women with different age, were the source of samples.

3.3. Study area

The study was conducted at Omdurman Maternity Hospital in Sudan.

3.4. Sampling

Sampling Technique:

Simple random sample

Sample size:

A total of ninety subjects (n=90) were enrolled in this study.

3.5. Selection criteria

3.5.1. Inclusion criteria

All pregnant women that visit clinic of Omdurman Maternity Hospital.

3.6. Ethical consideration

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

Permission to carry out the study was taken from Department of Virology, Laboratories Administration, State Ministry of Health, and Khartoum, Sudan. After explaining the study and its goals.

Written consent was taken from the study recruits before proceeding with the study and collecting samples.

3.7. Data collection and analysis

3.7.1. Interview with a Questionnaire

An interview with questionnaire to obtain the clinical data was used for each participant in this study.

3.7.2 Blood samples collection

Ethanol (70%) was used to clean the skin. Venous blood (3ml) were taken from each participant by standard procedures and put in plain containers then left to clot for 15 min and then centrifuged at 1500 rpm for 5 minutes. The serum was separated and kept at -20C °until used.

3.7.3 Methods

3.7.3.1 Detection of HBsAg by Sandwich Enzyme linked Immune-Sorbent Assay (ELISA)

Principle: For detection of HBsAg, Diagnostic Automation, Inc HBsAg ELISA uses antibody 'sandwich' ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum sample is added to the microwell together with a second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) and directed against a different epitope of HBsAg. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-cojugate, Chromogen solution containing tetramethyl-

benzidine (TMB) and urea peroxide are added to the well. In presence of the antibody-antigen-antibody (HRP)"sandwich" immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. Well containing samples negative for HBsAg remain colorless.

Assay Procedure

1-Reagent preparation: The reagents and sample were allowed to reach room temperature (18-30°C) for at least 15-30 minutes. Wash buffer concentrate was checked for the presence of salt crystals.

2- If crystal has formed in the solution, it was resolubilized by warming at 37°C until crystal dissolved. The stock wash buffer was diluted 1 to 20 with the distilled water.

3-Numbering Wells: The strip was set in the strip holder, and numbered the wells included three for the negative control, two for positive control and one blank.

4-Adding Sample and HRP-Conjugate: 50 µl of positive control, negative control, and specimen were added in to their respective wells, then 50 µl HR-conjugate was added to each well except the blank and mixed by tapped the plate gently.

5-Incubation: The plate was covered with the plate cover and incubated for 60 minutes at 37°C in incubator.

6-Washing: The plate content was discarded and each well was washed 5 times with diluted wash buffer. The microwell were allow to soak for 30-60 seconds. After the final washing cycle, the plate was turned down onto blotting paper, and tap it to remove any remainders.

7-Coloring: 50 µl of chromogen A and 50 µl chromogen B solution were dispensed into each well including the blank, and mixed by tapping the plate gently. The plate was incubated at 37°C for 15 minutes avoiding light. The enzymatic reaction between the

chromogen solution and the HRP-conjugate produced blue color in positive control and HBsAg positive sample well.

8-Stopping reaction: Using a multichannel pipette, 50 μ l stop solution was added in to each well and mixed gently. Intensive yellow color developed in positive control and HBsAg positive sample well.

9-Measuring the absorbance: The plate reader was calibrated with the blank well and read the absorbance at 450nm. Then Calculated the cut-off value and evaluated the result.

Quality control (assay validation)

The test results are valid if the quality control criteria are fulfilled:

The value of the blank well, which contains only chromogen and stop solution, is \leq 0.080 at 450nm.

The values of the positive control must be \geq 0.800 at 450nm/630nm or at 450nm after blanking.

The values of Negative control must be \leq 0.100 at 450/630nm or at 450nm after blanking.

3.7.3.2 Detection of HBcAb By competitive Enzyme Linked Immune Sorbent Assay (ELISA)

The system of the HBcAb ELISA test is founded on the solid phase, one- step incubation competitive principle. When anti-HBc is present, it compete with the monoclonal anti-HBc conjugated to horseradish peroxidase(HRP-Conjugate) for a fixed amount of purified HBcAg pre-coated in the wells. If no anti-HBc is present, HRP-conjugated anti-HBc will be bound together with antigens inside the wells. In the course of washing, any unbound HRP-Conjugate is removed. After chromogen solutions A and B are added into the wells and during incubation, a blue-colored product appears when the colorless chromogens are hydrolyzed by the bound HRP-Conjugate. After the reaction is stopped

with sulfuric acid, the blue color turned yellow. A presence of antibodies to HbcAg in the sample is indicated by low color, or no color present at all.

