Prevalence of Hepatitis B Surface Antigen among the Surgical Patients in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynecology (2013)

*Mohamed alhassan Taha Said*


A Dissertation

Submitted to the University of Gezira in Partial Fulfillment of the Requirements for the Award of the Degree of Medical Doctorate in

Clinical Pathology

Department of Pathology

Faculty of Medicine

January 2014
Prevalence of Hepatitis B Surface Antigen among the Surgical Patients in *Wad Medani* Teaching Hospital and *Wad Medani* Hospital for Obstetrics and Gynecology (2013)

Mohamed alhassan Taha Said

**Supervision Committee:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Sumia Elhag Mohmed ElAmin</td>
<td>Main Supervisor</td>
<td>..........</td>
</tr>
<tr>
<td>Prof. Ahmed Abd alla Mohamedani</td>
<td>Co-supervisor</td>
<td>..........</td>
</tr>
</tbody>
</table>

**Date:** 16/1/2014
Prevalence of Hepatitis B Surface Antigen among the Surgical Patients in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynecology (2013)

Mohamed alhassan Taha Said

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Ahmed Abd alla Mohamedani</td>
<td>Chair person</td>
<td>...............</td>
</tr>
<tr>
<td>Prof. Mohmed Sir alkhaim Ali</td>
<td>External Examiner</td>
<td>...............</td>
</tr>
<tr>
<td>Dr. Hassan Ali Musa</td>
<td>Internal Examiner</td>
<td>...............</td>
</tr>
</tbody>
</table>

Date of Examination: 4/2/2014
To the memory of my father.

To my mother, and to my wife.

To everyone who believed in my abilities, and supported me in my intention to make some of my dreams come true.
I wish to acknowledge a considerable debt to many people who helped me in this study. I am especially grateful to Dr. Sumia El haj for her close supervision offering invaluable guidance during the course of this work.

I am deeply grateful to my co-supervisor Prof. Ahmed Aba alla Mohammedani for his help and suggestions.

I am also grateful to my colleagues, laboratory staff at Wad Medani Obstetrics & Gynaecology Hospital; and to the nurses of the surgery wards at Wad Medani Teaching Hospital.

My thanks and deep gratitude is also extended to Dr. Isam Elkhidir, Mr. Mohamed Omer Babikir and Mr. Qiaus Elhadi Babikir.
Prevalence of Hepatitis B Surface Antigen (HBsAg) among the Surgical Patients in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynecology Diseases (2013)

Mohamed alhassan Taha Said

Abstract

Hepatitis B is a viral infection that attacks the liver, and can cause both acute and chronic diseases. The severe pathological consequences of this infection include the development of cirrhosis and hepatocellular carcinoma (HCC). It is a major health problem in many countries of the world, especially those in Asia, Middle East, and Africa. This is a prospective descriptive cross-sectional study aimed to determine the prevalence of hepatitis B surface antigen (HBsAg) among the surgical patients in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynecology Diseases, using the rapid immunochromotograpy (ICT) test and an enzyme-linked immunosorbent assay (ELISA). One hundred seventy three patients, planned for different surgical operations from the two hospitals were enrolled in this study. Out of these patients 10 were positive by the rapid ICT test giving 5.7 % prevalence and 11 were positive by ELISA giving 6.4 % prevalence. The prevalence of HBsAg is higher in males than that in females. The virus is common among the surgical patients of age group between 30 - 45 years, and also common in those patients who have lower education level. The common risk factors associated with HBsAg seropositivity among the surgical patients in the two hospitals showed that; tattooing and cauterization , history of previous surgery or dental procedure and blood transfusion. The rapid ICT device used in this study showed 90.9% sensitivity, and 100% specificity when compared to the ELISA as a gold standard technique for the detection of HBsAg, so the rapid ICT kits can be a good choice for the screening of the patients specially in rural areas where laboratory facilities are not available, and also it can be used in the screening of the patients before the emergency operations. Considering this prevalence of HBsAg among the surgical patients in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynecology Diseases, this study recommended that routine pre-operative screening of all patients for hepatitis B should be mandatory and all the preventive measures should be adopted to prevent further spread of this virus infection. Hepatitis B vaccination should be given to high risk groups especially to health care workers so as to protect them and to reduce the further transmission of this virus to patients.
انتشار المستضد السطحي لفيروس الكبد البائي وسط مرضى العمليات الجراحية بمستشفى ودمدني التعليمي ومستشفى ودمدني لأمراض النساء والولادة (2013 م)

محمد الحسن طه سيد

ملخص الدراسة

فيروس الكبد البائي هو حمى حموي (فيروسي) يصيب الكبد، مما يؤدي إلى إلتهاب حاد أو إلتهاب مزمن. يسبب الفيروس تشمع الكبد وسرطان الخلايا الكبدية. يعتبر هذا الفيروس من أعظم المشاكل الصحية المنتشرة في العالم، بكونه خطرًا على الصحة للذكور والإناث، وهو خطر أكبر في المناطق الريفية البعيدة، وخصوصًا في المناطق الشرقية. أجريت هذه الدراسة لتحديد نسبة إنتشار المستضد السطحي لفيروس الكبد البائي وسط مرضى العمليات الجراحية في مستشفى ودمدني التعليمي ومستشفى ودمدني لأمراض النساء والولادة في فترة من فبراير وحتى الأول من مايو 2013 م. شملت الدراسة 173 مريضاً خضعوا للعمليات الجراحية في مستشفى ودمدني التعليمي ومستشفى ودمدني، وشملت بيانات النتائج على 7.7% (نسبة 10 من المرضى) من المرضى حاملين للمستضد السطحي لفيروس الكبد البائي، و4.6% (نسبة 11 من المرضى) حاملين للمستضد السطحي لفيروس الكبد البائي، وذلك باستخدام اختبار الاستشراب المناعي السريع، الذي يعتبر من اختبارات المراقبة المتميزة للكشف عن المستضد السطحي لفيروس الكبد البائي. كما تحقق نسبة 91.9% ونسبة الدقة 11% مقارنة مع اختبار المقاييس المناعي (إليزا). اعتماداً على هذه النتائج، أوصت الدراسة بضرورة إجراءات الوقاية من إنتشار الفيروس، خاصة في المناطق الريفية البعيدة، حيث لا يتوفر فيها خدمات التشخيص والعلاج. كما تشير الدراسة إلى أهمية استخدام الفحص السريع كفحص بديل للكشف عن المستضد السطحي لفيروس الكبد البائي، والذي يمكن أن يكون فعالًا في المناطق الريفية البعيدة، حيث لا يوجد فحص إلإيزا. كما يمكن استعماله قبل العمليات الجراحية المستجدة.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supervision committee</td>
<td>ii</td>
</tr>
<tr>
<td>Examination committee</td>
<td>iii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgment</td>
<td>v</td>
</tr>
<tr>
<td>Abstract in English</td>
<td>vi</td>
</tr>
<tr>
<td>Abstract in Arabic</td>
<td>vii</td>
</tr>
<tr>
<td>Table of contents</td>
<td>viii</td>
</tr>
<tr>
<td>List of contents</td>
<td>ix</td>
</tr>
<tr>
<td>List of tables</td>
<td>xi</td>
</tr>
<tr>
<td>List of figures</td>
<td>xii</td>
</tr>
<tr>
<td>List of abbreviations</td>
<td>xiii</td>
</tr>
</tbody>
</table>

