1. Introduction

1.1 Background

Hepatitis B virus is a DNA virus, causing acute as well as chronic liver disease including cirrhosis and hepatocellular carcinoma. Carriers of Hepatitis B are asymptomatic individuals without manifest disease harboring infection and capable of transmitting infection. Transmission of infection occurs through blood transfusion, needles, body fluids and sexual intercourse (Mohan *et al*, 2013). HBV is a partially double-stranded, enveloped virus of the Hepadnaviridae family, which replicates in the liver and causes hepatic dysfunction (Xiaodong and Lihong, 2006). The first clue in unraveling this mystery was the discovery of an enigmatic serum protein named Australia antigen 50 years ago by Baruch Blumberg. Some years later this was recognized to be the HBV surface antigen (HBsAg) (Wolfram, 2013). The hepatitis B virus (HBV) is one of the most prevalent blood-borne viruses worldwide and is a major cause of chronic liver disease and hepatocellular carcinoma. It is estimated that two billion people worldwide have been exposed to the hepatitis B virus and more than 240 million have chronic (long term) liver infections. Approximately 600,000 people die annually due to hepatitis B infection (WHO, 2013). HBsAg is found on the surface of the virus and is also produced in excess amounts, circulating in the blood as 22-nm spherical and tubular particles. HBsAg acts as a marker for the diagnosis of HBV infection (Marion, 1988). Can be identified in serum 30 to 60 days after exposure to HBV and persists for variable periods (Klingmüller and Schaller, 1993).

Detection of hepatitis B surface antigen (HBsAg) in blood is diagnostic for infection with HBV and in the blood banks screening for HBsAg is carried out routinely to detect HBV infection (Bhattacharya *et al*, 2007). The most important laboratory test for the detection of early HBV infection is the immunoassay for HBsAg. HBsAg appears during the incubation period and is detectable in most patients during the prodrome and acute disease (Levinson, 2010). Vaccination programmes have been successful in reducing the percentage of HBV carriers in some parts of the world such as Taiwan and Alaska. Alpha-interferon has been used for some years to treat HBV-infected persons. This treatment does not eliminate the infection, but it results in a significant reduction in viraemia in about 20–30 per cent of cases (Carter and Saunders, 2007).

1.2.Ratianale

HBV infection is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) (Surendra *et al.*, 2008). Transfusion of blood and blood components, as a specialized modality of patient management saves millions of lives worldwide each year and reduces morbidity. The use of unscreened blood transfusion keeps the patient at risk of acquiring many transfusion transmitted infections (TTI) like hepatitis viruses (HBV). The risk of transfusion-transmitted HBV infection has been reduced by screening all blood donations for HBV surface antigen (HBsAg) since 1970 (Allian, 2004). Rapid tests may yield false test out-comes due to the prozone effect, especially during the initial phase after infection when the viral load is high and there are high antigen concentrations (Van de Perre *et al.*, 1988; Pujol *et al.*, 1993), and due to genotype variations that may influence test sensitivity (Huy *et al.*, 2008). Serological tests, such as enzyme immunoassays (EIA),

have high accuracy in detection of serological markers, such as HBsAg. The detection of HBsAg has become necessary to find out antigen carrier rate among healthy blood donors. A number of methods for detection of HBsAg have been reported in the diagnostics, which vary in their sensitivity as well as specificity. Immunochromatographic method is being used for the screening of HBsAg which has a very low sensitivity as compared to Enzyme linked immunosorbent assay (ELISA). Data from this study could be helpful in order to evaluate the diagnosis of HBV infections among blood donors.

1.3. Objectives

1.3.1. General objective

To assess the rapid test based on an Immunochromatography technique (ICT) in relation to ELISA in the diagnosis of HBV among blood donors.

1.3.2. Specific objective

To detect HBsAg using ICT

To detect HBsAg using ELISA

To compare the specificity and sensitivity of ICT versus ELISA

2. Literature review

2.1 Hepatitis B virus (HBV)

(HBV) is one of the transfusion transmission infectious agents of public health relevance. Its prevalence varies across the globe. Establishing the sero-prevalence of the disease is critical to informing the direction of preventive and control strategies (Walana *et al.,* 2014). Infection with hepatitis B virus (HBV) may lead to acute or chronic hepatitis. HBV infections were previously much more frequent but there are still 240 million chronic HBV carriers today. 620,000 die per year from the late squeal liver cirrhosis or hepatocellular carcinoma. Hepatitis B was recognized as a disease in ancient times, but it etiologic agent was only recently identified (Wolfram, 2013).