Assay Procedure

Step 1 Reagents preparation: The reagents and sample were allowed to reach room temperature (18-30°C) for least 15 -30 minutes. Dilutes the stock wash buffer 1 to 20 with the distilled water.

Step 2 Numbering wells: The strip was set in the strip holder, and numbered the wells included three for the negative control, two for positive control and one blank.

Step 3 Adding Sample and HRP-Conjugate: 50 µl of positive control, negative control and specimen were added in to their respective wells. Add 50 µl of HRP-conjugate to each well except in to the blank and mixed by tapped the plate gently.

Step 4 Incubating: the plate was covered with the plate cover and incubated for 60 minutes at 37°C in dry incubator.

Step 5 Washing: The plate content was discarded and each well was washed 5 times with the dilutes wash buffer. Each time , the micro wells allowed to soak for 30-60 seconds. After the final washing cycle , the plate was turned onto blotting paper or clean towel, and tapped to remove any remained liquids.

Step 6 Coloring : 50 µl of chromogn A and chromogen B solution was dispensed into each well including the blank and Incubated the plate at 37°C for 25 minutes, avoiding light. The enzymatic reaction between the chromagen solution and HRP-Conjugate produced blue color in negative control and anti-HBc.

Step 7 Stopping the reaction: use a multichannel pipette, 50 µl stop solution into each well and mixed gently. Intensive yellow color developed in negative control and anti-negative sample well.

Step 8 measuring the absorbance: The plate reader was calibrated with the blank well and read the absorbance at 450nm.

Quality Control range:

The test results are valid if the quality control criteria are verified. It is recommended that each laboratory must establish appropriate quality control material similar to or identical with the patient sample being analyzed.

1-The optical density (OD) value of the blank well, which contains only chromogen and stop solution, is less than 0.080at 450nm.

2-The (OD) value of the negative control must be equal to or greater than 0.800 at 450/630nm or at 450 after blanking.

3-The (OD) value of the positive control must be less than 0.100 at 450nm after blanking.

3.8 Statistical analysis

The data obtained were analyzed and presented using Statistical Package for Social Science (SPSS) computer software version 11.5 for windows 7.(Significance level were set as $P \leq 0.05$).

CHAPTER FOUR

4. Results

A total of ninety subjects (n=90) were enrolled in this study, age range was from 17 to 43 years (mean: 29.66 years), eighty one (90%) from urban and nine (10%) from rural. And no one is vaccinated against HBV (100%).The mean of previous miscarriage 35(38.8%), history of previous transfusion 22(24.4%) and history of jaundice is 22 (24.4%).Table (4.4) and (4.5).

4.1. Detection of HBsAg among pregnant women

One out of 90 pregnant women was found HBsAg positive (1.1%) Table (4.1) and Fig (4.1). 89(98.9%) of them is HBsAg negative.

Table .4.1: The percentage of HBsAg among pregnant women

		Frequency	Percent
Valid	positive	1	1.1
	negative	89	98.9
	Total	90	100.0

HBsAg

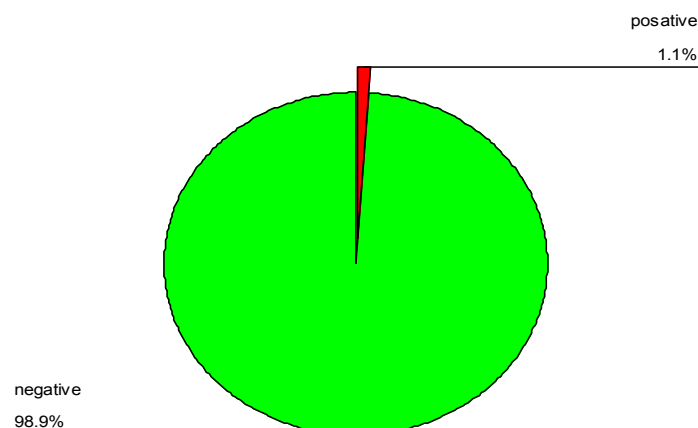


Fig .4.1: The frequency of HBsAg among pregnant women

4.2. Detection of HBcAb among pregnant women

Six out of 90 pregnant women showed HBcAb positive (6.6%).Table(4.2) and Fig(4.2).