## CHAPTER ONE
### INTRODUCTION

1.1 Introduction

## CHAPTER TWO
### LITERATURE REVIEW

2.1 Historical notes
2.2 Classification
2.43 Structure and composition
2.4 Viral genome
2.5 Serotypes
2.6 Genotypes
2.7 Virus mutants
2.8 Stability
2.9 Virus replication
2.10 Pathogenesis
2.11 Pathology
2.12 Clinical features
2.12.1 Clinical features of acute hepatitis B
2.12.1.1 Fulminant hepatitis
2.12.2 Clinical features of chronic hepatitis B
2.12.2.2 Chronic HBV and hepatocellular carcinoma
2.12.3 Extrahepatic manifestations of hepatitis B
2.12.4 Coinfection or superinfection with hepatitis D virus
2.12.5 Occult HBV infection
2.13 Laboratory diagnosis
2.13.1 Hepatitis B surface antigen
2.13.2 Anti- Hepatitis B surface antigen
2.13.3. Hepatitis B core antigen
2.13.4 Anti-hepatitis B core antigen
<table>
<thead>
<tr>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.13.5 Hepatitis B e antigen</td>
<td>18</td>
</tr>
<tr>
<td>2.13.6 Anti-hepatitis B e antigen</td>
<td>18</td>
</tr>
<tr>
<td>2.13.7 Hepatitis B x antigen</td>
<td>18</td>
</tr>
<tr>
<td>2.13.8 HBV DNA</td>
<td>18</td>
</tr>
<tr>
<td>2.13.9 HBV DNA polymerase</td>
<td>19</td>
</tr>
<tr>
<td>2.14 Treatment</td>
<td>21</td>
</tr>
<tr>
<td>2.15 Epidemiology</td>
<td>23</td>
</tr>
<tr>
<td>2.16 Prevention</td>
<td>23</td>
</tr>
<tr>
<td>2.16.1 Passive immunization</td>
<td>25</td>
</tr>
<tr>
<td>2.16.2 Active immunization (vaccine)</td>
<td>25</td>
</tr>
<tr>
<td>2.17 Rationale</td>
<td>27</td>
</tr>
<tr>
<td>2.18 Research objectives</td>
<td>28</td>
</tr>
<tr>
<td>2.18.1 General objective</td>
<td>28</td>
</tr>
<tr>
<td>2.18.2 Specific objectives</td>
<td>28</td>
</tr>
<tr>
<td>3.1 Study design</td>
<td>29</td>
</tr>
<tr>
<td>3.2 Study area</td>
<td>29</td>
</tr>
<tr>
<td>3.3 Study setting</td>
<td>29</td>
</tr>
<tr>
<td>3.4 Study population</td>
<td>29</td>
</tr>
<tr>
<td>3.5 Inclusion criteria</td>
<td>29</td>
</tr>
<tr>
<td>3.6 Exclusion criteria</td>
<td>29</td>
</tr>
<tr>
<td>3.7 Sampling</td>
<td>30</td>
</tr>
<tr>
<td>3.7.1 Sample size</td>
<td>30</td>
</tr>
<tr>
<td>3.7.2 Sampling technique</td>
<td>30</td>
</tr>
<tr>
<td>3.8 Data collection tools</td>
<td>30</td>
</tr>
<tr>
<td>3.9 Variables</td>
<td>30</td>
</tr>
<tr>
<td>3.10 Methods and materials</td>
<td>31</td>
</tr>
<tr>
<td>3.10.1 HBsAg detection by ICT</td>
<td>31</td>
</tr>
<tr>
<td>3.10.1.1 Principle</td>
<td>31</td>
</tr>
<tr>
<td>3.10.1.2 Procedure3</td>
<td>31</td>
</tr>
<tr>
<td>3.10.1.3 Interpretation of the result</td>
<td>32</td>
</tr>
<tr>
<td>3.10.2 HBsAg detection by ELISA</td>
<td>32</td>
</tr>
<tr>
<td>3.10.2.1 Principle</td>
<td>32</td>
</tr>
<tr>
<td>3.10.2.2 Assay procedure</td>
<td>32</td>
</tr>
<tr>
<td>3.10.2.3 Interpretation of the result</td>
<td>33</td>
</tr>
<tr>
<td>3.11 Data management</td>
<td>34</td>
</tr>
<tr>
<td>3.12 Ethical consideration</td>
<td>34</td>
</tr>
<tr>
<td>4.1 Results</td>
<td>35</td>
</tr>
<tr>
<td>4.2 Discussion</td>
<td>43</td>
</tr>
<tr>
<td>TITLE</td>
<td>PAGE</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>CHAPTER FIVE</td>
<td></td>
</tr>
<tr>
<td>CONCLUSION AND RECOMMENDATIONS</td>
<td></td>
</tr>
<tr>
<td>5.1 Conclusion</td>
<td>46</td>
</tr>
<tr>
<td>5.2 Recommendations</td>
<td>47</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>47</td>
</tr>
<tr>
<td>APPENDIX</td>
<td></td>
</tr>
<tr>
<td>Questionnaire</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 2.1  Serological Markers of HBV infection .....................Page 21

Table 4.1  Demographic criteria of the patients in this study........ Page 37

Table 4.2  The ICT parameters compared to ELISA ................. Page 42
LIST OF FIGURES

Figure 2.1 A diagram of complete HBV particle……………………Page 4

Figure 2.2 Hepatitis B virus genome organization …………………… Page 6

Figure 2.3 The outcome of hepatitis B infection …………………… Page 14

Figure 2.4 Typical course of hepatitis B infection ……………………Page 20

Figure 2.5 Worldwide prevalence of hepatitis B carriers and primary hepatocellular carcinoma…………………………… Page 24

Figure 4.1 Sex distribution of the patients enrolled in this study …..Page 38

Figure 4.2 Prevalence of HBsAg by ICT……………………………. Page 39

Figure 4.3 Prevalence of HBsAg by ELISA……………………………Page 39

Figure 4.4 Sex distribution among HBsAg positive patients……… Page 40

Figure 4.5 Age distribution among HBsAg positive patients……… Page 41
LIST OF ABBREVIATIONS

Anti-HBs   Anti Hepatitis B surface
Anti-HBc   Anti Hepatitis B core
ALT       Alanine aminotransferase
AST       Aspartate amino transferase
BP        Base pair
DNA       Deoxyribo Nucleic Acid
Ds DNA    Double Stranded DNA
ELISA     Enzyme linked-immunosorbent assay
HBV       Hepatitis B virus
HBcAg     Hepatitis B core antigen
HBcAb     Hepatitis B core antibody
HBsAg     Hepatitis B surface antigen
HBsAb     Hepatitis B surface antibody
HBeAg     Hepatitis B e Antigen
HBeAb     Hepatitis B e antibody
HBx       Hepatitis B x antigen
HAV       Hepatitis A virus
HCV       Hepatitis C virus
HDV       Hepatitis D virus
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEV</td>
<td>Hepatitis E virus</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HCWs</td>
<td>Health care workers</td>
</tr>
<tr>
<td>ICT</td>
<td>Immunochromatography</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>OBI</td>
<td>Occult Hepatitis B infection</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

Hepatitis is a medical condition defined as inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. The name is derived from the Greek word hepar, being hepat-, meaning liver.\(^1\)

The liver is a vital organ that processes nutrients, filters the blood and fights infections. When the liver is inflamed or damaged, its function can be affected.\(^2\) Many illnesses and conditions can cause hepatitis for example drugs, alcohol, chemicals and autoimmune diseases.\(^3\)

Viral hepatitis is reserved for infection of the liver caused by a group of viruses having a particular affinity for the liver. It may present in acute or chronic forms. The most common causes of viral hepatitis are the five hepatotropic viruses: Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D and Hepatitis E.\(^4\)

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus.\(^5\) It is also called type B hepatitis, serum hepatitis, and homologous serum jaundice.\(^6\)

It is estimated that more than one third of the world's population has been infected with HBV. About 5% of the populations are chronic carriers of HBV, and nearly 25% of all carriers develop serious liver diseases such as chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma (HCC). HBV infection causes more than one million deaths every year.\(^6\)

Infection with HBV is primarily blood borne (parenteral transmission). Routes of parenteral transmission include contaminated blood and blood products, sharing unsterilized injection needles for intravenous drug use, reuse of contaminated razors by barber, haemodialysis, and acupuncture needles,
tattooing devices, and contaminated medical instruments. Sexual and perinatal) vertical) transmission from mother to child usually results from mucous membrane exposure to infectious blood or body fluids.

