HEPATITIS B VIRUS INFECTION AND VIRUS GENOTYPES AMONG PREGNANT WOMEN IN AL FASHIR TOWN - NORTH DARFUR STATE

A thesis Submitted in partial fulfillment of the academic requirements for the Ph.D. degree in Medical Microbiology

By

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Dedication

To my parents

The soul of my father

My mother

husband

daughters

and friends

With love and appreciation
# List of Contents

Dedication  
Table of contents  
Acknowledgements  
Abstract (English)  
Abstract (Arabic)  
Abbreviations  
List of figures  
List of tables  

## Chapter 1: Introduction

1. 1 Background on HBV Epidemics  
2. 2 Viral Hepatitis  
1. 3 HBV Infection during pregnancy  
1. 4 History of HBV  
1. 5 HBV Evolution  
1. 6 Classification of HBV  
1. 7 Morphology and Structure of HBV  
1. 8 Distinctive properties  
1. 9 Subtypes and Serotypes  
1. 10 HBV Genetic Diversity  
1. 11 Transmission  
1. 12 Replication  
1. 13 Pathogenesis and Immunity  
1. 14 Clinical Findings  
1. 15 Laboratory Diagnosis  
1. 16 Treatment  
1. 17 Rationale  
1. 18 Objectives  

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>i</td>
</tr>
<tr>
<td>Table of contents</td>
<td>ii, iii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>Abstract (English)</td>
<td>v, vi</td>
</tr>
<tr>
<td>Abstract (Arabic)</td>
<td>vii</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>viii</td>
</tr>
<tr>
<td>List of figures</td>
<td>ix</td>
</tr>
<tr>
<td>List of tables</td>
<td>x</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1. 1 Background on HBV Epidemics</td>
<td>2</td>
</tr>
<tr>
<td>2. 2 Viral Hepatitis</td>
<td>4</td>
</tr>
<tr>
<td>1. 3 HBV Infection during pregnancy</td>
<td>5</td>
</tr>
<tr>
<td>1. 4 History of HBV</td>
<td>7</td>
</tr>
<tr>
<td>1. 5 HBV Evolution</td>
<td>8</td>
</tr>
<tr>
<td>1. 6 Classification of HBV</td>
<td>10</td>
</tr>
<tr>
<td>1. 7 Morphology and Structure of HBV</td>
<td>10 - 12</td>
</tr>
<tr>
<td>1. 8 Distinctive properties</td>
<td>13</td>
</tr>
<tr>
<td>1. 9 Subtypes and Serotypes</td>
<td>18</td>
</tr>
<tr>
<td>1. 10 HBV Genetic Diversity</td>
<td>14 - 17</td>
</tr>
<tr>
<td>1. 11 Transmission</td>
<td>19</td>
</tr>
<tr>
<td>1. 12 Replication</td>
<td>20</td>
</tr>
<tr>
<td>1. 13 Pathogenesis and Immunity</td>
<td>21</td>
</tr>
<tr>
<td>1. 14 Clinical Findings</td>
<td>23</td>
</tr>
<tr>
<td>1. 15 Laboratory Diagnosis</td>
<td>23</td>
</tr>
<tr>
<td>1. 16 Treatment</td>
<td>25</td>
</tr>
<tr>
<td>1. 17 Rationale</td>
<td>26</td>
</tr>
<tr>
<td>1. 18 Objectives</td>
<td>27</td>
</tr>
</tbody>
</table>
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Abstract

Background: Sudan is a highly endemic country for hepatitis B virus (HBV). Pregnant women with HBV represent a major reservoir of the virus in the community. Of the epidemiological studies carried in different regions of Sudan few are available regarding pregnant women. In Darfur region such data is absent. The study aimed to evaluate prevalence of HBV, risk factors and genotypes circulating among pregnant women attending antenatal care in Al Fashir town.

Methods: This descriptive cross sectional hospital based study conducted during the period from June 2013 to July 2016. A total of 900 pregnant women were enrolled. Most of the studied women age range were 15 to 50(86%), mostly were housewives (87%) and 90% of low class income. Socio-demographic, obstetrics and medical data were collected using structured questionnaires. Blood samples were collected; plasma separated and tested for the HBV markers (HBcAb, HBsAg, HBeAg, HBeAb) using ELISA. DNA was extracted and genotypes were identified by multiplex- nested PCR.

Results: HBcAb was detected in 46% with higher exposure in those of the age range 15 – 25 years. 162 (18%) were HBsAg positive. HBeAg and HBeAb were found in 2.6% and 37.7% respectively. There was significant association between residence, occupation, income, bloodletting and ear piercing with HBV infection (P < 0.05). Of the 162 HBsAg positive pregnant women, 148(91%) samples were further studied for genotypes characterization. Genotype specific bands were detected in 97 (65.5%) samples while the remaining 51(34.5%) samples remained untypable. 31 samples (31.9%) were classified as genotype D, 30(30.9%) as genotype E and 3(3.1%) as genotype A.
Mixed genotypes were detected in 33(34.0%) samples as {A+D; 17(17.5%), D+E; 13(13.4%), A+D+E; 3(3.1%)}.

**Conclusion:** The results of the study suggest that HBsAg has higher prevalence among the pregnant women in Al Fashir town, North Darfur State. So, to minimize the burden of the disease antenatal women must be routinely screened for HBV. A high percentage of the pregnant women infected with mixed HBV genotypes, therefore, it is an important consideration for the clinician to adopt better strategies for prevention and cure of HBV infection.
الخلاصة

الخلفية: السودان كان أكثر استثماراً بفيروس التهاب الكبد الوبائي الباطني. وتعتبر النساء الحوامل الحالات للفيروس مصدر لانتشاره في المجتمع. من بين الدراسات الوبائية التي اجريت للمصابين بهذا الفيروس في مناطق مختلفة من السودان، قليل منها أجريت في الحوامل. ونسبة لعدم توفير الدراسات في منطقة دارفور أجري هذا البحث لتقييم مدى انتشار هذا الفيروس. عوامل الاختبار ذات الصلة والنوع الجنسي وسط النساء الحوامل بمرافقة الرعاية الصحية بمدينة الفاشر، ولاية شمال دارفور.

طريقة البحث: هذه الدراسة وصفية أجريت في الفترة ما بين 2013 - 2016، شملت تسعة هئة (900) امرأة حامل، حيث تراوحت أعمارهن ما بين 15 - 35(86%)، اغلبهن برات منزلي (87%) و90% منهن ذو الدخلك البسيط.

تم جمع المعلومات على الممارسة الطبية من النساء بتنظيم استبانات. بعد جمع العينات تم فصل بلازما الدم وحفظها تحت التجميد لحين إجراء الفحوصات السيرولوژية. استخدم فحص الأليزا لتحديد الإيجابية السيرولوژية لعدوى الالتهاب الكبد الوبائي الباطني (ELISA) وعيارات الإصابة بالفيروس (HBcAb, HBsAg, HBeAg, HBeAb) للتعريف على الاناعوجينية. استخدمت طريقة الـ Multiplex-Nested PCR بعد استخلاص المادة الجينية من بلازما الدم.

النتائج: أظهرت الدراسة أن 46% من النساء قد تعرضن للاصابة بالفيروس و18% منهن كن موجbies المستضدي (s)، من بينهن 2.6% من موجbies المستضدي (e) و37.7% من موجbies المستضدي (e). كما وجد أن هناك ارتباط ذو دالة إحصائية بين الإصابة بالفيروس وكل من السكن، المهنة، مستوى الدخلك، الحجاجة وفترة القرو (قيمة P<0.05).

تم إجراء التحليل الجيني على 148(91%) من العينات موجbies المستضدي (s). اظهرت 97 (65.5%) منها الأحماض الجينية بينما بقيت 34.5% (51) منها بدون تمييز. اظهرت نتيجة التحليل الجيني أن الاناعوجينية وسط مجموعة الدراسة تشمل النوع الجيني (A) (%31.9) و (D) (%30.9) ، النوع الجيني (E) (%31.9) و (D) (%30.9). خليط من الجينات الثلاثة في 33 عينة (34.0%) (A+D; 17(17.5%), D+E; 13(13.4%), A+D+E; 3(3.1%)).

الاستنتاج: الدراسة تعكس مدى اصابة النساء الحوامل بمصر المرض التهاب الكبد الوبائي الباطني مع التنوع الجيني للفيروس بمدينة الفاشر، حيث أن نسبة النساء الحوامل اللائي يحملن الفيروس كانت كبيرة. إذا يجب تفعيل الفحص الدوري لتقدير نسبة المصابات بالفيروس. كما أن النتائج تشير إلى أن الوضع يحتاج إلى المزيد من الجهود ووضع الاستراتيجيات المناسبة للمقاومة والعلاج من المرض.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HAV</td>
<td>Hepatitis A virus</td>
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<td>HCV</td>
<td>Hepatitis C virus</td>
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<td>HDV</td>
<td>Hepatitis D virus</td>
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<tr>
<td>HEV</td>
<td>Hepatitis E virus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>HBcAb</td>
<td>Hepatitis B core antibody</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
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<td>HBeAg</td>
<td>Hepatitis B e antigen</td>
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<tr>
<td>HBeAb</td>
<td>Hepatitis B e antibody</td>
</tr>
<tr>
<td>HBsAb</td>
<td>Hepatitis B surface antibody</td>
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<tr>
<td>ELISA</td>
<td>Enzyme – linked immunosorbent assay</td>
</tr>
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<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetra acetic acid</td>
</tr>
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<td>HRP</td>
<td>Horseradish Peroxidase – conjugate</td>
</tr>
<tr>
<td>ORFs</td>
<td>Open reading frames</td>
</tr>
<tr>
<td>PC</td>
<td>Precore</td>
</tr>
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<td>BCP</td>
<td>Basal core promotor</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>MTCT</td>
<td>Mother to child transmission</td>
</tr>
<tr>
<td>rc DNA</td>
<td>Relaxed circular DNA</td>
</tr>
<tr>
<td>m RNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>ccc DNA</td>
<td>Covalent closed circular DNA</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>CTL</td>
<td>Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction fragment polymorphism</td>
</tr>
<tr>
<td>Inno – LipA</td>
<td>Line probe assay</td>
</tr>
<tr>
<td>TSP – PCR</td>
<td>Type – specific primers PCR</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
<tr>
<td>ACOG</td>
<td>American College of Obstetricians and Gynecologists</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
</tbody>
</table>
List of Figures

<table>
<thead>
<tr>
<th>Figure No</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure I</td>
<td>Structure of HBV</td>
</tr>
<tr>
<td>Figure II</td>
<td>Organization of HBV genome</td>
</tr>
<tr>
<td>Figure III</td>
<td>Replication cycle of HBV</td>
</tr>
<tr>
<td>Figure IV</td>
<td>Assay Scheme for Enrolled Subjects</td>
</tr>
<tr>
<td>Figure V</td>
<td>HBcAb frequency in the study population</td>
</tr>
<tr>
<td>Figure VI</td>
<td>HBsAg frequency among the study population</td>
</tr>
<tr>
<td>Figure VII</td>
<td>Frequency of HBeAg in the tested HBsAg positive samples</td>
</tr>
<tr>
<td>Figure VIII</td>
<td>Frequency of HBeAb in the tested HBsAg positive samples</td>
</tr>
<tr>
<td>Figure IX</td>
<td>Frequency of the HBsAb in the tested samples</td>
</tr>
<tr>
<td>Figure X</td>
<td>Genotypes frequency among the HBsAg positive samples</td>
</tr>
<tr>
<td>Figure XI</td>
<td>Genotypes frequency among the HBsAg positive samples</td>
</tr>
<tr>
<td>Figure XII</td>
<td>Genotype analysis with respect to HBeAg</td>
</tr>
<tr>
<td>Figure XIII</td>
<td>Genotype analysis with respect to HBeAb</td>
</tr>
<tr>
<td>Figure IV</td>
<td>Gel electrophoresis product of PCR for amplified genotypes</td>
</tr>
</tbody>
</table>
List of Tables

<table>
<thead>
<tr>
<th>Table No</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Primer sequences used for the first PCR</td>
</tr>
<tr>
<td>Table 2</td>
<td>Primer sequences used for the nested PCR (Mix A)</td>
</tr>
<tr>
<td>Table 3</td>
<td>Primer sequences used for the nested PCR (Mix B)</td>
</tr>
<tr>
<td>Table 4</td>
<td>Sociodemographic characteristics of the study population</td>
</tr>
<tr>
<td>Table 5</td>
<td>Prevalence of the HBV markers tested among the study population</td>
</tr>
<tr>
<td>Table 6</td>
<td>Prevalence of HBsAg in the different age groups</td>
</tr>
<tr>
<td>Table 7</td>
<td>Prevalence of HBsAg in the resident areas</td>
</tr>
<tr>
<td>Table 8</td>
<td>Prevalence of HBsAg in the different educational status</td>
</tr>
<tr>
<td>Table 9</td>
<td>Prevalence of HBsAg in the different income classes</td>
</tr>
<tr>
<td>Table 10</td>
<td>Prevalence of HBsAg in the different occupational categories</td>
</tr>
<tr>
<td>Table 11</td>
<td>P – value and Odds ratio analysis of the possible risk factor for HBsAg</td>
</tr>
<tr>
<td></td>
<td>among the study population</td>
</tr>
<tr>
<td>Table 12</td>
<td>HBsAg and Caesarian Section</td>
</tr>
<tr>
<td>Table 13</td>
<td>Genotype analysis with respect to HBeAg</td>
</tr>
<tr>
<td>Table 14</td>
<td>Genotype analysis with respect to HBeAb</td>
</tr>
</tbody>
</table>
Chapter One

Introduction
1.1 Background on HBV Epidemics

Hepatitis B Virus (HBV) is a hepatotropic virus that causes acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). It is one of the most significant global health problems. It was estimated that approximately two billion people have serological evidence of past or present HBV infection and more than 350 million have chronic infections. These chronically infected persons are at high risk of death from cirrhosis of the liver and hepatic cancer, diseases that kill about one million people each year.\(^1\) The prevalence of chronic hepatitis B infection is variable throughout the world, ranging from < 1% in areas of low endemicity up to 30% in highly endemic areas.\(^2\) HBV infection is estimated to be the cause of 30% of cirrhosis and 53% of liver cancer in the world.\(^3\) The age at which HBV infection occurs is one of the main factors that predispose to the acquisition and frequency of the chronic carrier status. Almost 90% of infants born to HBV surface antigen (HBsAg) and hepatitis B e antigen (HBeAg)-positive mothers and approximately 30% of children infected before 6 years of age become chronic carriers, compared to less than 10% of older children or adults.\(^4; 5\) Even if they are not infected during pregnancy, children of HBV-infected mothers have a high risk of acquiring HBV infection by horizontal transmission during the first years of life.\(^6\) In addition, maternal transmission of HBV predisposes infected newborns to liver cirrhosis and hepatocellular carcinoma in young adulthood.\(^7\) Sudan is classified among the countries with a high HBsAg endmicity of more than 8%.\(^8\) The reported prevalence of HBV infection, characterized by varied detectable levels of HBsAg as 6.8% up to 18.7% in central Sudan.\(^9; 10\) Exposure to HBV infection ranges from 47% to 57% \(^9\) it is also found that hepatitis infections seem to be important risk factors for HCC in Sudan and about 57% of all HCC cases can be attributed to this viral infection.\(^12\)
The prevalence of HBV among pregnant women worldwide is approximately 5%, ranging from 0.6% in low-endemic regions to > 20% in high-endemic areas in the Far East and Africa.\cite{13} Although much recent data are available on the epidemiology of HBV among pregnant women in different countries,\cite{14,15} few published data are available from Sudan. Such data are fundamental for health planners and care givers for evidence-based intervention. I select pregnant women to draw attention to this sector of the community to strengthen the existing health program specially health education program in this part of the world. Eight genotypes of HBV have been identified (A through H) as well as several subgenotypes.\cite{16} The global distribution of genotypes varies by geography, such that A, F, G, and H occur most frequently in western countries while genotypes B, C, and E are most commonly encountered in Asia and Africa. Genotype D is most prevalent in the Mediterranean region.\cite{16,17}

Africa is one of the highly endemic regions for HBV, with five HBV genotypes (A–E) predominating.\cite{18} Previous studies conducted in several African countries showed that HBV genotype E is the most prevalent genotype, spreading in western Africa from Senegal to Namibia, subgenotype A1 is dominant in southern and eastern Africa, subgenotype A3 is present in central and West Africa, and genotype D is dominant in northern Africa.\cite{19} However, to date data about molecular epidemiology of HBV infection in Sudan is scarce. The current study was conducted at Al Fashir new hospital (a reference hospital for obstetrics and gynecology) in addition to peripheral health care centers in Al Fashir city, to investigate the seroprevalence of HBV, associated risk factors, and genotypes circulating. A total of 900 pregnant women were enrolled for screening with ELISA and positive samples
were further analyzed to determine HBV (A-F) by multiplex - nested PCR using type specific primers.