Table 4.2: The percentage of HBcAb among pregnant women

		Frequency	Percent
Valid	positive	6	6.7
	negative	84	93.3
	Total	90	100.0

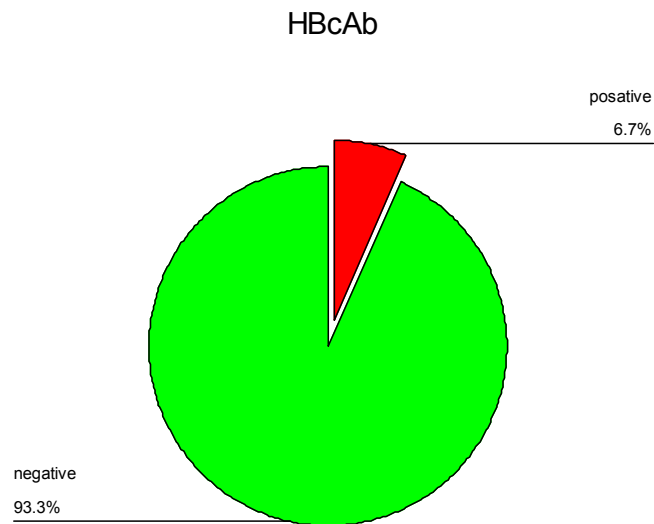


Fig. 4.2: The frequency of HBcAb among pregnant women

4.3. Relation between HBsAg and HBcAb among pregnant women

Out of 90 there was one positive HBsAg which positive for HBcAb.

While from remainder 89 negative HBsAg there was 5 positive for HBcAb.

Table.4.3: The percentage of both HBsAg and HBcAb among pregnant women

			HBcAb		Total
			positive	negative	
HBsAg	positive	Count	1	0	1
		% of Total	1.1%	.0%	1.1%
	negative	Count	5	84	89
		% of Total	5.6%	93.3%	98.9%
Total	Count		6	84	90
	% of Total		6.7%	93.3%	100.0%

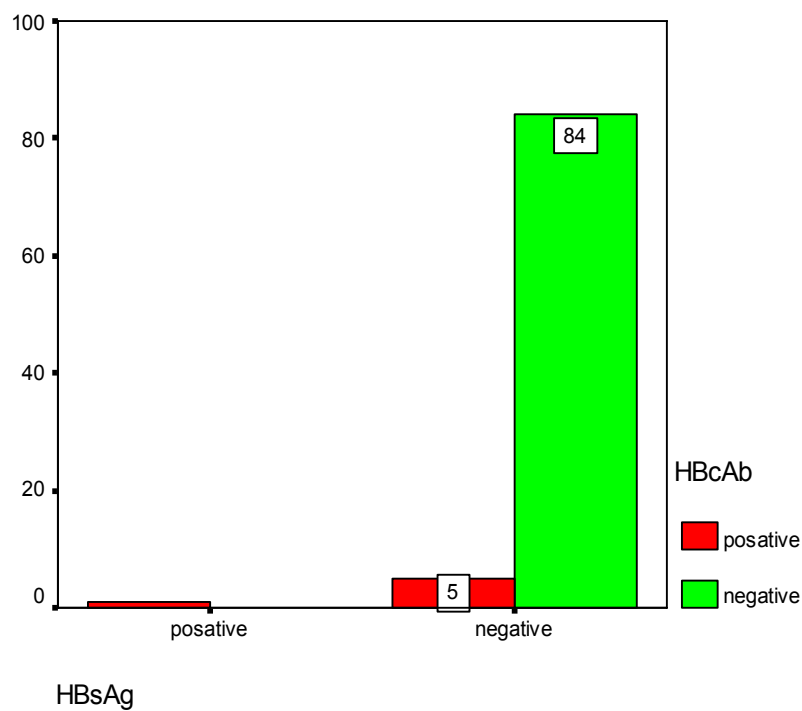


Fig. 4.3: The frequency of both HBsAg and HBcAb among pregnant women

Table 4.4: Distribution of HBsAg and HBcAb according to risk factors

Risk factor	HBsAg positivity	HBcAb positivity	p.value HBsAg positivity	p.value HBcAb positivity
History of PBT	1/22 4.5%	3/22 3.6%	0.07	0.13
History of Jaundice	1/22 4.5%	2/22 9.0%	0.07	0.6
History of Miscarriages	0/35 0.0%	2/35 5.7%	0.42	0.77

PBT- Previous Blood Transfusion

($\chi^2 \cdot 0.05, P \cdot 0.05$)

This association was statistically not significant

Table 4.5: Distribution of HBsAg and HBcAg according to the age

Age group	HBsAg		HBcAb	
	No	%	No	%
17-25	0	0.0%	2	33.4%
26-34	1	100%	3	50%
35-43	0	0.0%	1	16.6%
Total	1	100%	6	100%

CHAPTER FIVE

CHAPTER FIVE

Discussion

The prevalence of HBsAg in this study was (1.1%). This result was agreement with those obtained from Egyptian pregnant women (1.75%) (Mortada,2006), and pregnant women from Port Harcourt, Nigeria (2.8%) (Obi *et al.*, 2006). That may be due to similar environmental condition and culture. Other studies that slightly similar in prevalence to this study obtained from Greece pregnant women (4%) (Elefsiniotis *et al.*,2009), Jazan “Kingdom of Sudia Arabia”(4%) (Ibrahim,2012) and pregnant women in southern Turkey (2%),(Nilgun, 2011), and finally pregnant women in North America gave (3.6%)(Med ,2011). Similarity obtain from previous studies when compare with this study (1.1%) may be due to small sample size because is not possible result obtained from first world countries with best care and hygienic behavior gave similar result to studies conducted in third world countries.