HBV is stable on environmental surfaces for at least 7 days, an indirect inoculation of HBV can occur via inanimate objects. HBV is 100 times more infectious than HIV, and 10 times more infectious than HCV.\textsuperscript{[6]}

Health care personnel and laboratory workers are at risk from patients. Surgery, dental surgery, obstetrics and gynecology often involve working with sharp instruments, often in restricted spaces. Operators may injure themselves and inoculate the patient's blood. The spilling of patient's blood will pose a threat if there is contamination of unprotected skin, with abrasions, or mucous membranes. Patients are also at risk from staff, and episodes have been identified in which a surgeon, gynecologist, dentist, or other staff member has transmitted the virus to patients invasive procedures.\textsuperscript{[7]}
CHAPTER TWO

LITERATURE REVIEW

2.1 Historical notes:

Early Mesopotamian civilizations thought that liver was the basis of the life. The manifestations of liver disease such as hepatitis B included jaundice, characterized by Hippocrates and found to be infectious as early as the 8th century. By 1885, hepatitis was found to be transmissible through blood transfusions and syringes when epidemics of jaundice broke out during the wars of the 17th - 19th centuries. During World War II, a series of out breaks occurred after vaccination for measles and yellow fever, implying further that the virus was blood-borne. In 1947, the Mac Callum classified viral hepatitis into two types: viral hepatitis A, or infectious hepatitis, and viral hepatitis B, or serum hepatitis. In 1965, Baruch Blumberg, discovered the Australia antigen (later known to be Hepatitis B surface antigen, or HBsAg) in the blood of aborigines. He had been studying samples of multiply transfused haemophilicas for polymorphic antibodies, and showed that HBsAg had high presence in leukemia and Down's syndrome patients. Later in 1968, Prince and Okochi isolated the Australia antigen in hepatitis B patients and from this information, along with the discovery of Dane particle HBV particle (complete in the 1970), the first vaccine for hepatitis B was produced in 1981 and licensed as ((Heptavax)).[8]

2.2 Classification:

The hepatitis B virus is classified as the type species of the Orthohepadna viruses. The genus is classified as part of the Hepadnaviridae family.[9]

2.3 Structure and composition:

Ultra structural examination of sera from hepatitis B patients shows three distinct morphological forms. The predominant form is small, spherical particle
with a diameter of 22 nm. Tubular or filamentous forms, which have the same diameter but may be over 200 nm long. Both types of particle are composed of lipid, protein and carbohydrate; they are not infectious and consist solely of surplus virion envelope. They contain HBsAg polypeptides. The third type of particle, the virion or *Dane particle* has a diameter of 42 nm and consists of an outer envelope which contains HBsAg. The envelope surrounds the nucleocapsid core (HBcAg) which surrounds the viral genome acid and the DNA polymerase. HBeAg is also a part of the core. (Fig.2.1).

![Figure 2.1 A diagram of complete HBV particle.](image)

There may be as many as $10^{13}$ of the small particles and filaments per milliliter. The virions are present in much smaller number, usually by a factor of $10^3$ or more, and the proportion varies considerably in different stages of the disease. (7)

### 2.4 The viral genome:

Electron microscopy gave the initial views of the hepatitis B genome. The genome appears partially double-stranded circular DNA, approximately 3200 bp in length. The full-length DNA minus strand (L or long strand) is complementary to all HBV mRNAs; the positive strand (S or short strand) is variable and between 50 and 80% of unit length. (10) The minus strand contains four open reading frames (ORF): pre-S/S, pre-C/C, P and X. The P gene, which compromises 80% of the genome, encodes a polymerase with three distinct enzymatic functions (DNA polymerase, reverse transcriptase, and RNase H) and
also encodes the terminal protein primer. Gene X spanning the cohesive ends of
the genome, encodes a transactivating protein that up-regulates transcription.
The C gene has two initiation sites that divide it into a pre-C and C region,
producing two distinct proteins hepatitis B e antigen (HBe Ag) and HBcAg,
respectively. The pre-S/S gene encodes the envelope protein, S, which occurs in
three forms: a large (L) protein, translated from the first of the three in-phase
initiation codons, is a single polypeptide encoded by the pre-S1, pre-S2, plus S
regions of the genome and occurs in the envelope of infectious virions; a
middle-sized (M) protein comprises the product of pre-S2, plus S ; and finally,
the most abundant product is the S protein the basic constituent of noninfectious
HBsAg particles, comprising only the product of the S ORF.\textsuperscript{[10]} (Fig. 2.2).

2.5 Serotypes:

Traditionally, HBV is classified into 4 subtypes or serotypes [adr,adw, ayr, and ayw]
based on antigenic determinants of the HBsAg. These subtypes can be further classified into 9
serotypes.\textsuperscript{[11]} These virus-specific markers are useful in epidemiologic studies because they
are concentrated in certain geographic areas.\textsuperscript{[9]}

2.6 Genotypes:

HBV is characterized by a high genetic variability resulting in the recognition of eight
well-established genotypes (A-H) based on >7.5% intergroup divergence in the
complete genome, and several subgenotypes within these genotypes. Subgenotypes are
defined by more than 4% intra-genotypic divergence but not greater than 7.5%.\textsuperscript{[11]}

The genotypes show a distinct geographical distribution; genotype E is found
predominantly in sub-Saharan Africa, where as genotypes B and C are found in
the far East, and Genotypes F and H in Central and South America.\textsuperscript{[7]} Recently,
a ninth genotype tentatively termed “I” was proposed.\textsuperscript{[12]}
Figure 2.2 Hepatitis B virus genome organizations.
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.¹³

In Khartoum, Sudan, phylogenetic analysis showed that genotype E was dominant, followed by genotype D. Strains of other genotypes such as the European A2 or the Asian B genotypes were occasionally detected.¹⁴

2. 7 Hepatitis B virus mutants:

Using refined molecular biological methods in recent years, more and more HBV mutants have been found with one or more amino acid exchange in certain proteins.¹⁵ A mutation rate of 10 times has been reported compared with other DNA viruses. These mutations can occur naturally as well as due to selective pressure from antiviral therapy.¹⁶

2.8 Stability of HBV:

It is difficult to assess the stability of HBV owing to the lack of a suitable laboratory culture system.⁷ HBsAg is stable at −20°C for over 20 years and stable to repeated freezing and thawing. The virus is also stable at 37 °C for 60 minutes and remains viable after being dried and stored at 25º C for at least one week.

HBV is sensitive to higher temperatures (100 °C for 1 minute) or to longer incubation periods (60º C for 10 hours). HBsAg is stable at ph 2.4 for up to six hours, but hepatitis B virus infectivity is lost.

Treatment with hypochlorite or 2% gluteraldehyde for 10 minutes will inactivate the virus.⁷ HBsAg is not destroyed by ultraviolet irradiation of plasma or other blood products, and viral infectivity may also resist such treatment.¹⁰
2.9 HBV replication:

The infectious virion attaches to cells and becomes uncoated. In the nucleus the partially double-stranded viral genome is converted to covalently closed circular double-stranded DNA (cccDNA). The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The pregenome RNA becomes encapsidated with newly synthesized HBcAg. Within the cores, the viral polymerase synthesizes by reverse transcription a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell.\cite{10}

2.10 Pathogenesis:

The specificity of HBV for liver cells is based on two properties: virus-specific receptors located on the hepatocytes cell membrane (facilitate entry) and transcription factors found only in the hepatocytes that enhance viral mRNA synthesis (act post-entry). After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens. The pathogenesis of hepatitis B is probably the result of this cell-mediated immune injury, because HBV itself does not cause a cytopathic effect.

Lifelong immunity occurs after the natural infection and is mediated by humoral antibody against HBsAg. HBsAb is protective because it binds to surface antigen on the virion and prevents it from interacting with receptors on the hepatocyte.\cite{17}

2.11 Pathology:

All types of viral hepatitis produce similar changes at the histological level. In the acute stage there are signs of inflammation in the portal triads: the
infiltrate is mainly lymphocytic. In the liver parenchyma, single cells show ballooning and form acidophilic (Councilman) bodies as they die. In healthy carriers, the inflammatory response is mild, and the affected hepatocytes are pale staining and glassy.

In chronic hepatitis, damage extends out from the portal tracts, giving rise to the piecemeal necrosis appearance. Some lobular inflammation is also seen. As the disease progress fibrosis develops and, eventually, cirrhosis.[7]

2.12 Clinical features:

The course of hepatitis B may be extremely variable.[18] HBV infection has different clinical manifestations depending on the patient’s age at the infection, the immune status and the stage at which the disease is recognized. The infecting dose of the virus correlates with the severity of acute or chronic hepatitis B.