1.2 Viral Hepatitis

Viral hepatitis is a term commonly used for several clinically similar yet etiologically and epidemiologically distinct diseases. Hepatitis A (formerly called infectious hepatitis) and hepatitis B (formerly called serum hepatitis) have been recognized as separate entities since the early 1940s and can be diagnosed with specific serologic tests. Delta hepatitis is an infection dependent on the HBV. It may occur as a coinfection with acute HBV infection or as superinfection of HBV carriers.[20]

Several diseases of the liver, collectively known as hepatitis, are caused by a group of viruses known as the hepatitis viruses cause most cases of hepatitis worldwide, but hepatitis can also be due to toxins (notably alcohol, certain medications and plants), other infections and autoimmune diseases.[21] Viruses that cause hepatitis include, hepatitis A, B, C, D and E viruses. The hepatitis viruses comprise a range of unrelated and often highly unusual human pathogens.[22]

All of these viruses can cause an acute disease that may last for several weeks including yellowing of the skin and eyes (jaundice), dark urine, nausea, vomiting and abdominal pain. It can take several months to a year to feel fit again. Virus can cause chronic infection in which the patient never gets rid of the virus and may develop cirrhosis of the liver or liver cancer.[1]

Viral hepatitis has emerged as a major public health problem throughout the world affecting several hundreds of millions of people. It is a cause of considerable
morbidity and mortality in the human population, both from acute infection and chronic sequelae which include, in the case of hepatitis B, C and D, chronic active hepatitis and cirrhosis. HCC which is one of the ten most common cancers worldwide is closely associated with hepatitis B, and at least in some regions of the world with hepatitis C virus.\textsuperscript{[22]}

Infection by enterically-transmitted viruses (HAV and HEV) causes acute hepatitis and is generally benign compared to the disease caused by chronic infections which may lead to HCC. Some types of HDV have also been associated with a high frequency of fulminant hepatitis. In addition to these viruses, other viruses have been discovered and initially proposed as causative agents of hepatitis, like GB virus type C (GBV-C), TT virus (TTV) and Sev virus (SenV). However, the association with hepatitis was later discarded.\textsuperscript{[23]}

HBV is the most serious type of viral hepatitis causing chronic hepatitis for which a vaccine is available and according to WHO reports (2000) in much of the developing world (sub-Saharan Africa, most of Asia and the pacific) most people become infected with HBV during childhood, and 8% to 10% of people in the general population become chronically infected. In these regions liver cancer caused by HBV figures among the prime cause of death by cancer in men.\textsuperscript{[24]}

1.3 HBV Infection during Pregnancy

Pregnant women with HBV represent a major reservoir of the virus in the community. Mother to child transmission of HBV is the main transmission route and contributes significantly to chronic HBV infection.\textsuperscript{[25]} Of the estimated 350 million individuals chronically infected with HBV worldwide, it is generally accepted that at least 50% acquired their infections either perinatally or in early childhood, especially
in countries where HBV is endemic.\textsuperscript{[26]} Therefore, investigating seroprevalence of HBsAg in pregnancy in different settings is needed to prevent mother to child transmission.

Early studies pointed that an increase in fulminance and mortality rates with acute HBV infection during pregnancy has been demonstrated in some HBV-endemic areas \textsuperscript{[27; 28]}, although other investigators in western countries have suggested that these adverse outcome were related more to health care conditions and maternal malnutrition.\textsuperscript{[29; 30]}

Women with hepatitis have an increased risk for complications during pregnancy.\textsuperscript{[31]} As confirmed by Ka Yu et al (2005) there is association between HBsAg carrier state and gestational diabetes mellitus, antepartum haemorrhage, and threatened preterm labour which may be related to the chronic inflammatory state in these subjects.\textsuperscript{[32]} Whereas Oguntola (2008) observed that hepatitis is one of the diseases in pregnancy that causes jaundice in women and if left untreated may lead to babies with low intelligence quotient.\textsuperscript{[33]}

Children may be infected horizontally in early childhood or perinatally from carrier mothers. Three mechanisms of HBV transmission from HBsAg-positive mothers to infants were suggested: (i) vertical transmission (ii) transmission during delivery by contact with maternal infected fluids in the birth canal and (iii) postnatal transmission from mothers to infants during childcare or through breast feeding.\textsuperscript{[34; 35]} Perinatal vertical transmission is the most common mode of transmission worldwide.\textsuperscript{[36]} High maternal viral load and maternal serum HBV envelope antigen (HBeAg) positivity increase the risk of perinatal transmission.\textsuperscript{[37]}

The studies on transplacental transmission of HBV suggested two possible mechanisms (1) hematogenous route: where certain factors can make the placental
microvascular broken, thus the high titer HBV maternal blood leak into fetus circulation.\textsuperscript{[38]} (2) cellular transfer: where the placental tissue is infected by the high-titer of HBV in maternal blood and then step by step, HBV reaches the fetus circulation through the villous capillary endothelial cells.\textsuperscript{[39; 40]}

1. 4 History of HBV

HBV was originally recognized as the agent responsible for “serum hepatitis”, the most common form of parenterally transmitted viral hepatitis, and an important cause of acute and chronic infection of the liver. Hepatitis B has also been called type B hepatitis, serum hepatitis, homologus serum jaundice.\textsuperscript{[41; 42]}

Epidemic jaundice was described by Hippocrates in the 5th century BCE. The first recorded cases of “serum hepatitis” or hepatitis B are thought to be those that followed the administration of smallpox vaccine containing human lymph to shipyard workers in Germany in 1883. In the early and middle parts of the 20th century, serum hepatitis was repeatedly observed following the use of contaminated needles and syringes. The role of blood as a vehicle for virus transmission was further emphasized when Beeson (1943) described jaundice that had occurred in seven recipients of blood transfusions.\textsuperscript{[23]}

Australia antigen, later called hepatitis B surface antigen (HBsAg), was first discovered by Blumberg et al (1965) in the blood of Australian aboriginal people.

D.S. Dane (1970) and others discovered the virus particle when serum from Australia antigen-positive patients was studied by electron microscopy, vast numbers of spheres and filaments of 22 nm in diameter were seen, but also larger particles of 42 nm with a central nucleocapsid and an outer coat,\textsuperscript{[43]} hence named Dane particle. These Dane particles were subsequently shown to constitute the complete virion and the smaller filaments and spheres were found to be excess Australia antigen or
HBsAg. HBV has since been characterized into different antigenic subtypes and later into nucleotide divergence-based genotypes. Identification of serologic markers for HBV infection followed, which helped clarify the natural history of the disease.\textsuperscript{[43]}

Between 1981 and 1982, the first vaccines were produced by harvesting the HBsAg from plasma of chronic HBsAg carriers and contained highly purified 22 nm HBsAg particles inactivated through a combination of urea, pepsin, formaldehyde and heat.\textsuperscript{[44]}

In the mid 1980s, recombinant DNA hepatitis B vaccines containing HBsAg expressed in HBV transfected yeasts (i.e. \textit{Saccharomyces cerevisiae}), the so-called “second” generation hepatitis B vaccine, were commercialized. This new technology offered the potential of unlimited production, which allowed the hepatitis B vaccine to become one of the most widely used in the world.\textsuperscript{[45]}

Before the advent of PCR, the molecular characterization of HBV was a cumbersome process, as it was shown very early that HBV would not grow in cell culture and was highly species specific, only infecting humans and some other primates. HBV was the first member to be discovered of a family of viruses, later designated Hepadnaviridae. These are hepatotropic, partially double-stranded DNA viruses. Their replication strategy is unique for animal DNA viruses, in that they use RNA intermediate and a reverse transcription step.\textsuperscript{[46]}

Since the introduction of the PCR (1986) for amplification of viral DNA, investigation of the sequence heterogeneity of the HBV genome and its role in pathogenesis and viral protein expression has become a focus in hepadnavirus research. Later on mutant variants were extensively studied.
1.5 HBV Evolution

Viruses just do not evolve in a manner comparable to that of animals and plants. Constraints on the evolution of certain viruses, imposed perhaps by possessing single-stranded genomes, extensively overlapping reading frames or regulatory elements embedded in coding sequences, may lead to constraints on sequence change considerably different from those operating on animal and plant genomes. As a result, many of the models and assumptions that underlie phylogenetic reconstruction of animal and plant evolution may arguably not apply to at least some viruses.\[^{[47]}\]

The origin of HBV is still an unsolved question.\[^{[48]}\] The reduced size of HBV genome, together with the high degree of overlapping of its open reading frames, has impeded the drawing of an evolutionary picture of this virus. With the advent of sequences from several HBV strains circulating in non human primates, many hypothesis have been proposed, of which Devesa and Pujol (2007) suggested that human HBV genotypes might have emerged through several zoonotic introductions from simian strains, both at the Old and New World.\[^{[49]}\]

Since HBV replication involves an error prone reverse transcription step, the HBV genome evolves quickly over time, with an estimated nucleotide substitution rate between 1.5 and $7.9 \times 10^{-5}$ substitution per site per year. This unique replication strategy accounts for the majority of the point mutations, deletions and insertions observed in the HBV genome. The lengthy evolution of HBV has led to the current existence of various genotypes, subgenotypes, mutants, recombinants, and even quasispecies of HBV.\[^{[50, 51]}\]

The absence of proof reading capacity of the HBV reverse transcriptase leads to a high mutation rate. On the other hand, the extreme overlapping of the open
reading frames of this small viral genome reduces the viability of many of these mutations. For these opposite characteristics, the substitution rate of HBV is intermediate between RNA and DNA viruses.

1.6 Classification of HBV

HBV is the main representative of the family of hepadnaviruses, Hepadnaviridae. The name of the family is an acronym of the disease caused by the virus and its genomic type. It causes a sometimes chronic form of liver inflammation (hepatitis) and its genome consists of partially double-stranded DNA (hepadnavirus = hepatitis DNA virus). The hepadnaviridae are subdivided into mammalian and avian hepadnaviruses. The mammalian hepadnaviruses include human hepatitis B virus (HBV) woodchuck hepatitis virus (WHV) and the ground squirrel HBV (GSHV). The duck hepatitis B virus (DHBV) and the heron hepatitis B virus (HHBV) belong to the avian hepadnaviruses.

The hepadnaviridae share the following features:

- A partially double-stranded genomic DNA comprising a complete coding strand (negative strand) and an incomplete non-coding strand (positive strand).
- A RNA- dependent DNA polymerase.
- A high degree of species and tissue specificity.

1.7 Morphology and Structure of HBV

Human HBV is the prototype; enveloped virion with icosahedra nucleocapsid core containing a partial double stranded circular DNA and forms a circle of around 3,200 bases. 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.[56] Although surrounded by a host cell-derived envelope, HBV is remarkably stable to organic solvents. It is also heat and pH resistant. The genome is associated with the P (polymerase) protein and this complex is, in turn, surrounded by the core antigens (HBcAg and HBeAg). These two proteins have most of their sequence in common and most of the HBeAg is secreted since it is processed differently from the HBcAg and thus not assembled into progeny virus. Embedded in the surface lipid bilayer is the surface antigen (HBsAg). The HBsAg (Australia antigen) is made up of three glycoproteins that are encoded by the same gene. (Figure I)

![Figure I: Structure of HBV](http://www.google.com/images/Figure/Structure of Hepatitis-B-virus.)
1.7.1 Organization of the HBV Genome

Analysis of the coding potential of the genome reveals four overlapping open reading frames (ORFs) which are conserved between all of virus isolates.

- S, coding for the viral surface envelope proteins,

- C, coding for the capsid and e antigen proteins,

- P, coding for the polymerase, functionally divided into the terminal protein domain, which is involved in encapsidation and initiation of minus-strand synthesis; the reverse transcriptase (RT) domain, which catalyzes genome synthesis; and the ribonuclease H domain, which degrades pregenomic RNA.

- X, coding for a protein with multiple functions, including signal transduction, transcriptional activation, DNA repair, and inhibition of protein degradation.\textsuperscript{[57]} (Figure II).
**Figure II: Organization of the HBV Genome**

The outer lines represent the different classes of transcripts; the bold inner circles the DNA genome as present in the virion. The four major ORFs (preC/C, preS1/preS2/S, P and X) are indicated in the centre.

http://www.Klinikum.uni-heidelberg.de/zentrum-fuer-infektiologie/molecular-virology/research-area/hbv/1-morpholgy-genome-organization#

1. **Distinctive properties**

Hepatitis B is distinguished from the other viral hepatitides by its long incubation period (1-6 months), by the presence of extrahepatic symptoms in up to 20% of patients (arthralgia, rash, and myalgia thought to be a result of antigen – antibody complex deposition)\(^{58, 59}\) and eventually, by the detection of HBV-specific
serum markers. The virus persists in 5 to 10% of immunocompetent adults, and in as many as 90% of infants infected perinatally. Persistent carriage of hepatitis B is defined by the presence of HBsAg in the serum for more than six months. The pathology is mediated by the responses of the cellular immune response of the host to the infected hepatocytes. Long term continuing virus replication may lead to progression to cirrhosis and HCC.

1.9 Serotypes and Subtypes

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins. Classically, HBV strains were distinguished by the presence of two pairs of mutually exclusive serotype determinants ‘d’y’ and ‘w’r’, in the HBsAg along with the main antigenic determinant ‘a’, which led to the description of the 4 serotypes. Additional serotypes were subsequently characterized leading to the description of nine serotypes namely ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+ and adrq- and a distinct geographical pattern for the distribution of serotypes was also documented.\[53\]

Subtyping of HBV strains was used for epidemiological purposes and, in some cases, to trace nosocomial chains of infection or to find correlations between disease and a particular subtype. Over the last decade, however, with the advent of molecular biological techniques and advanced computational methods for the phylogenetic analysis of complete viral genome sequences, HBV genotypes and subgenotypes have been described, that have largely replaced the classical serotype based classification of HBV strains.

1.10 HBV Genetic Diversity

High genetic variability is a characteristic feature of the HBV. A number of variants of this virus have been described.\[22\] Random errors/variations in the HBV
genome, occurring due to long periods of persistence and immune selection pressures operating at the population level have led to the emergence of distinct genotypes and their subgenotypes in specific geo-ethnic populations, and being transmission competent these variants stably circulate within the given geo-ethnic population. In addition, certain mutations may also emerge under medical pressures (vaccine, or antiviral therapy), which are selected at the individual level.

