**Sudan University of Science and Technology**

**College of Graduate Studies**

# Seroprevalence of Hepatitis B Virus Infection among Sudanese Men Whipped During Wedding Ceremonies in Al-Gadarif State

# الكشف المصلي لإلتهاب الكبد الفيروسي B وسط الرجال الذين يُجلدون أثناء حفلات الزواج بولاية القضارف

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

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## الآيـــــــــــة

**قال تعالي: (اقْرَأْ كِتَابَكَ كَفَى بِنَفْسِكَ الْيَوْمَ عَلَيْكَ حَسِيبًا)**

**صدق الله العظيم**

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# DEDICATION

**To my Mother, Father, Brothers,**

**Teachers and Friends**

## ACKN0WLEDGEMENT

Firstly my gratitude and prayers to the ALMIGHTY ALLAH for his mercy and guidance throughout the long path of this research. I owe so much to my supervisor **Dr. Ahmed Ibrahim Hashim** for his close supervision, valuable advices and stimulating suggestions. His pleasant personality made it easy for me to do this project and complete the research. I really do appreciate the assistance and patience of the College of Medical Laboratory science staff. Last but not least, I would like to thank everyone who contributed by any means in this study from the beginning, during the processing of the specimens or through the final stage. Finally, I would like to thank all the participants for their kind cooperation.

## Abstract

Whipping ritual during Sudanese wedding ceremonies could be associated with blood-borne infection that results from viruses such as hepatitis B virus, HCV and HIV. This study was aimed to investigate hepatitis B virus infection among men whipped during Sudanese wedding ceremonies using enzyme-linked immunesorbant Assay (ELISA) for detection of HBs Ag. This was a comparative cross-sectional study which was carried out during the period from January to April 2017. A total of hundred rural participants living in Al-Gadarif State were recruited in the study. The men recruited in this study were divided into whipped men (n=50) this involved those who strip off their clothes and get whipped (lashed) during wedding ceremonies. The other group (n=50) included those men who have never participated in this whipping ritual. The results revealed that 9(18%) were positive for HBs Ag, among those whipped during ceremonies, while 41(82%) were negative. In the other group 1(2%) was positive for HBs Ag and 49(98%) were negative. The comparison between HBs Ag and the population yielded significant statistical relationship ($p > 0.05$). The highest frequency of HBs Ag was 6 (12%) among age group (29-37) yrs old. While the frequency was 3 (6%) in the age group (20-28) Yrs old among whipped groups. This study revealed that HBV infection was more prevalent among the elder age groups. The frequency of whipping ritual has an impact on prevalence of the HBV infection since the study showed that men whipped frequently between (10-17) years were the most infected 6(12%) out the total 9(18%) among the whipped group. Further studies are required to validate these results.

## المستخلص

عادة الجلد أثناء حفلات الزواج السودانية ربما تساهم في الاصابة بالامراض التي تنتقل عن طريق الدم مثل فيروس الكبد الوبائي نوع (بي), فيروس الكبد نوع سي وفيروس نقص المناعة المكتسبة.
هذه الدراسة تهدف للكشف عن الاصابة بفيروس الكبد نوع (بي) وسط الرجال الذين يجلدون في حفلات الزواج السوداني بالقضارف, باستخدام الإليزا لكشف الانتيجين السطحي لفيروس الكبد نوع (بي) وكان تصميم الدراسة مقارنة مسحية . والتي تم اجراؤها في الفترة ما بين فبراير حتى أبريل 2017 , وقد تم جمع 100 عينة من القرويين بالقضارف المشاركين مقسمة الى (50) شخص من الممارسين لعادة الجلد و (50) من غير الممارسين لعادة الجلد وتم تضمين كلتا المجموعتين في الدراسة حيث وجد ان 9 (18%) من مجموع (50) كانت ايجابية في مجموعة الممارسين لعادة الجلد بينما 41(82%) من مجموع (50) كانت سالبة. و كانت 1(2%) من مجموع (50) ايجابية في المجموعة في المجموعة الأخرى و 49(98%) من مجموع (50) كانت سالبة. إعتمادا على قيمة ($p > 0.05$) فإنه وجدت علاقة احصائية في الدراسة .
وكان أعلى تردد لانتجين فيروس الكبد بي السطحي 6 , (12%) من مجموع في الفئة العمرية (29-37) سنة. في حين كان التردد 3، (6٪ ) في الفئة العمرية (20-28) سنة. وقد تبين أن فيروس التهاب الكبد الوبائي أكثر انتشارا في المجموعة الاكبر سنا بين المارسين لعادة الجلد. وقد كان تردد عادة الجلد له تأثير على إنتشار الاصابة بين الرجال الذين ترددوا على عادة الجلد لفترة بين (10-17) سنة كانوا اكثر المصابين بنسبة 6 (21%) من المجموع 9(18%) وسط مجموعة الممارسين لعادة الجلد. ويلزم إجراء مزيد من الدراسات للتحقق من صحة هذه النتائج.

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# CHAPTER ONE

## INTRODUCTION

### 1.1 Background

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

Hepatitis B virus (HBV) infection is a major global public health problem. Of the approximately 2 billion people who have been infected worldwide, more than 350 million are chronic carriers of HBV Approximately 15–40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC). HBV infection accounts for 500 000 to 1.2 million deaths each year and is the $10^{th}$ leading cause of death worldwide (Lavanchy, 2004).

The earliest recognition of the public health importance of hepatitis B virus infection is thought to have occurred when it appeared as an adverse event associated with a vaccination campaign (WHO, 2011). More than 300 million people have chronic liver infections globally and about 600,000 people die annually from acute or chronic complications of hepatitis B infection. The highest prevalence of hepatitis B infection is in sub-Saharan Africa and East Asia Majority of the people in these regions become infected during childhood and between 5–10% of the adult population is chronically infected (WHO, 2009).The hepadnaviridae are a family of hepatotropic DNA virus with a unique life cycle involving an RNA (Ribo Nucleic Acid) intermediate and the use of a viral polymerase enzyme with reverse transcriptase activity. The virion of HBV is 42nm double-shelled particle known as Dane particle. The outer envelope of the virion is formed by hepatitis B surface antigen (HBs Ag) the inner core, 27nm in diameter; consist of hepatitis B core antigen (HBc Ag) which encloses the viral genome DNA and polymerase. The viral DNA is about 3200

nucleotides long and is circular in configuration (Greenwood *et al.*, 2012). More than 400 million people worldwide are chronically infected by the hepatitis B virus. The virus is responsible for more than 300000 cases of liver cancer every year and for similar numbers of gastrointestinal hemorrhage and ascites. Major breakthroughs have been achieved in diagnosis and treatment of this virus. Hepatitis B vaccine reduces incidence of liver cancer. As with hepatitis C, advances have been made in molecular virology, especially for naturally occurring and treatment-induced mutant viruses. The clinical significance of low viral load and genotypes are also under investigation. Currently available monotherapies interferon, lamivudine, and adefovir dipivoxil—very rarely eradicate the virus, but greatly reduce its replication, necro-inflammatory histological activity, and progression of fibrosis. Lamivudine, and presumably other nucleoside analogues, can reverse cirrhosis of the liver (Lai *et al.*, 2003).

Studies related to blood-borne disease was accomplished in tattooing in Brazil, HBV infection is blood-borne disease can transmitted by contaminated tools Tattoos have been shown to be associated with transfusion-transmitted diseases (TTDs), particularly hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Very little is known about the association between different categories of tattoos and TTDs they studied 182 individuals with tattoos and assessed the odds of testing positive for a TTD according to tattoo type, number, design and performance conditions. Major rending were significant associations between an increasing number of tattoos and HBV infection. (Nishioka *et al.*, 2002).

In Sudan high prevalence of HBV in 2012-2014 was in South Kordofan, White Nile, and Al-Gadarif State respectively. From 2012-2014, the Northern and Nahr Alnile states had the lowest prevalence of HBV (Abdo *et al.*, 2015).