HBV has a long incubation period, varies usually between 45 and 180 days with an average of 60 to 90 days. Most cases of HBV are subclinical.[6]

2.12.1 Clinical features of acute hepatitis B:

The course of acute viral hepatitis is conventionally divided into three phases: (1) preicteric, (2) icteric, and (3) post icteric.[19]

The preicteric (prodromal) phase commences with malaise, lethargy, anorexia, and commonly nausea, vomiting, and pain in the right upper abdominal guardant. A minority of patients develop at this time a type of serum sickness characterized by mild fever, urticarial rash and polyarthritis.[7,10,19]

The icteric phase of acute viral hepatitis begins usually within 10 days of the clinical symptoms with the appearance of dark urine (bilirubinuria) followed by pale stools and jaundice. It is accompanied by hepatomegaly and splenomegaly.[6]
The *convalescent* phase may be long and drawn out, with malaise and fatigue lasting for weeks. Most adults’ patients recover uneventfully following complete regeneration of damaged liver within 2-3 months, but some may progress to chronic infection.\[6,19\]

The outcome depends upon several factors, including the virulence of the virus and the immunocompetence and age of the patient as well as some genetic factors.\[20\]

### 2.12.1.1 Fulminant hepatitis:

Fulminant hepatitis B is a rare condition that develops in about 1% of cases. The hepatic insufficiency progresses from onset of symptoms to hepatic encephalopathy within 2 to 3 weeks. A less rapid course, extending up to 3 months, is called *subfulminant failure*.\[4\] It is caused by massive necrosis of liver substance and is usually fatal.\[6,19\]

Fulminant hepatic failure may present as jaundice, encephalopathy, and fetor hepaticus. Life – threatening extra hepatic complications include coagulopathy and bleeding, cardiovascular instability, renal failure, adult respiratory distress syndrome, electrolyte and acid-base disturbances, and sepsis.

Survival in adults is uncommon, prognosis for children is rather better.\[6\] In most patients who survive, complete restoration of the hepatic parenchyma and normal liver function is the rule.\[10\]

Patients infected with precore mutants often manifest severe chronic hepatitis, early progression with cirrhosis, and a variable response to interferon therapy. It may have an association with fulminant hepatic failure.

Genetic heterogeneity of HBV, coinfection or superinfection with other viral hepatitis agents, or host immunological factors, may be associated with the development of fulminant hepatitis B. A rapid fall in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in patients with fulminant hepatic
failure may be erroneously interpreted as a resolving hepatic infection, when in fact hepatocytes are being lost and the outcome is fatal.\textsuperscript{[6]}

2.12.2 Clinical features of chronic hepatitis B:

Although most adults patients recover completely from an acute episode of hepatitis B, in a significant proportion, 5 to 10%, the virus persists in the body.

Chronic HBV is a prolonged (more than 6 months) infection with persistent serum levels of HBsAg and IgG anti HBeAg and the absence of anti-HBsAg antibody response. HBV DNA and HBeAg are often detectable at high concentrations.\textsuperscript{[6]} It occurs in:

- 5-10% of adult’s cases.
- in 30% of childhood cases (< 6 years).
- in 90% of new born infections.
- more frequently in males.
- more often in immunocompromised.\textsuperscript{[7]}

Three forms of chronic hepatitis B differentiated:

- A symptomatic carrier state: they usually have anti-HBe antibodies and little or no infectious virus in their blood. Later progression of liver damage or recurrence of acute episodes of hepatitis is rare in such patients.
- Chronic persistent hepatitis (CPH): are asymptomatic most of the times but have a higher risk of reactivation of disease, and small fraction does progress to cirrhosis.
- Severe chronic hepatitis (formerly, chronic active hepatitis): results in more frequent exacerbations of acute symptoms, including progressive liver damage, potentially and/or HCC. These symptoms are accompanied
by active virus replication and the corresponding presence of HBeAg in the blood. (Figure 2.3) \[21\]

There are four phases of chronic HBV infection and the host immune response in each phase determines the outcome of infection and severity of liver injury. These phases are \[22\]:

1 - **Immune tolerance phase:**

This initial phase is characterized by hepatitis B e antigen (HBeAg) positivity, high HBV DNA levels (>20,000IU/ml), normal ALT levels and minimal liver injury. It is prevalent in those who acquired the infection vertically. This phase may persist for decades and is associated with a low risk of progression to advanced liver disease.

2 - **Immune clearance phase:**

The liver injury in HBV is determined by the immune response to the virus. This phase, also called the immunocompetence phase, is characterized by fluctuating HBV DNA and ALT levels as an active, immune-mediated cytotoxic response to the infected liver cells. Active inflammation and eventually fibrosis can be found.\[22\]

3 - **Immune control phase (inactive carrier of the infection):**

Liver inflammation is minimal, HBV DNA is undetectable or at a low level (< 2000IU/ml) and liver function tests (LFTs) are normal. These patients are at low risk of developing advanced liver disease and its related complication.\[22\]

4 - **Immune escape phase (HBeAg- negative chronic hepatitis B):**

This phase is characterized by negative HBeAg, positive anti-HBe and detectable viral load (HBV DNA> 2000 IU/ml). It is often termed pre-core mutant HBV because a mutation in precore region of the DNA results in a lack of HBeAg production.\[22\]
2.12.2.1 Chronic HBV and hepatocellular carcinoma (HCC):

A number of HBV patients with chronic hepatitis will develop HCC. Persons at increased risk of developing HCC are those who contracted hepatitis B in early childhood. The mechanism of oncogenesis is still unclear.\textsuperscript{[7,10]} It is possible that in man HBV is not carcinogenic by a direct viral mechanism. Instead, the role of HBV may be to cause liver cell damage with associated inflammation and liver regeneration that continues for many years. This pathological process, especially when leading to cirrhosis, may be carcinogenic.\textsuperscript{[6]}

Integration of the viral DNA with the host-cell chromosomal DNA does appear to have a major role in carcinogenesis.

There is evidence to implicate inactivation of p53 induced apoptosis by protein X, allowing accumulation of abnormal cells and, eventually, carcinogenesis.\textsuperscript{[20]}

The incidence of HCC varies with geography, race, age, and sex. Male: female ratio is 4:1.

HCC is responsible for 90% of primary malignant tumor of the liver observed in adults. It is almost always fatal, and is one of the ten most common tumors in the world. There may be an interval of 30-40 years between infection and tumor development, although shorter intervals are seen.\textsuperscript{[7]}
HBV INFECTION

90-99% in adults and older children
5-20% in neonates and infants

Acute infection
- Sub clinical hepatitis
- Icteric hepatitis
- Fulminant hepatitis

Chronic infection
- Asymptomatic hepatitis
- Chronic persistent hepatitis
- Chronic active hepatitis

Cirrhosis

Hepatocellular Carcinoma

**Figure 2.3** The outcome of hepatitis B infection
2.12.3 Extrahepatic manifestations of hepatitis B:

Extra hepatic manifestations of hepatitis B are seen in 10-20% of patients as:

- **Transient serum sickness-like syndrome** with fever, skin rash, and polyarthritis. Symptoms usually precede the onset of jaundice by a few days to 4 weeks and subside after the onset of jaundice and may persist throughout the course of the disease. Immune complexes (e.g. surface antigen antibodies) are important in the pathogenesis of other disease syndromes characterized by severe damage of blood vessels.\(^6\):
  - **Acute necrotizing vasculitis** (polyarteritis nodosa),
  - **Membranous glomerulonephritis**, is present in both adults and children.
  - **Papular acrodermatitis of childhood** (Gianotti-Crosti syndrome), characterized by skin lesions and papular eruptions localized to face and extremities, last 15-20 days. The disease is accompanied by generalized lymphadenopathy, hepatomegaly and acute an icteric hepatitis B.\(^6\)

2.12.4 Coinfection or superinfection with HDV:

Hepatitis Delta virus (HDV) is a defective virus that is only infectious in the presence of active HBV infection. Fulminant hepatitis may follow.\(^6\)

2.12.5 Occult HBV infection (OBI):

Is generally defined as the detection of HBV-DNA in the serum or liver of subjects who have negative test for HBsAg, it has been a matter of debate for years, but its existence and clinical relevance are supported by Chemina and Trepo.\(^{23, 24}\)

The prevalence of OBI will be maximum with an insensitive HBsAg assay and very sensitive genomic amplification method. In addition to test sensitivity, there is a growing body of evidence suggesting that HBV genotype is a factor influencing the frequency of OBI.
This infection may persist in individuals for years without emerging symptoms of overt HBV infection. Co-infection, drug abuse or immunosuppression can trigger an enhancement of HBV DNA levels without an increase of HBsAg.\textsuperscript{[25]}

OBI may follow recovery from infection, displaying antibody to hepatitis B surface antigen (anti-HBs) and persistent low-level viraemia, escape mutants undetected by the HBsAg assays, or healthy carriage with antibodies to hepatitis B e antigen (anti-HBe) and to hepatitis B core antigen (anti-HBc).\textsuperscript{[23]}

Published evidence indicates that OBI is spread widely throughout the world, its frequency varies considerably according to the prevalence of the infection.\textsuperscript{[26,27]}

A wide range of OBI (0–36\%) has been reported in hemodialysis patients, among blood donors, and in patients with HCC.