2.2 History of HBV

Progress with hepatitis B began with the serendipitous discovery of the envelope protein of the virus, hepatitis B surface antigen (HBsAg), originally termed Australia antigen. This was discovered in 1965 by Blumberg, who precipitated an antigen–antibody complex by immunodiffusion of sera from a multiply transfused patient and an Australian aboriginal, during a study of blood and leukocyte antigens. The protein was later recognized to be associated with transmission of hepatitis and could be detected in the sera of a proportion of patients with viral hepatitis. Electron microscopic studies in the late 1960s led to the discovery of particles of around 20 nm diameter that are now known to be composed of membrane embedded HBsAg, secreted from the hepatocytes as non infectious, subviral particles. In 1970, Dane visualized larger, 42 nm particles with electron dense cores. Originally termed ‘Dane particles’, these are the hepatitis B virions and were shown later to contain a

small circular DNA genome that was partially single-stranded and associated with a DNA polymerase activity that could render the molecule fully double-stranded (Mahy and Van Regenmortel, 2008).

2.3. Epidemiology

HBV infection is the tenth leading cause of death worldwide and results in 500,000 to 1.2 million deaths per year due to chronic hepatitis, cirrhosis, and HCC. About 1.25 million Americans are chronically infected with HBV. Approximately 15–40% of patients chronically infected with HBV will develop cirrhosis, liver failure, or HCC. HCC accounts for 320,000 deaths per year. About 5–15% of persons in tropical countries are chronically infected with HBV, whereas only 0.1–0.5% of persons in the United States are chronically infected (Chamberlain, 2009). The prevalence of HBV is highest among the developing countries of Asia, Africa and the Pacific Islands and lowest among the developed countries of America, Europe and Australia (Hasnain, 1994). Countries are classified on the basis of endemicity of HBV infection into high (≥8%), intermediate (2 to 7%) or low (≤2%) incidence countries. The prevalence of chronic HBV infection in India ranges from 2 to 10%. India therefore comes under the intermediate to high endemicity category (Karandeep *et al.*, 2009). Chronic HBV infection occurs in 90% of infants infected at birth, 30% of children infected at ages 1–5 years, and 6% of persons infected after 5 years of age (Chamberlain, 2009). Hepatitis B is endemic in parts of Asia and Africa where greater than 8% of the adult population are estimated to have chronic hepatitis B infection. High rates of chronic infections are also found in the Amazon and the southern parts of eastern and central Europe. In the Middle East and Indian subcontinent, an estimated 2–7% of the general population is

chronically infected. Less than 1% of the population in Western Europe and North America is chronically infected (WHO, 2013).

2.3.1. Epidemiology in Sudan

Hepatitis virus infections are the most common cause of liver disease worldwide. Sudan is classified among the countries with high hepatitis B virus seroprevalence. Exposure to the virus varied from 47%–78%, with a hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in Southern Sudan(Mudawi, 2007). Studies pointed to infection in early childhood in Southern Sudan while there was a trend of increasing infection rate with increasing age in Northern Sudan. Recent introduction of screening of blood and blood products for hepatitis B virus infection and the introduction of hepatitis B virus vaccine as part of the extended program of immunization is expected to reduce the infection rate of these viruses in the Sudan (Mahgoub *et al.,* 2011). Recent studies showed that the seroprevalence of HBV is 5.1 and 5.6% among blood donars in northern and central Sudan, respectively (Nagi *et al.,* 2007; Elsheikh *et al*., 2007). In Sudan there is high incidence rate of hepatocellur carcinoma (Omer *et al.,* 2001).

2.4. HBV structure

HBV is a member of the hepadnavirus family. It is a 42-nm enveloped virion, with an icosahedral nucleocapsid core containing a partially double-stranded circular DNA genome (Levinson, 2010). The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity. The virus is one of the smallest viruses, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core which is referred to as "Dane particles. The HBV core gene codes for two distinct protein products: a 21.5-

k Da protein that assembles to form nucleocapsid particles designated HBcAg ,which is mature virions contain the viral DNA as well as the viral polymerase and RNase H and non-particulate e- antigen (HBeAg). Mature (HBeAg) is C-terminally truncated at HBcAg amino acid position 149 and retains 10 precore amino acids. HBcAg and HBeAg are distinctly recognized by antibodies but are highly cross-reactive at the T cell level (Milich *et al*., 1987).

2.4.1. Noninfectious particles

An unusual and intriguing feature of HBV infection is the presence in the blood of not only virions, but also large quantities of non-infectious particles that have been released from infected liver cells. These particles are composed of lipid and virus envelope proteins, but they do not contain nucleocapsids. Some of the particles are spheres and some are filaments; both have diameters of 22 nm and the filaments have variable lengths up to200 nm (Carter and Saunders, 2007).