Whipping or «Al-botan » is one of the habits established by some Sudanese tribes, it is the ritual that accompanies occasions of weddings at those tribes, where Young people volunteer to flog in front of girls and women, the

bridegroom flogs the Volunteers whips on their backs amid the singing and singing of women. "Al-Botan" is spread among the tribes of the Ja'aleen, Al-Manasir, Al-Kawahla and Bedouins Northern Sudan, and in Al-Botana area in the Eastern part of Sudan. "Al-Botan" is a manifestation of courage and patience. The Botan is one the Sudanese marriage ritual that is practiced by young and adult men. In this ritual men expose their backs and get lashed\ whipped in front of the girl on the rhythm of “Aldallookah” (Sudanese musical instrument. The men are usually lashed \ whipped frequently for many times until they get wounds on their back (see figure 1.1).

They continue the lashing\ whipping during wedding ceremonies and several men lashed with same tool. The men usually achieved selected Sudanese idioms and phrases such as “ I am the brother of the girls” and they continue saying that while they setting whipped as a sign of their braveness and patience. Although many see this ritual as harmful to the health of the men, many tribes continue this ritual and thinking it as an essential part of the wedding ceremony.

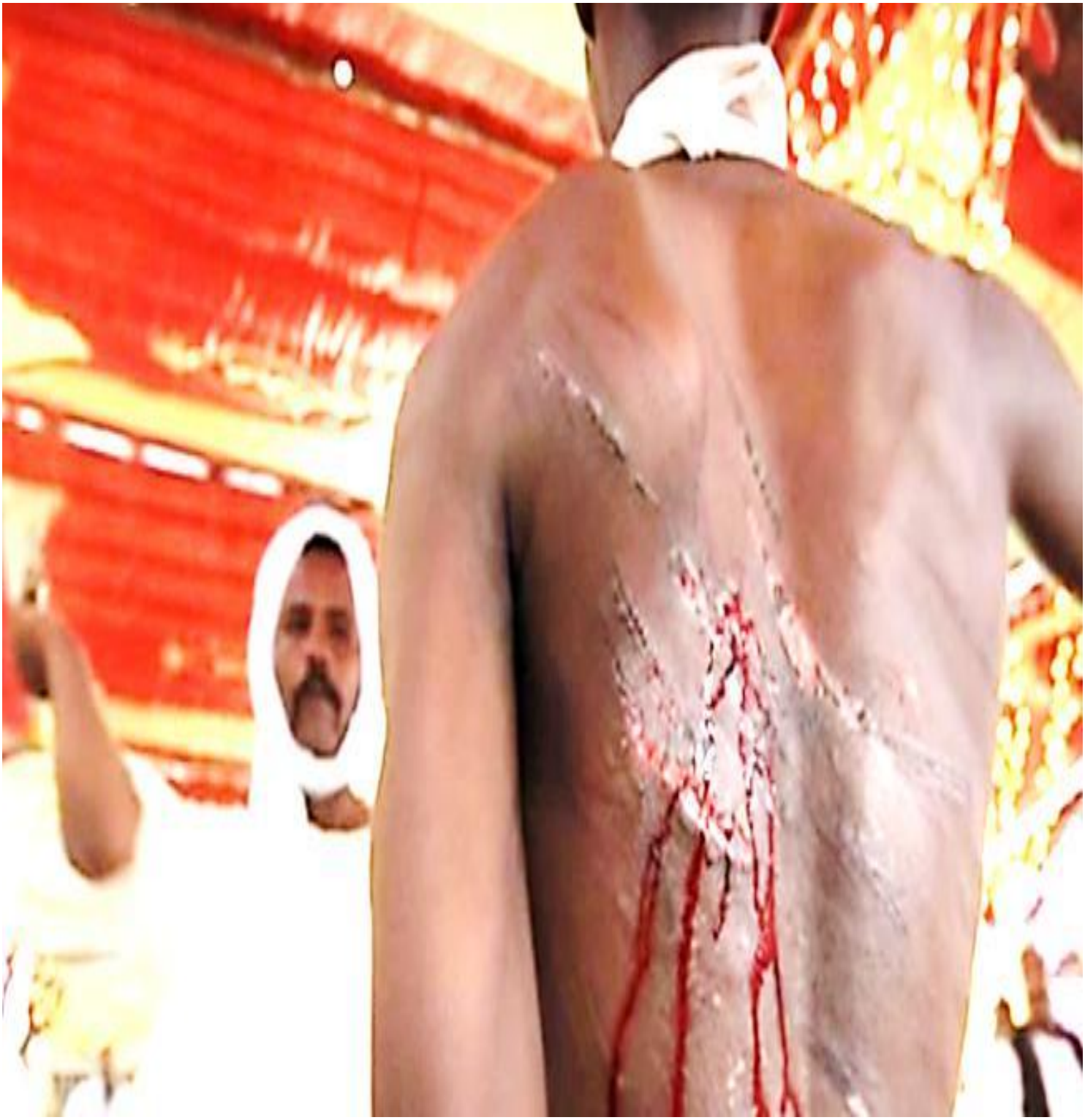

**Fig1. 1** Sudanese whipping ritual available online from URL [www.doratalsudan.com, 2014]

## 1.2 Rationale

HBV infection is a major health problem and causes significant morbidity and mortality. The observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary to transmit the disease. Prevalence of disease is associated with a proper understanding of the mode of transmission of the disease. HBV is blood-borne disease in transmission and any tools contaminated with blood could assist in the transmission of this infection among the community. Whipping ritual or lashing with leather material could be a source of transmission of viruses and blood borne diseases. Since the tool used for whipping is used by number of men during the ceremonies while it is contaminated with traces of blood, it could be a source of transmission because the men usually get wounds during whipping and the viruses could be introduced through these wounds to those men. Hence; it was necessary to screen those men for HBV infection in order to determine whether there is association between whipping ritual and HBV infection. This mechanism of transmission is relatively similar to those who get infection during tattooing.

## 1.3 Objectives

### 1.3.1 General objective

To determine seroprevalence of HBV infection among men whipped during wedding ceremonies in Al Gadarif rural community.

### 1.3.2 Specific objective

1. To detect HBs Ag in men whipped during ceremonies in Al-Gadrif rural area.

2. To determine the possible association between whipping during wedding ceremonies and transmission of hepatitis B virus infection.

3. To compare between HBV infection and the impact of frequency of whipping.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1.1 Historical background of HBV

Hepatitis B virus (HBV) is a common viral pathogen that currently infects an estimated 4 million people worldwide, including 400 million who have chronic infection. Persons with chronic HBV infection are at a lifelong risk of developing hepatocellular carcinoma (HCC) or cirrhosis or both. Many persons with HBV are unaware that they carry the infection, and, of those who are chronically infected, only a minority receives routine, scheduled follow-up to monitor their disease status. Persons from high-risk populations, especially immigrants from nations where hepatitis B is highly endemic, should be tested for HBV sero-markers and should be vaccinated if they are found to be negative. The natural history of chronic HBV is a dynamic one: patients can fluctuate between periods of active liver inflammation and periods of inactive disease. Progression of disease is influenced by various factors, including viral genotype, specific mutations, demographic features, concurrent viral infections and social and environmental factors. Recent data suggest that antiviral therapy can decrease the risk of liver decomposition and liver-related death and reduce the risk of HCC in selected individuals with active liver disease and severe fibrosis. Persons identified with chronic HBV infection need lifelong, regular monitoring for the development of active liver disease and HCC (Mahon, 2005)

## 2.1.2 Classification and Structure

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. Hepadnaviruses have a strong preference for infecting liver cells, but small amounts of hepadnaviral DNA can be found in kidney, pancreas, and mononuclear cells. However, infection at these sites is not linked to extra hepatic disease. HBV virions are double-shelled particles, 40 to

42 nm in diameter. With an outer lipoprotein envelope that contains three related envelope glycoprotein (or surface antigens) within the envelope is the viral nucleocapsid, or core. The core contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase that is responsible for the synthesis of viral DNA in infected cells. DNA sequencing of many isolates of HBV has confirmed the existence of multiple viral genotypes, each with a characteristic geographic distribution In addition to virions, HBV-infected cells produce two distinct sub viral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter These HBs Ag particles contain only envelope glycoprotein's and host-derived lipids (Ganem *et al.*,2004)

