

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**

**College of Graduate Studies**

**Serodetection of Hepatitis B and C Virus among Blood  
Donors at the National Laboratory for Public  
Health**

الكشف المصلي الفيروسي لالتهاب الكبد (ب) و (ج) لدى المتبرعين بالدم في المعمل القومي  
للصحة العامة

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بسم الله الرحمن الرحيم

قال تعالى: (اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ  
وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)).

صدق الله العظيم

سورة العلق- الآيات (1-5)

## **DEDICATION**

I dedicate this research to the soul of my father.

To my mother, husband, brother and sister.

## ACKNOWLEDGEMENTS

Firstly, thanks to ALMIGHTY ALLAH for giving me strength to carry out this research.

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## **ABSTRACT**

The objectives of this study were determined the serodetection and the possible risk factors of hepatitis B and C virus infections among the blood donors attending the National Laboratory for Public Health, Khartoum, Sudan.

Hundred (100) subjects were investigated during the period from September to December 2013. All the subjects examined were males, their age ranging from 20 to 59 years. The hepatitis B Surface Antigen (HBs Ag) and hepatitis C virus antibodies (Anti-HCV Abs) were detected among blood donors using both Immunochromatographic test (ICT) and the enzyme linked Immunosorbent assay (ELISA). Data were collected by structured questionnaire.

Hepatitis C virus (HCV) specific antibodies (anti-HCV Abs) was detected among (3.0%) of blood donors and hepatitis B Surface Antigen (HBs Ag) was detected among (6.0%) of blood donors. There was no significant difference ( $p<0.05$ ) between the prevalence of HBs Ag or HCV Abs among married blood donors compared to the single donors.

Subjects of younger ages 20 to 30 years, had been significantly higher infection rate ( $p<0.05$ ) with HBV (4 cases) and HCV (2 cases) when compared to other groups. In all subjects examined, infection with both viruses was never detected. Both techniques were, equally, sensitive in detection of both serological markers. History of jaundice was found to be a significant ( $p<0.05$ ) finding to both viral infections. However, no other risk factors (e.g. Previous blood transfusions, needle stick injuries, alcohol intake and previous surgical operations) were found to be significant ( $p<0.05$ ) to contract both diseases.

## المستخلص

هدفت هذه الدراسة بصورة أساسية إلى تحديد مدي انتشار الإصابة بالتهاب الكبد الفيروسي "ب" و "ج" وتحديد عوامل الخطر المحتملة الممهدة للإصابة بها وسط المتبرعين بالدم بالمعمل القومي للصحة العامة الخرطوم – السودان.

شملت الدراسة 100 متبرعا بالدم خلال الفترة من سبتمبر حتى ديسمبر 2013 كل المتبرعين الذين تم فحصهم من الذكور ، تتراوح أعمارهم بين 20-59 سنة كانت نتيجة المستضد السطحي لفيروس الكبد "ب" والذي يعتبر المؤشر الرئيسي للإصابة بالتهاب الكبد الفيروسي "ب" ايجابية في (6%) من المتبرعين بالدم باستخدام كل من تقنيتي الكروماتوغرافي والاليزا . أما الأجسام المضادة لفيروس الكبد "ج" فكانت ايجابية في (3%) من المتبرعين باستخدام نفس التقنيات أعلاه . لم تظهر الدراسة أي فروقات ذات دلالة إحصائية ( $p < 0.05$ ) بين انتشار المستضد السطحي لفيروس الكبد "ب" والأجسام المضادة لفيروس الكبد "ج" وسط المتزوجين المتبرعين بالدم مقارنة بغير المتزوجين من المتبرعين .

أوضحت الدراسة أن المتبرعين الشباب الذين تتراوح أعمارهم بين 20-30 سنة أكثر عرضة للإصابة ( $P < 0.05$ ) بالتهاب الكبد الفيروسي "ب" (4 حالات) والتهاب الكبد الفيروسي "ج" (2 حالة) مقارنة بالفئات العمرية الأخرى، بينما لم تبين الدراسة أي إصابة مشتركة بكلا الفيروسين معا في جميع المتبرعين بالدم الذين تم فحصهم. كانت التقنيتين المستخدمتين علي السواء حساستين تجاه الكشف عن المؤشرات المصلية للفيروس . خلصت الدراسة الي أن تاريخ التعرض لليرقان كان عاملا ذو دلالة إحصائية ( $p > 0.05$ ) للإصابة بكلا الفيروسين. بينما لم تظهر الدراسة أي تأثيرات ذات دلالة إحصائية ( $p > 0.05$ ) لعوامل الخطر الأخرى (التاريخ السابق لنقل الدم، وحوادث الطعن بالإبر الملوثة، إدمان الكحول، العمليات الجراحية السابقة) للإصابة بالفيروسين.

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## **LIST OF ABBREVIATIONS**

**ALT**= Alanine Aminotransferase.

**AST**= Aspartate Aminotransferase.

**Au Ag**= Australia Antigen.

**EIAs**= Enzyme Immunoassays.

**ELISA**= Enzyme-Linked Immunosorbent Assay.

**HAV**= Hepatitis A Virus.

**HBc Ag**= Hepatitis B core Antigen.

**HBe Ag**= Hepatitis B e Antigen.

**HBIG**= Hepatitis B Immunoglobulin.

**HBs Ag**= Hepatitis B surface Antigen.

**HBV**= Hepatitis B Virus.

**HCV**= Hepatitis C Virus.

**HCV Abs.** = Hepatitis C Virus Antibodies.

**HD**= Haemodialysis.

**HD Ag**= Hepatitis D Antigen.

**HDV**= Hepatitis D Virus.

**HEV**= Hepatitis E Virus.

**HGV**= Hepatitis G Virus.

**HRP**= Horse Radish Peroxidase.

**ICT**= Immunochromatographic Test

**NANBH**= Non-A, Non-B Hepatitis.

**PCR** = Polymerase Chain Reaction.

**RT**= Reverse Transcriptase

**CHAPTER ONE**  
**INTRODUCTION AND OBJECTIVES**

## CHAPTER ONE

### INTRODUCTION AND OBJECTIVES

#### 1.1. Viral hepatitis

Hepatitis means inflammation and damage to the liver, and has different etiologies including non – infectious multi system conditions and drug toxicity as well as infectious agents. The latter include viruses and less commonly bacteria e.g. *Leptospira sp.* and other microorganisms (Mims *et al.*, 2004). Five different human hepatitis viruses have been recognized and characterized in details and a sixth agent has just been discovered. The five established viral agents are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV), the sixth agent is hepatitis G virus (HGV) (Reid and Dienstag, 1997).

All human hepatitis viruses are RNA viruses except hepatitis B which is DNA virus. Hepatitis viruses produce acute inflammation of the liver, resulting in clinical illness characterized by fever, gastrointestinal symptoms such as nausea vomiting and Jaundice (Brooks *et al.*, 2007). Although blood transfusions save millions of lives worldwide each year, recipients of blood have increase risk of becoming infected with blood – borne pathogens.

Viral hepatitis has become an issue of global importance, In Sudan, HBV infections still remain a public health problem. Determination of prevalence of HBV and HCV in population in general, and among blood donors in particular, will certainly help in reviewing the screening procedures and in making health policy decisions easier.

## **1.2. Rationale**

Blood transmitted diseases are the most serious complication of blood transfusion particularly viral infection. Thus blood screening prior to transfusion is becoming the most important procedure to minimize the risk of blood transfusion related to viral infections. This, in population general and among blood donors in particular, will certainly help in reviewing the screening procedures.