The frequency of detection of HBV DNA is higher in liver tissue than in serum. Because of the low amounts of HBV-DNA, sensitive PCR assays are increasingly used.\textsuperscript{[26]}

\subsection*{2.13 Laboratory diagnosis:}

Diagnosis of hepatitis B is made by biochemical assessment of liver function. Initial laboratory evaluation should include: total and direct bilirubin, ALT, AST, alkaline phosphatase (ALP), prothrombin time, total protein, albumin, globulin and coagulation studies.\textsuperscript{[6]}

The virology laboratory can test for antigens (produced by the virus) and antibodies (produced by the host).\textsuperscript{[7,28]} The main serum markers of hepatitis B are; HBsAg and anti- HBs, anti- HBe IgM and anti-HBc IgG, HBeAg and anti-HBe. The standard screening test is for HBsAg, which if present, indicates that the patient is infected with HBV, either as a recent infection or as a carrier.\textsuperscript{[7]}

The specimen of choice for the diagnosis of HBV infection is blood.\textsuperscript{[29]} The most useful detection methods are the enzyme-linked immunosorbent assay (ELISA) for HBV antigens and antibodies and polymerase chain reaction (PCR) for viral DNA.\textsuperscript{[7]}
The diagnosis of HBV infection can also be made by the detection of HBsAg or HBcAg in liver tissues by immunohistochemical staining.[30]

The serological markers vary depending on whether the infection is acute or chronic.

2.13.1 Hepatitis B surface antigen (HBsAg):

Is the surface protein of HBV. It is a reliable marker of HBV infection, and a negative test for HBsAg makes HBV infection very unlikely but not impossible. HBsAg appears in the blood late in the incubation period and before the prodromal phase of acute type B hepatitis, it may be present for only a few days, disappearing even before jaundice has developed, but usually lasts for 3-4 weeks and can persist for up to 5 months. It is also an indicator of chronic infection if it persists for more than 6 months.[13,31] HBsAg can exist in blood, saliva, breast milk, sweat, tears, nasal secretions, semen, and vaginal secretions.

2.13.2 Anti- Hepatitis B surface (anti-HBs):

This is the specific antibody to HBsAg. Its appearance 1-4 months after onset of symptoms indicates clinical recovery and subsequent immunity to HBV. Anti-HBs can neutralize HBV and provides protection against HBV reinfection.[6] It may persist for many years and perhaps permanently. Anti-HBs implies either a previous infection, in which case anti- HBc is usually also present, or previous vaccination if anti- HBc is not present.[3]

2.13.3 Hepatitis B core antigen (HBcAg):

Is derived from the protein envelope that encloses the viral DNA, and it is not detectable in the blood stream.[6]

2.13.4 Anti-Hepatitis B core (anti-HBc):

This is the specific antibody to HBcAg. Antibodies to HBc are of class IgM and IgG. They do not neutralize the virus.[6] The presence of IgM identifies an early acute infection. IgM, anti-HBc is the sole marker of HBV infection.
during widow period between disappearance of HBsAg and the appearance of anti-HBsAg. IgG anti-HBc persists along with anti-HBs in patients who recover from acute hepatitis B. It also persists in association with HBsAg in those who progress to chronic HBV infection.[30]

2.13.5 Hepatitis B e antigen (HBeAg):

Hepatitis B e antigen (HBeAg) is a secretory protein that is processed from the precore protein. It is generally considered to be a marker of HBV replication and infectivity. The presence of HBeAg is usually associated with high levels of HBV DNA in serum and higher rates of transmission of HBV infection from carrier mothers to their babies and from patients to health care workers.[30]

2.13.6 Anti-Hepatitis B e (anti-HBe):

This is the specific antibody to HBeAg. During the acute stage of the infection the seroconversion from HBeAg to HBeAb is prognostic sign and indicates resolution of infection.[6] Seroconversion of HBeAg to anti-HBe occurs early in patients with acute infection, prior to seroconversion of HBsAg to anti-HBs. However, HBeAg seroconversion may be delayed for years to decades in patients with chronic HBV infection. Seroconversion from HBe Ag to anti-HBe is usually associated with a decrease in serum HBV DNA.[30]

2.13.7 Hepatitis B x antigen (HBxAg):

Is detected in HBeAg positive blood in patients with acute and chronic hepatitis. HBxAg is a HBV transactivator protein. It is a potential viral oncoprotein.[10]

2.13.8 HBV DNA:

HBV DNA is detectable by PCR as soon as one week after initial infection. Qualitative and quantitative tests for HBV DNA in serum have been developed to assess HBV replication.[31]
HBV DNA levels in the blood are essential for the diagnosis, to detect mutants, decision to treat and monitoring of patients. High levels may be present in carriers with no evidence of liver damage.\textsuperscript{[34]}

\textbf{2.13.9 HBV DNA polymerase:}

Is detected during incubation period and early in the disease, i.e. during the viraemia.\textsuperscript{[17]}
Figure 2.4  Typical course of hepatitis B virus infection.

A: Acute infection.  B: Chronic infection (From Lippincott's: Illustrated Microbiology)
### Table 2.1 Serological markers of HBV infection

<table>
<thead>
<tr>
<th>Marker</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>Marker of current infection</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Marker of active infection</td>
</tr>
<tr>
<td>HBsAb</td>
<td>Marker of recovery or immunity</td>
</tr>
<tr>
<td>HBeAb</td>
<td>Marker of inactive virus</td>
</tr>
<tr>
<td>HBcAb</td>
<td>IgM: Marker of infection during widow period</td>
</tr>
<tr>
<td></td>
<td>IgG: Marker of past infection or chronic infection</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>Measure viral activity</td>
</tr>
<tr>
<td></td>
<td>Marker of treatment response</td>
</tr>
<tr>
<td>HBV polymerase</td>
<td>Detected during incubation period and early in the disease</td>
</tr>
</tbody>
</table>

### 2.14 Treatment:

Currently, there is no treatment available for acute hepatitis B. Symptomatic treatment of nausea, anorexia, vomiting and other symptoms may be indicated.\textsuperscript{[6]}

#### 2.14.1 Treatment of chronic HBV infection:

Patients with HBsAg, HBeAg and HBV DNA in the serum with abnormal aminotransferases and chronic hepatitis on liver biopsy should be treated. The aim of treatment is to eliminate the HBeAg and HBV DNA from the serum, with consequent reduction in inflammatory necrosis of hepatocytes. This seroconversion occurs spontaneously at a rate of 10-15% per year, and this varies in different population.\textsuperscript{[20]}
There are two main classes of treatment:

- Immunomodulators: aimed at helping the human immune system to mount a defense against the virus.
- Antivirals: aimed at suppressing or destroying HBV by interfering with viral replication.[6]

Historically, α - interferon (α-IFN) has been the principal drug used for the treatment of chronic HBV infection.[10] To be eligible for IFN, patients should have infection documented for at least 6 months, elevated liver enzymes and an actively dividing virus in their blood (HBeAg, and/ or HBV DNA positive tests).[6]

Peglyated α – 2 β interferon (100 µg once a week subcutaneously) gives a response rates of 25- 45% after 6 months of treatment.[20] Side effects are common and include influenza-like symptoms, mild exacerbation of hepatitis (often a favorable indication of subsequent response) and granulocytopenia.[7]

Several antiviral drugs are available for use against chronic hepatitis B infection, with nucleoside and nucleotide analogue.[10] The most widely used nucleoside analogue is lamivudine (EPIVIR®), a potent inhibitor of HBV DNA synthesis. At a dose of 100-300 mg daily, lamivudine leads to a marked reduction or elimination of detectable HBV DNA in plasma and normalization of alanine aminotransferase levels in approximately 40% of individuals. Over a treatment period of one year, about 30% of those who are HBeAg positive become anti-HBe positive.[7]