### 2.1.3 Replication

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

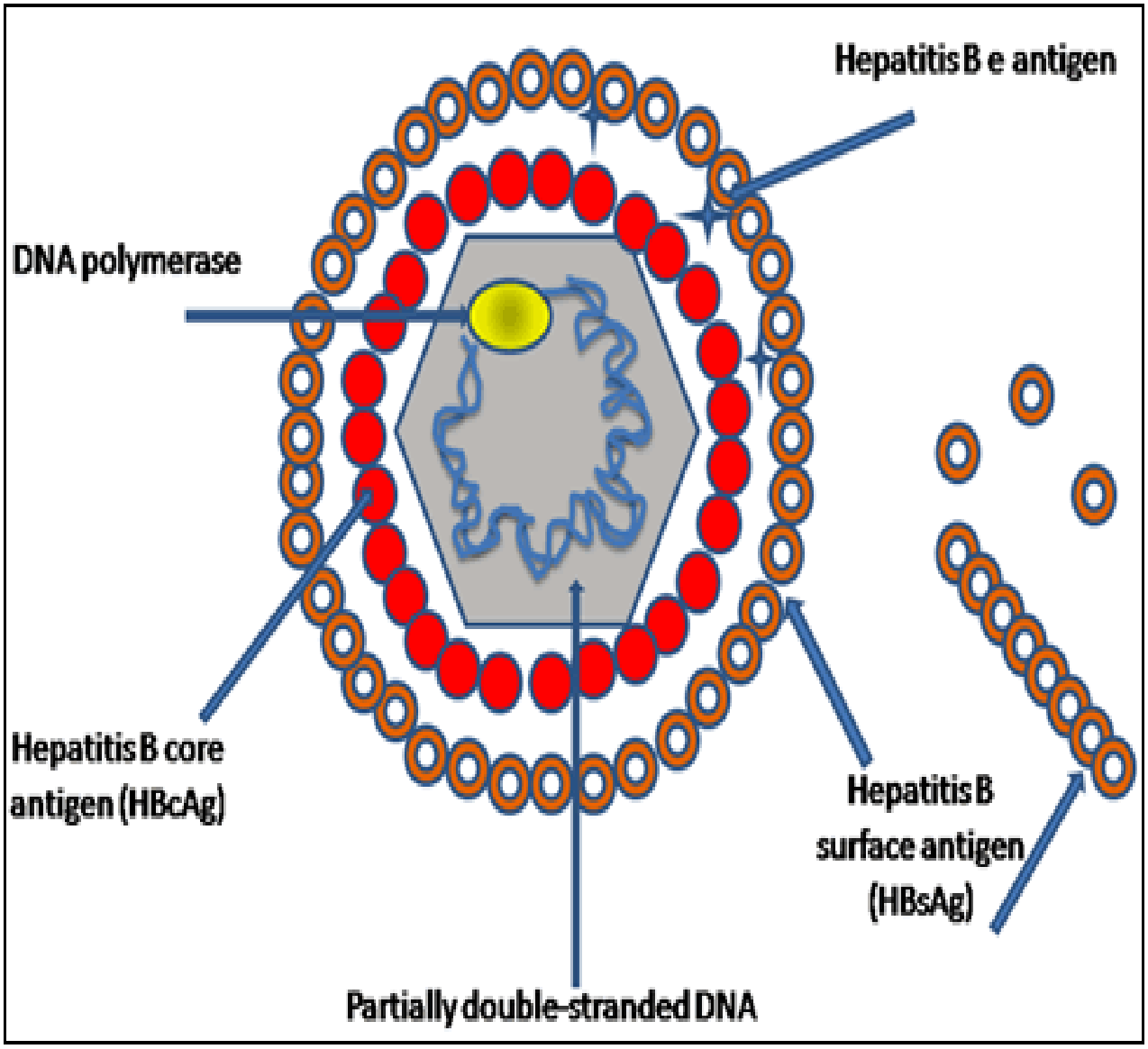


**Fig 2.1 HBV structure available from:**

[www.hepatitisbvirusinformation.weebly.comlhbv-viral-structure.html, 2015]

### 2.1.4 Serotypes and genotypes

For vaccine purposes HBV has one serotype based on HBs Ag, however, for epidemiological purpose there are four subtype of HBs Ag based on a group specific Ag (a) was marked and to sets of mutually exclusive based on these antigenic epitopes which are presented on its envelope proteins, and in to 8 genotype (A-H) according to overall nucleotide sequence variation of the genome. The genotype has a distinct geographical distribution and is used in tracing the evaluation and transmission of the virus. Differences between genotype affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.

Genotypes differ by at least 8% of their sequences and were first reported in1988 when six were initially described (A-F). Tow further types have been described (G and H) (Baumert *et al.,* 2007).

### 2.1.5 Occult hepatitis B

Occult hepatitis B is defined by the presence of hepatitis B virus DNA in serum or liver in the absence of HBs Ag. Serum HBV level usually less than 104 copies/ml. Although occult HBV infection has identified in patient with chronic liver disease two decades ago. Occult HBV infection has been found in patient with hepatocellular carcinoma (HCC), past HBV infection, or chronic hepatitis C virus, and individual without hepatitis B serological markers, the frequency of the diagnosis depends on the relative sensitivity of HBV DNA assay and the prevalence of HBV infection in the population. Collectively around 30% to 35% of HBs Ag negative. Subject with chronic hepatitis with or without HCC have positive serum HBV DNA (range from 5% to 55%) (Mumtaz *et al.,* 2011). Clinical significance of occult HBV infection remains unclear. Occult infection represents a potential transmission source of HBV via blood transfusion or organ transplantation. Some studies suggested that occult might affect responsiveness of chronic hepatitis C to interferon therapy and disease progress (Jinlin *et al.,* 2005).

### 2.1.6 Mutantion

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

### 2.1.7 Physical properties

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

### 2.1.8 High risk groups

The health care workers (HCWs) and medical students are at risk of infection with HBV through occupational exposure to blood and infection body fluids and HBV is also the most easily transmitted blood borne pathogens (Amini *et al.,* 2008). The following groups represent the high risk group: Infants from mother who are infected at the time of delivery, Partners or individuals living in close house hold contact with an infected person, Individual with multiple sex partner (past or present), Individual who has been diagnosed with a sexually transmitted disease, Illicit drug user (injection, inhaling, snorting, popping pills), Men who have sex with men, Individual who receive a blood transfusion, Individual who get tattoos or body piercing, Individual who travel to countries where HBV is common, Individual with early kidney disease or undergoing kidney dialysis, Individual who use blood products for medical conditions (i.e. hemophilia) (Amini *et al.,* 2008).

### 2.1.9 Pathogenesis

Among all viral hepatitis, the immune-pathogenesis of hepatitis B has been studied most extensively. The existence of inactive hepatitis B carriers with normal ten liver histology and function suggest that the virus is not directly cytopathic. The fact that patients with defects in cellular immune competence are more likely to remain chronically infected rather than to clear the virus is cited to support the role of cellular immune responses in the pathogenesis of hepatitis B related liver injury. The model that has the most experimental support involves cytolytic T cells sensitized specifically to recognize host and hepatitis B viral antigens on the liver cell surface (Ganem and Princ, 2004). 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, and inflammation and necrosis occur. Immune attack against viral antigens on infected hepatocytes is mediated by cytotoxic T

cells. The pathogenesis of hepatitis B is probably the result of this cell-mediated immune injury, because HBV itself does not cause a cytopathic effect. Antigen–antibody complexes cause some of the early symptoms (e.g., arthralgias, arthritis, and urticaria) and some of the complications in chronic hepatitis (e.g., glomerulonephritis, cryoglobulinemia, and vasculitis) (Levinson, 2014). Fully differentiated hepatocytes are the primary cell type infected by HBV. The primary cause of hepatic cell destruction appears to be the cell-mediated immune response, which results in inflammation and necrosis. The cells involved are cytotoxic T cells, which react specifically with the fragments of nucleocapsid proteins (HBcAg and HBeAg), expressed on the surface of infected hepatocytes. This response also contributes to control of the infection by eliminating virus-producing cells. Enhanced natural killer cell activity, as well as production of interferon-γ also contributes to limiting the extent of infection. Anti-HBsAg antibody, which is the neutralizing antibody, does not appear until well into the convalescence period, when it may aid in clearing any remaining circulating free virus (Harvey and Cornelissen, 2007). Four different stages have been identified in the viral life cycle of hepatitis B: The first stage is immune tolerance. The duration of this stage for healthy adults is approximately 2-4 weeks and represents the incubation period. For newborn, the duration of this period is often decades. Acute viral replication is known to continue despite little or no elevation in the amino transfer's levels and no symptoms of illness (Ganji *et al.*, 2011).