1. 10.1 HBV Genotypes

The definition of HBV genotypes occurred when Okamoto et al (1988) suggested that the traditional subtypes could be complemented or replaced by a classification of different HBV strains into genetic subgroups. Comparing the full nucleotide sequences of 18 HBV strains, they found that these clustered into four groups, A to D, with more than 8% divergence between the groups. This degree of divergence has since become the definition for HBV genotype. Eight human HBV genotypes (A–H) have been described, based on a minimum divergence of 8% of the complete genome sequences. Some of the HBV genotypes are divided into subgenotypes, based on a divergence of more than 4%. Seven subgenotypes are described at the moment for genotype A, 9 for genotype B, 12 for genotype C, 7 for genotype D and 4 for genotype F. No subgenotypes have been found at present inside genotypes E, G and H. This might be due to the fact that these genotypes might be more recent than the other ones. In addition, a new genotype I has been proposed for a recombinant of genotypes A, C, and G mainly found in Laos and Vietnam, genotype J for a recombinant strain between human and ape viruses. Indeed, several studies have pointed that recombination seems to play an important role in shaping the evolution of HBV. The exact mechanism of recombination of HBV genomes is not clear, but it seems more likely to occur in the
nucleus, by illegitimate replication, or by recombination with integrated HBV DNA. Thus ten HBV genotypes (A - J) and 34 HBV subgenotypes have been identified.

Sequence differences between HBV genotypes can lead to structural differences at the level of the pregenome and can also lead to dramatic differences at the translational level when specific and commonly occurring mutations occur. There is increasing evidence that the clinical picture, the response to treatment and the long-term prognosis may differ depending on which genotype has infected the patient. The consideration of traditional serological patterns in a patient must therefore take the genotype of the infecting strain into account. Nucleotide variability between HBV strains has been used in several studies to trace routes of transmission and, since it is becoming increasingly clear that the differences between HBV genotypes are important, the need for reliable and easy methods of differentiating HBV genotypes has arisen.

The genotypes show a distinct geographical distribution between and even within regions. HBV genotype A is mainly found in Northwestern areas of Europe and North America. Some genotype A strains have also been found in the Philippines, possibly reflecting the close contact with North America, especially over the last century. A similar epidemiological link would explain the genotype A strains isolated from patients in Hong Kong and South and Eastern Africa. Genotype B and C strains belong to the indigenous population of Southeast Asia. Their distribution is fairly intermixed, with a tendency towards more genotype C strains being found in the Northern mainland regions and in mainland Japan. However, genotype C especially is also found in the populations of the South Pacific islands, where the prevalence of HBV carriers is sometimes very high.
Genotype D is the most widely distributed genotype and has been found universally, with its highest prevalence in a belt stretching from Southern Europe and North Africa to India, in West and South Africa and among intravenous drug users on all continents.

Genotype E is the most similar to genotype D genetically and has been interpreted as a subset of genotype D when using the X gene for phylogenetic analysis. It is found in West and South Africa. The most divergent genotype, F is found in South and Central America. It shares some structural features with genotype A strains. Genotype G has been found in France and the USA. The distribution of genotype G is not fully known. This genotype exhibit several interesting characteristics. A low intragenotypic variability has been found among different isolates from different countries. Genotype H is found in Central America and is phylogenetically very closely related to genotype F.

In many countries where well-known waves of migration have occurred over time, the prevalence of different HBV genotypes reflects the origin of the immigrants and other patterns of migration. This is exemplified by South Africa, where the most prevalent genotypes, A and D, correlate with migration from Northwestern Europe (UK and the Netherlands), Southern Europe and India. Not only migration but also behavioral patterns may change the prevailing genotype in a given region. As found by Koibuchi et al. that Japanese homosexual men coinfected with HIV were unexpectedly found to harbor HBV genotype A instead of C or B, which are the prevailing genotypes in Japan.

1.10.2 Genotype and Clinical Outcome

Structural and functional differences between genotypes can influence the severity, course and likelihood of complications, HBeAg seroconversion and response
to treatment of HBV infection and possibly vaccination against the virus.\cite{94} There have been substantial efforts to link genotypes to different clinical outcomes. HBV variability seems to play a role in HCC development. Pathogenic differences in causing HCC have been reported not only among HBV variants, but also among genotypes. HBV genotype C is associated with a more severe disease,\cite{95, 96} and genotype D seems to evolve worse than genotype A.\cite{98} HBV genotype F was associated to a higher frequency of HCC development at younger age in Alaskan individuals.\cite{98} However, the risk of HCC may differ among subgenotypes.\cite{99} Genotype B has been associated with an earlier and a higher rate of HBeAg seroconversion than genotype C.\cite{94, 100, 101} On the other hand, genotype B has also been associated with a higher rate of severe icteric flares as compared with patients in Hong Kong carrying genotype C.\cite{102} In comparison with C2, subgenotype C1 has been associated with a higher frequency of developing basal core promoter mutations.\cite{103} Genotype A has been associated with a higher tendency to cause chronic infection and for the better, transition into the inactive carrier state after HBeAg seroconversion, in comparison with genotype D.\cite{104, 105} However, in an European study comparing genotype A and D, no difference was found in the degree of liver damage.\cite{104} Subgenotype D1 has been associated with higher frequency of chronic liver disease compared to other D subgenotypes.\cite{106} Clinical impact of genotypes on treatment efficiency has also been studied, Genotype A and B has been associated with a better response to interferon treatment compared to genotype C and D.\cite{107, 108, 109} Although the number of studies on these genotypes has increased dramatically during recent years, much remains to be learnt about their full implications.
1. 11 Transmission

Humans are the sole reservoir of HBV. Transmission is parenteral, either with blood or body fluids containing HBV that come into contact with mucosa, lesions, or microlesions in the skin. The three main modes of transmission are via blood, during sexual intercourse and perinatally from mother to newborn. Routes of transmission include vertical (mother to child), early life horizontal transmission (through bites, lesions, and sanitary habits), and adult horizontal transmission (through sexual contact, intravenous drug use, and medical procedure exposure) and are evident to varying degrees in every country.

In transmission by blood, the tiniest amounts contaminating syringe needles, ear-piercing needles, tattooing instruments…, are suffice to produce an infection. All blood samples must be considered potentially infectious and handled only with disposable gloves. High-risk group includes all healthcare workers with regular blood contact, patients receiving multiple transfusions or dialysis and addicts who inject drugs with needles.

Sexual transmission of HBV is a major source of infection in all areas of the world, especially in the low endemic areas, such as North America. Hepatitis B is considered to be a sexually transmitted disease (STD). For a long time, homosexual men have been considered to be at the highest risk of infection due to sexual contact (70% of homosexual men were infected after 5 years of sexual activity). However, heterosexual transmission accounts for an increasing proportion of HBV infections. In heterosexuals, factors associated with increased risk of HBV infection include duration of sexual activity, number of sexual partners, history of sexually transmitted disease, and positive serology for syphilis. Sexual partners of injection drug users,
prostitutes, and clients of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk for infection.\textsuperscript{[110]}

1. 12 HBV Replication

The life cycle of HBV is complex. Hepatitis B is one of a few known non-retroviral viruses which use reverse transcription as a part of its replication process. The life cycle of hepadnaviruses is characterized by the synthesis of a $\sim 3$-kb partially double-stranded, relaxed-circular DNA (rcDNA) genome by reverse transcription of an RNA intermediate, the pregenome.\textsuperscript{[111; 112]} In contrast, early events of the viral life cycle, including entry, uncoating, and delivery of the viral genome into the cell nucleus, is not well understood. This is, in part, due to the absence of cell lines that are susceptible to hepadnavirus infection.\textsuperscript{[113]}

During initiation of infection the viral rcDNA (or linear DNA) genome, with a protein (the viral reverse transcriptase) attached to the 5′ end of the minus strand and a short RNA attached to the 5′ end of the plus strand, is converted into covalently closed circular DNA (cccDNA). During this process, both the protein and the RNA are removed. The cccDNA serves as the template for transcription of viral mRNAs. One of these, the pregenome, serves as the mRNA for the synthesis of core protein (nucleocapsid subunit) and the viral reverse transcriptase. The reverse transcriptase binds to the 5′ end of its own mRNA template, and the complex is then packaged into nucleocapsids, where viral DNA synthesis occurs. Once partially double-stranded DNA has been produced, nucleocapsids can undergo a maturation event that facilitates their acquisition of an outer envelope via budding into the endoplasmic reticulum (ER). These nucleocapsids can also migrate to the nucleus to increase the copy number of cccDNA. Since cccDNA does not undergo semiconservative
replication, all cccDNA is derived from viral DNA made in the cytoplasm via the reverse transcription pathway.\textsuperscript{[114]} Accumulation of viral envelope proteins prevents excessive cccDNA formation, which can be toxic to hepatocytes.\textsuperscript{[114; 115]} (Figure III).

\textbf{Figure III: Replication cycle of HBV}


\textbf{1.13 Pathogenesis and Immunity}

HBV primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. HBV virions (DANE particle) bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. PreS and IgA receptors are accused of this interaction. HBV-preS specific receptors are primarily expressed on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells.\textsuperscript{[116; 117]} During HBV infection, the host immune response causes both hepatocellular damage and viral clearance.
Although the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes.\textsuperscript{118} Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver. Antigen- Antibody complexes cause some of the early symptoms e.g. arthralgias, arthritis, cryoglobulinemia, and vasculitis.\textsuperscript{24}

Long term continuing virus replication may lead to progression to cirrhosis and HCC. In the first phase of chronicity, virus replication continues in the liver, and replicative intermediates of the viral genome may be detected in DNA extracted from liver biopsies.\textsuperscript{22} Markers of virus replication in serum include HBV DNA, the S1 proteins (HBsAg) and a soluble antigen, HBeAg which is secreted by infected hepatocytes. In those infected at a very young age, this phase may persist for life but, more usually, virus levels decline over time.\textsuperscript{22} Eventually, in most individuals, there is immune clearance of infected hepatocytes associated with seroconversion from HBeAg to anti-HBe. During the period of replication, the viral genome may integrate into the chromosomal DNA of some hepatocytes and these cells may persist and expand clonally.\textsuperscript{22} Rarely does seroconversion to anti-HBs follow clearance of virus replication but, more frequently, HBsAg persists during a second phase of chronicity as a result of the expression of integrated viral DNA.\textsuperscript{22}
1.14 Clinical Findings

Many HBV infections are asymptomatic and are detected only by the presence of antibody to HBsAg. The mean incubation period for hepatitis B is 10 to 12 weeks. Symptoms common to all forms of acute hepatitis (jaundice, nausea, weight loss, flu-like illness etc), and patients with acute HBV infection typically also suffer from fever, urticaria and arthralgia. These symptoms generally subside within a few weeks along with disappearance of HBV DNA and seroconversion from HBeAg to anti-HBe. Most patients with acute hepatitis B are HBsAg positive at presentation, but the critical test is IgM anti-HBc, which confirms acute HBV infection. If HBsAg is not lost within 6 months, the patient is considered to be a chronic carrier. Acute hepatitis may in some cases progress to fulminant hepatitis leading to liver failure.

1.15 Laboratory Diagnosis

The tests, called assays, for detection of HBV infection involve serum or blood tests that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is complex. The HBsAg is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. The appearance of HBsAg usually predates any clinical symptoms by 4 weeks, on average, and remains detectable for 1 to 6 weeks in most patients. In 90% to 95% of patients in whom chronic infection does not develop, HBsAg titers decrease as symptoms diminish. The appearance of HBsAb defines the absence of the carrier state; titers increase slowly during the clinical recovery period and may continue to increase up to 10 to 12 months after HBsAg is no longer detectable. In most patients with self-limited, acute hepatitis B, HBsAb is detectable only after HBsAg titers in
serum disappear. A "window " of time has been described in which a patient still with clinical hepatitis is negative for both HBsAg and HBsAb. During this time, HBV infection still can be diagnosed by the detection of HBcAb, which begins to appear 3 to 5 weeks after HBsAg does. HBcAb titers may decrease in the first 1 to 2 years after infection, although the antibody is still detectable years after acute disease in most patients. A person negative for HBsAg but positive for anti-HBs have either cleared an infection or have been vaccinated previously.

The appearance of HBeAg parallels that of HBsAg; in self limited infections, HBeAb is detectable soon after the time that HBeAg disappears. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, some variants of the HBV do not produce the 'e' antigen, so this rule does not always hold true. During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (anti-HBe) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication.

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers. Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver, as revealed by biopsy. Carriers who have seroconverted to HBeAg negative status, particularly those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others. PCR tests have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment. Individuals with high viral loads, characteristically have
ground glass hepatocytes on biopsy. Dane particles also can be identified in the serum from these patients through electron microscopy.\textsuperscript{[125]}

1.16 Treatment

Acute HBV infection during pregnancy is treated mainly by supportive measures, as in the nonpregnant state. In early symptomatic phase, encouragement is necessary to maintain adequate nutrition balance, and liver metabolized drugs, if not avoidable, need to be monitored carefully through blood levels. Phenothiazine may be used, if needed, to control nausea and vomiting.\textsuperscript{[126; 127]} Early antiviral treatment may only be required in less than 1% of patients, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy.\textsuperscript{[128]}

Common drugs used include:

1 - Alpha interferon is clinically useful for treatment of chronic hepatitis B infections. Response to treatment differs between the genotypes. Although no specific mutations have been associated to interferon resistance, some genotypes are more susceptible to this immunomodulator, like genotypes A and B, compared to D and C. \textsuperscript{[128]}

2 - Some nucleoside analogues such as Lamivudine (thiacytidine), that inhibit the reverse transcriptase of HIV, also are effective against HBV, others include Adefovir, Entevavir, Telbivudine and Tenefovir. Drug resistance mutations emerge during treatment with these drugs, consisting of point mutation in one of the five domains of the HBV polymerase.\textsuperscript{[129]} These drugs reduce hepatic inflammation and lower the levels of HBV in chronic carriers.\textsuperscript{[24]}
1. Rationale

Surveillance of carriers of viral hepatitis is essential to assess the burden of the disease in population and it is of great importance to the program managers and health planners.

In Darfur State seroprevalence studies of HBV in the general population is unknown. Also nothing is known about the prevalence and genetic diversity of HBV in pregnant women in Sudan as general and in this region as specific.

Eight genotypes of HBV designated A-H, have been known. In Sudan little is known about the molecular diversity of HBV, but in Darfur, no such data is available on the prevalent HBV genotypes. Therefore, this study was also conducted to determine HBV genotypes in Darfur population.

Most of the available published data of the epidemiology of viral hepatitis in Sudan are just screening. So the study was conducted to find that if HBV infection is a health problem among the pregnant women in Al Fashir town?
1. 18 Objectives of the Study: -

General Objectives:

The study was conducted to assess the prevalence of HBV among the pregnant women in Al Fashir town, genotypes circulated and to raise awareness about HBV infection in the study area, by screening blood samples to investigate seroprevalence of HBV.

Specific Objectives:

1. To determine the prevalence of HBV exposure and markers.
2. To determine the pattern of distribution of the socioeconomic and cultural factors regarding the traditional practices.
3. To determine the genotypes circulating among the pregnant women.
4. To make recommendations to all parties concerned about changes that should be made and how.
Chapter Two
Literature Review
2. Epidemiology

Epidemiology of HBV in a population is of great importance to identify estimates. Seroprevalence of HBV among pregnant women may be a good indicator of general population prevalence and a determinant of vaccination policy.\[130;131\]

Screening for Hepatitis B during pregnancy may help to decide on appropriate antiviral therapy and the institution of steps to minimize vertical transmission to the newborn infants. Although, screening for HBsAg in pregnancy is recommended by the Royal College of Obstetricians and Gynecologists (United Kingdom), American Congress of Obstetricians and Gynecologists, as well as many colleges of obstetrics and gynecology worldwide,\[132;133\] it does not represent in most of the countries where the HBV infection is endemic.