The study is conducted to obtain information about seroprevalence of Hepatitis B and C viruses in blood donors attending the National Laboratory for Public Health, Khartoum, Sudan.

## **1.3. Objectives**

### **1.3.1. General objective**

To determine the seroprevalence of HBV and HCV among Sudanese blood donors.

### **1.3.2. Specific objectives**

- 1- To detect HBs Ag and anti-HCV Abs in the blood donors.
- 2- To evaluate the possible risk factors and profounder associated with HBV and HCV seropositivity.



**CHAPTER TWO**  
**LITERATURE REVIEW**

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1. History**

The understanding of viral hepatitis has evolved more rapidly in the last 30 years than at any other time in history. Although epidemics of Jaundice were recognized as early as the middle ages, the notion that such out – breaks were the result of hepatitis caused by infectious agents was not known until the years at World War 2 (Reid *et al.* and Dienstag, 1997).

Much of the agents and pathogenesis of viral hepatitis were derived from inoculation experiments with human volunteers in various countries during World War 2, and subsequently in the United States, particularly at the Willow brook State School, New York (Duguid *et al.*, 1983). The virus of type B hepatitis first came into view with the serendipitous discovery of so called Australia Antigen (Au Ag) by Blumberg and colleagues in 1965 (Wright *et al.*, 1997).

They were interested in serum protein polymorphisms as genetic markers in human population. To identify such polymorphisms, they screened sera from multiple-transfused individuals for antibodies to normal human serum proteins. Such screening led to the detection of distinctive antigen, initially termed Australia antigen (AuAg) because it was first recognized in the blood of an Australian Aborigine (Wright *et al.*, 1997).

AuAg was found to be present in high levels in many other sera from leukemic, dialysis patients, institutionalized retarded children and other groups .The specific relationship of AuAg with hepatitis B virus emerged from examinations of sera

specimens generated from a human inoculation experiment conducted at the Willowbrook State School (Duguid *et al.*, 1983).

Testing sera from this experimental study revealed that AuAg was uniquely associated with the long incubation period, parenterally transmissible form of the disease now known as hepatitis B (Wright *et al.*, 1997).

### **2.3. Hepatitis B Virus**

Hepatitis B virus is classified as hepadnavirus, also known as "Dane Particles". HBV establishes chronic infections, especially in those infected as infants. It is a major factor in the eventual development of liver disease and hepatocellular carcinoma in those individuals (Brooks *et al.*, 2007).

#### **2.3.1. Structure**

Hepatitis B virus is a small, enveloped, DNA virus with several unusual properties. Specifically, the genome is a small, circular, partly double – stranded DNA of only 3200 bases. Although DNA virus, it encodes a reverse transcriptase and replicates through an RNA intermediate (Murray *et al.*, 2002).

Electron microscopy of HBs Ag – positive sera reveals three morphologic forms (Brooks *et al.*, 2007). The virion is 42 nm in diameter. The virions are usually stable for an enveloped virus. They resist treatment with ether, a low PH, freezing, and moderate heating.

HBV virion includes a polymerase with reverse transcriptase and ribo – nuclease H activity, and a P protein attached to the genome, which is surrounded by the hepatitis B core antigen (HBc Ag), and an envelope containing the glycoprotein, hepatitis B

surface antigen (HBs Ag). Hepatitis B e antigen (HBe Ag) protein is minor component of the virion.

The HBe Ag and HBc Ag proteins share most of their protein sequence (Murray *et al.*, 2002). HBs Ag contains a common 'a' antigen, and two sets of mutually exclusive determinants, 'd' or 'y' and 'w' or 'r' giving the four main types – adw, dr, ayw and ayr, the letter being associated with different HBV genotypes. Fortunately, the invariant 'a' determinant is the main target for the protective antibody response to infection, and immunity induced by infection or immunization with one HBV genotype will cross – protect against infection with other (Greenwood *et al.*, 1996).

### **2.3.2. Properties of Hepadna viruses**

The defining features of the family are:

Relaxed circular, partially duplex DNA genome of approximately 3kb, enveloped virus of 40 nm with an outer lipoprotein envelope and an inner nucleocapsid or core, a virion – associated polymerase activity in the viral core, Production or export of 20 – 22 nm sub viral lipoprotein particles composed of envelope protein in the absence of other viral components, and relative but not absolute hepatotropism (Wright *et al.*, 1997).

### **2.3.3. Stability of the virus**

It is difficult to assess the stability of HBV due to the lack of suitable laboratory culture system (Greenwood *et al.*, 1996).

Indirect evidence has been obtained from the study of recipients of blood products treated in various ways and chimpanzee inoculation experiments. Thus it was established that:-

Heating to 60<sup>0</sup> C for 10 hours inactivates the virus. 2-Treatment with hypochlorite. Or 2% glutaraldehyde for 10 min will inactivate the virus.

Studies based on the survival of HBs Ag show that it is much more resistant to destruction (Greenwood *et al.*, 1996).

#### **2.3.4. Epidemiology of HBV**

Hepatitis B virus is worldwide in distribution. Chronic hepatitis B is a common disease with an estimated global prevalence of over 300 million HBs Ag carriers, or approximately 5% of world population (Duguid *et al.*, 1983). In 2004, an estimated 350 million individuals were infected world wide (Brooks *et al.*, 2007).

There are wide ranges in the prevalence of HBV infection in different parts of the world:-

Highest rates HBs Ag 10 - 15%, anti – HBs Abs 70 - 90% were reported in South - East Asia, China, Equatorial Africa, Oceania and South America. Vertical and horizontal transmission are both common.

Intermediate rates HBs Ag 5%, anti- HBs Abs 30 - 90% in Eastern Europe, around Mediterranean, Russia, Japan, South American and Middle East.

Lowest rate HBs Ag 0.1 - 0.5%, anti-HBs Abs not more in Western Europe, North America and Australia ( Greenwood *et al.*, 1996).

According to World Health Organization (WHO), an estimated 600,000 people die every year related to infection. In the United States about 19,000 new cases occurred in 2011.

Transmission modes and response to infection vary, depending on the age at time of infection. Most individuals infected as infants, develop chronic infection. As adults

they are subject to liver disease and are at high risk of developing hepatocellular carcinoma (Brooks *et al.*, 2007).

HBV is present in the blood and also body fluids such as semen, vaginal secretions and saliva, although the concentration is only about 1 in 1000 of that in blood.

However, this may still represent a large number of infectious virions. The presence of HBV in blood underlines the original association of infection with blood transfusion or the use of blood product post-transfusion serum hepatitis, and infections associated with needle-stick injuries. Sexual transmission is also recognized, as is that which occurs between family members, siblings, peers and residents in institution for learning – impairment (Greenwood *et al.*, 1996).

In these circumstances there will be frequent contact with blood and saliva, the virus will gain entry through cuts and abrasions or across mucus membranes.

Vertical transmission from mother to child is one of the most important routes. Transmission occurs when maternal blood contaminates the mucus membranes of the baby during birth. Transplacental infection is thought to be quite rare.

Health care personnel and laboratory workers are at risk from patients, although the degree of risk varies with the place and nature of their work, the care with which it is performed and their immune status (Greenwood *et al.*, 1996).

#### **2.3.4. High risk groups for HBV infection**

These include people from endemic regions (such as China, parts of Africa, Alaska, and Pacific Islands, babies borne to mothers with chronic HBV, close family contacts of case or carrier, individuals who get tattoos or body piercing, intravenous drug abusers, people with multiple sex partners, hemophiliacs and other patients, requiring

blood and product treatments, health care workers, who have contact with blood, hemodialysis patients, and blood and organ recipients (Murray *et al.*, 2002).