Very high prevalence observed from third world countries that may be due to very bad health care with pregnant women and very bad hygienic behavior in addition to lack of possibilities to gave educated doses to community about infection, transmission and prevention of Hepatitis B disease, this counteris was Nigeria from which pregnant women reported prevalence of HBsAg (44%) (Esan *et al.*,2014). Flowed by pregnant women in the Makurdi (11%) (Mbaawuaga *et al.*, 2008), and pregnant women in Buea Health District, Cameroon, (9.7%).Then flowed by pregnant women assisted at the public maternity hospitals of São Luís, Maranhão, Brazil. (7.4%) (Marinilde *et al.*, 2012) and finally pregnant women attending State Specialist Hospital, Maiduguri (6.5%) (Isa,2015).

Very low prevalence of HBsAg was reported from two studies one achieved by (Seyed *et al.*, 2011), in Lorestan West of Iran (0.2%), suggest that due to best care with pregnant women. And other carried out in pregnant women who attended the antenatal clinic of

Obstetrics Gynecology Unit, Bhaskar Medical College and Hospital Andhra Pradesh, India 0.6% (Shazia *et al.*, 2012).

On another hand this study showed that 6 out of 90 pregnant women were HBcAb positive (6.7%) by using ELISA. This is a bit higher than that reported by (Seyed *et al.*, 2011), in Lorestan West of Iran is (3.4%).

This study (1.1%) when compared with local previous study (5.6%) conducted in Omdurman Maternity Hospital, Khartoum. Sudan (Rasha *et al.*, 2007), showed lower result that may referred to best health care with pregnant women in few years ago or due to small sample size used in this study.

As regards to age, in present study high HBs Ag and HBcAb rate in pregnant women 3(50%) was found in age group 26-34, 2(33.3%) between 17-25, and 1(16.6%) between 35-43 years. Pregnant women in this study were interviewed for the history of jaundice 22(24%), blood transfusion 22(24%) and Miscarriage 35(38%). Out of 22 pregnant women who had history of jaundice, 1 (4.5 %) were HBs Ag positive and, 1(4.5%) out of 22 with blood transfusion and nobody had history of previous miscarriages. On another hand, out of 22 pregnant women who had history of jaundice, 2 (9%) were HBcAg positive and, 3(13%) out of 22 with blood transfusion, and 2(9%) out of 35 had history of previous miscarriages.

Though, the risk factors were founded in this study, but statistically significant association was not observed between the above this risk factors and HBs Ag positive or HBcAb positive pregnant women ($\chi^2 \cdot 0.05$, $P > 0.05$). This association was statistically not significant ($\chi^2 \cdot 0.05$, $P > 0.05$) in this study that may be due to small sample size used. The future expectation of HBV disease in Sudan among pregnant women will be decrease if adopted protocols to managing it.

5.1 Conclusion:

The prevalence of HBV infection among pregnant women attending Omdurman Maternity Clinic did not exceed the estimated worldwide prevalence. However, that not means to ignore this low prevalence and giving special attention to the prevention of hepatitis B.

5.2 Recommendations:

- 1- Pregnant mothers should receive prenatal screening for hepatitis B.
- 2- Reproductive female must have HBsAg vaccine.
- 3- Further more study need, with increase sample size.