2.1 Global Epidemiology of HBV

HBV infection remains a major health problem causing considerable morbidity and mortality despite the availability of vaccine and antiviral treatment. The prevalence of HBV infection varies markedly throughout regions of the world.\[134\] HBV infection is globally ubiquitous, but its distribution is very heterogeneous, with prevalence of serological markers in various nations ranging from less than 1% to more than 90% and it is found that the prevalence of infection is largely determined by a feedback mechanism that relates the rate of transmission, average age at infection and age-related probability of developing carriage following infection.\[135\] The prevalence of HBV infection occur with significant burdens in Asia, the Pacific Islands, sub-Saharan Africa, the Amazon Basin, and Eastern Europe.\[136\] Up to 45% of the world’s population resides in these endemic areas where the rate of HBsAg seropositivity is over 8%. The HBV disease burden is generally classified as
percentage of HBsAg carriers in the population and categorized as low (<2%), intermediate (2-7%) or high (>8%) endemicity regions. The major concern is about high endemicity countries, especially in Asia and Africa, where the most common routes of infection remain vertical transmission from mother to child and horizontal transmission between children. \[^1\]

High endemicity areas include South East Asia, China, sub-Saharan Africa and the Amazon Basin. In these areas, 70–95% of the population shows past or present serological evidence of HBV infection. 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 adults are high. \[^26\]

Intermediate endemicity includes part of Eastern and Southern Europe, the Middle East, Japan, and part of South America. Between 10–60% of the population have evidence of infection, and 2-7% is chronic carriers. Acute disease related to HBV is common in these areas because many infections occur in adolescents and adults; however, the high rates of chronic infection are maintained mostly by infections occurring in infants and children. \[^137\] In these areas, mixed patterns of transmission exist, including infant, early childhood and adult transmission.

The endemicity of HBV is low in most developed areas, such as North America, Northern and Western Europe and Australia. In these regions, HBV infects 5–7% of the population, and only 0.5–2% of the population is chronic carriers. \[^138\] In these areas, most HBV infections occur in adolescents and young adults in relatively well-defined high-risk groups, including injection drug user, homosexual males, and health care workers, patients who require regular blood transfusion or hemodialysis.
Worldwide different estimates of HBV seroprevalence among pregnant women were reported. In IRAN a cross-sectional survey conducted in a period of one month in four areas of Zahedan (a city in Southeast of Iran), to detect the prevalence of HBsAg among pregnant women. Out of the 200 who selected randomly, (6.5%) were HBsAg positive and all were housewives. [139]

In another cross-sectional study carried between 2007 and 2008, all pregnant women who attended the reproduction section of rural and urban health care centers in Lorestan province, west of Iran, were recruited to determine the prevalence of HBV and HCV infection among them. Anti-HBc was found in 28 of 827 pregnant women (overall prevalence, 3.4 %; 14 of 523 in urban areas, 2.7%; 14 of 304 in rural areas, 4.6%). Of the 28 positive samples, 6 (0.7%) were positive for HBs-Ag. [140]

In Turkey, a cross sectional study was carried during the period between June 2003 and February 2005 to determine the frequency of HBV carriers in pregnant women registered at the Gaziantep Maternity Hospital. The records of a total of 11,840 pregnant women were investigated retrospectively. HBsAg was detected in 252 (2.1%) none of whom were aware of their condition. The prevalence of HBV infection in pregnant women in the southern region of Turkey was considered as an intermediate level. [141]

In India a study was carried out during 2006 and 2007, at M LN Medical College, Allahabad, to investigate the seroprevalence of HBsAg in pregnant women and possible risk factors for perinatal HBV transmission. Of 4,000 women screened, 1,800 (45%) were from urban areas and 2,200 were from rural areas (55%). Seroprevalence of HBsAg was found to be 0.9% (37/4,000). The highest prevalence rate was observed in the age group of 21–25 years (21/1,824; 1.15%) followed by the
26–30 year age group (15/1,753; 0.86%). The difference in HBsAg prevalence rates in different age groups was not significant. Assessment of risk factors revealed history of tattooing in 29/37 (78.4%) women. HBeAg in this study was positive in 21 of 37 (56.8%) women.\textsuperscript{[142]}

A survey chronic HBV infection among pregnant women and their infants was conducted in Shenyang, China to analyze the reason for immunoprophylaxis failure. A total of 4,536 pregnant women aged 16 – 45 years were screened for HBsAg. The prevalence of HBsAg among pregnant women was 5.49% (249/4536). Genotypes B and C were present among 165 pregnant women and genotype C was present in 85 pregnant women.\textsuperscript{[143]}

Africa is globally classified as a high HBV prevalence area, although hepatitis B endemicity may vary greatly from a region to another. Based on HBsAg positivity, HBV is hyperendemic (>8%) in some sub-Saharan countries such as Nigeria, Namibia, Gabon and Cameroon. Other countries (Kenya, Zambia, Côte d'Ivoire, Liberia, Sierra Leone and Senegal) are considered areas of intermediate endemicity (2–8%), while Egypt, Tunisia, Algeria and Morocco, located at the North of the continent, are low endemicity (<2%) regions. Prevalence of (anti-HBc), a serological marker for previous HBV exposure, is extremely high (>80%) in various African populations.\textsuperscript{[144]}

Data on viral hepatitis during pregnancy are not readily available in many African and Arab countries. In their investigation of the prevalence and possible risk factors for HBV and HCV infection among the pregnant women in African and Arab countries, Gasim and co-workers (2013) pointed to the weaknesses of regional studies in these countries, where most of the epidemiological studies are performed in
specific groups such as blood donors, health-care employees, or patients undergoing hemodialysis. Seroprevalence studies of HBV conducted among pregnant women in these countries vary greatly, with different viral genotype patterns reported in some countries.\textsuperscript{[145]}

In Egypt pregnant mothers, who were attending outpatient clinic for routine antenatal care at Cairo University Hospital were evaluated in a cross-sectional study for the prevalence of HBV infection. 2,000 pregnant women were included aged between 16 and 46 years. HBV infection was positive in 1.6% of the whole study population. It was founded that a pregnant woman with a family history of HBV infection had a greater chance of acquiring HBV infection.\textsuperscript{[146]} The genetic diversity was evaluated among HBV carriers in Egypt, by Saudy and co-workers (2003), when they determined the genotypes of HBV isolated from 105 serum samples. Using serologic and genetic methods all serum samples were classified into HBV genotype D indicated that it is most prevalent in Egypt.\textsuperscript{[147]}

In Libya during April 2001 to 2002 March, a study was conducted to determine the seroprevalence of HBV infection and to estimate the risk of perinatal transmission in women positive for HBsAg. Blood samples from 1,500 pregnant women who delivered at Tripoli Medical Center, was tested for HBsAg using ELISA techniques. The prevalence of HBsAg was 1.5%, HBeAg was detected in 21.7% of HBsAg-positive pregnant women.\textsuperscript{[148]}

In Algeria a survey was conducted in 715 pregnant women in different regions. Serologic markers of hepatitis B (HBsAg, anti-HBc) were investigated. HBsAg seropositivity was detected in 1.6% and anti-HBc was found in 11.1% of pregnant women.\textsuperscript{[149]} The molecular features of the viral strains circulating in Algeria were investigated when Khalifa and Thibault, (2008) studied 75 chronic hepatitis B
patients from north-east Algeria. The characteristics of HBV strains were determined by use of serological and molecular testing. Genotype D was predominant (93%) followed by genotype A (5%) and E for one patient. It was founded that Algerian strains clustered independently from other genotype D reference sequences, suggesting a possible new D subtype.\textsuperscript{[150]}

In Tunisia a survey was conducted by Ayed and co-workers (2007) to determine the frequency of genotype in 164 HBV infected individuals. Genotypes were studied using Inno-LiPA and Multiplex-PCR and PCR-RFLP methodology. The 164 genotypes were distributed as follows: 1 genotype A (0.6%), 1 genotype B (0.6%), 3 genotype C (1.82%), 139 genotype D (84.75%), and 20 mixed genotypes (12.2%). The results confirmed the prominence of genotype D in Tunisia.\textsuperscript{[151]}

A novel subgenotype D7 was described in Tunisia, by Meldal and co-workers who characterized HBV for the first time in Tunisia, they performed viral load quantification and phylogenetic analyses of full genome or pre-S/S sequences on 196 HBsAg-positive plasma samples from Tunisian blood donors. 59 strains formed a novel subgenotype D7, 41 strains clustered in subgenotype D, and seven strains in subgenotype A2 and one strain in genotype C. The study concluded that HBV/D is dominant in asymptomatic Tunisian HBsAg carriers and a novel subgenotype, D7, was the most common subgenotype found in this population.\textsuperscript{[152]}

In Morocco a study was carried to find out the pattern of HBV genotypes circulating in chronic hepatitis B patients. Viral genotypes were determined in 221 chronic carriers using INNO-LiPA HBV assay and hemi-nested PCR. Phylogenetic analysis was performed in 70 samples, and multiplex PCR method was used to confirm some
genotyping results. The genotype distribution was D in 90.45% of cases, A (5.9%), E (1 case), and mixed genotypes (5 A/D and 2 D/F) in 3.17% patients.\textsuperscript{[153]}

In Mauritania, (2012) a cohort of 1020 pregnant women visiting for routine checkups were screened for HBV infection. Demographic, epidemiological, ethnic, clinical, and biological data were recorded. HBV genotypes were determined by sequencing and phylogenetic analyses. The prevalence of HBsAg (10.7%) and HBcAb (66.3%) indicated high HBV endemicity. Exposure to HBV was significantly associated with education level, ethnicity, blood transfusion, and occupation. The most frequent HBV genotypes were: HBV/D, 53%; HBV/E, 35%; and HBV/A. The study confirmed the high prevalence of HBV infection in Mauritania and demonstrated the high genetic diversity of HBV in this country.\textsuperscript{[154]}

In a study conducted between 2000 and 2003, in 7 West African countries (Mali, Burkina Faso, Togo, Benin, Nigeria, Cameroon, and the Democratic Republic of Congo). Serum samples were obtained from healthy individuals, patients with measles, or known HIV-infected individuals. Serum samples containing viral DNA were selected for sequence analysis, by which Mulders and co-workers investigated the PreS1/PreS2/S genes of 127 viruses. Genotype E was most prevalent and showed a conspicuously low genetic diversity. The study concluded that HBV/E was by far the most prevalent genotype found across most of this vast crescent, except for Cameroon, where most of the strains belonged to HBV genotype A (HBV/A). A single sporadic case of HBV/A was also detected in Burkina Faso.\textsuperscript{[155]}

In Cameroon to form the basis of infection management policies in pregnancy, due to viruses that share common modes of transmission, such as the sexual route, a study was undertook including 384 rural pregnant women, to investigate the
prevalence of antibodies and other markers of the hepatitis A, B, C, and D viruses, (HIV-1) and Treponema pallidum were sought, using ELISA. Regarding HBV, it was tested for the (HBsAg), (anti-HBs) and (anti-HBc). The incidence of HBV markers was as follows: 5.4% for HBsAg, 61.3% for anti-HBs, and 84.6% for anti-HBc.\textsuperscript{[156]}

In Ivory Coast, a retrospective survey estimating the prevalence of HBV and HCV was conducted on samples taken from 1,002 African pregnant women (501 diagnosed as HIV-1 positive and 501 HIV-1 negative) participating in a clinical trial program conducted in Abidjan, Hepatitis B markers studied were (HBsAg), and if positive, HBeAg /anti-HBe antibodies and HBV DNA. HBsAg was found in a similar proportion among HIV-positive (9.0%) and HIV-negative (8.0%).\textsuperscript{[157]}

The distribution of HBV genotypes in Ivory Coast was determined by Suzuki and his colleagues (2003), in a study carried out to elucidate the distribution of HBV genotypes and to clarify the genotype-related characteristics of genotype E. Using serological and genetic methods, among 48 HBV carriers, the distribution was 6.3% for genotype A, 6.3% for genotype D, and 87.4% for genotype E. The results indicate that: (1) genotypes A, D, and E of HBV exist in Ivory Coast and genotype E is the most prevalent; (2) genotype E spread with low genetic diversity over the complete genome in West Africa.\textsuperscript{[158]}

In Ghana a cross-sectional analysis of HBV serological and molecular parameters was conducted in pregnant women age ranged (15 - 48 years) by Candotti and co-workers (2007). Plasma samples from pregnant women and corresponding cord blood were collected at delivery in the Department of Obstetrics and Gynecology, Komfo Anokye Teaching Hospital, and Kumasi. HBsAg was detected
in 174 plasmas and confirmed by EIA in 173 (12.6% of total). No difference in age distribution was observed between HBV carriers and non-carriers.\textsuperscript{[159]}

The genetic diversity of HBV in Ghana was investigated before that when Candotti (2006), conducted study of 117 blood donor samples. S and the basic core promoter-precore regions (BCP/PC) sequencing was used to identify genotypes and variants relevant to HBV. Genotype E (87%) was dominant with little genotypes A (10%) and D (3%).\textsuperscript{[160]}

In Nigeria (2007) a hospital based cross sectional survey was conducted to study the epidemiology of HBV infection among pregnant women attending antenatal clinic at General hospital Minna, Nigeria State. 261 consenting pregnant women were included; seroprevalence of HBsAg was 12.3%. With respect to age, the results showed that there is increase in HBsAg titres with increase in age up to 30 years followed by a decline. Statistically, however, there was no significant association between age and seroprevalence of Hepatitis B infection. Also it revealed that there is inverse relationship between educational attainment of the women and seroprevalence of Hepatitis infection. Details showed that women with high prevalence of the infection were illiterates (15.90%) while those with some levels of education had lower prevalences even though there was no significant association. Similarly, the result revealed that housewives had higher prevalence (13.60%) than the other women considered in the study. Despite this observation, no significant association between infection and the occupation of the women.\textsuperscript{[161]} A similar findings was found by Ugbebor\textsuperscript{1} (2011) in a study conducted at the University of Benin Teaching Hospital (UBTH) located in the heart of Benin City, Edo state Nigeria, included 5760 pregnant women who attended the antenatal clinic, HBsAg was detected in (12.5%).\textsuperscript{[162]} Earlier to that Odemuyiwa and co-workers demonstrated that all the viruses circulated in
Nigeria belonged to the genotype E, when they studied isolates of HBV collected from 20 acute and chronic hepatitis patients in a highly endemic region of Nigeria, and phylogeneticaly analyzed the complete pre-S2/S (large S) genes.[163]

In Guinea Plasma samples from random asymptomatic carriers of HBsAg in Conakry, were studied by Garmini and co-workers (2009). In a total of 117 HBsAg+ samples the complete genome sequences of 81 strains were obtained in addition, three samples from Kumasi, Ghana, were also included in the analysis. Phylogenetic analyses confirmed the dominance of genotype E (95.1%).[164]

In Sierra Leone to determine the necessity and likely benefits of a scheme aimed at the vaccination of children of seropositive mothers who can afford the cost until mass immunization is possible. A study was carried to find out the prevalence of HBV markers amongst pregnant women of middle and high socio-economic class. A total of 302 women were enrolled. The seroprevalence rate formed in this study population was 6.2%. The low anti-HBs found in this population was surprising (5.1%).[165]

In Burkina Faso the epidemiology of HBV was evaluated in different studies. In Bobo Dioulasso, the prevalence rate and the risk factors for the carriage of hepatitis B markers in pregnant women were investigated by Narco and his colleagues (2000). Out of 917 pregnant women recruited during antenatal care, 98 (10.7%) were HBsAg positive. Among these ones, 18.2% carded HBeAg, 66.7% antiHBe antibodies and 95.6% antiHBc antibodies.[166] In another study carried to assess the antenatal transmission of HBV in a context of moderate prevalence of HIV, at the university hospital, in Ouagadougou, (Burkina Faso) by Sangare (2009). Among 360 counselled pregnant women for HIV and HBV testing, 307 were voluntarily enrolled at their last antenatal clinic. Blood samples were collected from all the 307 mothers and tested for
HBsAg, HBeAg and analysed. HBsAg were found in 35 (11.4%) mothers, including 7 with HBeAg. It was founded that HBeAg significantly associated with mother-to-child transmission (MTCT) of HBV.\cite{167}