### **2.3.5. Pathogenesis and immunity**

Hepatitis B virus can cause acute or chronic, symptomatic or asymptomatic disease. Which of these occurs seems to be determined by the person's immune response to the infection (Murray *et al.*, 2002).

Detection of both the HBsAg and the HBc Ag components of the virion in the blood indicate the existence of an ongoing active infection. HBs Ag particles continue to be released into the blood even after virion release has ended and until the infection is resolved (Murray *et al.*, 2002). The virus starts to replicate with 3 days of its acquisition, but symptoms may not be observed for 45 days or longer, depending on the infectious dose, the route of infection and gender.

All types of viral hepatitis produce similar changes to the histological level. In the acute stage there are signs of inflammation in the portal triads and the infiltrate is mainly lymphocytes. 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 (Greenwood *et al.*, 1996).

In chronic hepatitis, damage extends out from the portal tracts, giving the piecemeal necrosis appearance. Some lobular inflammation is also seen and as the disease progresses fibrosis develops and eventually, cirrhosis.

Cell – mediated immunity and inflammation are responsible for causing symptoms and affecting resolution of HBV infection by eliminating the infected hepatocytes.

Interferon most likely initiates the response by enhancing major histocompatibility complex antigen expression and the display of peptides from the HBs, HBc and HBe antigens to cytotoxic T-cells. Episodes from HBc antigen are prominent T-cell response to the infection generally results in the occurrence of mild symptoms, an inability to resolve the infection and the development of chronic hepatitis (Murray *et al.*, 2002).

Progress in understanding the nature of immune response to HBV and their role in pathogenesis has been hindered by the absence of experimental systems for HBV infection that allow direct immunologic manipulation (Timbury *et al.*, 1997). Nonetheless, great progress has been made by careful description of the cellular and humoral responses of infected humans and correlation of these responses with the pathologic outcome of infection.

These studies show that in individuals experiencing acute, self-limited hepatitis B, strong T-cell responses to many viral antigens are readily demonstrable in the peripheral blood. These responses include both MHC classII restricted CD4+ cells and MHC class I-restricted CD8+ cells, the latter population comprising the bulk of the cytotoxic T lymphocytes (CTLs). The anti-viral CTL response is polyclonal and multi-specific, the breadth of this response presumably limits the likelihood of escape mutants that can evade CTL recognition. CTLs have been identified against multiple episodes with HBc core, polymerase, and envelope proteins, strong helper T cell (Th) responses to C and P proteins have been demonstrated in acute Infection. The responses to envelope proteins are much less vigorous, for reasons that are still unclear but may be related to the large circulating load of HBs Ag. In contrast, in



chronic HBV carriers, such virus specific T-cell response both CD4+ and CD8+ are greatly attenuated, at least as assayed in the peripheral blood. Recent experiments suggest that some inflammatory cytokines, notably IFN $\gamma$ , TNF $\alpha$  and interleukin-2 (IL-2) can have additional antiviral effects independent of their contributions to chronicity (Timbury *et al.*, 1997).

#### **2.3.5.1. Clinical picture**

Hepatitis B virus infection is characterized by a long incubation period and an insidious onset. The incubation period varies widely from 40 days to 6 months. A dose-related effect has been observed as shorter incubation periods have been associated with the inoculation of large doses of virus as in the transfusion of infected blood (Greenwood *et al.*, 1996).

#### **2.3.5.2. Persistence of HBV**

Persistence of HBV is indicated by the continued presence of HBs Ag and HBV DNA in blood for more than 6 months (Greenwood *et al.*, 1996). This occurs:

In 5-10 % of adult cases, in 30% of childhood cases, in 90% of newborn infections, more frequently in males, and more often in immunocompromised patients.

#### **2.3.5.3. Carrier states**

It is not yet clear, what determines an individual progress to the carrier state. There may be genetic factors, but it is clear that the absence of relative inefficiency of the immune system is important, as shown by the increased likelihood of the carrier state in the very young and immunocompromised (Greenwood *et al.*, 1996).

#### **2.3.5.4. Chronic persistent hepatitis**

Chronic persistent hepatitis is a benign and self-limiting disease which can follow hepatitis B; there are mild inflammatory signs in the liver, but symptoms are minor or absent (Timbury, 1997).

#### **2.3.5.5. Fulminant viral hepatitis**

Fulminant hepatitis occurs in approximately 1% of icteric patients, and may be fatal. It is marked by more severe symptoms and indications of severe liver damage, such as ascites and bleeding (Murray *et al.*, 2002).

Evidence of a prolonged prothrombin time should raise concern regarding the potential development of hepatic failure. If clinical symptoms of hepatic failure develop, the patient should be referred for consideration of liver transplantation. Fulminant hepatitis occasionally develops during acute viral hepatitis, leading to hepatic encephalopathy within the first 8 weeks of disease in patients without preexisting liver disease (Brooks *et al.*, 2007).

#### **2.3.5.6. Acute hepatitis**

Hepatitis B virus replicates in the hepatocytes which are reflected in the detection of viral DNA and HBc Ag in the nucleus and HBs Ag in the cytoplasm and at the hepatocyte membrane (Greenwood *et al.*, 1996).

The incubation period from acute exposure to clinical symptoms ranges from 60 to 180 days.

In acute infection, HBs Ag and markers of active viral replication become detectable approximately 6 weeks after inoculation, prior to the onset of clinical symptoms or biochemical abnormalities (Wright *et al.*, 1997).

The biochemical diagnosis of acute hepatitis largely depends on measurements of serum bilirubin and aminotransferase. Serum alanine aminotransferase (ALT) is typically higher than serum aspartate aminotransferase (AST), and elevations of both aminotransferase are usually 500 U/L or greater (Timbury, 1997).

#### **2.3.5.7. Chronic active hepatitis**

It follows HBV infection in around 3% of cases (Wright *et al.*, 1997).

Other studies showed that chronic hepatitis occurs in 5-10% of people with HBV infection, usually after mild or in apparent initial disease (Murray *et al.*, 2002).

When infection progresses to chronicity the early clinical biochemical events are very similar to those in acute infection. However, in chronic infection, HBs Ag, HBe Ag and HBV DNA remain positive for 6 months or longer. Anti-HBc IgM titers typically fall to undetectable, again, during reactivation of infection. Anti-HBc IgG persists indefinitely (Timbury, 1997). Chronically infected people are the major source of spread of the virus and are at risk for fulminant disease if they become co-infected with HDV (Murray *et al.*, 2002).

#### **2.3.6. Lab diagnosis**

Current serologic assays for diagnosis of acute and chronic HBV infection (HBs Ag and HBe Ag) are both sensitive and specific. HBs Ag, anti HBc and HBe antibodies are detected by standardized ELISA.

While the detection of HBs Ag indicates active HBV infection, detection of HBe Ag indicates active viral replication and increased infectivity. Characteristic serologic changes develop in relationship with clinical symptoms and biochemical

abnormalities in acute resolving infection and in acute followed by chronic infection (Timbury, 1997).

During the symptomatic phase of infection, detection of antibodies to HBe Ag and HBs Ag is obscured because the antibody is complexed with the antigen in the serum. The best way to diagnose a recent acute infection, especially during the period when neither HBs Ag nor anti-HBs antibodies can be detected, is to measure anti- HBc IgM antibodies (Murray *et al.*, 2002).