The other licensed antiviral in clinical use is adefovir. This is an orally administered nucleoside analogue. It is effective in lamivudine- resistant chronic hepatitis B. Treatment should be continued until adequate seroconversion has occurred. It is continued for life in patients with decompensated liver disease or cirrhosis.[33]
The emergence of drug-resistant virus mutant in long term therapy is a major problem, so the combination therapy can be used.\[7\]

A number of other nucleoside analogues are in phase II or III trials. These include entecavir, emtricitabine, telbivudine and clevudine. These work in a similar way to lamivudine.\[7\]

For end-stage liver disease, liver transplantation is necessary. However the risk of reinfection on the graft is at least 80%, presumably from extrahepatic reservoirs in the body.\[10\] Attempts have been made to prevent reinfection of the graft with HBV through the administration of anti-HBs immunoglobulin, and more recently this has been combined with a nucleoside analogue such as lamivudine.\[7\]

2.15 Epidemiology:

More than 2000 million people alive today have been infected with HBV at some time in their lives. Of these about 350 million remain infected chronically and become carriers of the virus. Every year there are over 4 million acute cases of HBV, and about 25% of the carriers, one million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer.\[6\] Of the 350 million people in the world chronically infected with HBV, 65 million reside in Africa with 12% of the world’s population, carries approximately 18% of the global burden of HBV infection, with hepatocellular carcinoma and cirrhosis accounting for 2% of continuous annual deaths.

The world can be divided in to three areas where the prevalence of chronic HBV infection is: high (> 8%), intermediate (2- 8%) and low (< 2%).\[6\]

High endemicity areas include south-east Asia, and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the central Asia Republics, and some countries in the Eastern Europe. In these areas about 70-90% of the population becomes HBV-infected before the age of 40, and 8-20% of the people are HBV Carriers.
Low endemicity areas include North America, Western and Northern Europe, Australia, and parts of the South America. The carrier rate here is less than 2%, and less than 20% of the population is infected with HBV.

The rest of the world falls in to the intermediate range of HBV prevalence, with 2 to 8% of a given population being HBV carriers.\[6\]

Sudan is classified among the African countries with high HBV endemicity.\[14\]

![Worldwide prevalence of hepatitis B carriers and primary hepatocellular carcinoma. (Source WHO)](image)

**Figure 2.5** Worldwide prevalence of hepatitis B carriers and primary hepatocellular carcinoma. (Source WHO)

### 2.16 Prevention:

Prevention of HBV infection has become a high priority in the global community.\[6\] It depends on avoiding risk factors, such as shared needles, multiple male homo-sexual partners and prostitutes. Standard safety precautions in laboratories or hospitals must be enforced strictly to avoid accidental needle punctures and contact with infected body fluids.\[20\] It is essential of course, that blood for transfusion is screened.
However, there are limits to these approaches, and immunization, both passive and active offers many advantages.\[^7\]

### 2.16.1 Passive immunization:

HB immunoglobulin (HBIG), is prepared from the blood of donors having a high titer of anti- HBs antibody. Immediate administration of HBIG is recommended as the initial step in preventing infection of individuals accidentally exposed to HBV contaminated blood by needle-stick or other means and of those exposed to infection by sexual contact with an HBV positive partner. It is also strongly recommended that pregnant women should be screened for HBsAg. Infants born to mothers who are HBV positive are given HBIG plus hepatitis B vaccine at birth, followed by additional doses of vaccine at one and six months.\[^21\] HBIG must be given as soon as possible after an injury and preferably within 48 hours. If the victim has not been vaccinated, HBIG should be used and a course of active immunization started, injecting the two materials into different body sites to provide immediate protection with passively acquired antibody followed by active immunity generated by the vaccine.\[^7,10\]

The adults dose is 500 IU of HBIG, and to newborns is 200 IU given intramuscularly.\[^20\]

### 2.16.2 Active immunization (vaccine):

A vaccine for hepatitis B has been available since 1981. The initial vaccine was prepared by purifying HBsAg associated with the 22-nm particles from healthy HBsAg-positive carriers and treating the particles with virus inactivating agents (formalin, urea, heat). Preparations containing intact 22-nm particles have been highly effective in reducing HBV infection.\[^10\] Currently available vaccines are produced by cloning the surface gene in yeast cells.\[^7\]

HBV vaccine is 95 % effective in preventing HBV infection and its chronic consequences, and is the first vaccine against a major human cancer.
Groups at high risk are: all health care personnel, members of emergency and rescue teams; morticians and embalmers; children in high-risk areas; people with haemophilia; patients in some psychiatrics units; patients with chronic renal failures; long-term travelers; homosexual and bisexual men and prostitutes and intravenous drug abusers.\[^{20}\]

In 2010, 179 countries reported that they had included the hepatitis B vaccine in to their national infant immunization program’s.\[^{4}\]

The vaccine is administered with alum as adjuvant. Three injections at 0, 1 and 6 months are given by intramuscular injection in the anterolateral aspect of the thigh (infants) or deltoid muscle (older).\[^{20}\]

The vaccine is free from major side effects; local swelling and reddening may occur in up to one in five recipients, with slight fever in only a few cases.\[^{7}\]

Following the primary course of vaccination, blood test must be taken 1- 4 months to establish if there was been adequate response, which is defined as an anti- HbsAg level above 100 mIU/ ml. Such a full response occurs in about 85-90% of individuals.

An antibody level between 10 and 100 m IU/ml is considered a poor response, and these people should receive a single booster vaccination at this time. People who fail to respond (anti-HBs antibody level below10 mIU/ml) should be given a repeat course of 3 vaccinations, followed by further testing 1- 4 months after the second course.

Poor responses are mostly associated with being over the age of 40 years, obesity and smoking and also in alcoholics, especially if with advanced liver disease. Patients who are immunosuppressed or on renal dialysis may respond less well and require larger or more frequent doses of vaccine.\[^{28}\]
2.17 RATIONALE:

Hepatitis B is a major health problem in many countries of the world, especially those in Asia, Middle East and Africa.

The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the virus for many years.

Screening for HBV is not routinely carried out in our hospitals. Precautions against HBV are taken only when a known positive person is operated.

Surgeons, obstetricians, theater staff, nurses, and other health care workers (HCWs) have significantly increased risks of infection along with further transmission of virus, if preoperative screening and standard precautions are not followed strictly. This makes the preoperative screening for HBV one of the most important precautions. Unfortunately, little is known about the current prevalence rate of HBV among patients presenting for surgery in Sudan, and no published data on seroprevalence of HBV among surgical patients in Gezira area.
2.18 RESEARCH OBJECTIVES:

2.18.1 General objective:

- To determine the seroprevalence of HBsAg among patients who undergo different elective surgical interventions in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynecology Diseases, using an ICT and an enzyme linked immunosorbent assay (ELISA).

2.18.2 Specific objectives:

- To illustrate the various risk factors related to infection with HBV among the surgical patients who are positive for HBsAg.

- To explore the potential benefits of introducing HBsAg as a routine test for screening all patients who are planned for surgical interventions.

- To determine the sensitivity and specificity of the ICT which is widely used for HBsAg screening; compared to ELISA technique in blood screening for HBV.
CHAPTER THREE
METHODOLOGY

3.1 Study design:
This was a prospective descriptive hospital-based study conducted from 1\textsuperscript{st} February to 1\textsuperscript{st} of May 2013.

3.2 Study area:
Wad Medani; Gezira State - Central Sudan.

3.3 Study settings:
\textit{Wad Medani} Teaching Hospital and \textit{WadMedani} Hospital for Obstetrics and Gynecology Diseases.

These are two tertiary care hospitals that serve the whole Gezira and nearby states. A round 2000 and 6530 operation per year is done in these two hospitals respectively, which reflects the tremendous surgical load.

3.4 Study population:
Patients planned for different surgical operations at \textit{WadMedani} Teaching Hospital, and \textit{WadMedani} Hospital for Obstetrics and Gynecology Diseases, during the study period.