In Gabon, between January and March 2005, seroprevalence of HBV and HDV was evaluated in a large population cohort of pregnant women in the five main cities of the country, with characterization of the circulating genotypes, a total of 1,186 samples were obtained from pregnant women (age range, 14 to 40 years). HBsAg was detected (9.2%). 10% had the HBeAg and 89.9% had anti–Hbe antibodies. The prevalence of HBsAg did not differ significantly by age. However, the prevalence of HBeAg positivity was significantly higher in the 14- to 20- year old age group than in the other age groups. Phylogentic analysis showed that the HBV genotypes circulating in pregnant women belong to HBV subgenotype A3 or genotype E.\cite{168}

In Brazzaville (Congo), the risk of perinatal transmission of HBV was studied in 292 of pregnant women who came as outpatients or to deliver in 3 health centers in Brazzaville, for three months samples were screened systematically for HBsAg. Positive sera for HBsAg were also tested for the other markers of HBV, except for specific DNA. The seroprevalence of HBV among these women was 6.5%. The overall prevalence of HBs was 57.8%; the prevalence of the profile HBsAg + HBeAg was 2.05%.\cite{169}

In Uganda and Rwanda a retrospective survey to estimate the prevalence of HBV and HCV infections was conducted on the samples of 247 African HIV-1 positive pregnant women who had participated to a mother-to-child prevention trial carried out in urban settings in Kampala, Uganda and Kigali, Rwanda. Hepatitis B
markers studied were HBsAg and, if positive after confirmatory testing, HBe antigen/anti-HBe antibodies and HBV DNA. HBsAg was found in 10/246 women seroprevalence (4.1%), 8/164 (4.9%) in Uganda and 2/82 (2.4%) in Rwanda. HBe Ag was found in 33% of HBsAg-positive patients.\textsuperscript{[170]}

To determine whether HBV strains in the Central African Republic (CAR) belong predominately to the homogeneous West-African genotype E, Bekondi (2007) analyzed serum samples randomly collected from 196 patients admitted with symptoms of acute or chronic hepatitis to the Central Hospital in Bangui. Thirty complete and 36 partial sequences of HBV strains were obtained. 94% (62/66) of the strains belonged to genotype E, while genotype A1, most closely related to a strain from Tanzania and genotype D were detected in only one and three samples, respectively. The study concluded that genotype E is predominant in CAR with little overlap with genotypes from Eastern Africa, extending the West-African HBV genotype E crescent further to the East.\textsuperscript{[171]} Similarly to study how far the genotype E crescent extends to the East, Hubschen (2009), analyzed a large number of HBV strains from Rwanda. Phylogenetic analysis of 45 S fragment sequences revealed strains of genotypes A (30), D (10), C (4), and B (1). Despite this exceptional genetic variability, genotype E virus was not found indicating that this country does not belong to the genotype E crescent, but is east of an emerging African genotype E/A1.\textsuperscript{[172]}

In Kenya a study was undertaken to determine HBV genotypes circulated. Seropositive HBV blood samples from a blood donor setting were used in the study. HBV genotypes were determined in 52 nucleic acid-positive samples using specific primer in a nested PCR and sequencing employed in the HBV genotyping. The study
showed presence of HBV variants with genotypes A (88%), E (8%) and D (4%). It was concluded that HBV genotype A is the most predominant genotype in Kenya with both subgenotype A1 and A2 present. Genotype D and E are also present in the population, which demonstrates that there could be a high genetic diversity of HBV in Kenya.\cite{173}

In Ethiopia, Gondar a prospective cross-sectional study was performed from August 01 to December 30, 2006, involving pregnant women attending antenatal clinic at Gondar Health Center, to determine the seroprevalence of HIV, HBV, HCV and syphilitic infections. Data on socio-demographic characteristics and sexual behaviors were collected using structured questionnaire. Blood was collected and serum was tested for the presence of HBsAg, antibodies to HIV, HCV and Treponema pallidum. Among the 480 ANC attendees, the seroprevalence of HBV was 7.3%.\cite{174}

Another study was conducted from February to April 2014 at Dessie referral hospital, Ethiopia, to determine the prevalence and predictors HBV infections among pregnant women attending for antenatal care clinic. A total of 385 pregnant women were enrolled in the study. Serum samples were tested for HBsAg using rapid test kits. The overall prevalence of HBV infection was 4.9%. Multiple sexual practices, nose piercing and history of abortion were found to be significant predictors of prevalence of HBV. The study showed an intermediate prevalence of HBV infection among pregnant women.\cite{175}

In Tanzania a cross-sectional study was conducted between September-December 1999 to describe the seroprevalence of hepatitis C and B viruses and their association with HIV and other sexually transmitted diseases (STDs) among women aged 15-49 years, attending three primary health care clinics in Moshi urban,
Tanzania. A total of 382 consenting women were enrolled in the study. The seroprevalence for HBsAg was 4.2%. It concluded that HBsAg was not more prevalent in HIV-infected women. Public preventive efforts should thus focus on HBV immunization.\textsuperscript{[176]}

In Malawi a descriptive study using serum samples collected between 1993-1995 from pregnant women in the Shire valley in rural Malawi, was conducted to describe the seroprevalence of (HBV) and (HCV) infection in HIV-positive and HIV-negative pregnant women. Fifty HIV-positive and 100 HIV-negative samples were selected randomly from 153 HIV-positive and 443 HIV-negative women delivering in the hospital. Evidence of HBV infection was found in 71.7\% of women. Chronic carriage of HBV (HBsAg positive) is high (13\%). Exposure to HBV probably occurred well before adulthood as the prevalence of anti-HBc antibody was high in young mothers <20 years of age (81\%). HBV infection was highly endemic in rural Malawi.\textsuperscript{[177]} The genotype of HBV was determined in 20 serum samples from Malawian chronic HBV carriers, when Sugauchi and his colleagues sequenced two complete genomes and 13 entire pre-S2/S genes directly. Genotype A HBV isolates were found in all of the samples.\textsuperscript{[178]}

In Luanda (Angola), HBV genotypes was investigated in a study carried to determine the prevalence and risk factors associated with HBV infection among staff, visitors and patients of a public hospital in Luanda. 508 individuals were investigated. Serum samples were screened by (ELISA) for the presence of HBsAg and other HBV markers. Total nucleic acids were extracted from HBsAg-positive samples and HBV-DNA detection was performed by amplification of the pre-S/S region of the genome using a semi-nested PCR assay. RFLP analysis, sequencing and Phylogenetics
analyses were performed for the detection of HBV genotypes in 40 out of the 41 HBV-DNA-positive samples. Thirty-five samples belonged to genotype E, four belonged to genotype A and one belonged to genotype D.\textsuperscript{179}

In Zimbabwe, Gulube and his colleagues (2011) determined the HBV genotype distribution in blood donors from different geographical locations. Using RFLP assay, sequencing of the basic core promoter/ precore region and of the complete S open reading frame showed that 29 HBV isolates belong to subgenotype A1.\textsuperscript{180}

In South Africa within the routine screening cohort of urban obstetric population, 3,469 urban pregnant women, HBsAg was found in 42 (1.21%) patients. Only 2 (4.6%) patients were HBeAg-positive (0.06% of the entire cohort), whereas the remaining 40 were identified as HBeAb-positive.\textsuperscript{181}

In Western Cape, South Africa a retrospective cross-sectional study aimed to determine whether HIV co-infection will change the epidemiology of HBV both by increasing infectivity and by favoring the escape of viruses bearing phenotypically altered HBsAg. Antenatal samples collected for the 2008 Antenatal Sentinel HIV and Syphilis Prevalence Survey were used. The study showed a trend toward loss of immune control of HBV in HIV-infected women with 3.4% of samples containing HBsAg, 18.9% contained HBeAg. In contrast, 2.9% of samples from HIV-uninfected women contained HBsAg and 17.1% of these HBeAg. Genotyping showed 63/68 samples belonged to genotype A and the remainder genotype D.\textsuperscript{182}

Seroprevalence of HBV was also reported from non African Arab countries with variation in estimates and in sometimes with genotypic diversity.
In Gulf States in June–July 2000, a cross-sectional study in nine centers across Oman, Qatar and United Arab Emirates, was conducted to evaluate the prevalence of HBeAg and HBsAg in pregnant women. A total of 1710 pregnant women aged 15–45 years were enrolled in the survey. Serology results were available for 1694 women. A total of 7.1% of the women in Oman, 1% in Qatar and 1.5% in UAE were HBsAg-positive. Three (0.5%) women in Oman were HBeAg-positive. Risk factors identified for being HBsAg-positive were younger age, being a national (i.e. not an expatriate) and residing outside the city. The results have shown that HBV prevalence in pregnant women was of intermediate endemicity in Oman and of low endemicity in Qatar and UAE.[183]

In Oman the molecular feature of HBV genotypes was investigated in various hospitals represented the whole Sultanate. 179 chronically HBV-infected outpatients were included in the study. Serum samples were collected from the HBsAg positive patients. HBV genotypes were determined by sequencing and phylogenetic analysis. Of all circulating HBV genotypes, genotypes D (130/170; 76.47%) and A (32/170; 18.28%) are predominant in Oman. HBV genotypes C (2/170; 1.18%) and E (2/170; 1.18%) were less frequent and the HBV genotypes B, F, G, and H were not detected. Noteworthy, four patients (2.35%) showed HBV genotype mixtures implying double infections. Three of these patients were infected with HBV genotypes A and D, and one patient was infected with HBV genotypes C and D.[184]

In Saudi Arabia (2005), HBsAg prevalence rate among pregnant women was evaluated 12 years later following the beginning of the vaccination program (1990), in 5 regions of the country, using multistage sampling, 2664 pregnant Saudi women were recruited. Blood samples were tested for HBsAg; positive samples were also tested for HBeAg. In all 2.44% were positive for HBsAg and 4 (0.15%) were also
positive for HBeAg. HBsAg prevalence was highest in Gizan (4.2%) and lowest in Tabuk (1.4%). Positivity for women ≤ 20 years of age was 0.5% compared with 2.6% for older women. Concluded that the overall HBsAg prevalence rate was lower than previously reported.[185]

In (2012) another study was conducted to determine the prevalence and associated risk factors of HBV infection in pregnant women in Jazan region KSA. A random sample of 537 collected from pregnant females who attended Jazan general hospital and randomly selected health care centers. Prevalence of HBsAg was found to be 4.1%, past history of hospitalization and jaundice are important risk factors for transmission of the infection.[186]

In Yemen (Sanaa), during November–December 2011, a cross-sectional study was conducted, to investigate the seroprevalence and associated risk factors for markers of HBV (HBsAg) and anti-HCV antibody among pregnant women at the Al-Thawra hospital in Sana’a, Yemen. 400 pregnant women were enrolled in the study; the prevalence of HBsAg was (10.8%). The results of the study suggested that HBsAg have high prevalence among pregnant women.[187]

2.2 Epidemiology of HBV in Sudan

Sudan is considered highly endemic for HBsAg, with prevalence about 16%–20% in the general population.[188] Epidemiological Studies in different regions of Sudan were carried out in different groups of the general population reporting varied prevalence estimates of hepatitis in Sudan.

Early serosurveys were carried out by McCarthy (1987) in Port Sudan and Suakin, eastern Sudan to determine the prevalence and risk factors associated with the transmission of hepatitis B. A total of 990 study subjects including different
population groups were enrolled. Serologic markers for hepatitis B were detected in 68% of the entire study population.\textsuperscript{[189]} Another survey was conducted among 773 male soldiers living in five urban locations. 78% of the study population had serologic evidence of past hepatitis B infection. It was founded that sexual promiscuity is a risk factor for hepatitis B transmission in Sudan.\textsuperscript{[189]}

In central Sudan a study was carried to determine prevalence and risk factors in rural residence of Gazira by surveying two villages (Khalawaat and Saleim). 851 subjects were enrolled (age 1-89 years; mean age 24.6 years) of equal sex distribution, 408 from Khalawaat and 443 from Saleim, HBsAg was found in 18.7% and HBeAg was present in 70% of HBsAg-positive women of childbearing age. Age, residence in Khalawaat, crowding, and tattooing were predictive of seropositivity for any hepatitis marker.\textsuperscript{[10]} Another survey was carried in Dec 2000 in the population of Um Zukra village, Gazira State, an area endemic for schistosomiasis and malaria. To determine the prevalence and risk factors for transmission of HBV infection, HBsAg and HBcAb were reactive in 6.9% and 47.5% of subjects respectively; there was no statistically significant difference regarding infection rate in different age groups. Exposure to HBV infection was highest in those over the age of 50 years (68%) and lowest in those under the age of 10 years (12.5%). The only significant risk factors for HBV exposure were a previous history of parenteral antischistosomal therapy and increasing age.\textsuperscript{[190]}

Blood donors and laboratory technical staff from the Gezira area were investigated when Elshafie studied the prevalence of HBsAg in a total of 110 donors, 19 (17.3%) were found to be carriers of the antigen and 4 of 33 (12.1%) technical staff were also found to be carriers.\textsuperscript{[191]}
In Khartoum State a cross sectional, facility-based study carried out in 2004 by El Mukashfie and co-workers to examine the sero-prevalence of HBV markers among the health care workers in the Public Teaching Hospitals in Khartoum. Among the 843 subjects tested for all HBV markers (Anti-HBc, HBsAg, HBsAb, and HBeAg), the prevalence of Anti-HBc, HBsAg, HBsAb, and HBeAg was found to be 57%, 6%, 37%, and 9% respectively, concluded that seroprevalence of all HBV markers was found to be significantly high.[11]

A similar study from March 2006 to March 2007 was also conducted in Khartoum to determine the seropositivity of hepatitis B infection, associated risk factors and history of vaccination among staff in 3 teaching hospitals. A total of 245 health workers were participated in the study, 168 (68.6%) females and 77 (31.4%) males. They included 23 surgeons, 37 laboratory staff, 6 dentists, 73 nurses and 106 domestic staff. Twelve (4.89%) of the participants tested positive for HBsAg.[192]

Another epidemiological study was conducted in Khartoum during April 2008 to 2011 to estimate the prevalence of HBsAg and anti-HCV antibodies among patients who undergo different surgical interventions in Al-Shaab Teaching Hospital. The study involved 3172 patients from all ages and both sexes. The prevalence of HBsAg is slightly higher in males (5.46%), than females (4.04%); however, it is statistically insignificant.[193]

In Darfur State the first epidemiological study was conducted during the period from May to July 2007, as a hospital based study to determine the seroprevalence of HBV and HCV infections and the possible risk factors among blood donors in Nyala, South Darfur State, which has never been studied before, a total of 400 subjects were included in the study. The study concluded that the seroprevalence
of HBV was in an intermediate (6.5%) and unprotected sexual activities was the
major risk factor for infection in the population.\[194\]

The first published study documenting seroprevalence of HBV and HCV
among pregnant women in Sudan, was a cross-sectional study conducted at
Umdurman maternity hospital, during the period of March–June 2006 carried out by
Elsheikh et al, 728 pregnant women were enrolled in the study. HBsAg was detected
in 41 (5.6%) out of 728 women, all of them were not aware of their condition. None
of the expected risk factors (parity, age, history of blood transfusion, dental
manipulations, tattooing and circumcision) had been found to be associated with
HBsAg sero-positivity.\[195\]