### 2.1.10 Transmission of HBV Infection

Hepatitis B virus is present in the blood, saliva, semen, vaginal secretions, menstrual blood, and to a lesser extent, perspiration, breast milk, tears, and urine of infected individuals. A highly resilient virus, HBV is resistant to break-down, can survive outside the body, and is easily transmitted through contact with infected body fluids. In areas of high endemicity, the most common route of transmission is prinatal or the infection is acquired during the preschool

years. In areas of intermediate endemicity, transmission is either perinatal or horizontal. The route of transmission has important clinical implications, due to very high probability of developing chronic hepatitis B (CHB) if the infection is acquired prenatally or in the preschool years. The use of unsafe injections poses a particular public health problem in developing countries. Contaminated needles cause 8–16 million HBV infections each year. In areas of low endemicity, most HBV infections are acquired by horizontal transmission in early adult life, i.e. through intravenous drug use or unprotected sexual activities. Blood transfusions were once a common route of transmission, but improved diagnostic tests and progressively broader screening for HBV infection in recent years, such as occurred in Latin American countries from 1994 to1997, has dramatically reduced the risk of acquiring HBV infection through transfusion. Other sources of infection include contaminated surgical instruments and donor organs. Health care workers, dentists, and others who have frequent contact with infected blood or blood products are at highest risk (Lavanchy, 2004).

### 2.1.11 Stages of infection

An individual can develop hepatitis B infection that is acute and achieve complete immune clearance of virus yielding lifelong immunity; however an alternate fate of the host is the development of chronic hepatitis B. There are three stages of HBV infection based on viral-host interaction, namely, the immune tolerant phase, the immune clearance phase, and the inactive carrier phase with or without reactivation. After acute infection of HBV, some patients may remain HBeAg positive with high levels of serum HBV DNA, little or no symptoms, normal ALT levels and minimal histological activity in the liver, this phenomenon is known as the immune tolerance phase. This phase is typical of infection in children and young adults. It usually lasts for 2-4 weeks, but can last for years in those who acquired the infection during the perinatal period. Individuals in this group are highly contagious and can transmit HBV easily.

When the tolerogenic effect is lost during the immune tolerant phase, immune-mediated lysis of infected hepatocytes become active and patients enter the second stage defined as immune clearance phase, the HBV DNA level decreases and ALT level increases. The duration of clearance phase lasts from months to years. This is followed by the carrier stage, in which sero-conversion of HBeAg to HBeAb occurs, HBV DNA becomes non-detectable or at low level and ALT is usually normal, reflecting very low or no replication of HBV and mild or no hepatic injury. The inactive carrier stage may last for years or even lifetime. Patients in this stage can have spontaneous resolution of hepatitis B and develop HBsAb, but a portion of them may undergo spontaneous or immunosuppressant-induced reactivation of chronic hepatitis, featuring elevated ALT, high level of DNA, moderate to severe liver histological activity, and with or without HBeAg sero-reversion(Pan and Zhang, 2005).

### 2.1.12 Clinical Symptoms

Many of HBV infection are asymptomatic and are detected only by presence of antibody to HBsAg. The mean incubation period for hepatitis B is 10-12 weeks. The clinical appearances of acute hepatitis B are fever, anorexia, nausea, vomiting and typical jaundice. Dark urine, pale feces and elevated transaminase level are seen they tend to be more severe than. Most chronic carriers are asymptomatic but some have chronic active hepatitis which lead to cirrhosis and death (Levinson, 2004).

### 2.1.13 Epidemiology

Worldwide, two billion people have been infected with hepatitis B virus (HBV), 360 million have chronic infection, and 600,000 die each year from HBV-related liver disease or hepatocellular carcinoma. This comprehensive review of hepatitis B epidemiology and vaccines focuses on definitive and influential studies and highlights current trends, policies, and directions. HBV can be transmitted vertically, through sexual or household contact, or by unsafe injections, but chronic infections acquired during infancy or childhood account

for a disproportionately large share of worldwide morbidity and mortality. Vaccination against HBV infection can be started at birth and provides long-term protection against infection in more than 90% of healthy people. In the 1990s, many industrialized countries and a few less-developed countries implemented universal hepatitis B immunization and experienced measurable reductions in HBV-related disease. For example, in Taiwan, the prevalence of chronic infection in children declined by more than 90%. Many resource-poor nations have recently initiated universal hepatitis B immunization programs with assistance from the Global Alliance for Vaccines and Immunization. Further progress towards the elimination of HBV transmission will require sustainable vaccination programs with improved vaccination coverage, practical methods of measuring the impact of vaccination programs, and targeted vaccination efforts for communities at high risk of infection (Shepard *et al.*, 2006)**.**

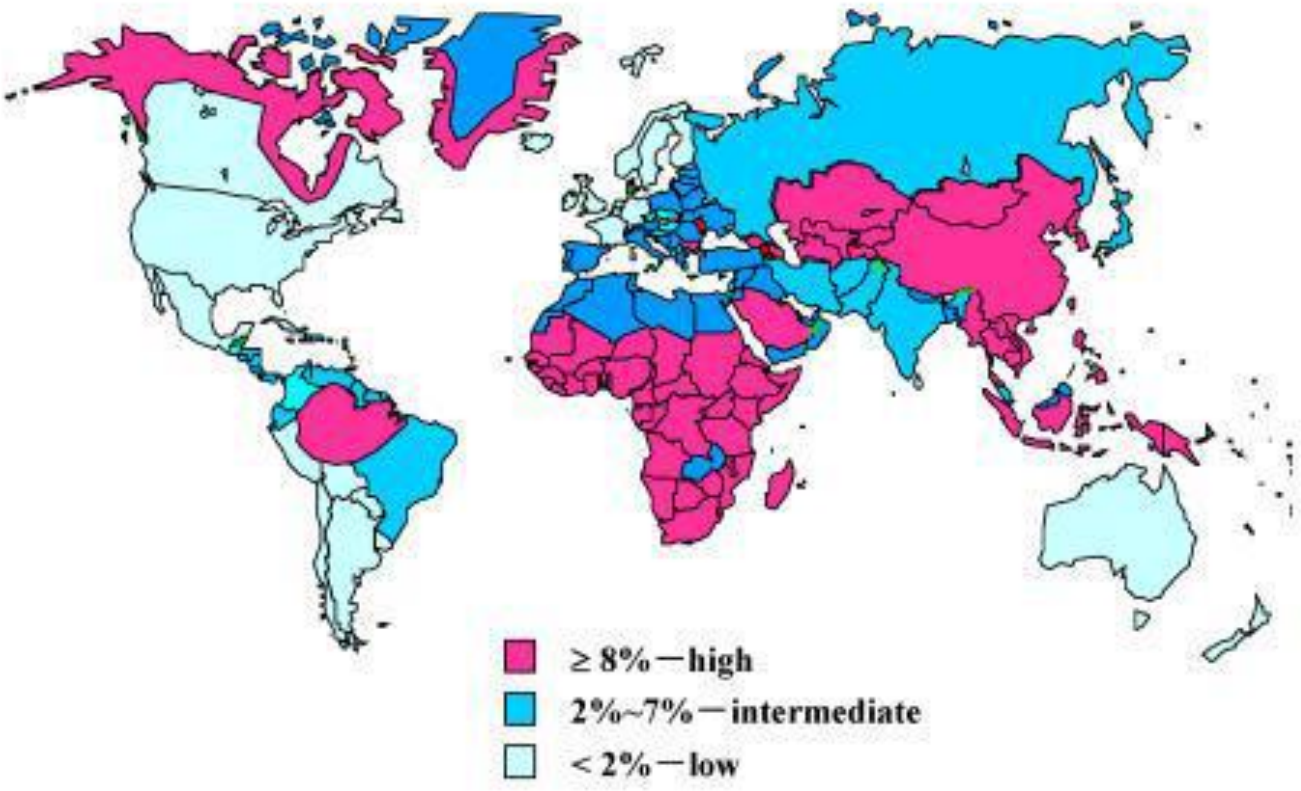


**Fig 2.2** Prevalence of HBV in the world available from:

[www.medsci.org/v02p0050.htm, 2013]