REFERENCES

1. **American College of Obstetricians and Gynecologists. ACOG.** (2007). Practice Bulletin No. 86: *Viral hepatitis in pregnancy. Obstet Gynecol.* Oct;110:941-56.
2. **Andreas A .**(2014). Prevalence of HBsAg and knowledge about hepatitis B in pregnancy in the Buea Health District, Cameroon: a cross-sectional study. *BMC Research Notes*, 7:394.
3. **Benhenda S, Drucroux A, Rivire L, Sobhian B, Michael D.** (2013). Methyltransferase PRMT1 Is a Binding Partner of HBx and a Negative Regulator of Hepatitis B Virus Transcription. *Journal of Virology* **87**: 4360–4371.
4. **Beck J and Nassal M .** (2007). Hepatitis B virus replication. *World J. Gastroenterol.* **13**: 48–64.
5. **Bouchard MJ and Schneider RJ.** (2004). The enigmatic X gene of hepatitis B virus. *J. Virol.* **78**: 12725–34.
6. **Bruss V.** (2007). Hepatitis B virus morphogenesis. *World J. Gastroenterol.* **13** : 65–73.
7. **Briggs GG, Freeman RK, Yaffe SJ.** (2005) *Drugs in Pregnancy and Lactation*, 7th edition, Baltimore, p 836 -838.
8. **Centers for Disease Control and prevention.** (2012). Basic information about HIV and AIDS. Available at: <http://www.cdc.gov/hiv/topics/basic/index.htm>, accessed at 10/6/2013.
9. **Centers for Disease Control and Prevention,** (2006) *Sexually transmitted diseases treatment guidelines*. 1-94.
10. **Centers for Disease Control.** (1990) Protection against viral hepatitis. *Recommendations of the Immunization Practices Advisory Committee (ACIP)*. 1-26

11. **Chai N, Chang HE, Nicolas E, Han Z, Jarnik M, Taylor J.** (2008). Properties of subviral particles of hepatitis B virus. *J. Virol.* **82**: 7812–7.
12. **Chisari FV, Ferrari C.** (1997). Viral Hepatitis In: Nathanson N et al., eds. *Viral Pathogenesis*, Philadelphia, Lippincott – Raven p:745-778.
13. **Dupinay T, Michel ML, Benkirane M, Semmes OJ, Buendia MA, Neuveut C.** (2013). Discovery of naturally occurring transmissible chronic hepatitis B virus infection among *Macaca fascicularis* from Mauritius Island.. *Hepatology*, **58**. pp. 1610–1620.
14. **Drexler JF, Geipel A, König A, Corman VM, Van Rie LD, Leijten LM, et al.** (2013). Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes. *Proc. Natl. Acad. Sci. U.S.A.* **110**: 16151–6.
15. **Esan A.J, Omisakin CT, Ojo-Bola T, Owoseni MF, Fasakin KA, et al.** (2014). Sero-Prevalence of Hepatitis B and Hepatitis C Virue Co-Infection among Pregnant Women in Nigeria." *Am. J. Biomed. Res.* Volume 23: p11-15.
16. **Elefsiniotis I ,Glynou I, Zorou I, Magaziotou I, Brokalaki H, et al.** (2009). Surveillance for hepatitis B virus infection in pregnant women in Greece shows high rates of chronic infection among immigrants and low vaccination-induced protection rates ,*Euro surveill.* Volume 14. P:5-7.
17. **Guo GH, Tan DM, Zhu PA, Liu F.** (2009). Hepatitis B virus X protein promotes proliferation and upregulates TGF-beta1 and CTGF in human hepatic stellate cell line, LX-2". *Hbpd Int* **8**: 59–64.
18. **Ganem D and Schneider RJ.** (eds) .(2001) *Hepadnaviridae: The Viruses and Their Replication.* In: *Knipe DM et al. Fields Virology*, 4th ed. Philadelphia, *Lippincott Williams & Wilkins*,:2923-2969.
19. **Hatim MY.** (2008). Epidemiology of viral Hepatitis on sudan . *Clinical and Experimental Gastroenterology*. volume 1, p.9-13.

20. **Hepatitis B Foundation.** (2011) discovery of hepatitis B virus {online} A violable from :[http:// hepb.Org](http://hepb.Org) Accessed:1/8/2015.
21. **Hernández S, Venegaset M, Brahm J, Villanueva RA, et al.** (2014). Full-genome sequence of a hepatitis B virus genotype flb clone from a chronically infected Chilean patient. *Genome Announc* 2: e01075–14
22. **Hieber JP, Dalton D, Shorey J, Combes B.** (1977) Hepatitis and pregnancy. *J Pediatr*. 545-9.
23. **Hiratsuka M, Minakami H, Koshizuka S, Sato I.** (2000). Administration of interferon-alpha during pregnancy: *effects on fetus*. 28:372-6.
24. **Hollinger FB, Liang TJ.** (2001) Hepatitis B Virus. In: Knipe DM *et al.*, eds. *Fields Virology*, 4th ed. Philadelphia, Lippincott Williams & Wilkins, :2971-3036.
25. **Horvart RT and Tegtmeier GE.** (2011). Hepatitis B and D viruses. In Versalovic J, Carrol KC, Funke G, Jorgensen JH, Landry ML, and Warnock DW (eds). *Manual of clinical Microbiology*, 10th ed, volume 2. ASM Press, Washington DC, p.1659-1676.
26. **Howard CR.** (1986). The biology of hepadnaviruses. *J. Gen. Virol.* 67: 1215–35.
<http://www.cdc.gov/ncidod/diseases/hepatitis/b/Bserology.htm>
27. **Ibrahim B.** (2012). Prevalence and risk factors of Hepatitis B Virus among Pregnant Women in Jazan Region-Kingdom of Saudi Arabia. *Int. J. TRO. Dis & Health*, ISSN: 2278 – 1005, Vol.3.
28. **Isa. M. A.** (2015). Sciences Seroprevalence of Hepatitis B virus among pregnant women attending state specialist hospital Maiduguri. *Int. J. Adv. Res. Biol*, P: 133–137.
29. **John B and Venetia A.** (2007). *Virology Principal and Application*, p214-226.
30. **Jonas MM.** (2009). Hepatitis B and pregnancy: *an underestimated issue*. *Liver Int.* Jan;29 Suppl 1:133-9