A cross-sectional study was conducted at Khartoum Teaching Hospital, during
the period of March to June, 2010 to determine the prevalence of hepatitis B infection
in pregnant women and to evaluate the risk factors of hepatitis B infection. A total of
160 pregnant women who presented to the labor ward or antenatal clinic of Khartoum
Teaching Hospital were enrolled. The seropositivity of HBsAg among the studied
population was 7.5%. The significant relationship between some risk factors and HBV
infection was found, including surgery, jaundice and blood transfusion.\[196\]

At Wad Madani hospital, during the period June through December 2011, a
cross sectional study was carried to investigate the prevalence of HCV, HBV and HIV
infection among pregnant women. In 396 pregnant women recruited 5.1% were
seropositive for HBsAg using ELISA. Home delivery was found to be the only
significant risk factor for seropositivity of HBsAg.\[197\]

Most of the above studies were done just as screening procedure for either
HBsAg or in sometimes combined with other serological markers, without the use of
modern technology for detection of exposure to HBV. The first study done as a
molecular epidemiology was between February and August 2008 when Mahgoub and co-workers aimed to assess the question of blood safety in Sudan in asymptomatic blood donors from Khartoum, local evidence was collected on the prevalence of anti-HBc- and HBsAg-negative/HBV DNA-positive donations. 404 plasma samples were randomly collected from blood donors in Federal Hospitals in Khartoum state, Khartoum Teaching Hospital (KTH), and Radiation Isotopes Centre Khartoum (RICK), Federal Ministry of Health. Samples initially tested HBsAg negative were investigated further for the presence of anti-HBc, anti-HBs, and HBV DNA. Anti-HBc was detected in 145 samples (36%). Anti-HBs was successfully quantified in 43/77 (56%). Pre-S/S and/or whole genome sequences were obtained from 47 randomly selected HBsAg-positive donors. Genotype E was identified in 27 strains (57.5%), genotype D in 19 strains (40.5%), and genotype A2 in 1 strain (2%). Two outlier strains within genotype D ultimately were identified as recombinants of genotypes D and E with identical recombination points, suggesting circulating, infectious, recombinant strains.\[^{198}\]

Another study to molecularly characterize HBV was from patients with liver disease in Sudan, done by Yousif et al (2013). Of the 99 patients, 77 were males and 22 were females. All sera were HBsAg- and anti-HBc-positive; 12 were HBeAg-positive/anti-HBe-negative, 75 were HBeAg-negative/anti-HBe-positive, and 12 had neither HBeAg nor anti-HBe. All patients were HBV DNA-positive. It was founded that Sudanese HBV carriers were mainly infected with genotypes D or E, with patients infected with genotype E having higher HBeAg-positivity and higher viral loads.\[^{199}\]
Chapter Three
Methodology
1. Ethical Clearance

The study was approved by the Ethics committee of Alneelain Institute for Medical Research, Faculty of Medicine, Alneelain University. Informed consent was obtained from all participants with assurance that all information obtained would be treated as confidential, and would be used for the purpose of this study only. A separate permission was taken from the participating hospitals.

2. Study Design

The study was a hospital based, descriptive cross sectional survey conducted between June, 2013 and July, 2016. The health centers Include Al Fashir New Hospital, a reference hospital for obstetrics and gynecology. Peripheral health care centers and some private clinics in Al Fashir town were also included.

3. Study Area

The study was conducted in Al Fashir town, capital of North Darfur State – Western Sudan. North Darfur is one of the 26 states of Sudan. It is one of the federal states composing the Darfur region. North Darfur located between 24 and 27 degree latitude east 12 and 20 latitude north. It has an area of 296,420 Km² and an estimated population of approximately 1.400.000. The main feature of the physical geography is the presence of plains, low hills of sandy soils known as (goz) and sandstone hills. To the north the goz is overtaken by the desert sand of the sahra. A second feature is the valleys, seasonal watercourses that flood only occasionally during the wet season. Climate is ranging from desert to semi-desert and poor savanna to the south. The rainy season is from June through September. Average rainfall range between 100-60 ml. The economy is primarily based on agriculture, producing cereal crops and tobacco as well as livestock. “http://en.wikipedia.org/wiki/Darfur”
4. Study Population

Women in any trimester of pregnancy who attend to the health care centers in the town for antenatal care were the target group of this study.

5. Study Sample

Only consenting attendees were recruited and included in the study. On every antenatal day of the refer clinic, the pregnant women were interviewed. Participation in the study was voluntary, so when a woman refused to participate, she was substituted by another attending woman.

5.1 Selection Criteria

5.1.1 Inclusion Criterion
Any pregnant woman who gave consent to participate in the study.

5.1.2 Exclusion Criterion
Pregnant woman who did not accept to be part of the study.

5.2 Sample size
The sample size estimate using the most common formula \( N = \frac{Z^2 \times p \times (1-p)}{d^2} \) was an obstacle because of the unknown \( p \) (a similar pre-published data) in the area. So it was decided that a large number of pregnant women to be interviewed (1100) and the serological tests were available for 900 pregnant women.

6. Demographic Data Collection
The purpose and research procedure were first explained to each subject. Demographic data were collected from each woman who agrees to participate by interviewed questionnaire. Respondents were interviewed verbally in the local language by the researcher assisted by nurses and technicians. The questionnaire was designed to obtain socio-demographic data such as age, residence, occupation, educational status and income.
Risk factors information were also obtained and these included, history of surgery, dental manipulation, tattooing, unsafe injection, caesarian section, abortion, jaundice, blood transfusion, ear piercing, bloodletting and history of vaccination.

7. Collection of blood samples / plasma preparation

On days of antenatal refer clinic, at the hospitals general laboratory blood samples were collected aseptically by venepuncture using five ml sterile disposable hypodermic syringes and needles and dispensed into relabeled Ethylenediamine tetra-acetic acid (EDTA) tubes; the samples were then centrifuged at 3,000 rpm for five minutes to separate the plasma. Using micropipette each plasma sample was divided into two 2.5 ml cryotubes of equal volumes (labelled A and B), one for serology and the other for molecular testing. Samples then transported on ice bags to Alneelain Institute for Medical Research, Khartoum and stored at -20°C until required. The serological testing was carried out at Alneelain Institute for Medical Research Centre, Faculty of Medicine, Alneelain University, and the genotyping was carried at the Central Research Laboratory, Ministry of Higher Education - Khartoum.

8. Procedure for detecting HBV markers

Serological markers for HBV profiles for HBV antigens (HBsAg, HBeAg) and antibodies (anti-HBc, anti-HBe) were determined using the in vitro enzyme-linked immunosorbent assay (ELISA) diagnostic kit. Nine hundreds (900) samples were first tested for HBeAb. Those which were positive for anti-HBc were tested for HBsAg. Samples that were positive for HBsAg were further tested for HBeAg and HBeAb respectively.
8.1 ELISA Technique

Semi automated ELISA machine was used to detect the presence of HBV antigens and antibodies in plasma samples.

8.2 Equipments

- ELISA Microplate Washer (BioTek, USA).
- ELISA Microplate Reader (BioTek, USA) controlled by gen5™ software (version 5.1). Gen5™ software serves as the operating system for the BioTek reader for data collection, analysis, exporting and reporting.
- Incubator 37°C.
- Dispensing system (single/multichannel Automatic Pipette).
- Vortex tube mixer.
- Timer
- Absorbtent paper.

Competition ELISA was carried out for the detection of HBcAb and HBeAb markers ELISA kits manufactured by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. China. This kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies or antigens for HBV in human serum or plasma.

8. 3 Principle of the Assays

The kit is based on solid phase, one step incubation competitive principle ELISA method.

8. 4 Assay Procedure

Assays were carried out at room temperature. The samples were removed from the freezer and left at room temperature to thaw. All samples were first screened for anti-HBc. Reagents allowed to reach the room temperature. Wash buffer checked for the presence of salt crystals and diluted. The plate with the test strips was removed from their foil pouches. On a paper equal numbers of wells were numbered including three negative controls and two positive controls and one blank. 50 µL of positive control, negative control, and specimen were added into their respective wells using a separate disposal pipette tip for each. 50 µL of Horseradish peroxidase- Conjugate (HRP) was added to each well except into the blank and mixed by tapping the plate gently and incubated for 1 hour. This allowed antibody in the sample to compete with the HRP-conjugated to anti-HBc or anti-HBe for a fixed amount of purified HBcAg or HBeAg pre-coated in the wells in order to form antigen–antibody complex. Plates were
washed with washing buffer 5 times. 50 µL of each Chromogen A and Chromogen B solutions were dispensed into each well including the blank and incubated for 15 minutes. Blue color developed in Negative control and anti-HBc or anti-HBe negative sample wells. The reaction was stopped by adding 50 µL of sulphric acid into each well and mixed gently. Intensive yellow color developed in Negative control and anti-HBc or anti-HBe negative sample wells. The colour was read as optical density (OD) and the Cut-off value (C.O.) was calculated in order to determine the result of the test.

8. 5 Quality Control

Control materials a negative control and positive control was used to validate the test result. Quality control criteria were verified as follows:

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

2- The OD value of the Negative control must be equal to or greater than 0.800 at 450nm after blanking.

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

8.6 Calculation of the results

The results were calculated by relating each sample (OD) value to the (C.O.) value of the plate.

Calculation of the (C.O.) value = Ne*× 0.5

Ne* = the mean absorbance value for three negative control.

8. 7 Interpretation of the results

(S = the individual absorbance (OD) of each specimen)

- Negative Results (S/C.O. > 1): samples giving an absorbance greater than the C.O. value were considered negative.
- Positive Results (S/C.O. ≤ 1): samples giving an absorbance less than, or equal to the C.O. value were considered positive.
- Borderline (S/C.O. = 0.9 – 1.1): samples with absorbance to C.O. ratio between 0.9 and 1.1 were considered borderline samples and were retested.

9. Hepatitis B virus Antigens Assays

A solid phase qualitative enzyme immunoassay ELISA based on a sandwich principle was used for detection of HBsAg and HBeAg in the serum samples, in which antibody-antigen-antibody (HRP)”sandwich”complex formed in case of presence of HBsAg or HBeAg in the samples.

9.1 Assay Procedure for HBsAg

HBsAg was assayed using Foresight HBsAg EIA Test Kit (San Diego, CA92121, USA). Prior to testing all samples and reagents were allowed to reach the room temperature. Working wash buffer was prepared by diluting the concentrated Wash buffer to 1:25. The plate with the test strips was removed from their foil pouches. On a paper equal numbers of wells were numbered including two negative control, two positive controls and one blank. 100 µL of positive control, negative control, and specimen were added into their respective wells using a separate disposal pipette tip. 50 µL of Horseradish peroxidase- Conjugate (HRP) was added to each well except the blank well. Mixed gently by swirling the microwell plate on the flat bench for 30 seconds, covered with the plate sealer and incubate at 37°C for 60 minutes. The plate sealer was removed, washed 5 times with 350 µL washing buffer and any residual wash fluid was tapped out by turned the microwell plate upside down on absorbent tissue. 50 µL of each Chromogen A and Chromogen B solutions were dispensed into each well including the blank, mixed then covered and incubated at 37°C for 10 minutes. Blue color developed in positive control and HBsAg positive
sample wells. The reaction was stopped by adding 50 µL of sulphric acid into each well. Intensive yellow color developed in positive control and HBsAg positive sample wells. The color was read as optical density (OD) at 450nm.

9.2 Quality Control

Control materials were used and their mean absorbance was calculated to validate the results matching the following criteria:

- Blank well absorbance should be < 0.050.
- Negative Control Mean absorbance after subtraction of Blank absorbance should be < 0.100.
- Positive Control Mean absorbance after subtraction of Blank absorbance should be > 1.00.

9.3 Calculation of the results

Cut-off value = NC*+ 0.070

NC*(Mean absorbance of negative control-Blank absorbance).

9.4 Interpretation of the Results

- Non-Reactive: Specimens with absorbance less than the cut-off value are non reactive for HBsAg and may be considered negative.
- Reactive: Specimens with absorbance greater than or equal to the cut-off value are considered positive.

10. Assay Procedure for HBeAg

HBeAg was assayed using (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. China). The samples and reagents were allowed reaching the room temperature. Working wash buffer was prepared by diluting the concentrated Wash buffer1 to 20. The plate with the test strips was removed from their foil pouches. On a paper equal numbers of wells were numbered including three negative controls and
two positive controls and one blank. 50 µL of positive control, negative control, and specimen were added into their respective wells using a separate disposal pipette tip for each. 50 µL of Horseradish peroxidase-Conjugate (HRP) was added to each well except into the blank, mixed by tapping the plate gently and incubated at 37°C for 1 hour. Plates were washed with washing buffer 5 times. 50 µL of each Chromogen A and Chromogen B solutions were dispensed into each well including the blank and incubated at 37°C for 15 minutes. Blue color developed in positive control and HBeAg positive sample wells. The reaction was stopped by adding 50 µL of sulphric acid into each well and mixed gently. Intensive yellow color developed in positive control and HBeAg positive sample wells. The colour was read as optical density (OD) and the Cut-off value (C.O.) was calculated in order to determine the result of the test.

10.1 Quality Control

A negative control and positive control materials were used to validate the test result. Quality control criteria were verified as follows:

1. The (OD) value of the Blank well which contains only chromogens and stop solution is less than 0.080 at 450nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450nm after blanking.

10.2 Calculation of the results

The results were calculated by relating each sample (OD) value to the (C.O.) value of the plate.

\[
\text{Calculation of the Cut-off value (C.O.)} = \text{Ne}^* \times 2.1
\]

\text{Ne}^* \text{= the mean absorbance value for three negative control.}
10.3 Interpretation of the results

(S = the individual absorbance (OD) of each specimen)

- Negative Results (S/C.O. < 1): samples giving an absorbance less than the C.O. value were considered negative.

- Positive Results (S/C.O. ≥ 1): samples giving an absorbance greater than or equal to the C.O. value were considered positive.

- Borderline (S/C.O. = 0.9 – 1.1): samples with absorbance to C.O. ratio between 0.9 and 1.1 were considered borderline samples and were retested.

11. DNA Extraction

HBV DNA was extracted from 200 μl plasma of pregnant women using the innuPREP Virus DNA/RNA Kit (analytikjena, Germany). Following the manufacturer’s instructions, before starting RNase-free water was incubated at 70°C until the elution step and Lysis Solution CBV / Carrier Mix was prepared. A mixture of 200 μl sample and 20 μl Proteinase K was added to 200 μl Lysis Solution CBV / Carrier Mix in a reaction tube, vortexed and incubated at 70°C, then centrifuged to remove condensate.