#### **2.3.6.1. Enzyme linked Immunosorbent Assay (ELISA)**

Enzyme linked immunosorbent assay techniques are becoming increasingly used in the diagnosis of microbial infections. They are sensitive, specific and require only a small amount of specimen. The results of quantitative ELISA techniques can be read visually. Large numbers of specimens can be tested at one time and the ELISA can be easily automated for use in epidemiological surveys (Cheesbrough, 2000). As the name suggests, the enzyme linked immunosorbent assay uses an enzyme system to show the specific combination of antigen with its antibody; the enzymes system consists of:

An enzyme which is labeled, or linked, to a specific antibody or antigen, and a substrate which is added after the antigen antibody reaction. This substrate is acted on by the enzyme attached to the antigen antibody complexes, to give a color change. The intensity of the color gives an indication of the amount of bound antigen or antibody (Cheesbrough, 2000). There are two ways of performing ELISA. There are double antibody techniques to detect antigen, and indirect technique, to detect and assay antibody (Cheesbrough, 2000). The application of one variant of ELISA, the

double-antibody sandwich for the assay of antigen (e.g hepatitis B antigen), uses two antibodies:-

The first antibody (e.g antibody specific for HBs Ag) is coated on a plastic surface (polystyrene plate). The second antibody (i.e enzyme linked anti-HBs Ag-specific antibody). Any excess conjugate is removed by washing and finally the enzyme substrate is added to detect the presence of the enzyme (Shimotohno and Fienstone, 1997).

#### **2.3.6.2. Molecular diagnosis**

The principle behind the early molecular assays is the hybridization of a characterized nucleic acid probe to a specific nucleic acid sequence in a test specimen followed by detection of the paired hybrid. For example, single stranded probe DNA (or RNA) is used to detect complementary RNA or denatured DNA in a test specimen (Brooks *et al.*, 2007).

Methods of HBV DNA detection can be broadly classified into those that are dependent on hybridization of labeled probe to the DNA with quantitation achieved by comparing the result with known standards.

(Genostics assay , Abbott laboratories , Abbott park , Chicago, ILJ ) and branched DNA assays ( Chiron corporation, Emeryville , CA) and polymerase chain reaction ( RCR) – based assays , in which the viral DNA is amplified so that the amplification product can be readily detected by gel electrophoresis (Wright *et al.*, 1997).

#### **2.3.7. Prevention and Control of HBV**

Simple environmental procedures can limit the risk of infection to health care workers, laboratory personal and others (Brooks *et al.*, 2007).

Transmission of HBV in blood or blood products has been greatly reduced by screening donated blood for the presence of HBs Ag and anti-HBc Abs (Murray *et al.*, 2002).

Three main strategies exist for the prevention of HBV infection these include: Behavior modification to prevent disease transmission, passive immunoprophylaxis, and active immunization (Wright *et al.*, 1997).

#### **2.3.7.1. Behavior modification:**

Changes in sexual practices in response to HIV infection have probably contributed to declining incidence of HBV in United States. The use of condoms appears to reduce the risk of sexual transmission. Other primary preventive measures, such as needle exchange programs in injection drug users, are more difficult to implement.

Behavior modification is unlikely to be beneficial in developing countries where neonates and children in early childhood are at great risk of acquiring infection. In these groups, immunoprophylaxis, both passive and active will be the most effective measure for preventing infection (Wright *et al.*, 1997).

#### **2.3.7.2. Passive immunoprophylaxis**

Passive immunoprophylaxis is used in four situations these are:

Neonates born to HBs Ag positive mothers, after needlestick exposure, after sexual exposure, and after liver transplantation in patients who are HBs Ag positive pretransplantation.

Studies on passive immunization using specific hepatitis B immunoglobulin (HBIG) have shown protective effect if it is given soon after exposure. HBIG is not recommended for pre-exposure prophylaxis because the HBV vaccine is available and

effective (Brooks *et al.*, 2007). Immunoglobulin isolated from plasma by the cold ethanol fractionation methods has not been documented to transmit HBV, HAV, or HIV, through transmission of HCV infection by such a preparation occurred in the USA in 1994.

Immunoglobulins prepared outside the USA by other methods have been implicated in outbreaks of hepatitis B and C (Brooks *et al.*, 2007). Current dosing recommendations are 0.13 ml/Kg of anti-HBs Ig immediately after delivery or immediately there after up to 12 hours after birth, in combination with recombinant vaccine. This combination results in a greater than 90% level of protection against perinatal acquisition of HBV. Between 3% and 15% of infants, still acquire HBV infection perinatally from HBV-infected mothers (Wright *et al.*, 1997).

Failure of passive and active immunoprophylaxis in this setting may be due to in utero transmission of HBV infection, perinatal transmission related to high inoculums; or the presence of surface gene escape mutants (Wright *et al.*, 1997).

#### **2.3.7.3. Active immunization**

Vaccine for hepatitis B has been available since 1982 (Brooks *et al.*, 2007). The HBs Ag is used to prepare vaccines conferring protection, because it is the antibody to this virion component that neutralizes infectivity (Harvey; *et al* 2001).

Recombinant DNA techniques made it possible to clone the gene for HBs Ag in yeast, and this is now the source of a virtually unlimited supply of vaccine. Thus immunization has become feasible on a population- wide scale. HBV vaccination is now recommended as one of the routine infants immunizations, as is immunization of adolescents who were not given the vaccine as infants.

Vaccination programs targeting at patients at high risk for infection have field for two reasons difficulty in identifying and accessing patients at risk, and noncompliance with vaccination regimens once instituted (Reid and Dienstag, 1997). Even in knowledgeable patients at high risk of infection, such as health care workers, compliance with vaccine programs is only 50% and compliance in other risk groups is even lower (Wright *et al.*, 1997).

## **2.4. Hepatitis C virus**

Hepatitis C virus was identified by molecular biologic means in 1989 by screening infected chimpanzee blood for a viral RNA. HCV is the predominant cause of non- A, non B hepatitis (NANBH) virus infections and was the major cause of post transfusion hepatitis before routine screening of the blood supply for HCV (Murray *et al.*, 2002).

HCV is a positive – sense single stranded RNA virus, classified in the family flaviviridae, genus Hepacivirus. Most new infections with HCV are subclinical. The majority of HCV patients develop chronic hepatitis, and many are at risk of progressing to chronic active hepatitis, cirrhosis and hepatocellular carcinoma (HCC). Incubation period 6-8 weeks, does not grow in cell culture.

### **2.4.1. Diagnosis of HCV**

#### **Serology**

ELISA: HCV-specific IgG indicates exposure, not infectivity.

Western blot: confirmatory test (Line Immune Assay "LIA", Recombinant Immune Assay "RIA").

PCR: detects viral genome in patient's serum and genotyping.



#### **2.4.2. Epidemiology of HCV**

It is estimated that 150-200 million People, or about (3%) of the World's population, are living with chronic hepatitis C. About 3-4 million people are infected per year, and more than 350,000 people die yearly from hepatitis related disease. During 2010 it is estimated that 16,000 people died from acute infections while 196,000 deaths occurred from liver cancer secondary to the infection. Rates have increased substantially in the 20th century due to a combination of intravenous drug abuse and reused but poorly sterilized medical equipment.

Rates are high (>3.5% population infected) in Central and East Asia, they are intermediate(1.5%-3.5%) in South and Southeast Asia, Sub-Saharan Africa, Andean, Central and Southern Latin America, Caribbean, Oceania, Australasia and Central, Eastern and Western Europe, and they are low (<1.5%) in Asia Pacific, Tropical Latin America and North America ( Brooks *et al.*, 2007).