### 2.1.14 Diagnostic method of HBV

#### 2.1.14.1 Immunochromatographic assay for screening

Simple, rapid and accurate assays for hepatitis B surface antigen (HBs Ag) and hepatitis B e antigen (HBe Ag) are helpful for clinical diagnosis and field epidemiological surveys. A commercially developed, rapid immune-chromatographic test for simultaneous detection of HBs Ag and HBe Ag was evaluated using a total of 2463 selected samples (827 frozen sera, 1011 fresh sera, and 625 whole blood samples). Results of the rapid test were compared with standard enzyme immunoassay (EIA) methods for HBs Ag and HBe Ag detection. The accuracy of the rapid test was excellent and was similar for frozen sera, fresh sera and whole blood. The overall sensitivity and specificity for the detection of HBs Ag were 95 and 100%, and the corresponding positive and negative predictive values were 100 and 99.7%, respectively. The sensitivity and specificity for the detection of HBe Ag were slightly less than that for HBs Ag, and were 80 and 98%, with positive and negative predictive values of 91 and 94%, respectively. Thus, compared with the EIA method, the rapid test was highly sensitive and accurate for the detection of HBsAg although somewhat less sensitive and specific for detection of HBeAg. Because of its speed, simplicity and flexibility, the rapid test is ideally suited for HBs Ag and HBeAg screening in population-based epidemiological studies and in low risk populations, particularly in regions of the world where hepatitis B is endemic(Lemon *et al.*, 2003)

#### 2.1.14.2 Molecular Detection of HBV

Quantitative of hepatitis B virus (HBV) DNA in serum is a useful method for the monitoring of HBV replication. It is attempted to develop a quantitative assay system for HBV DNA that is more sensitive, accurate, and reproducible than existing systems. We detected HBV DNA by real-time detection PCR (RTD-PCR) based on Taq Man chemistry. The efficacy of this assay was evaluated by quantitatively measuring sequential levels of synthetic DNA and

DNA in clinical serum samples. The detection limit of this system was as 10 DNA copies/ reaction. A linear standard curve was obtained between 101 and 108 DNA copies/ reaction. The coefficient of variation for both intra- and inter-experimental variability indicated remarkable reproducibility. This system detected HBV DNA in 100% of chronic hepatitis B patients tested and never detected HBV DNA in healthy volunteers who were negative for HBV markers. These observations suggest that RTD-PCR is an excellent candidate for a standard HBV quantification method (Abe *et al.*, 1999).

### 2.1.14.3 Advances in Molecular Diagnosis of HBV Infection and Drug Resistance

Serological markers are key elements in diagnosing acute hepatitis B virus (HBV) infection and determining its possible evolution towards chronicity. Once treatment of chronic HBV is initiated with approved anti-hepadnaviral agents, such as lamivudine, interferon-alpha, or adefovir dipivoxil, the measurement of HBV DNA in serum can not only help monitor treatment efficacy but also indicates breakthrough infection should drug resistance emerge. Advances in the molecular diagnosis of drug resistance using highly sensitive methodologies such as DNA hybridization assays can further pinpoint the type of mutation responsible and, more importantly, detect upcoming viral resistance at an early stage when the variant represents only a minor fraction of the total viral population. Such new tools are especially relevant for patients at high risk for disease progression or acute exacerbation. Recent diagnostic developments including HBV genotyping and precore/core promoter assays that could well play important future roles in HBV patient management are also reviewed (Sablon and Shapiro, 2005).

### 2.1.14.4 Liver biopsy

Liver biopsy is usually the most specific test to assess the nature and severity of liver diseases. In addition, it can be useful in monitoring the efficacy of various treatments. There are currently several methods available for obtaining liver

tissue: percutaneous biopsy, transjugular biopsy, laparoscopic biopsy or fine-needle aspiration guided by ultra sonography or computed tomography (CT). Each of these methods has advantages and disadvantages. Liver biopsy provides an accurate diagnosis in approximately 90 percent of patients with unexplained abnormalities revealed on liver-function tests (Bravo *et al.*, 2001)

**2.1.14.5 Immunization against hepatitis B infection**

The cornerstone of prevention for patients who are HBV seronegative is immunization. Currently available HBV vaccines are very safe and have an efficacy of >90% in immune-competent young individuals. Non-response is associated with a number of factors including genetically determined resistance, advanced age, obesity, chronic liver disease, smoking, male gender and miscellaneous systemic diseases including renal failure Although universal vaccination of newborns has been implemented worldwide according to World Health Organization recommendations, it will take several decades until the majority of the world's adult population will be immune. Delaying administration of the third dose in healthy individuals (up to 1 year) may increase anti-HBs antibody levels. Frequently, in haemato-oncological patients, urgent administration of chemotherapy does not allow completion of the three-dose regimen. In such cases, an effort should be made to immunize patients with at least two doses within a 3– 4 week interval. The third dose can then be given a few months after chemotherapy is completed (Sheppard *et al*., 2006). The world's first universal vaccination program for HBV infection was launched in 1984 in Taiwan (Ni *et al*., 2001). In 1999, vaccination rates were 80–86% for young children and higher than 90% for older children; the prevalence of HBsAg was reduced to 0.7% for children younger than 15 years of age (Ni *et al.*, 2001). To evaluate the long-term efficacy of hepatitis B (HB) vaccination in newborns, one of the longest HB vaccine follow-up studies in the world was conducted in Shanghai, China (Zhou *et al*., 2003).

### 2.1.14.6 Serology

**Diagnosis is confirmed by demonstration in sera of specific Antigens**:

Three clinical useful antigen systems have been identified for hepatitis B:

Hepatitis B surface antigen **(**HBs Ag), hepatitis B core antigen (HBc Ag) and hepatitis B e antigen (HBe Ag**)**.

HBs Ag can be detected in the serum from several weeks before onset of symptoms two months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious (Mahoney and Kane, 1999). Acute hepatitis patients who maintain a constant serum HBsAg concentration, or whose serum HBe Ag persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver. The presence of HBeAg is associated with relatively high infectivity and severity of disease.

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

**Core antigen (HBeAg) and its antibody (anti-HBe):** Naked DNA strands and associated proteins make up HBeAg.The presence of HBeAg in serum indicates active viral replication, and it persists in patients with chronic disease, its presence correlating with infectivity. As the patient with acute hepatitis B recovers, HBeAg disappears, and anti-HBe appears. Seroconversion from HBeAg to anti-HBe usually corresponds with the disappearance of hepatitis B virus DNA from the serum (Pommerville, 2004).

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

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

**2.1.15. Prevention &control**

Broadly there are two approaches to the prevention of infection with HBV modification of risk behavior and immunization. Measures for the former include avoiding unprotected sexual contact by the use of condoms and reducing needle-sharing among injecting drug users through needle exchange schemes implementation of sensible infection control policies can reduce the risks considerably to healthcare workers and patients .it is essential that blood for transfusion and organ donors for transplantation are screened (Greenwood *et al.*, 2012).

The whipping ritual or back-lashing using leather whip, a traditional ritual by many tribes of Sudan " Jalabia " are stripped off by young male Sudanese and flogged by the groom and other family members, to show endurance and braveness to empress ladies, available from [www.nerdygaga.com, 2014].