- 31.**Kay A and Zoulim F.** (2007). Hepatitis B virus genetic variability and evolution. *Virus Res.* **127**: 164–76.
- 32.**Kazim SN, Wakil S M, Khan LA, Hasnan SE, Savin SK, et al.**(2002). Vertical transmission of hepatitis B virus despite maternal lamivudine therapy. *Lancet*; 1488-9.
- 33.**Kramvis A, Kew M, François G.** (2005). Hepatitis B virus genotypes. *Vaccine* **23**: 2409–23.
- 34.**Kurbanov F,Tanaka Y, Kramvis A, Simmonds P, Mizokami M.**(2008). When should "I" consider a new hepatitis B virus genotype? . *J. Virol.* **82**: 8241–2.
- 35.**Lavanchy D.**(2004). Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat.* 11:97-107.
- 36.**Lin HH, Wu WY, Kao J H,Chen DS,** (2006). Hepatitis B post-partum e antigen clearance in hepatitis B carrier mothers: correlation with viral characteristics. *J Gastroenterol Hepatol.*21:605-9.
- 37.**Locarnini S.** (2004). "*Molecular virology of hepatitis B virus*". *Semin. Liver Dis.* **24** (Suppl 1): 3–10.
- 38.**Magnius LO, Norder H.** (1995). Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. *Intervirology* **38**: 24–34.
- 39.**Mahgoub S, Candotti D, Ekiaby E M, Allain J.** (2011).Hepatitis B virus (HBV) infection and recombination between HBV genotypes D and E in asymptomatic blood donors from Khartoum,Sudan .*J Clin Microliol.*49:298-306.
- 40.**Mahoney FJ and Kane M (1999).** Hepatitis B vaccine. In: Plotkin SA and Orenstein WA, eds. *Vaccines*, 3rd ed. Philadelphia, W.B. Saunders Company,p:158-182.

41. **Mahtab MA, Rahman S, Khan M, Karim.** (2008) Hepatitis B virus genotypes: an overview. *Hbpd Int* 7: 457–64.
42. **Marinilde T, Taina L, Max D, Alessandro S, Veral L ., et al** (2012). Prevalence of hepatitis B among pregnant women assisted at the public maternity hospitals of São Luís, Maranhão, Brazil. *Braz. J. infect dis* [online]. volume 16, p.7.
43. **Mason, W.S, Gerlich WH, Taylor JM, Kann M, Mizokami T, et al.** (2008). 00.030. Hepadnaviridae. ICTVdB Index of Viruses. International Committee on Taxonomy of Viruses.
44. **Mbaawuaga, Enenebeaku, Okopi and Damen.** (2008). Hepatitis B Virus (HBV) Infection among Pregnant Women in Makurdi, Nigeria. *Afr. J. Biomed Re*, Vol. 11; 155 – 159.
45. **Med N Am J.** (2011). The prevalence of hepatitis B and C viral infections among pregnant women. *North Am. J. Med Sci.* Volume3: 238–241.
46. **Mortada El and Mohamed F** (2006). Prevalence of Hepatitis B Virus Infection among Egyptian Pregnant Women - A Single Center Study. *Afr. J. Clin Exp. Microbiology*. Vol 7, No 2.
47. **Mudawi H.** (2008). Epidemiology of viral hepatitis in sudan. *Clin Exp Gastroenterol*. 1:9-13.
48. **Nilgun C .** (2011). Seroprevalence of hepatitis B among pregnant women in southern Turkey. *JPMA Journal of Medical Association*. 61:176-7.
49. **Obi RK, Umeh SC , Okurede OH, Iroagba II** (2006). Prevalence of hepatitis B virus infection among pregnant women in an antenatal clinic in Port Harcourt, Nigeria.