Among those chronically infected the risk of cirrhosis after 20 years varies between studies but has been estimated at (1-5%) for women. The reason for this difference is not known. Once cirrhosis is established, the rate of developing hepatocellular carcinoma is (1-4%) per year. Rates of new infections have decreased in the Western World since the 1990s due to improved screening of blood before transfusion.

In the United States, about (2%) of people have hepatitis C, with the number of new cases per year stabilized at 17,000 since 2007. The number of deaths from hepatitis C has increased to 15,800 in 2008 and by 2007 had overtaken HIV/AIDS as a cause of death in the USA. In Europe the percentage of people with chronic infections has been estimated to be between (0.13 and 3.26%) (Brooks *et al.*, 2007).

The total number of people with this infection is higher in some countries in Africa and Asia. Countries with particularly high rates of infection include Egypt (22%), Pakistan (4.8%) and China (3.2%). It is believed that the high prevalence in Egypt is linked to a now-discontinued mass-treatment campaign for schistosomiasis, using improperly sterilized glass syringes (Brooks *et al.*, 2007).

#### **2.4.2.2. Transmission**

Hepatitis C virus is transmitted by blood transfusions, blood products. Organ donation /transplantation. Intravenous drug abusers, sexual intercourse and vertical transmission (Murray *et al.*, 2002).

#### **2.4.2.3. Risk Factors**

The major groups at risk of HCV infection there are (Shimotohno, 1997) recipients of blood and blood derivatives, intravenous drugs users, health – care workers, in countries where tattooing and folk medicine are practiced, and hemophiliacs.

#### **2.4.3. Control and prevention of HCV infection**

There is no vaccine for hepatitis C, to reduce risk of getting hepatitis C, although several candidate vaccines are undergoing tests. Despite the suggestive evidence of the presence of neutralizing antibodies in infected patients, HCV quasispecies and frequent mutation of viral genome during the course of hepatitis raise the possibility that virus may escape from host humoral immunity (Brooks *et al.*, 2007).

Control measures focus on prevention activities that reduce risks for contracting HCV. These include screening and testing blood plasma, organ, tissue, and semen donors;

virus inactivation of plasma – derived products; counseling of persons with high – risk drug or sexual practices; implementation of infection control practices in health-care and other setting, and professional and public education (Brooks *et al.*, 2007).

## **2.8. Viral hepatitis in Sudan**

Many studies have been carried out to determine the prevalence of viral hepatitis in Sudan (Nagi *et al.*, 2007) and the possible risk factors for hepatitis B and C virus infections among the blood donors attending National Laboratory for Public Health, 100 subjects were investigated. The HBs Ag was detected among (6%) blood donors using both ICT strips and ELISA.

HCV specific antibodies were detected among (3%) blood donors using ICT strips and ELISA. In all subjects examined dual infection with viruses was never detected. Previous blood transfusion was found to be a significant predisposing risk factor to both viral infections. However, no other possible risk factors (e.g. drug abuse, previous surgical operations, needle injures) were found to be significant to contract both diseases. There was no significant difference between the prevalence of HBs Ag among married blood donors compared to the single donors.

In another study conducted by Al Arabi *et al.*, (1987) to determine the viral etiology of acute hepatitis in Omdurman , Sudan , 119 patients , at least 13 years of age, with acute hepatitis were studied to determine the prevalence of Non – A , Non –B hepatitis. Acute hepatitis Non – A, Non – B was diagnosed in 88 patients (73.9%), hepatitis B in 15 patients (12.6%), delta infection in 15 (12.6%), and hepatitis A in just one patient (0.8%).

This study indicated that hepatitis Non – A, Non – B could be the major cause of acute hepatitis in adults in this area of Sudan.

In a study carried by Elsheikh *et al.*, (2007) to investigate the seroprevalence and possible risk factors for HBV and HCV among antenatal care attendants in central Sudan, HBs Ag was detected in 41 (5.6%) out of 728 women, anti-HCV was detected in 3 (0.6%) out of 423 women. All of them were not aware of their condition. Age, parity, gestational age, residence, history, of blood transfusion, dental manipulations, tattooing and circumcision did not contribute significantly to increase HBs Ag seropositivity. In a study performed in Sudan in mid-1985, when outbreaks of hepatitis were occurring at the refugee camps in Somalia, there were reports of an increase in cases of acute jaundice in Eritrean and Tigrean refugees from Ethiopia residing in refugee camps in Eastern Sudan. The investigation of this outbreak included intensified surveillance in four large reception centers. Active case detection by expatriate health staffs, refugee health workers, and refugee organizations revealed an increase in cases of acute illness with sclera icterus among refugees during June-October (beginning approximately 6 weeks after the onsets of heavy rains in Eastern Sudan). The majority of patients were adults 15 years of age (66%); only 6.3% were children 5 years of age. There were almost twice as many cases reported among males as among females. Reported fatality rates ranged from 1.3% - 4.7% and averaged (3.1%) in the four camps; 11 out of 63 persons (17.5%) who died were pregnant women.

Prevalence of HCV seropositivity among the hemodialysis (HD) population in Sudan was estimated to be around 34%. In cross-sectional study in two major HD centers in

Khartoum, Sudan prevalence of HCV seropositivity was 23.7%. All the children were HCV seronegative. HBV seropositivity was seen in 20 patients; all of them were dialyzed. There was no significant association between HCV seropositivity and gender. There was significantly higher HCV seropositivity among patients who were over 30 years. There was no significant association between HCV sero-positivity and hepatitis B virus seropositivity. Other risk factors, namely previous jaundice, shared needles and tattooing, were not significantly associated with HCV seropositivity (Al-Amin *et al.*, 2007).

To determine the prevalence and risk factors for hepatitis B infection in rural Sudan, 2 villages in the Gezira were surveyed by Hyams *et al.*, (1989) where 851 subjects, aged 1 - 89 years of equal sex distribution were investigated. HBs Ag was detected in 18.7%, and seropositivity for any hepatitis marker (HBs Ag, anti-HBs or anti-HBc-Abs) was found in 63.9%. The prevalence of HBs Ag was highest in subjects of less than 5 years of age (32.3%). Seropositivity for any hepatitis marker increased from (48.4%) in subjects less than 5 years to 88.5% in persons less than 50 years of age. HBe Ag was present in 70% of HBs Ag- positive women of childbearing age. Residence in Khalawaat village and parenteral therapy for malaria were found to be independent risk factors for HBs Ag- positivity. Age, residence in Khalawaat village crowding, and having had a tattoo were predictive of seropositivity for any hepatitis marker. The reason for increased markers of hepatitis B in Khalawaat compared to Saleim village was not clear.

In another study by Hyams *et al.*, (1991) to determine acute sporadic hepatitis in Khartoum State; 80 cases of acute viral hepatitis and 80 control selected from a public

pediatric clinic were enrolled in the study. Study subjects were 14 years of age or younger and were mainly from a low socioeconomic level. Non –A, B hepatitis was diagnosed by exclusion in 35 (43.8%) patients, hepatitis A in 27 (33.8%), acute hepatitis B in 8 (10.0%) and dual hepatitis A and B infection in 1 (1.3%) patient. Eight acute cases (10%) were positive for HBs Ag but negative for anti- HBc IgM and anti-HAV IgM. Delta hepatitis was not identified in any study subject (Hyams *et al.*, 1991). Household case of jaundice and acquaintance with an individual outside of the household with jaundice during the prior 6 months were associated with Non-A, Non-B hepatitis. There was no association between potential exposure and Non- A, Non - B hepatitis. These finding suggest that enterically transmitted Non - A, Non - B hepatitis may be a major cause of acute sporadic hepatitis in children in this area, as well as cause of epidemic hepatitis (Hyams *et al.*, 1991).