3.5 Inclusion criteria:
Patients from all ages and both sexes undergoing different surgical operations.

2.6 Exclusion criteria:
- Patients known as positive for HBsAg.
- Patients on regular dialysis.
- Those who received HBV vaccination.
3.7 Sampling:

3.7.1 Sample size: Calculated from the following formula:

\[
N = \frac{z^2 \cdot p \cdot (1-p)}{d^2}
\]

\(N = \text{Sample size}\)

\(Z = \text{Confidence interval}\)

\(P = \text{Prevalence (6.8\%, from a published study)}\)

\(D = \text{Desired margin error (I will take it as 4)}\)

\[N = (1.96)^2 \times 6.8 \times 93.2 \div 16\]

173 blood samples was taken as sample size

3.7.2 Sampling technique:

The systemic random technique was used.

3.8 Data collection tools:

- Questionnaire: it includes personal Informations, socioeconomic data, and past history of exposure to high risk procedures or behavior.
- Laboratory blood tests.

3.9 Variables:

- Independent variables: age, sex, locality, occupation,…etc.
- Dependent variables: HBsAg positive by ICT and ELISA.
3.10 Methods and Materials:

5 ml of blood was collected under aseptic technique from the antecubital vein of the arm of every selected patient by and transferred to a plain tube. The Blood was allowed to clot and the serum was separated by centrifugation for 3 minutes at 5000 rpm, then, kept in containers and labeled according to the serial number.

Serum samples were stored in deep freezers at -20°C till use and analysis.

3.10.1 HBsAg detection by ICT:

Test devices used were from Biorex Diagnostics Ltd (Expiry date: November 2014).

3.10.1.1 Principle:

The HBsAg rapid test device (serum/plasma) is a qualitative, solid phase; two-site sandwich for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg on the test line region of the device. During testing, the serum or plasma specimen reacts with anti-HBsAg conjugated particles. The mixture migrates upward on the membrane by capillary action to react with anti-HBsAg on the membrane and generate a coloured line. The presence of this coloured line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that the device is working.

3.10.1.2 Procedure:

The device and buffer were allowed to equilibrate to room temperature prior to testing. Then, the test device was removed from the sealed foil pouch and used. Then the dropper was held vertically, and 75 µl of specimen were transferred to well (S) of the device. The result was read after 15 minutes.
3.10.1.3 Interpretation of the Results:

**Positive results:** Two distinct coloured lines appear. One line should be in the control region (C) and another line should be in the test region (T).

**Negative results:** One coloured line appears in the control region (C). No apparent coloured line appears in the test region (T).

3.10.2. HBsAg detection by ELISA:

All the serum samples were screened for the HBsAg using ELISA kits from **Biorex** Diagnostics Ltd.

3.10.2.1 Principle:

The test is an enzyme-immunoassay based on a sandwich principle. Microtiter wells are coated with monoclonal anti-HBs, which constitute the solid-phase antibody.

The test sample is incubated in such a well; HBsAg, if present in the sample, will bind to solid-phase antibody. Subsequently guinea–pig anti-HBs, which has been labeled with the enzyme horseradish peroxidase (HRP), is added. With a positive reaction this labeled antibody becomes bound to any solid-phase antibody HBsAg complex previously formed. Incubation with enzyme substrate produces a blue color in the test well, which turns yellow when the reaction is stopped with sulphuric acid. If the sample contains no HBsAg, the labeled antibody cannot be bound specifically and only a low background color develops.

3.10.2.2 Assay procedure:

- The reagents and samples were allowed to stabilize at room temperature for 30 minutes before use. Then the reagents were gently shaken. The stock Wash Buffer was then diluted 1 to 20 with distilled water.

- Then the strips were placed in strip-holder as follows: three strips for negative controls, two positive controls and one strip for Blank (neither samples nor HRP conjugate was being added).
- Then 50 µL of positive control, negative control and specimen were added into their respective wells.
- Then 50 µL of HRP-conjugate were added to each well (except the blank), and mixed by tapping the plate gently.
- The plate was then covered and incubated for 60 minutes at 37°C, and then each well was washed automatically for 5 times with diluted wash buffer and after the final washing cycle, the plate was turned down onto blotting paper and tapping to remove any remainder.
- 50 µl of chromogen A and 50 µl of chromogen B were dispensed into each well including the blank, and mixed by tapping the plate gently. The plate then was incubated at 37°C for 15 minutes avoiding light. A blue color developed in positive control and HBsAg positive sample wells.
- 50 µl of stop solution were added to each well and mixed gently. Intensive yellow color developed in positive control and HBsAg positive sample wells.
- The plate absorbance was read at a plate reader of 450 nm. Cut-off value was calculated as the mean absorbance value for three negative controls, multiplied by 2.1.

3.10.2.3 Interpretation of the Results:
- The results were calculated by relating each sample’s optical density (OD) value to the cut-off value (C.O).
- **Negative results:** samples giving an absorbance less than the cut-off value were considered negative.
- **Positive results:** samples giving an absorbance greater than or equal to the cut-off value were considered reactive samples initially.
3.11 Data management:

Data was entered and analyzed by using Statistical Package for Social Science software (SPSS) version 16. \( P \leq 0.05 \) was considered statistically significant.

3.12 Ethical consideration:

Ethical approval was obtained from the research committee – University of Gezira.

A separate permission was taken from the general directors of the hospitals. The study cases were informed about the study objectives and consented prior filling the questionnaire. Confidentiality and privacy of suspected cases will be guaranteed.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results:

This study included a total of 173 patients undergoing different surgical operations at Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynaecology Diseases. Out of studied patients, 54 males (31.21%) and 119 (68.79%) were females (Figure 4.1) with ages ranging from 17-75 years. The mean age being 37 years.

Demographic criteria of the participants are shown in table 4.1.

Initially those 173 patients were screened for HBsAg by ICT, and 10 of them were found to be positive, giving the seroprevalence of 5.78% (Figure 4.2).

The samples were also processed by ELISA which indicated that, 11 out of the total number of patients were reactive for HBsAg giving the prevalence of 6.4% (Figure 4.3).

Among the 11 HBsAg reactive patients, 7 (63, 64%) were males and 4 (36, 36%) were females which is statistically significant (P value of .016) (Figure 4.4).

Results of the current study indicated that 90.9% of the HBsAg positive patients were married.

The highest prevalence rate of HBsAg (63.64%); was detected in the age group of 30 – 45 years, followed by 27.3 prevalence % in patients of more than 45 years of age, and the least prevalence (9.1%) was found in the age group of less than 30 years. (Figure 4.5).

The result showed that HBV infection was common (81.8%) in those who had a primary level of education. (Table 4.1)

9.1% of the patients studied had a family history of past infection with hepatitis B infection, i.e. history of contact.
The possible risk factors associated with HBsAg seropositivity included; tattooing or cauterization are the common risk factor followed by history of previous surgery or dental procedure, and blood transfusion.

18.2% of patients had past history of schistosoma infection.

Only 9.1% of the participants in this study had a history of accidental injection by a reused needle.
<table>
<thead>
<tr>
<th>Variable</th>
<th>% of patients</th>
<th>% of positive HBsAg</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31.2</td>
<td>63.6</td>
<td>0.016</td>
</tr>
<tr>
<td>Female</td>
<td>68.8</td>
<td>36.4</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30 years</td>
<td>36.4</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>30-45 years</td>
<td>43.4</td>
<td>63.64</td>
<td>0.016</td>
</tr>
<tr>
<td>&gt; 45 years</td>
<td>20.2</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td>Level of Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>22</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>36.5</td>
<td>81.8</td>
<td>0.014</td>
</tr>
<tr>
<td>Secondary</td>
<td>25.4</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Higher</td>
<td>16.2</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>13.9</td>
<td>9.1</td>
<td>0.635</td>
</tr>
<tr>
<td>Married</td>
<td>86.1</td>
<td>90.9</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.00</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

(N=173)
Figure 4.1 Sex distribution of the patients enrolled in this study.
Figure 4.2 Prevalence of HBsAg by ICT

(N = 173)

Figure 4.3 Prevalence of HBsAg by ELISA

(N = 173)
Figure 4.4  Sex distribution among HBsAg positive patients.
Figure 4.5 Age distribution among HBsAg positive group of patients.
Table 4.2 The ICT parameters compared to ELISA. (n=173)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>True positive / True positive+ False negative</td>
</tr>
<tr>
<td>(True positive rate )</td>
<td>= 90.90%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>True negative / True negative+ False positive</td>
</tr>
<tr>
<td>(True negative rate )</td>
<td>= 100%</td>
</tr>
<tr>
<td><strong>Positive predictive Value</strong></td>
<td>True positive / True positive+ False positive</td>
</tr>
<tr>
<td>(PPV)</td>
<td>= 100%</td>
</tr>
<tr>
<td><strong>Negative predictive Value</strong></td>
<td>True negative / True negative+ False negative</td>
</tr>
<tr>
<td>(NPV)</td>
<td>= 99.39%</td>
</tr>
</tbody>
</table>
4.2 DISCUSSION

The spread of HBV infection continues to be at an alarming rate worldwide. This study gave a highlight on HBV prevalence among the surgical patients in Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynaecology Diseases. It demonstrated an intermediate prevalence (6.4%) of HBsAg among the studied patients.