- 50.**Olinger CM, Jutavijittum P, Hubschen JM, Yousukh A,Thammavong T., et al.** (2008). Possible new hepatitis B virus genotype, southeast Asia. *Emerging Infect. Dis.* 14: 1777–80.
- 51.**Palumbo E.** (2007). Hepatitis B genotypes and response to antiviral therapy: a review. *Am J Ther* 14: 306–9.
- 52.**Robinson WS.**(eds) (1994). Hepatitis B viruses. General Features (human). In: Webster RG and Granoff A,. *Encyclopedia of Virology*, London, Academic Press Ltd:554-569.
- 53.**Robinson WS.**(eds) (1995) Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, Dolin R. *Principles and Practice of Infectious Diseases*, 4th ed. New York, Churchill Livingstone:1406-1439.
- 54.**Recommendations of the Immunization Practices Advisory Committee Prevention of Perinatal Transmission of Hepatitis B Virus** (1988): *Prenatal Screening of all Pregnant Women for Hepatitis B Surface Antigen MMWR* . 37;341-351.
- 55.**Rasha M E, Ahmed A, Mohamed A, Mubarak S, Ishag A,** (2007). Hepatitis B virus and C virus in pregnant Sudanese women, *virology* [online] volume 4,4:104.
- 56.**Seyed R, Sanati A, Cheraghipour K, Mohammad N, Shalmani H, Zali M,** (2011). Hepatitis C and Hepatitis B Virus Infection: *Epidemiology and Risk Factors in a Large Cohort of Pregnant Women in Lorestan. West of Iran* ;11:736-739
- 57.**Shazia P, Shyamala R, Rao JR, Rao RMV,** (2012). 345Sero-prevalence of Hepatitis B surface antigen among pregnant women attending antenatal clinic in a teaching hospital. *J. Microbiol Biotechnol. Res.* 2:343-5.
- 58.**Shepard TH.**(1998). *Catalog of Teratogenic Agents* pp 1309. 9th ed.Baltimore, MD: Johns Hopkins University Press .p1309

59. **Söderström A, Norkrans G, Lindh M.**(2003). Hepatitis B virus DNA during pregnancy and post partum: aspects on vertical transmission. *Scand J Infect Dis.*;35:814-9.
60. **Sookoian S.**(2006) Liver disease during pregnancy: acute viral hepatitis. *Ann Hepatol.* 5:231.
61. **Van Hemert F, Vande klundert M, Lukashov V, Kootstra N, Berkhout B, Zaaijer H,** (2011). Protein X of hepatitis B virus: origin and structure similarity with the central domain of DNA glycosylase. *PLoS ONE* 6: e23392.
62. **Van Zonneveld M, Van Nunen A, Niester H, Deman R, Schalm S, Janssen H,** (2003) treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. *J Viral Hepat.*10:294-7.
63. **Viral Hepatitis Prevention Board** (1996). Prevention and control of hepatitis B in the community. *Communicable Disease Series*, p: 1.
64. **Wang Z, Zhang J, Yang H, Li X, Wen S, Guo Y, Sm J, Nou J.**(2003) Quantitative analysis of HBV DNA level and HBeAg titer in hepatitis B surface antigen positive mothers and their babies: HBeAg passage through the placenta and the rate of decay in babies. *J Med Virol.* 71:360-6.
65. **World Health Organization.**(1993) *Laboratory Biosafety Manual*, 2nd ed. Geneva, WHO.
66. **World Health Organization.**(2001) Introduction of hepatitis B vaccine into childhood immunization services. Geneva, WHO, (unpublished document WHO/V&B/01.31 available on request from Department of Vaccines and Biologicals, World Health Organization, 1211 Geneva 27, Switzerland).
67. **Zhou Y, Holmes EC** (2007). Bayesian estimates of the evolutionary rate and age of hepatitis B virus". *J. Mol. Evol.* 65: 197–205.

68. **Zuckerman AJ** (eds.) (1996). Hepatitis Viruses. *In: Baron's Medical Microbiology*, (4th ed.). Univ of Texas Medical Branch.

بحث بعنوان

APPENDIX

Study of hepatitis B among (HBV) pregnant women attending Omdurman Maternity
clinic in Khartoum

اعداد / عهد قمر الدين حامد محمد علي

اقرار بالموافقة في البحث

بعد ان تم لي شرح طبيعة الدراسة واعطيت فرصة كافية للاستفسار عنه وقد تم الاجابة علي كل اسئلتي بصورة كافية وفهمت ان لي الحق في التوقف عن هذه الدراسة في اي وقت من غير ان افقد الحق في الرعاية الطبية او اي حقوق اخري ، ووافق طوعية علي المشاركة في هذه الدراسة.

اسم المريض:.....

التوقيع:..... التاريخ:.....

شاهد اول:.....