Young Sudanese men lashed in traditional wedding ceremony to show bravery many: different countries have many different wedding traditions. And the one in the River Nile state in and many northern trips Sudan don't not seem to be very hospitable to the guests, the groom whipping the male guests. The practice, known locally as 'Al-botan', it is passed down through generations. It's seen as a sign of dignity to take part in the ceremony and young boys in particular are often keen to be chosen to be whipped to demonstrate their maturity and braveness. Wedding guests show their appreciation to the groom by stripping off their clothes and allowing him to lash them. Sometimes he doesn't stop until they bleed. It's taken as given that the groom will have been whipped many times before in previous ceremonies so this is his turn to do the lashing. "It is our tradition to have this lashing ceremony at all wedding celebrations,' said one

of Al-Ja'alieen groom.”Sometimes the lashing is considered a debt meaning that if you lashed somebody in one celebration you should expect to be lashed by that person the next time round," he said. In Um Ali Village wedding guests beat drums and sing to encourage boys to take part in the whipping ceremony. The boys dance and jump to express pride and dignity before they are whipped. "The lashing ceremony is a special Al-Ja'alieen tradition. The style of whip and the practice of whipping originated with the Al-Ja'alieen tribes in Shendi, Ed Damer and Barbar in Sudan," said Amir Bashir, a poet in the village. While men and boys are being whipped, the women and girls in the village sing and dance to show that they are proud of their men. Zahia, a woman from the Al-Ja'alieen tribe said: "Women encourage men to be whipped while men demonstrate their braveness by not flinching when they are being lashed in front of the crowd." Although whipping ceremonies originated in rural areas the practice has spread to cities and even the capital Khartoum, where well known musicians can be found beating out rhythms on ceremonial drums known as Aldallookah and singing traditional Sudanese folk songs about bravery and tribal ideals. Kamal Idriss, a well-known musician in Khartoum said: "If a man was told he was going to be whipped without all the music and celebrations then he might get angry but the songs and dancing help people to forget themselves and they take off their clothes without thinking ready to be whipped." The sense of hysteria is seen as particularly important in encouraging the youngest members of the tribe to get caught up in the celebrations and want to take part. "When I was younger if I wasn't whipped during a wedding ceremony I'd get angry and leave the party but if I was chosen to be whipped it would make me very happy," said El Sheikh Abbas, one of the older tribesmen. For the young boys, chivalry and braveness is a way to honor older generations and family traditions. They also look forward to one day when they are grooms themselves, to be on the other side of the whip and play their part in inspiring the next generation, available from URL [www.intouece.com, 2010].

**Table 2.1** Prevalence of HBV among blood donors in eight States in Sudan 2014 (Abdo *et al.*, 2015)

| State | Total No of donor | No of HBs Ag positive | Percentage |
|---|---|---|---|
| Khartoum | 90905 | 2505 | 2.5% |
| Al-Gezira | 27990 | 1470 | 5.2% |
| Sinar | 20171 | 863 | 4.2% |
| Kassala | 10910 | 499 | 4.5% |
| Red sea | 4214 | 187 | 4.4% |
| Al-Gadarif | 12042 | 1063 | 8.8% |
| Blue Nile | 6178 | 254 | 4.1% |
| White Nile | 10970 | 894 | 8.14% |

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study design

This study was a descriptive non probability comparative cross sectional study

## 3.2 Study area

This study was conducted in rural area of Al-Gadarif State

## 3.3 Study Population

Men whipped during wedding ceremonies from rural areas in Al-Gadarif State and others not whipped during wedding ceremonies from rural areas in Al-Gadarif

## 3.4 Study Period

The study was conducted during the period from January to April 2017

## 3.5 sample size

A total of hundred (50) whipped men and (50) not whipped were enrolled for this study

## 3.6 Inclusion criteria

Men whipped during wedding ceremonies and not whipped during wedding ceremonies

## 3.7 Exclusion criteria

Females, elders and refugees

## 3.8. Data collection

Personal data was obtained by interviewing the recruits and filling a structured questionnaire.

## 3.9. Ethical consideration:

Permission to carry out the study was taken from the College of Graduate Studies, Sudan University of Science & Technology. The aims of the study

were explained to participants before collection of the specimens and verbal consent was taken from them.

### 3.10. Laboratory work

#### 3.10.1 Sample processing

Three ml of venous blood were drawn from patients after disinfection of the area using 70% alcohol, the blood samples were left for 30 min to clot and then centrifuged at 3000 r.p.m for 10 min, the serum sample was separated into plain container and stored at -20℃until used.

All the separated serum samples were tested for presence of HBsAg using commercial available ELISA kit (Fortress diagnostic, high sensitive).

#### 3.10.2 Principle of assay

The test was the enzyme-immunoassay based on a (sandwich) principle. Polystyrene micro titer strip well have been coated with monoclonal anti-HBs Ag (antibody to HBs Ag).patient serum or plasma sample is added to the micro-wells. During incubation the specific immune complex formed in case of presence of HBs Ag in the sample, is captured on solid phase. After washing to remove sample serum proteins second antibody conjugated to enzyme HRP and direct against a different epitopes of HBsAg is added to the wells. During the second incubation step these HRP conjugate antibodies will be bound to any anti HBs Ag-HBs Ag complex previously formed during the first incubation and unbound HRP conjugate is then removed by washing after washing chromogen solutions containing TMB and Urea peroxides are added to wells .in presence of the antibody-antigen-antibody HRP sandwich immune-complex, the colorless chromogen are hydrolyzed by the bound HRP conjugate to blue coloured product. The blue colour turn yellow after stopping the reaction using the stop solution the colour intensity can be measured and it proportional to amount in the sample respectively. Well containing sample negative for HBsAg remain colorless.

### 3.10.3 Procedure

**Step 1-Reagent preparation**

They were allowed to reach room temperature (18-30) $C_o$ for (15-30) minutes. The stock wash buffer had been diluted 1: 20 with distilled water.

**Step 2- Numbering of Wells**

The strip needed were set in strip holder and sufficient number of wells including one blank(A1) two negative control(B1,C1)and one well as positive control (D1) were numbered.

**Step 3-Adding Sample Diluents**

Twenty ul of sample Diluents were added to each well except the blank and mix by taping the plate gently

**Step 4 adding sample**

Hundred ul of positive control, negative control and specimen were added into their respective wells except blank.

**Step 5 Incubation**

The plate was covered with the plate cover and incubated for 60 minutes at 37℃

**Step 6 Added HRP Conjugate**

Then 50ml of HRP-conjugate were also added to each well except to blank well and mixed by tapping the plate gently.

**Step 7- Incubation**

The plate was covered with the plate cover and incubated for 30 minutes at 37℃

**Step 8- washing**

After the end of the incubation the plate cover was removed ,each well was washed 5 time with diluted washing buffer each time the well were allowed to soak for 30-60 second. After the washing end the plates were turn down onto blotting paper to remove any remainders.

**Step 9- Coloring**

Fifty ul of chromogen A and 50ul of chromogen B were added into each well including blank and mixed by tapping plate. The plate was incubated at 37℃

for15 minutes with avoiding light. Blue color was developed in positive control and HBs Ag positive sample wells.

**Step 10- stopping the reaction**

Fifty ul of stop solution were added into each wells and mixed gently intensive yellow color was developed in positive control and HBs Ag positive Sample Wells.

**Step 11 –Measuring the Absorbance**

The absorbance was read at 450nm using the ELISA reader.

**Calculation of the result**

The result was calculated by relating each specimen absorbance (A) value to cutoff value (C.O) of the plate.

**Calculation of cut-off value**

(C.O)=NC×2.1 NC (the mean absorbance value to tow negative controls

**Quality control range**

The A value of blank well which contains only chromogen and stop solution were less than 0.080 at 450nm The A value of positive control was more than equal 0.800 at 450nm The A value of negative controls were less than 0.100 at 450 nm

**3.10.4 Interpretation of the results**

**Negative Results**

Sample giving a value less than cut-off value are negative for this assay which indicate that no HBs Ag antibodies have been detected with this HBs Ag ELISA kits therefore the patient is probably not infected with hepatitis B virus.

**Positive Results:**

Samples giving a value greater than or equal to cut-off value are considered initially reactive which indicate that HBV surface antigen has probably been detected with this HBs Ag ELISA kit.

**Borderline**

Sample with A value to cut-off ratio between 0.9 and 1.00 are considered borderline samples And retest is recommended. Repeatedly positive sample can be considered positive for HBs Ag.

### 3.10.5. Data Analysis

Collected data were analyzed by statistical package for social sciences (SPSS) version (16) value were considered significant at P. values<0.05.

# CHAPTER FOUR

# RESULTS

A total of hundred men 50 whipped and 50 not whipped were enrolled in this study, age range was from 20 to 37 years (mean: 28.12 years), all of them from rural areas where no one was vaccinated against HBV (100%). The mean of whipping duration period is (8.6) years, history of previous transfusion 3 (6%) and history of jaundice is 3 (6%) in 50 of the whipped men. Most of the infected men were in age group between (29-37) years.