**CHAPTER THREE**  
**MATERIALS AND METHODS**

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1. Type of study**

Descriptive, cross-sectional study.

##### **3.1.2. Study area**

This study was conducted in the National Laboratory for Public Health, Khartoum, Sudan.

##### **3.1.3. Sample size**

Apparently health male blood donors (n=100) in the National Laboratory for Public Health, were selected as study subjects aged (20 - 59years).

#### **3.2. Study duration**

The study was carried out during September to December 2013.

#### **3.3. Data Collection**

The data were collected by a questionnaire including, history of previous blood transfusions and other possible risk factors. The questionnaire also includes information's like age, sex and residence (Appendix1).

##### **3.3.1. Inclusion criteria**

Blood donors fit for donation that has passed the physical examination and laboratory investigation.

##### **3.3.2. Exclusion criteria**

Blood donors unfit for donation who do not passed the physical examination and/or laboratory investigation.



### **3.3.3. Ethical consideration**

The participant informed in their simple language about the disease, aim of research and the benefit from the study, and the donors consent was taken. The information that taken from the participant and results was confidential by gave it serial number during processing.

### **3.4. Collection of blood**

Under strict sterile conditions, 5mls of whole venous blood samples were drawn from each blood donor included in the study.

The specimens were collected in sterile plain containers (without anticoagulant) and left to clot. Serum was separated by centrifugation at 2000 rpm for 5 minutes. Serum samples were then stored at - 4<sup>0</sup> C until tested.

### **3.5. Methods**

#### **3.5.1. Laboratory tests**

Both immunochromatographic test (ICT) and enzyme-linked immunosorbent assay (ELISA) were used to detected HBs Ag and HCV antibodies (HCV Abs), in the collected sera.

##### **3.5.1.2. ICT for detection of HBs Ag**

##### **3.5.1.3. Principle**

The test reagent was obtained from ABON, P.R.China. The HBs Ag One Step Test (Serum/Plasma) is qualitative, lateral flow immunoassay for the detection of HBs Ag in serum or plasma, for in vitro diagnostic. The membrane is pre-coated with anti-HBs Ag antibodies on the test line region of the test. During testing, the serum specimen reacts with the particle coated with anti-HBs Ag antibody. The mixture migrates

upward on the membrane chromatographically by capillary action to react with anti-HBs Ag antibodies on the membrane and generate a colored line. The presence of colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen was added and membrane wicking.

#### **3.5.1.4. Procedure**

All the reagents and specimens were brought to room temperature before testing. The pouch was opened and the device was removed. The devices were labeled with the donor's numbers. 100µL of serum was added in the well marked "S". The results were interpreted after 15 minutes. When only colored band appeared at the control regions the results were negative. When a distinct colored band appeared at the test region "T" in addition to the control band, the test was positive.

### **3.5.2. ELISA for detection of HBs Ag**

#### **3.5.2.1. Principle**

The reagents were supplied by Biorex Diagnostics, United Kingdom, is an enzyme immunoassay based on a 'sandwich' principle.

Microtiter wells are coated with monoclonal anti-HBs (Ab to HBs Ag), which constitutes the solid-phase antibody. The test sample is incubated in such a well; HBs Ag, if present in the sample, will bind to the solid-phase antibody. Subsequently guinea-pig anti-HBs, which have been labelled with the enzyme horseradish peroxidase (HRP), are added. With a positive reaction this labelled antibody becomes bound to any solid-phase antibody HBs Ag 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 HBs Ag, the labelled antibody cannot be bound specifically and only a low background color develops.

### **3.5.2.2. Procedure**

The techniques were done according to Biorex diagnostics USA, instructions.

#### **Step 1- Reagents preparation**

The reagents and samples were brought to room temperature (18-30<sup>0</sup>C) for at least 15-30mins. The wash buffer concentrate was checked for the presence of salt crystals. The stock wash buffer was dilluted 1 to 20 with distilled or deionized water. And only clean vessels were used to dilute the buffer.

#### **Step 2- Numbering Wells**

The number of samples to be tested are initially, as certained (the number of wells required).

#### **Step3- Adding Sample and HRP- Conjugate**

50μL of the negative control was pipetted into 3 wells, and 50μL of the positive control into one well. The remaining wells were filled with 50μL of undiluted serum sample, and finally at the end of the test plate. Then 50μL of HRP-conjugate was added to each well (except blank) and the plates were sealed with foil, and immediately incubated at 37 <sup>0</sup> C for 60 minutes. After that the foil was removed and all wells were aspirated.

### **Step 5- Washing**

The Plates were then washed 5 times with washing buffer. Each time, they were soaked for 30-60 seconds. After the final washing cycle, the plate is turned by blotting paper or clean towel.

### **Step 6- colouring**

Then each well was filled with 50µL of working chromogen A, and 50µL chromogen B solution including the blank and the plates were resealed with fresh foil. The plates were incubated at room temperature for 15mins avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP- Conjugate were produced blue color in positive control and HBs Ag positive sample wells.

### **Step 7- Stopping Reaction**

Multi channel pipette was used; 50µL of stop solution (0.5% sulphuric acid) was added to each well and mixed gently. Intensive yellow color was developed in positive control and HBs Ag positive sample wells.

### **Step 8- Measuring the absorbance**

The results were read at 450 nm against a blank.

Cut-off value: C.O. = \*Nc \* 2.1

\*Nc = the mean absorbance value of three negative control values.

### **3.6. ICT for detection of HCV Abs**

The reagents were supplied by ABON, P.R.China. HCV is an in vitro, rapid, qualitative two site sandwich immunoassay used for the detection of antibodies to HCV in serum or plasma.

### **3.6.1. Principle**

ABON HCV utilizes the principle of lateral flow immunochromatography, a unique two site double antigen sandwich immunoassay on a membrane. As the test specimen flows through the test device, the colored HCV specific recombinant antigen-colloidal gold conjugate complexes with the gold conjugate complex moves further on the membrane to test region "T" where it is immobilized by the HCV specific recombinant antigen-colloidal gold conjugate complexes with HCV antibodies in the sample. This complex moves further on the membrane to test regions "T" where it is immobilized by the HCV specific recombinant antigens coated on the membrane leading to formation of a colored band, which confirms a positive test results. Absence of this colored band in the region indicates a negative result. The unreacted conjugate and unbound complex, along with rabbit IgG gold conjugate move further move further on the goat anti-rabbit antibodies coated on the membrane at the control region "C" forming a colored band. This colored band serves to validate the reagent and assay performance.

### **3.6.1.2. Procedure**

All the techniques were done according to ABON instructions. All reagents were brought to room temperature before testing. The pouch was opened and the device was removed and placed on the bench. The test device was labeled with donor's number. Two drops of serum (5 $\mu$ L) were added by the sample dropper, in the well marked "A". Three drops of sample running buffer (approximately 80 $\mu$ L) were added in the well marked "B" by the means of dropper vial. The test results were read after 10 minutes.

### **3.6.2. ELISA for detection of HCV Abs**

The reagents were supplied by Biorex diagnostics, United Kingdom, the fourth generation of ELISA. For qualitative detection of antibodies to hepatitis C virus in human serum or plasma.