400 μl Binding Solution SBS was added to the lysed sample and mixed by vortexing. In 2 ml Receiver tube with located Spin Filter the sample was applied and centrifuged. The Spin Filter was washed three times by adding Washing solution HS (500 μl, 650 μl, 650 μl) successively and centrifuged at (12.000 rpm) for 1 minutes. To remove all traces of ethanol centrifugation at (12.000 rpm) for 5 minutes was done. 60 μl pre-heat RNase-free Water(70°C) was added to the Spin Filter placed into a 1.5 ml Elution Tube which incubated at room temperature for 2 minutes and centrifuged at(10.000 rpm) for 1 minute. The extracted DNA was stored at -20°C until used.
12. Detection of HBV Genomes

Out of the 162 HBsAg positive pregnant women, one hundred forty eight (148) samples were further studied for genotype characterization. A genotyping system based on multiplex-nested PCR using type-specific primers was employed in assigning genotypes A through F based on pre-S1 through S genes of the Hepatitis B virus genome (Naito et al., 2001). The sequences of PCR primers used in this study are shown in Tables (1, 2 and 3). The P1 and S1-2 were universal outer primers. Primer B2 was used as the inner sense primer with a combination of other anti-sense primers for genotypes A, B, and C in a multiplexing system called “Mix A”. Primer B2R was used as the anti-sense inner primer with a combination of sense primers for genotypes D, E and F in a multiplexing system called “Mix B”. The genotype specific primers have been designed based on the conserved nature of those sequences within a genotype and poor homology with the sequences derived from other HBV genotypes. The first PCR was carried out in 45 μl reaction mixture containing 1μl(50μM) each outer primer, 1μl (10 mM) each dNTP (Intron/South-Korea), 10 μl of 5X PCR buffer, 3 μl MgCl2, 0,2μl of Taq DNA Polymerase (Intron/South-Korea), and 5 μl of extracted DNA. The thermocyclic parameters were 95°C for 5 min, followed by 40 cycles consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. Two second round PCRs were performed for each sample, one with the common universal sense primer (B2) and type specific primers for genotypes A, B, C in “Mix A” and the other with the common universal anti-sense primer B2R and type specific primers for genotypes D, E, F in “Mix- B”. Reaction mixtures of the second multiplexing PCR systems contained 5 μl of the extracted product, 1 μl of each primer, 1μl (10 mM) dNTP, 5X PCR buffer, 3 μl MgCl2 and 1 μl of Taq DNA Polymerase. The cyclic parameters were 94°C for 5 min, followed by 20 cycles
consisting of 94°C for 20 s, 58°C for 20 s and 72°C for 30 s for “Mix A” and 94°C for 20 s, 58°C for 20 s and 72°C for 30 s for “Mix B”. The two Mix have passed on only one program containing different parameters. Each sample was visualized on an Ethyldium bromide stained 2% agarose gel. Genotype of each sample was identified.

Table 1: Primer sequences used for the first PCR (Macrogen/South - Korea)

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Specificity</th>
<th>Position</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5’-TCA CCA TAT TCT TGG GAA CAA GA-3’</td>
<td>universal</td>
<td>nt 2823-2845</td>
<td>sense</td>
</tr>
<tr>
<td>S1-2</td>
<td>5’-CGA ACC ACT GAA CAA ATG GC-3’</td>
<td>universal</td>
<td>nt 685-704</td>
<td>antisense</td>
</tr>
</tbody>
</table>

Table 2: Primer sequences used for the nested PCR (Mix A) (Macrogen/South-Korea)

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Position</th>
<th>Specificity</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2</td>
<td>5’-GGC TCM ACT TCM GTA ACA GT-3’</td>
<td>nt 67 - 86</td>
<td>type A to E specific</td>
<td>sense</td>
</tr>
<tr>
<td>BAIR</td>
<td>5’- CTC CGG GAG ATT GAC GAG ATG T-3’</td>
<td>nt 113-134</td>
<td>type A specific</td>
<td>antisense</td>
</tr>
<tr>
<td>BBIR</td>
<td>5’- GGT CCT AGG AAT CCT GAT GTT G-3’</td>
<td>nt 165-186</td>
<td>type B specific</td>
<td>antisense</td>
</tr>
<tr>
<td>BCIR</td>
<td>5’- CAG GTT GGT GAG TGA CTG GAG A-3’</td>
<td>nt 2979 - 996</td>
<td>type C specific</td>
<td>antisense</td>
</tr>
</tbody>
</table>

Table 3: Primer sequences used for the nested PCR (Mix B) (Macrogen/South-Korea)

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Position</th>
<th>Specificity</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2R</td>
<td>5’- GGA GCC GTA TYT GCT GGC AA-3’</td>
<td>nt 3078 - 3097</td>
<td>type D to F specific</td>
<td>antisense</td>
</tr>
<tr>
<td>BD1</td>
<td>5’- GCC AAC AAG GTA GGA GCT -3’</td>
<td>nt 2979 - 2996</td>
<td>type D specific</td>
<td>sense</td>
</tr>
<tr>
<td>BE1</td>
<td>5’- CAC CAG AAA TCC AGA TTG GGA CCA -3’</td>
<td>nt 2955 - 2978</td>
<td>type E specific</td>
<td>sense</td>
</tr>
<tr>
<td>BF1</td>
<td>5’- GYT ACG GTG TAC GGT TAC CA – 3’</td>
<td>nt 3032 - 3051</td>
<td>type F specific</td>
<td>sense</td>
</tr>
</tbody>
</table>

Bands with distinct sizes according to the migration pattern of a 50 bp marker (Intron/South - Korea).

Mix A: Type A-68bp, Type B-281 bp, TypeC-122 bp;
Mix B: Type D-119 bp, Type E-167 bp, Type F-97 bp.

13. Data Analysis

Data from the questionnaire were multi-checked, coded, categorized, entered and analyzed using Statistical Package for Social Sciences (SPSS for Windows version 16.0). Income was calculated and the pregnant women were categorized in classes according to the international monthly income (USD) as follows [low (1500-2500), medium (2500-4000), high > 4000].
Descriptive statistics were carried out presenting results as counts (proportions) and mean with standard deviations (SD).

Significant statistics were used including Fisher Exact Probability and Chi – square to test associations between HBV infection and variables grouped in more than one category as in demographic data (age, residence, education, income and occupation). Using MedCalc Statistical Software prevalence odds ratio (OR) with their 95% confidence intervals (CI) was calculated to estimate the magnitude of the association between HBsAg positivity and study variables with data set constructed in 2× 2 table as for medical, obstetrical and the other possible risk factors. A p value ≤ 0.05 was considered significant.
Chapter Four

Results
Results

A total of nine hundreds (900) pregnant women were included in the study with age that ranged from 15 to 50 years, mean age of 26.93 ± 6.68, mostly was in the age group 15 – 35(774, 86%) and residing in the town (581, 64.6%). Most of the women were housewives 87%. The education level results of the women showed that most (55.7%) had only primary education and in low income class (90%). (Table 4)

Table 4: Socio-demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Age</th>
<th>No. (%)</th>
<th>Resid</th>
<th>No. (%)</th>
<th>Educ</th>
<th>No. (%)</th>
<th>Income</th>
<th>No. (%)</th>
<th>Occup</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25</td>
<td>428(47.6)</td>
<td>Urban</td>
<td>581(64.6)</td>
<td>H. educ</td>
<td>80 (8.9)</td>
<td>high</td>
<td>3 (0.3)</td>
<td>HW</td>
<td>783(87)</td>
</tr>
<tr>
<td>26-35</td>
<td>346(38.4)</td>
<td>Rural</td>
<td>234 (26)</td>
<td>P/S</td>
<td>501(55.7)</td>
<td>Medium</td>
<td>38 (4.2)</td>
<td>Medical</td>
<td>9 (1)</td>
</tr>
<tr>
<td>36-50</td>
<td>80 (8.9)</td>
<td>Others</td>
<td>33 (3.7)</td>
<td>Illiterate</td>
<td>271(30.1)</td>
<td>Low</td>
<td>810 (90)</td>
<td>Others</td>
<td>60 (6.7)</td>
</tr>
<tr>
<td>Missed</td>
<td>46 (5.1)</td>
<td>Missed</td>
<td>52 (5.7)</td>
<td>Missed</td>
<td>48 (5.3)</td>
<td>Missed</td>
<td>49 (5.4)</td>
<td>Missed</td>
<td>48 (5.3)</td>
</tr>
</tbody>
</table>


Of the 900 women enrolled in the study HBcAb was reactive in 46% (414/900).

(Figure I, Table 5)

Table 5: Prevalence of the HBV markers tested among the study population.

<table>
<thead>
<tr>
<th>HBV Marker</th>
<th>Positive No. (%)</th>
<th>Negative No. (%)</th>
<th>Total No</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBcAb</td>
<td>414(46.00)</td>
<td>54.00(486)</td>
<td>900</td>
</tr>
<tr>
<td>HBsAg</td>
<td>162(18.00)</td>
<td>252(28.00)</td>
<td>414</td>
</tr>
<tr>
<td>HBeAg</td>
<td>4(2.61)</td>
<td>149(97.39)</td>
<td>153</td>
</tr>
<tr>
<td>HBeAb</td>
<td>58(37.66)</td>
<td>96(62.34)</td>
<td>154</td>
</tr>
</tbody>
</table>

One hundred sixty two out of the 414 positive HBcAb pregnant women were confirmed positive for HBsAg (162/900) 18%. (Figure II, Table 5)
HBcAb was found in 2.6% (4/153) of the positive HBsAg pregnant women. Whereas HBeAb was found in 37.7% (58/154) of the positive HBsAg pregnant women (Figure III, IV, Table 5).
Figure III: Frequency of HBeAg in the tested samples

Figure IV: Frequency of HBeAb in the tested samples
Although HBsAg was high 42% (82/379) in pregnant women aged range 15 – 25, followed by decline in the other age groups 38.56% and 36%, the difference was statistically insignificant (P = 0.67). (Table 6)

Table 6: prevalence of HBsAg in the different age groups.

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Age</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 - 25</td>
<td>26 - 36</td>
</tr>
<tr>
<td>Negative</td>
<td>111(57.59%)</td>
<td>99(61.49%)</td>
</tr>
<tr>
<td>Positive</td>
<td>82(42.49%)</td>
<td>62(38.51%)</td>
</tr>
</tbody>
</table>

Residence was found to be significantly associated with the disease (P = 0.01), high HBsAg positive rate was found in 44.4% (114/257) of the women residing in the town. (Table 7)

Table 7: prevalence of HBsAg in the resident areas.

<table>
<thead>
<tr>
<th>HBV Marker</th>
<th>Residence</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>Urban</td>
<td>Rural</td>
</tr>
<tr>
<td>Negative</td>
<td>143(55.6)</td>
<td>67(63.81)</td>
</tr>
<tr>
<td>Positive</td>
<td>114(44.3)</td>
<td>38(36.19)</td>
</tr>
<tr>
<td>Total No. (%)</td>
<td>257(63.9)</td>
<td>105(26.1)</td>
</tr>
<tr>
<td>P - value</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Within the differed educational status, women with secondary education were found to be the highest carriers of HBsAg positivity 46.58% (34/73), however this relation is statistically insignificant (P = 0.69). (Table 8)
Furthermore income was significantly associated with the disease (P = 0.01). A high carrier of HBsAg was noticed in those who were in low income class 40.4% (145/359). However, 53% (8/15) of women in the medium income class were carriers of HBsAg their number in the study population are too small to make reasonable judgment. (Table 9)

Table 9: Prevalence of HBsAg in the different income classes

<table>
<thead>
<tr>
<th>HBV Test</th>
<th>Income</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>214(59.61)</td>
<td>7(46.67)</td>
</tr>
<tr>
<td>Positive</td>
<td>145(40.39)</td>
<td>8(53.33)</td>
</tr>
<tr>
<td>Total</td>
<td>359(95.73)</td>
<td>15(4.00)</td>
</tr>
<tr>
<td>P – value</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

In terms of occupation housewives and those who work in the medical field were more affected than other categories, HBsAg positive was found in 41.02% (137/334) and 60% (3/5) respectively, (P = 0.02). However, the number of women who work in medical field was too small to make reasonable judgment. (Table 10)
Ear piercing was found to be the only significant risk factor for the exposure (P = 0.00, Odd ratio = 1.9, 95% CI = 1.3 – 2.9). Significant association with the disease was also reported in cases of bloodletting (P = 0.02). (Table 11)

Table 10: Prevalence of HBsAg in the different occupational categories

<table>
<thead>
<tr>
<th>HBsAg Test</th>
<th>Occupation</th>
<th>Negative No.(%)</th>
<th>Positive No. (%)</th>
<th>Total No. (%)</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Housewives</td>
<td>207(58.15)</td>
<td>149(41.85)</td>
<td>356(88.56)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Medicals</td>
<td>2(40.00)</td>
<td>3(60.00)</td>
<td>5(1.24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>16(100.00)</td>
<td>0(0.00)</td>
<td>16(3.98)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unspecified</td>
<td>15(60.00)</td>
<td>10(40.00)</td>
<td>25(6.22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>240(59.70)</td>
<td>162(40.30)</td>
<td>402(100.00)</td>
<td></td>
</tr>
</tbody>
</table>

Table 11: P – value and odds ratio analysis of the possible risk factors for HBsAg among the study population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P - value</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational Level</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DentalManipulation</td>
<td>0.60</td>
<td>0.8</td>
<td>0.4 - 1.6</td>
</tr>
<tr>
<td>Tattooing</td>
<td>0.97</td>
<td>1.0</td>
<td>0.5 - 2.2</td>
</tr>
<tr>
<td>Surgery</td>
<td>0.32</td>
<td>0.7</td>
<td>0.3 - 1.4</td>
</tr>
<tr>
<td>Unsafe Injection</td>
<td>0.25</td>
<td>0.3</td>
<td>0.0 - 6.3</td>
</tr>
<tr>
<td>Caesarian Section</td>
<td>0.38</td>
<td>1.3</td>
<td>0.7 - 2.4</td>
</tr>
<tr>
<td>Abortion</td>
<td>0.22</td>
<td>0.8</td>
<td>0.5 - 1.2</td>
</tr>
<tr>
<td>Jaundice</td>
<td>0.82</td>
<td>0.9</td>
<td>0.5 - 1.6</td>
</tr>
<tr>
<td>Blood Letting</td>
<td>0.02</td>
<td>0.2</td>
<td>0.1 - 0.9</td>
</tr>
<tr>
<td>Ear piercing</td>
<td>0.00</td>
<td>1.9</td>
<td>1.3 - 2.9</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>0.25</td>
<td>0.6</td>
<td>0.3 - 1.4</td>
</tr>
<tr>
<td>Vaccination</td>
<td>0.80</td>
<td>0.9</td>
<td>0.3 - 2.5</td>
</tr>
</tbody>
</table>
No other socio-demographic or clinical characteristics were significantly associated with HBsAg seropositivity as (surgery, dental treatment, tattooing, unsafe injection, abortion, jaundice, and vaccination) \( (P \geq 0.05) \). (Table 11)

Although statistically there is no association between women having caesarian section and the disease \( (P = 0.38) \), HBsAg was positive in 46\% (23/50) of the women who had caesarian section. (Table 12)

Table 12: HBsAg and Caesarian Section

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>C Section</th>
<th>Total No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Negative No</td>
<td>199(60.49)</td>
<td>27(54.00)</td>
</tr>
<tr>
<td>Positive No</td>
<td>130(39.51)</td>
<td>23(46.00)</td>
</tr>
<tr>
<td>Total No</td>
<td>329(86.81)</td>
<td>50(13.19)</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.38</td>
</tr>
</tbody>
</table>

Out of the 162 HBsAg positive pregnant women, one hundred forty eight (148) samples were further studied for genotype characterization. HBV genomes were detected by nested PCR. Out of the 148 samples analyzed 97(65.54\%) showed genotype specific bands while the remaining 51(34.46\%) HBV positive samples were remained untypable. Thirty one Samples (31.96\%) were classified into genotype D, (30) were genotype E (30.93\%) and (3) as genotype A (3.09\%). (Figure V, VI)
Figure V: Genotype Frequency among the HBsAg positive Samples

In (33) (34.02%) HBsAg positive samples, mix genotypes were detected as \{A+D; 17(17.53%), D+E; 13(13.40%), A+D+E; 3(3.09%)\}. (Figure V)

Lane number (1) marker 50bp, Lane number (2)positive sample for hepatitis b genotype(A) 68bP, Lane number (3,4,5 and 9)positive sample for hepatitis b genotype(D) 119bp, Lane number (7,8)positive sample for hepatitis b genotype(E) 167bP, (6 and 11)positive sample for hepatitis b genotype(D) 160bp and(E) 120bp, Lane number (12) Negative control.

Figure VI: Gel electrophoresis for genotypes bands
The prevalence of genotypes was assessed further with respect to pregnant women age. Statistically there was a significant relationship (P = 0.001), however there was no trend found in the distribution of genotypes among various age groups. Genotype D was more prevalent in women with age range 15-25 years.