**Frequency of HBsAg among whipped men and not whipped**

This study revealed that 9 (18%) of whipped men were positive and 1 (2%) was positive in the other group.

**Table 4.1** Distribution of HBsAg positive among whipped and not whipped men during wedding ceremonies in Al-gadrif State.

| Results | | HBsAg Positive | HBsAg Negative | p.value |
|---|---|---|---|---|
| Study population | Whipped | 9 | 41 | 0.008 |
| | Not whipped | 1 | 49 | |

Pearson chi-square

(P value <0.05 consider significant).

**Distribution of frequency groups among positive results**

The result showed that 3(6 %) were positive in (3-9) yrs out of (18%) and 6 (12%) were positive in (10-17) yrs out of (18%) among whipped group.

**Table4.2** The relationship between HBsAg positive and frequency of whipping ritual among whipped men during wedding ceremonies in Al-gadrif

| Test | | Frequency of whipping | | Total |
|---|---|---|---|---|
| | | 3-9 | 10-17 | |
| HBsAg in whipped group | Positive | 3 | 6 | 9 |
| | Negative | 28 | 13 | 41 |

**Distribution of age groups among positive results**

Displays that distribution of age group in positive results, among age group (20-27) years 3(6%) out of (18%) were positive for HBs Ag and 6(12%) out of (18%) among age group (28-37).

**Table 4.3** Frequency of HBs Ag among age groups of whipped men during wedding ceremonies in Al-gadarif State.

| Test | | Age group | | Total |
|---|---|---|---|---|
| | | (20-28) yrs | (29-37) yrs | |
| HBsAg | Positive | 3 | 6 | 9 |
| | Negative | 18 | 23 | 41 |
| Total | | 21 | 29 | 50 |

**Odd ratio:**

Odd ratio = a d \ b c

9*49 \ 1* 41 = 10.7

**Interpretation of result**

The odds of whipping ritual are 10.7 times greater among those HBV infected than the odds of whipping ritual among those without HBV infection.

**Compare the prevalence of HBs Ag positive in person with whipped men:**

[a \ (a + b)] = 9\9+49 = 18 %

**Compare the prevalence of HBs Ag positive in person with not whipped men:**

[a \ (a + c)] = 9\9+1 = 2 %

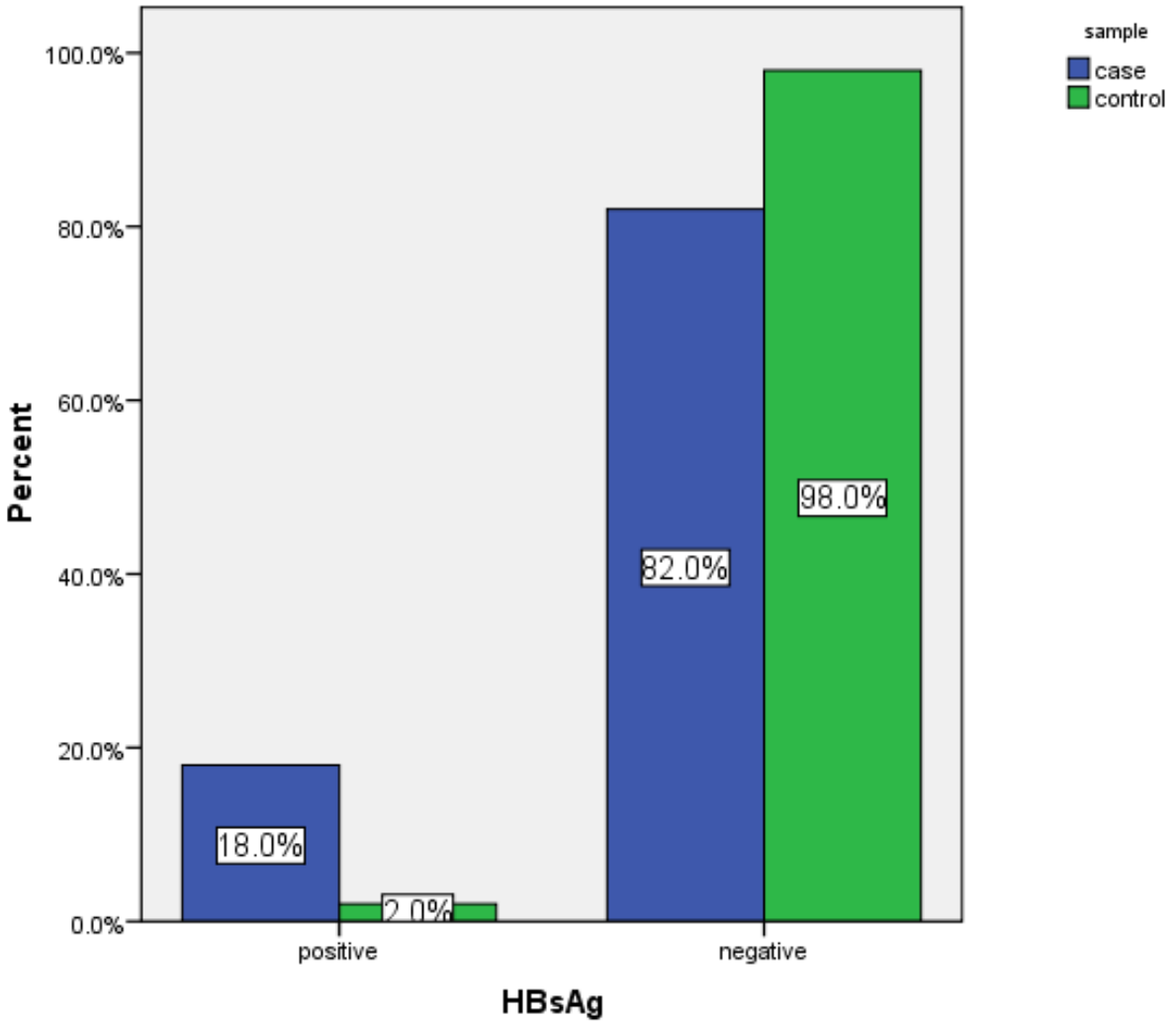


**Figure 4.1** Distribution of HBs Ag among whipped men during wedding ceremonies and not whipped men

**Table 4.4** The relationship between HBV and selected risk factors among men whipped during wedding ceremonies in Al-gadarif State

| Risk factors | | | | P value |
|---|---|---|---|---|
| Blood Transfusion | Yes | 1 | 3 | 0.704 |
| | No | 8 | 38 | |
| | No | 7 | 37 | |
| Age groups | 20-27 yrs | 3 | 18 | 0.561 |
| | 28-37yrs | 6 | 23 | |
| Whipping ritual Frequency groups | 3-9 yrs | 3 | 28 | 0.040 |
| | 10-17 yrs | 6 | 13 | |
| Residence | Al Gadarif | | | |
| Vaccination | All non-vaccinated | | | |
| Occupation | Farmers | | | |

# CHAPTER FIVE

# Discussion

## 5.1 Discussion

In the present study 100 men were tested 50 (whipped during wedding ceremonies) and 50 were not whipped. Both were tested for HBsAg, 9 (18%) were positive for HBV among those whipped during wedding ceremonies, while 1(2%) were positive for HBV in the other group. These results indicate high frequency of HBV among those whipped during wedding ceremonies compared to the other group. These results showed significant association between whipping ritual and Hepatitis B virus infection p-value was 0.008. Insignificant association between HBV and history of blood transfusion in whipped men was found in this study probably due to frequent donation processes rather than blood reception. Furthermore, there was significant association between the frequency of whipping and HBV infection; this could be attributed to the increase in exposure times of whipping which increase the possibility of infection transmission.

This study has shown that whipping ritual during wedding ceremonies using the same tool for lashing several men could be a potential source for the transmission of HBV and other blood borne infections. This study showed higher positive HBV than the study about people who got tattooing in Brazil (Nishioka *et al.*, 2002).

## 5.2 Conclusions

1. The study showed association between whipping during wedding ceremonies and hepatitis B infection.

2. The results showed significant association between positive HBs Ag and frequency of whipping ritual.

3. There were insignificant association between HBV infection and other possible risk factors, including residence, age and occupation.