#### **3.6.2.1. Principle**

Indirect ELISA method for detection of antibodies to HCV in two step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to core and non-structural regions of HCV during the first incubation, anti-HCV specific antibodies, if present, will be bound to solid phase pre-coated HCV antigens.

The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to HRP is added. During second incubation, these HRP-conjugated antibodies bound to any antigen-antibody (IgG) complexes formed and unbound HRP is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxidase are added to wells and in presence of the antigen-antibody-anti IgG (HRP) immunocomplex; the colorless chromogen is hydrolyzed by the bound HRP to a blue color product. The blue color turns yellow after stopping solution the reaction with sulphuric acid. The wells containing samples negative for anti-HCV remain colourless.

#### **3.6.2.2. Procedure**

The reagents were supplied by Biorex Diagnostics, and following techniques were according to its instructions.

### **Step 1- Reagent Preparation**

The reagent and samples were brought to reach room temperature (18-30<sup>0</sup> C) for at least 15-30minutes.

### **Step 2- Numbering Wells**

The strip-holder was taken with the required number of strips, including three negative controls, and two positive controls and one blank.

### **Step 3-Adding Diluent**

100μL of specimen diluents is was added into each well except the blank.

### **Step 4- Adding Sample**

10μL of positive control, negative control and specimen into their respective wells were added.

### **Step 5- Incubating**

The strips were covered with adhesive sealer, and incubated for 30 minutes at 37<sup>0</sup> C.

### **Step 6- Washing**

Each well was washed 5 times with diluted wash buffer.

### **Step 7- Adding HRP-Conjugate**

HRP-Conjugate 100μL was added to each well except the blank.

### **Step 8- HRP- Conjugate Incubating (2)**

The plate was covered with adhesive sealer, and incubated at 37<sup>0</sup> C for 30 minutes.

### **Step 9- Washing (2)**

The wells were then washed 5 times with diluted buffer.

**Step 10- Colouring**

50µL of chromogen A and 50µL of chromogen B were added into each well including the blank and mix gently, and incubated the plate at 37<sup>0</sup> C for 15 minutes. Produced blue colour in positive control and anti-HCV positive sample wells.

**Step 11- Stopping Reaction**

50µL of stop solution was added into each well and mix gently. Intensive yellow colour develops in positive control and anti-HCV positive sample wells. The absorbance of solution in the wells was read at 450 nm.

The cut-off value (C.O) =  $\frac{\sum Nc}{3}$  + 0.12\*  $\frac{\sum Nc}{3}$  = the mean absorbance value of three negative controls.



# **CHAPTER FOUR**

## **RESULTS**

## CHAPTER FOUR

### RESULTS

During September to December 2013 a total of 100 apparently healthy blood donors attending the National Laboratory for Public Health were screened to determine the prevalence of HBV and HCV infections, and to recognize the possible risk factors and profounders predisposing to the infection in the study group.

Fifty seven (57%) of blood donors were 20 -29 years old, thirty tow (32%) were 30 - 39 years old, ten (10%) were 40 – 49 years of age and only one (1%) was above 50 years (Table1).

The majority of blood donors were from Khartoum North city (76%). While only few (24%) were from outside Khartoum North .The married blood donors were higher in number (60%) compared to single blood donors (40%) (Fig. 1).

Six subjects (6%) were found to be sero-positive for HBs Ag ( $P>0.05\%$ ), while anti-HCV antibodies were detected in three (3.0%) blood donors when using both ICT and ELISA techniques (Table 2). No single blood donor was shown positive for both diagnostic markers.

The results revealed no significant difference ( $P>0.05$ ) between prevalence of HBs Ag and anti- HCV antibodies among the married blood donors (4 and 2) compared to single donors (2 and 1) respectively (Fig. 2).

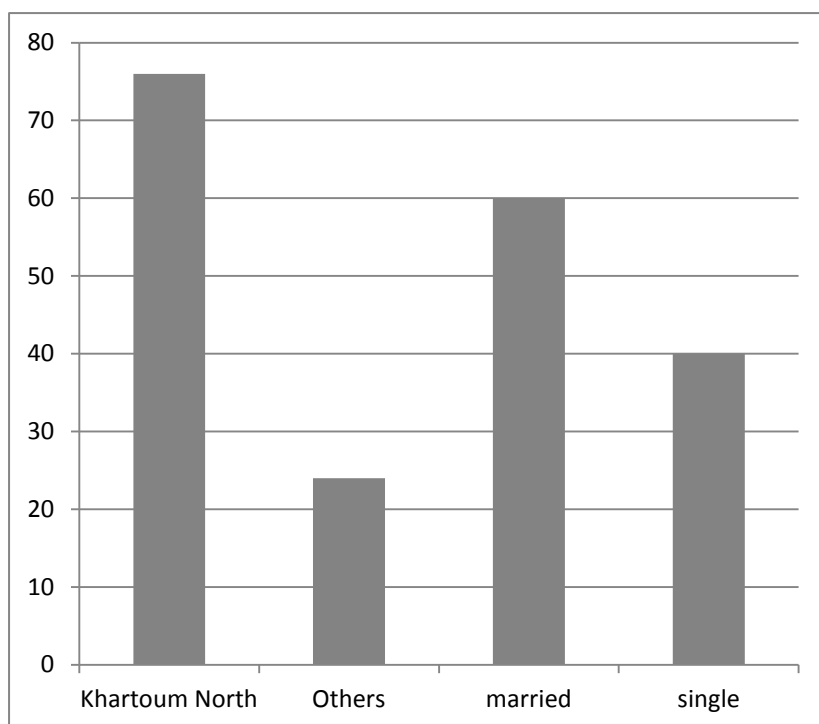
The analyzed data exhibited that the only possible profounder predisposing to infection with HBV and HCV, which had a significant effect ( $P<0.05$ ) was the previous Jaundice ( $P<0.05$ ) compared to other factors (blood transfusions, major or

minor surgery, and needle stick injury) that showed no effect to both viral infections (Table 3).

The results displayed in ( Table 4) revealed that while the highest positive cases for HBV infection was (3) and was among the age group 20 to 29 years , the positive cases for HCV infection was (2) and among the same age group.

**Table 1. Distribution of blood donors according to age**

Age group (years)	Frequency	%
20-29	57	57.0
30-39	32	32.0
40-49	10	10.0
50-59	1.0	1.0
Total	100	100.0



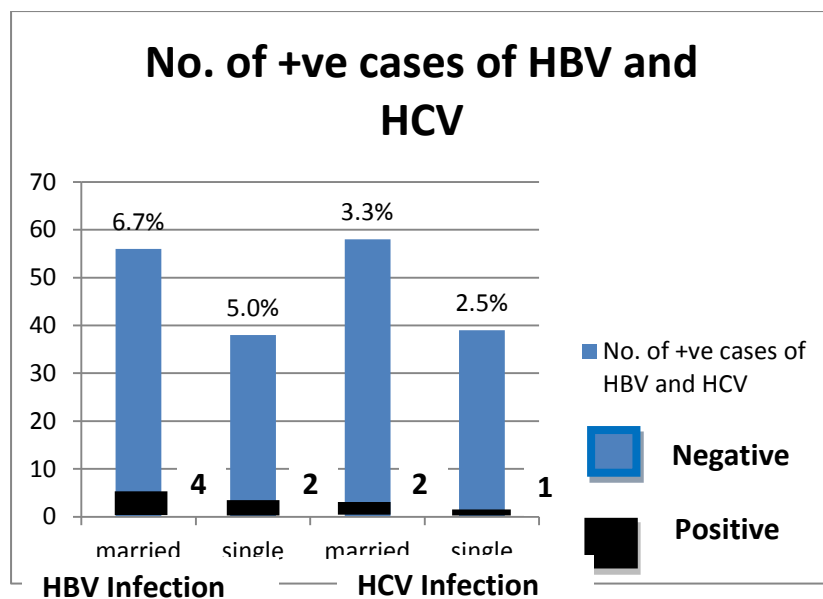
**Fig1. Distribution of blood donors according residence and marital status.**