In genotype analysis with respect to HBeAg it was noticed that distribution of the different typable genotypes is almost highly confide to HBeAg negative samples, although this relationship was found insignificant (P= 0.58). (Table 13, Figure VII)

Table 13: Genotype analysis with respect to HBeAg

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HBeAg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>D + E</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>A + D</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>A + D + E</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>3</td>
</tr>
</tbody>
</table>

P - value 0.58

Figure VII: Genotype analysis with respect to HBeAg
There was no relationship between genotypes distribution and HBeAb seropositive, seronegative (P=0.39). (Table 14, Figure VIII)

Table 14: Genotype analysis with respect to HBeAb

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HBeAb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>D + E</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>A + D</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>A + D + E</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>32</td>
</tr>
<tr>
<td>P - value</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

Figure VIII: Genotype analysis with respect to HBeAb
Chapter Five
Discussion
Discussion

Infection due to HBV is a significant health problem around the globe. Worldwide, viral hepatitis is the commonest cause of hepatic dysfunction in pregnancy. In the present study 900 pregnant women were investigated for the presence of hepatitis B virus infection and were evaluated for the risk factors for its acquisition. The study confirmed that extremely high HBV rates exist in antenatal women attending health care centers in Al Fashir town; as almost half 46.1(414/900) the pregnant women screened had evidence of exposure to HBV. These are believed to be the first data of HBV seroprevalence among pregnant women in North Darfur State.

High HB- anticore indicated high endemicity. In our study the reported HBcAb (46.1%) is more or less similar to that reported previously in Sudan (47.5%) by Mudawi and less than that found by El Mukashfie (57% ). \[190; 11\]

HBsAg is a marker of active HBV infection. The main finding of the present study was the high seroprevalence (18%) of HBsAg among the pregnant women in North Darfur. This result supports the WHO's report for Sudan as highly endemic area with prevalence greater than 8% for HBV. \[8\]

Seroprevalence of HBsAg among these women was different from that reported among the pregnant women in central Sudan, (5.6%) by Elsheikh in pregnant women visiting Umdurman Maternity Hospital and also to what was found by Abuelgasim and Baraka (7.5%) among pregnant women who presented to the antenatal clinic of Khartoum Teaching Hospital and by Osman et al (5.1%) among pregnant women at Wad Madani hospital. \[195; 196; 197\] Perhaps; the differences in the prevalence rate could be explained by either absence of blood screening among our study
population, or the screening of the rapid diagnostic tests which are less sensitive than ELISA.

Similar findings to our result (18%) were reported earlier from rural surveillance in El Gazira State by Hyams et al (18.7%) and (17.3%) from laboratory technical staff in the Gazira region by El shafie.\textsuperscript{[10; 191]} Seroprevalence of HBsAg among our women was higher than that reported among the Health care workers in Khartoum teaching hospital by Elduma and Saeed (4.89%), (6%) by El Mukashfie and to that reported by Osman et al (4.04%).\textsuperscript{[192; 11; 197]}

Seroprevalence of HBsAg among our population was higher than that reported from other population groups in different regions of Sudan; (6.25%) among blood donors in Nyala town by Abou et al and (6.9%) among population of Um Zukra village, in El Gazira State by Mudawi.\textsuperscript{[194; 190]} The probable explanation for the higher prevalence of HBsAg in our study in comparison to the previous reports in Khartoum; may be the improvement of blood screening procedures and the introduction of the HBV vaccination program among the health care workers in Khartoum State.

In our study, seroprevalence of HBsAg in pregnant women was as high as that in other African countries; for example, 10.7% in Mauritania, 12.5% in Nigeria, 12.6% in Ghana, 9.2% in Gabon, 11.4% in Burkina Faso.\textsuperscript{[154; 161; 159; 168; 167]} However, lower seroprevalence of HBsAg was reported from neighboring countries; like 1.6% in Egypt, 1.5% in Libya, 4.9% in Ethiopia, 1.6% in Algeria and 4.2% in Tanzania.\textsuperscript{[146; 148; 175; 149; 176]} Perhaps, the differences in the prevalence rate could be explained by effect of vaccination that was applied in some of these countries earlier than in Sudan. The high seroprevalence of HBsAg observed in this study could be an indication that pregnant women serve as a very important reservoir to fuel the HBV epidemic in the general population. However, HBeAg which is marker of infectivity and also an
indicator of transmissibility, \(^{[24]}\) was found in 2.6% of the HBsAg positive pregnant women. This is far lower than that (70%) reported by Hyams from his rural surveillance in El Gazira region but similar to that reported from Congo Brazzaville (2.05%). \(^{[10; 169]}\) Whereas, some neighboring countries reported higher HBeAg seroprevalence; like (21.7%) in Lybia, (10%) in Gabon, (33%) in Uganda and Rwanda and (18.2%) in Burkinafaso. \(^{[148; 168; 170; 166]}\) This may indicate that vertical transmission is less important in our population. On the other hand HBeAb which is recognized to be a clinical sign of recovery from the infection was found in (37.7%) of the women investigated. This is lower than that reported in Burkinafaso (66.7%) and in Gabon (89.9%). \(^{[166; 168]}\)

In our study the seroprevalence profile of the women investigated which were HBsAg\(^{-}\)/anti-HBc\(^{+}\) may indicate that either they had been in contact with the virus, recovered, and maintained detectable HB- anticore antibody levels after natural infection or if screened for containing HBV DNA, can meet the definition of occult HBV infection (OBI). Those women who were HBsAg\(^{+}\)/anti-HBc\(^{+}\) expected to be chronic carriers. As reported by Zukerman that; more frequently, HBsAg persists during a second phase of chronicity as a result of the expression of integrated viral DNA. \(^{[22]}\)

The presence of anti- HBe antibody is recognized to be a clinical sign of recovery the infection. In our study HBeAb is detected in 58/154(37.66%) of the study population. Samples with profile HBsAg\(^{+}\)/HBeAg\(^{+}\)/HBeAb\(^{+}\) indicate that clearance of HBeAg occur following the natural infection and antibodies raised. Whereas, demonstration of anti-HBe in samples without HBeAg as in the samples
with profile HBsAg+/HBeAg−/HBeAb+ may be an indication of presence of HBV mutants. [24]

Our data showed that only 1.78% of our studied women declared that they had previously received HBV vaccine. Some studies also reported absence of the vaccination program among their studied cases. [146] This indicates that vaccination program is also not available in some other countries as well.

Concerning socio-demographic status, the age at which HBV infection occurs is one of the main factors that Predispose to the acquisition and frequency of the chronic carriage status. [4] This is known to vary greatly throughout the world. Whereas, Mudawi reported that age pattern of exposure to HBV infection differed between various geographic locations in Sudan. [200]

Younger age was identified to be one of the risk factors for being HBsAg-positive among pregnant women in the Gulf States as reported by Al Awaidy. [189] In our study carriers of HBsAg was higher in those with age range 15-25 years (42.5%) then declined with increasing age range 26 -36 years (38.5% ) and 36 – 50 years (36%). However, the difference was statistically insignificant (P = 0.67). (Table 6) This is similar to what was reported by Hyams et al who found that the prevalence of HBsAg was highest in subjects less than 5 years of age. [10] This is in contrast to what was reported by Mudawi; that exposure to HBV infection was highest in those over the age of 50 years and lowest in those under the age of 10 years. [190] Other contrast studies reported that HBV infection in pregnant women increased with age like what was found by El Magrahe et al and EL Shabrawi et al in Libya and Egypt respectively. [148,146] The difference may be attributed to the young age marriage and pregnancy of women in rural Darfur as general.
In our study residence was associated with HBsAg seropositivity ($p = 0.01$). Some studies reported that socioeconomic conditions among the poor and less educated, and crowded living condition especially in the rural areas, may contribute to HBV exposure.\cite{201,202} Whereas Woodruff et al. concluded that the prevalence of HBV did not differ by educational level, occupation, or rural versus urban residence.\cite{203}

In this study the prevalence of HBsAg was higher in women from urban (44.4%) than in rural area (36.2%). As stated by Abongwa and Keneth; this may be due to the higher rates of risky life-style practices in most urban towns and the increased migration from rural to urban for purpose of education or employment.\cite{204} In this study 64% of the women residing in town; mostly were displaced and came to live on the periphery of the city following the conflict in this region. (Table 7)

The present study showed significant association between HBsAg seroprevalence and occupation ($P = 0.02$). Occupation is stated as a known predisposing factor for HBV infection.\cite{205} However, in our study most of the women were housewives (87%); and were more affected than other categories, HBsAg positivity was found in 41.9% (149/356) of them. This indicates that housewives need to be oriented about sexual diseases and their transmission. Higher rate was noticed in women who work in the medical field (60%) although they represent only 1% of the study population. (Table 10)

Regarding the level of education as a risk factor for acquisition of HBV, we found no significant association between the education level and the disease seroprevalence ($P = 0.69$). Women with primary and secondary education constituted more than half the study population 501(55.7%), HBsAg prevalence was (39.9%) in primary and (46.6%) in secondary levels. One third of the women were illiterate
271(30%), HBsAg prevalence was (37.9%), whereas in those with high education level which constituted 80(8.9%) of the study population, HBsAg prevalence was 11(36.7%). This is in contrast to what found by Abongwa and Kenneth; that the more educated the pregnant population, the lower the prevalence rates.\textsuperscript{[204]} (Table 8)

In this study significant association was found between income and the HBsAg seroprevalence (P = 0.01), since that 90% of our study population were in low income class, a high carrier rate of HBsAg was noticed among them [40.4% (145/359)]. However, 53% (8/15) of the women in medium income class were carriers of HBsAg, but their number in the study population was too low to make a reasonable judgment. (Table 9)

Pregnant women are considered at a higher risk due to increased exposure to risk factors (as blood transfusion, caesarian section).\textsuperscript{[206]} Although statistically there is no association between women who were having caesarian section and HBsAg seropositivity (P = 0.38), HBsAg was positive in 46% (23/50) of the women who answered that they had caesarian section during the interview, which may be due to insufficient sterilization of the surgical instruments. (Table 12)

Our study showed that ear piercing was a significant risk factor (Odd ratio = 1.9, 95% CI = 1.3 – 2.9, P = 0.003). During the interview most of the women answered that they use some plant thorns to make the ear piercing.

Significant association with HBsAg seropositivity was also found in cases of bloodletting (OR = 0.2, 95% CI = 0.1 – 0.9, P = 0.02). Bloodletting is a traditional healing practiced by many people in this area that is carried out without efficient sterilization of the equipments used. (Table 11)
Different contributing factors for HBV infection was reported in other studies. Surgery, jaundice, blood transfusion, was associated with HBsAg seropositivity. 

In this study no other clinical interventions as (surgery, dental procedure, tattooing, unsafe injection, abortion, jaundice, and blood transfusion) were significantly associated with HBsAg seropositivity ($P > 0.5$). The explanations for such observations need to be explored in the future. (Table 11)

Although HBV represents an important health problem in Sudan, data on the molecular diversity of HBV to assess features of the viral genotypes circulating is scarce. Several studies reported that different HBV genotypes have a significant role in determining the clinical outcome of liver diseases and the response to antiviral therapies. Accordingly; in this study we evaluated the HBV genotypes circulating in the area. This woman cohort could also be a representative of the general HBsAg positive population of North Darfur.

To investigate the distribution of HBV genotypes in our study population, we applied a multiplex PCR based methodology. Although the most common method for HBV genotyping is by PCR-RFLP technique, but it is reported that HBV genotyping by nested PCR with type specific primers is more sensitive than genotyping system using RFLP analysis. Africa is one of the highly endemic regions for HBV, with five HBV genotypes (A–E) predominating. In our study different genotypes were found including A, D and E, of which genotype D (31.96%) and genotype E (30.93%) have almost the same frequency and mixed genotypes were detected in 34% of the HBsAg positive pregnant women. (Figure V) This indicated that there could be a high genetic diversity of HBV in this area. These three genotypes (A, D and E) were
reported previously in central Sudan, in asymptomatic blood donors. It was found that 57.5% of these subjects were infected with genotype E, 40.5% with genotype D (9) and in patients with liver disease, 59% were infected with genotype D, 30% with genotype E, 8.5% with genotype A and 2.5% had putative D/E recombinant. [198; 199]

Depending on the fact that prevalence of HBV genotypes throughout the world is clearly linked to migration and the fact that this part of Sudan is sharing borders with some African countries and many of the people living in Darfur having origins from west African countries; it is not unexpected to find different viral types circulating. Similarly high genetic diversity was also reported from some neighboring countries. Genotype E in Central African Republic, genotypes A, D and E in Kenya, genotypes D, E and A in Mauritania and genotype D in Egypt. [171; 173; 154; 147]

Co-infection with different HBV-genotypes was reported to be associated with altered pathogenesis and clinical outcome, [208] thus; it is an important finding that 34% of our subjects were infected with mixed HBV genotypes. This is similar to a number of studies from different regions of the world. Mixed HBV genotypes infections were detected in different regions of the world e.g Egypt, Tunisia and Morocco in Africa [210; 151; 153] and Oman and Pakistan in Asia [184; 211] and in Europe. [212]

In our study, 51 (34.46%) HBV positive samples remained negative for HBV genotypes. It may be assumed that such samples represent recombinant or new genotypic variants present in our population that can be resolved after sequencing and further analysis. Because, some minor HBV genotypes as well as novel or distinct genotypic groups may be present in any population besides major genotypes. [73]
Limitations of the Study

Regarding the high prevalence of HBsAg in the current study; the population investigated consisted only of women who were able to access antenatal care, and the prevalence reported here may have underestimated the true prevalence among pregnant women in the larger community.

We investigate HBV infectivity based on HBeAg and HBeAb and we did not look for HBV viral load which is also important determinant of HBV transmission.
Chapter Six
Conclusions
Recommendations
References
Appendices
Conclusion

A total of 414/900 (18%) of the pregnant women studied were sero-positive for HBsAg indicating high prevalence of HBV infection in North Darfur. The three different genotypes A, D and E found circulating in the pregnant women indicated that there could be a high genetic diversity of HBV in North Darfur State. By Highlighting the burden of the disease in this region attention can be drawn to this sector of the community to strengthen the existing health program specially health education in this part of the world.1

Recommendations

- The results of the study suggest that HBV has a high prevalence among the pregnant women in Al Fashir town, North Darfur State. Therefore, the need to institute public health measures to reduce disease burden and transmission, including routine screening of all pregnant mothers for HBV infection.
- Also, the pregnant women were infected with more than one HBV genotypes; therefore, it is of paramount importance for clinicians to adopt better strategies for prevention of infection. We also suggest that HBV genotyping become a routine exercise in clinical medicine and molecular epidemiology.
- Since a specified group of subjects were employed in our investigation, we propose that large scale studies be conducted to substantiate our findings. Such studies could also provide more insight into the association between co-infection and disease exacerbations as well as shed light on the molecular, virological and host mechanisms underlying the pathogenesis of HBV-related disease.
References


100. Chu CM and Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. J Hepatol. 2005; 43(3):411-417.


Appendices
Questionnaire

1. Name.............................................................................................................
2. Age.............................................................................................................
3. Residence: - Urban...............................................................
   - Rural.................................................................
   - Others..............................................................
4. Occupation...................................................................................................
5. Education......................................................................................................
6. Income (SDG).................................................................
7. History of: (Yes / No)
   - Surgical Procedure..............................................
   - Dental Procedure..............................................
   - Tattooing...........................................................
   - Unsafe Injection.................................................
   - Caesarian Section.............................................
   - Abortion...........................................................
   - Jaundice............................................................
   - Blood Transfusion...........................................
   - Ear Piercing.....................................................
   - Blood Letting.....................................................
   - Vaccination.......................................................