## 5.3 Recommendations

1. The whipping ritual should be stopped by Health authorities of Sudan

2. Hepatitis B virus should be a routine investigation among men whipped during ceremonies.

3. Further studies with larger sample size and among bigger tribe is essential to validate the result of this study.

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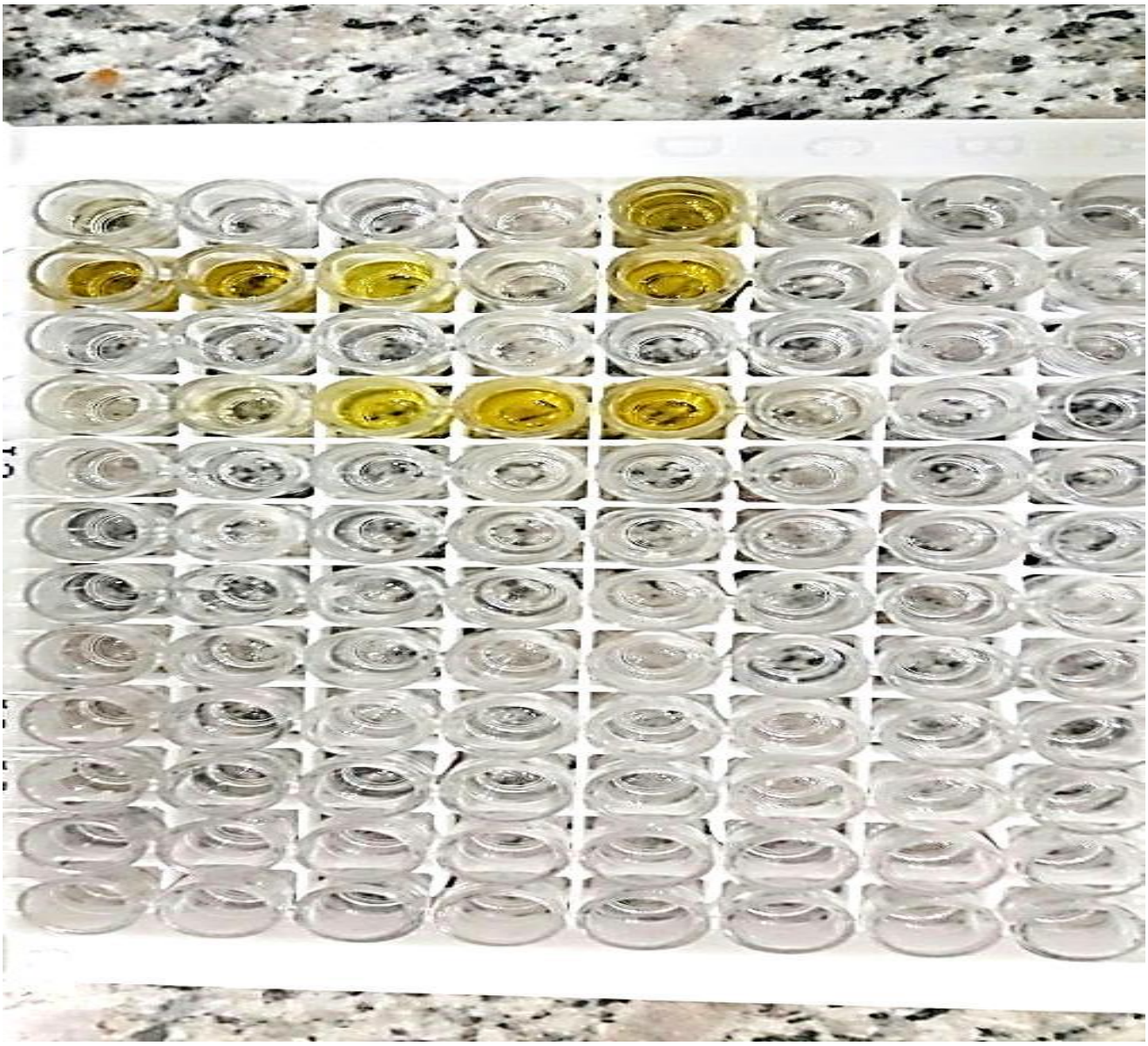
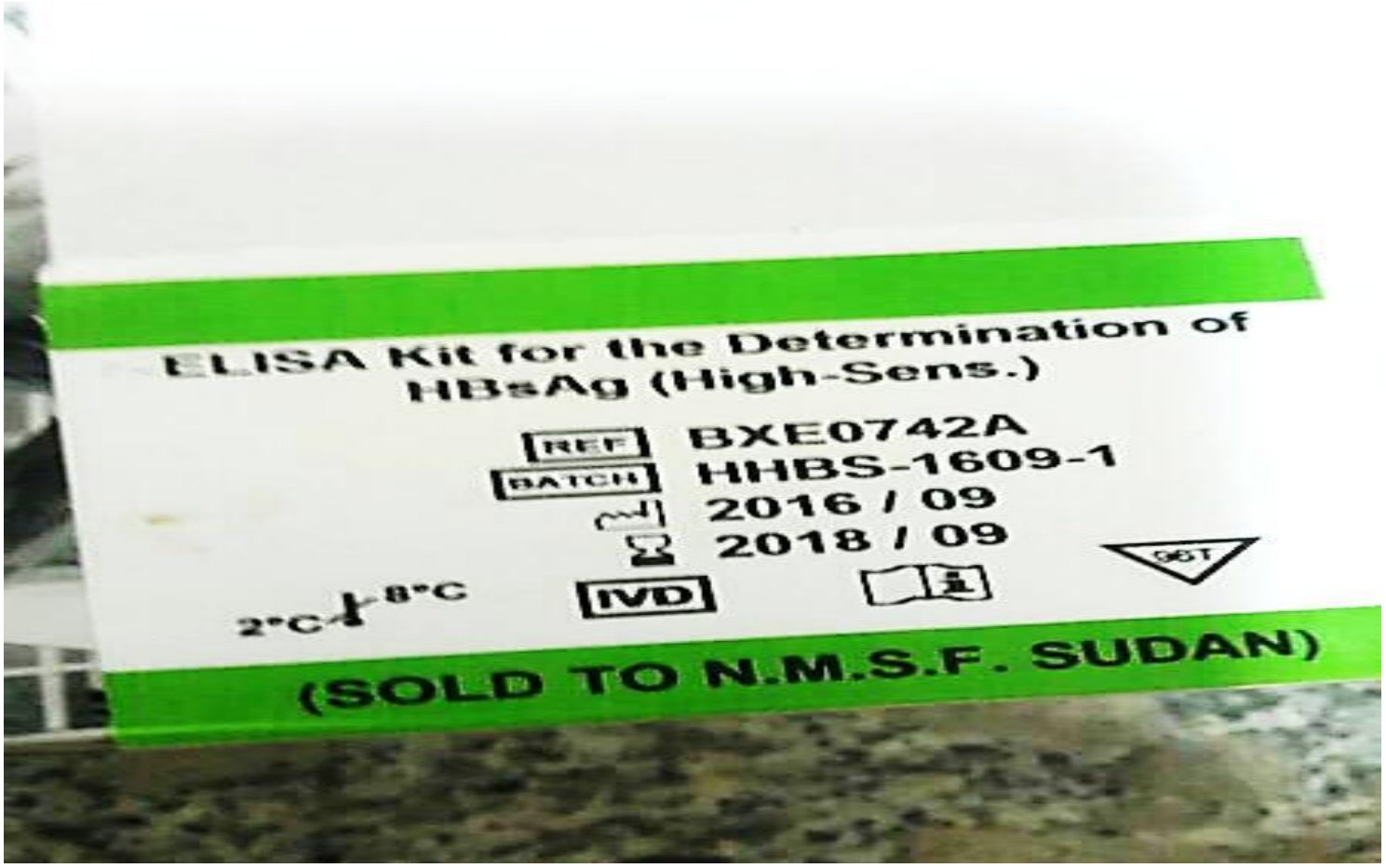
## *Appendixes*



**ELISA micro titer color plates see the result of HBsAg in population .**




**ELISA kits for determination of HBs Ag (high sens)**