**Table2. Detection of HBs Ag and anti-HCV antibodies among tested blood donors**

Serological markers	No. positive	%
HBs Ag	6	6.0
Anti- HCV Abs	3	3.0
Both markers	0.0	0.0

Chi-square for HBs Ag = 73.9

Chi- square for anti-HCV = 96.64



**Fig2. Number of positive cases of HBV and HCV infections among married blood donors compared to single ones.**

**Table 3. Possible risk factors and profounder predisposing to HBV and HCV infection in blood donors (n=100)**

Risk factors	No. Tested	No.positive	Percentage %
Blood transfusion	4	0	0.0
Needle stick injury	3	0	0.0
Major or minor operations	9	0	0.0
Previous jaundice	8*	2	25.0

Chi-square = 204



**Table 4. HBV and HCV positive cases in the different age groups**

Age group years	No. of donors	HBV+ve No (%)	HCV +ve No (%)
20-29	57	3 (5.26%)	2 (3.5%)
30-39	32	2 (6.25%)	1 (3.1%)
40-49	10	1(10.0%)	0 (0.0%)
<50	1	0 (0.0%)	0 (0.0%)
Total	100	6 (6.0%)	3 (3.0%)

## **CHAPTER FIVE**

### **DISCUSSION**

## CHAPTER FIVE

### DISCUSSION

In this study, the prevalence of HBV in blood donors was ( 6%) which is in accordance with that reported by Fayyaz *et al.*, (2000) in Pakistan (7.5%), Sallam *et al.*, (2003) in Aden, Republic of Yemen (6.7%) and Rodenas *et al.*, (2006) in Philippines (7.6%). However, these results are in disagreement with those observed by Sallam *et al.*, (2003) in Sana'a (15%) and Sogotra Island (26.3%), Republic of Yemen, and by Uneke *et al.*, (2005) in Nigeria (14.3%). In contrast, the prevalence of HBV observed in this study was higher than that reported by Vasconcelos *et al.*, (1994) in South Brazil (0.78%), Karki *et al.*, (2008) in Nepal (0.82%), El Beltagy *et al.*, (2006) in Saudi Arabia (0.3%) and Jalali *et al.*, (2007) in Iran (0.8%).

On other hand, the prevalence of HCV obtained in this study was (3.0%). These results were higher than those reported by Nagi *et al.*, (2007) in Sudan (1.3%), Ampofo *et al.*, (2002) in Ghana (0.9%), Gelaw and Mengistu, (2008) in Ethiopia (1.7%) and by Etard *et al.*, (2003) in Senegal (1.2%), Mauritania (1.4%) and Benin (1.1%). However, some previous studies conducted in Republic of Yemen among residents from an Africa ethnic minority in Sana'a and residents of Sogotra Island Sallam *et al.*, (2003) Showed HCV prevalence of (5.2%) and (5.1%), respectively.

These variations in results are possibly, due to the large sample size used by the previous investigators (e.g., 3192 subjects in Saudi Arabia, 5000 subjects in Brazil 6560 subject in Philippines). Furthermore these considerable differences could be complex multi-factorial due to possible ethnic background, socioeconomic status,

geographical locations, the health care services provided and the diagnostic techniques used in each separate study.

All subjects examined in this study, were males because females traditionally do not donate blood in Sudan. Culturally, people in Sudan believe that females are always in need for blood, even if they are healthy, especially during child-bearing age. This trend of male domination is common practice at Sudanese blood donation centers.

Concerning the distribution of age, the analyzed data displayed that usually young adult men (20-29 years), who can easily accept the idea of blood donation, were the highest age group (57%). Elder men (50-59 years) were lowest group (1%) to donate blood due to some social habits and considerations, rather than the health status of the individual.

The results of this study revealed that majority of the blood donors were replacement donors, who were relatives to the blood recipients and were living in Khartoum North city (76%). The National Laboratory for Public Health is located in the center of Khartoum North city close to residential areas, where it was convenient for the blood donors to help their relatives.

The results obtained in this study illustrated that previous jaundice was the only significant profounder predisposing to HBV infection ( $P < 0.05$ ) among the subjects examined. Jaundice persons should not donate blood and have might escape notice. This could be, due to the fact that the current regulation for blood donation and history taken by the physicians are not enough to detect the problem. Sometimes the donors themselves are not aware of their previous infections or diseases.

These results reflect that there are no standard vaccination protocols for HBV implemented in the country.

No significant difference could be observed ( $p < 0.05$ ) between the two techniques used for the detection of HBs Ag and anti-HCV antibodies (ELISA and ICT) in the results obtained by both methods, and they are both sensitive and reliable.

Co-infection with both HBV and HCV characterized by the detection of HBs Ag and anti-HCV antibodies were not observed in this study. Further in-depth studies using large sample size at different blood bank centers, and employing recent molecular characterization techniques (e.g, Real Time –Polymerase Chain Reaction RT-PCR) are strongly needed to resolve these clinically important viral infections in Sudan.

## **CONCLUSION**

1. The prevalence of HBV and HCV in apparently healthy blood donors was found to be fairly high, 6% and 3% respectively.
2. The obtained results emphasize the importance of screening programs for hepatic viral infection especially for blood donors.
3. The results can reflect the station of viral infections in Khartoum state.
4. The ICT and ELISA techniques were found to be efficient and reliable for detection of HBV and HCV infections.

## **RECOMMENDATIONS**

1. Effective health planning strategies are required to increase the public awareness of infection with viral diseases.
2. Wide spread educational and vaccination programs and rigid rules in donor selection are recommended.
3. Formulation of safe blood transfusion policy and implementation of standard laboratory screening procedures at all levels is strongly recommended.
4. Organized mass vaccination programs implemented in schools, clubs, communities, etc., will have a significant positive impact on reducing the prevalence of HBV and HCV infections.
5. Accurate identification and clinical management of both blood donors and family members are advised.
6. Further extensive epidemiological and community- based studies are urgently needed to investigate viral infections in the whole country using confirmatory techniques (PCR, Western blot and Southern blot Techniques) are essential.

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## Appendix

### Questionnaire

Serodetection of Hepatitis B and Hepatitis C viruses among the blood in the National Laboratory for Public Health

Name: ..... Serial No: .....

Sex: ..... Age: .....

Residence: .....

Marital Status:

Married (     )                      Single (     )

Previous blood transfusions:

Yes (     )                      No (     )

History of jaundice:

Yes (     )                      No (     )

History of Surgical operation:

Yes (     )                      No (     )

If yes:

Major (     )                      Minor (     )

History of Alcohol abuse

Yes (     )                      No (     )

History of intravenous drugs abuse

Yes (     )                      No (     )

History of needle syringe injury

Yes (     )                      No (     )

History of medical intervention

Yes (     )                      No (     )

If yes .....

Dialysis (     )                      Gastrointestinal endoscopy (     )

Catheterization                      Uroscopy (     )                      others (     )

Investigation result .....