التوقيع:..... التاريخ:.....

Kordofan University

Faculty of Medicine and Health Science

Questionnaire about:

**Sero prevalence of Hepatitis B virus among Pregnant Women Attending
Omdurman Maternity Hospital clinic**

الانتشار المصلي لالتهاب الكبد الفيروسي ب لدى الحوامل في عيادة مستشفى الولادة امدرمان

Date: / / 2015

NO.....

Age.....

Residence.....

1-Urban ()

2-Rural ()

1. History of blood transfusion:

Yes()

No()

2. History of jaundice:

Yes()

No()

3. History of previous miscarriage:

Yes()

No()

4. Immunization against HBV:

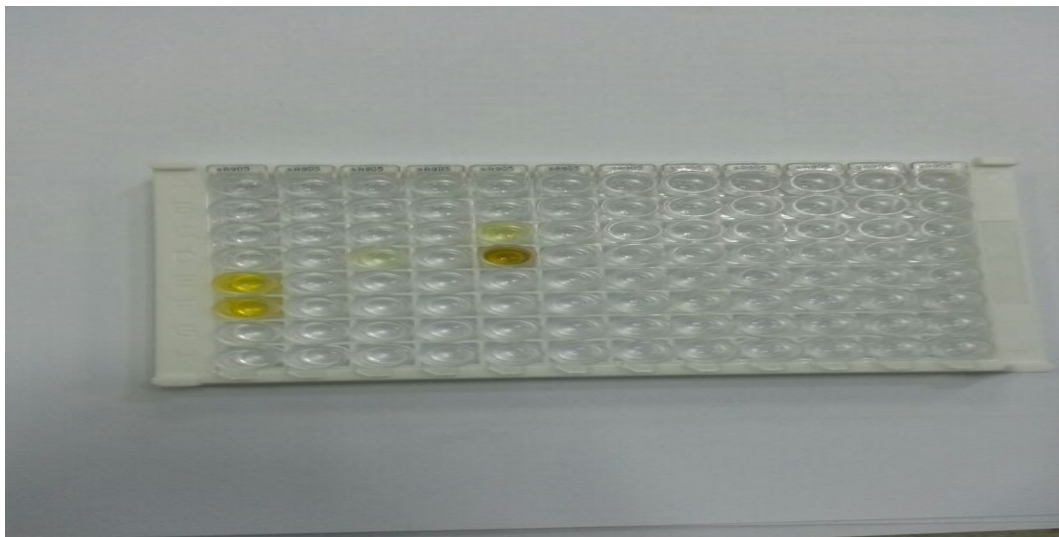
Yes()

No()

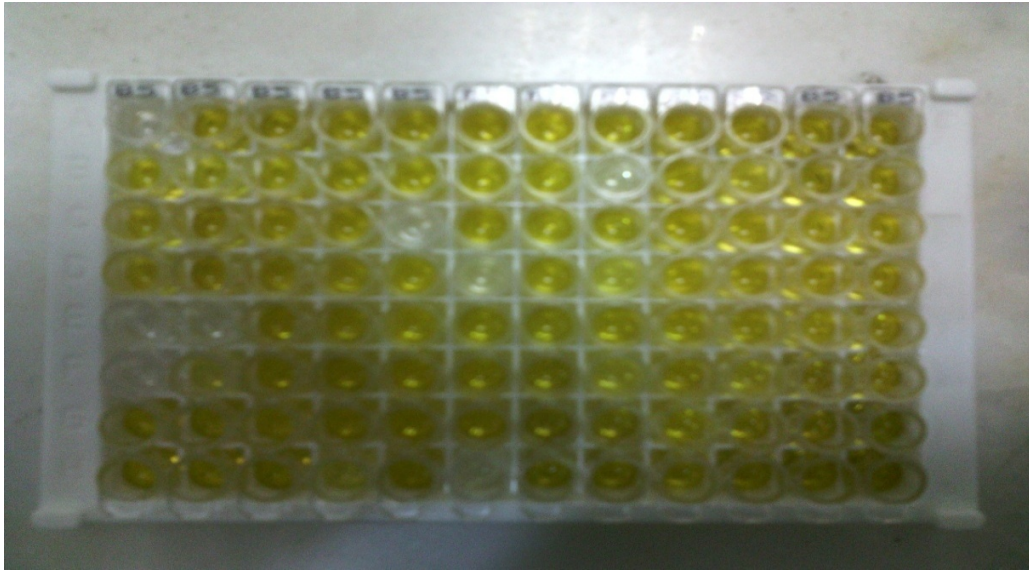
Color plate



Color plate 1: ELISA reader



Color plate 2: ELISA for HBsAg



Color plate 3: ELISA for HBcAb