Sudan has been classified among the countries with high HBV endemicity.\[34\] The prevalence differs from one area to another. Previous studies of HBV epidemiology were done in a selected group, such as pregnant women representing females at a child bearing age. Other studies are done among blood donors, and some studies were done in haemodialysis patients.

Earlier reports showed a high prevalence of HBV in Gezira. In 1989 study reported a prevalence of 18.7 and 22.3% % HBsAg positivity after a survey involved a two rural villages; Khalwaat and Saleim, respectively.\[35\] Furthermore, in 1992 another study in the Gezira reported HBsAg prevalence of 17.3% and 12.1% among blood donors and laboratory technical staff respectively.\[36\] A survey in the Gezira State found 6.9% prevalence HBsAg positivity among the population of Um Zukra village in December 2000.\[37\] Another study in 2001; reported 7.0% prevalence of HBsAg among healthy controls from Gezira and North Kordofan States. \[38\] The lower prevalence of HBsAg reported in this study in comparison to previous studies, is probably explained by the introduction of the screening of blood or blood products over the last ten years, and also the introduction of the HBV vaccination. Increasing awareness of people about the virus, and the uses of disposable syringes are also plays a major role in decreasing the prevalence of the virus.

A similar study done in Khartoum State among patients undergoing surgery at Al-Shaab Teaching hospital, reported that HBsAg seroprevalence was 4.91%, which is lower than the result of this study.\[39\]
In Nyala, South Darfur State one study revealed 6.2% prevalence among blood donors,[40] which is nearly similar to the prevalence reported in this study.

A 26% prevalence of HBsAg was reported in hospital outpatients from Southern Sudan,[41] which is very higher than the prevalence in Sudan.

Two studies similar to this study were done in Pakistan; one reported 4.3% prevalence of HBsAg among the surgical patients,[42] and the second one reported 2.02 % prevalence of HBsAg among patients admitted in department of ophthalmology.[43]

The current study revealed that; the patients who had history of tattooing, scarification or ear piercing are the common group affected, and the HBV infection is common among these groups of patients because these practices are carried most of times by persons who do not use sterilized equipments in these procedures. This finding provides support for the postulate that occasionally, out breaks of hepatitis have been traced to tattoo parlors or acupuncturists.[44] Health education will be a potent tool for reducing this health hazard.

This study demonstrated that, patients with a previous history of surgery or dental procedure are also commonly affected, this was in agreement with that people sharing unsterile medical or dental equipment are at high risk of contracting HBV.[45]

This different from USA and other developed countries in which the parenteral drug use was reported to be the major risk factor identified among the majority of HBV positive cases.[46]

In this study; the blood transfusion was also identified as a risk factor among the HBsAg positive surgical patients, this because the screening of donated was introduced throughout, and also there is a risk of the country in 2002, and also there is a residual risk of transfusing infected if it is donated during the window period even ELISA result is negative (occult hepatitis B infection).
This study showed that 2 patients had a history of schistosomiasis, and this result may support the hypothesis of the association between the HBV and hepatic schistosoma infection.

Analysis by gender revealed that, the seroprevalence of hepatitis was higher in males than that found in females, although the number of females in this study is very high in comparison to males. This is agree with same study done in Morocco, and no plausible explanation had been given, but probably due to higher exposure to HBV risk factors in men, or females clear the HBV more efficiently as compared to females.\[47\]

The study; shows high prevalence of HBV infection among those who had a lower education level. This is expected because of the lack of knowledge about the routes and the risk factors for transmission of the virus.

The 9.1% association between the HBsAg infection and afamily history of liver disease identified in this study.

The results of this study demonstrated that 9.1 % of HBV positive cases were accidentally subjected to a used needle. In another study, contaminated and inadequately sterilized syringes and needles had resulted in outbreaks of hepatitis B among patients in clinics and physicians.\[44\]

The two main tests commonly used for screening of HBsAg are the ICT and ELISA. All the tests positive by ICT were positive by ELISA, but ELISA detected one positive case which was not detected by the ICT, and this means that ICT is not sensitive test for detection of HBsAg. This agree with that ELISA technique is more superior to ICT in blood screening for infectious agents in the blood such as HBV.\[48\] According to recent literature, PCR is more sensitive than ELISA for detection of HBV.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion:

- This was a descriptive hospital-based study done among the patients planned for different surgical operations at Wad Medani Teaching Hospital and Wad Medani Hospital for Obstetrics and Gynaecology Diseases from 1st February to 1st May 2013.
- The result showed 6.4% positivity for HBsAg among the studied group of patients.
- The seroprevalence was higher in males than in females, common among patients of age group 30-45 years, and among those who have a primary level of education.
- The possible risk factors associated with HBsAg seropositivity are; tattooing or cauterization, history of previous surgery or dental procedure and blood transfusion.
5.2 Recommendations:

- Considering this prevalence of HBsAg among surgical patients, routine screening of all patients is recommended before surgery.
- ELISA technique should be the method of the routine screening, but the rapid ICT kits can be used where laboratory facilities are not available or in emergency operations.
- In order to protect the theater staff, it is further recommended that all should receive active immunization.
- Gloves must be worn during performance of any procedure, including taking blood from vein and insertion of intravenous cannulae.
- Masks and eyes protectants should be worn in the theater room to avoid exposure to blood.
- Needles which have been used for patients must not be resheathed.
- Equipments, notes and other articles must not be handled with contaminated gloves.
- Abrasions and cuts on hand of any HCWs must be covered to avoid infection.
- If a needle stick injury occurs, encourage bleeding, wash it with soap and running water and a single dose of HBV immunoglobulin combined with active immunization should be given immediately.
- Public are to be informed through media for HBV, its routes of transmission and HBV vaccination must be given to high risk groups especially to health care workers to reduce hepatitis B transmission.
- New techniques like PCR, which is more sensitive for the detection of HBV DNA, should be incorporated in further studies.
- Further studies in other hospitals all over the Gezira State are needed to determine the prevalence of HBV, HCV and HIV.
REFERENCES


17. Wareen L. Review of Medical Microbiology and Immunology. 9th ed. USA: Lange; 2006.


34. Expanded program on immunization, hepatitis B vaccine, making global progress. EPI update. WHO: October 1996.


48. Khan J.K et al. Evaluation of the Performance of Two Rapid Immunochromatographic tests for Detection of HBsAg and anti-HCV antibodies Using ELISA tested Samples. SPECIAL EDDITION ANNALS 2010; 16
University of Gezira
Faculty of Medicine- Pathology Department

QUESTIONNAIRE

Date: / / 2013 Patient number  Hospital ...........

Personal Data:

Name: .................................................................
Age: _______ Years Sex: Male [ ] Female [ ]
Residence: ............................................................
Marital status: Single [ ] Married [ ] Other [ ]
Level of education: Illiterate [ ] Read/write [ ] Secondary [ ] University [ ]
Occupation: ............................................................
Contact number: ........................................................

Clinical Data:

- Past history of screening for HBV? Yes [ ] No [ ]
- Family history of jaundice/ hepatitis? Yes [ ] No [ ]
- Past history of surgery or dental procedure? Yes [ ] No [ ]
- History of tattooing /cauterization/ear piercing? Yes [ ] No [ ]
- Past history of injection by re-used needle? Yes [ ] No [ ]
- Past history of blood transfusion? Yes [ ] No [ ]

if yes; how many times ? [ ]

-Past history of bilharzia infection? Yes [ ] No [ ]
**Investigations:**

- The result of HBsAg screening by ICT: Positive [ ] Negative [ ]
- The result of HBsAg screening by ELISA: Positive [ ] Negative [ ]