2.4.2. HBVgenome

The hepadnaviruses are especially fascinating for two reasons. First they have very small genomes, which are used with great economy to encode the virus proteins and to control expression of the virus genes. Second, their DNA genomes are replicated via an RNA intermediate. In other words, their replication involves reverse transcription, so they are very different from DNA viruses that replicate their DNA directly to DNA (Carter and Saunders, 2007). The HBV genome comprises a partially double stranded 3.2 kb DNA organized into four open-reading frames. The longest open-reading frame encodes the viral polymerase (Pol open-reading frame).

The envelope open-reading frame is located within the Pol open-reading frame in a frame shifted manner. Partially overlapping with the envelope open-reading frame are the core (C) and the X open-reading frames. The covalently closed circular DNA (ccc DNA) is the template that is transcribed to generate four major RNA species: the 3.5-, 2.4-, 2.1-, and 0.7-kb viral RNA transcripts. Expression of these four transcripts is directed by the enhancer II/basal core, large surface antigen (L), major surface antigen (S), and enhancer I/X gene promoters, respectively. There are four overlapping genes coding for the core, surface and polymerase protein and an X protein that may act as an activator of transcription. The hepatitis e antigen (HBeAg) is translated from the HBcAg gene using an upstream initiating codon. The protein is secreted from infected cell into plasma, especially at time when there is active viral replication reflected by release of Dane particles into the circulation. The surface antigen gene is transcribed to produce three mRNAs,L, M and S. The L product is present only in the virion, while the M and S proteins found in each type of particle. (Greenwood *et al.*, 2002).There are four open reading frames (ORFs) that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional transactivator (X), and a large polymerase (P) protein that includes DNA polymerase, reverse transcriptase, and RNase H activities (Brooks *et al.*, 2010). The P ORF has several functions in virus replication, such as RNA pregenome encapsidation, priming of DNA synthesis, reverse transcriptase, and (+) strand DNA-polymerasation. The multifunction nature of HBV polymerase gene is unique *Hepadnaviridae* (Sattler and Rbinson, 1979).

2.4.3.Hpatitis B antigens and antibodies

During HBV infection, many virus particles are released from infected liver cell, resulting in large amount of viral antigen entering the blood. HBsAg is present in the blood about 2 weeks before the onset of symptoms, and persists throughout the clinical course of the disease. At the start of recovery it declines and is no longer detectable after 4 – 5 months. Persistence of HBsAg beyond 6 months indicates chronic infection/carrier state **(**Cheesbrough, 2007). HBV is a double strand circular DNA virus composed of an outer envelope containing hepatitis B surface antigen (HBsAg) and an inner nucleocapsid consisting of hepatitis B envelope antigen (HBeAg) and hepatitis B core antigen (HBcAg). Corresponding antibodies to each of these antigens are Hepatitis B surface antibody (anti- HBs or HBsAb), Hepatitis B envelope antibody (anti-HBe or HBeAb) and hepatitis B core IgM and IgG antibody (anti-HBc or HBcAb). The viral core also contains double strand DNA genome and DNA polymerase. the serological markers of Hepatitis B Virus HBsAg, anti-HBs, HBcAg, anti-HBc(IgM and IgG,HBeAg), HBeAg, anti-HBe and HBV DNA, these are important as they can be used in the diagnosis of the infection and to determine the severity of the infection (Gitlin, 1997). Following infection by Hepatitis B Virus (HBV), the first serological markers to appear in the blood is the HBV DNA, followed by HBsAg, the DNA polymerase, and HBeAb. Thereafter, the antibodies to the Hepatitis B antigens (HBcAb, HBeAb and HBsAb) can be detected (Kumar *et al.*, 2007). IgM antibody to hepatitis B core antigen (anti-HBc IgM) appears only in acute infection and is a useful marker of recent infection. The presence of IgG antibody to HBcAg(anti-HBc IgG) in the absence of IgM,

indicare past infection. Antibody to HBsAg (anti-HBs) is the last serological markers to form, appearing in the convalescence stage. It indicates recent infection or past immunization. Antibody to HBeAg(anti-HBe) may be found in the convalescence stag and and often in chronic hepatitis and the carrier state**(**Cheesbrough, 2007).

2.5. HBV Genotype

HBV genotypes can be localised into different geographical areas and are classifed into 10 recognised genotypes (A-J) (Tatematsu *et al*., 2009). These genotypes have discrete biological distributions with subtype adw found in genotypes A, B, C, F and G, while both adr and ayr occur in genotype C alongside adw (Echevarría and Avellón, 2006).

2.6. Classification

Collectively, the HBV-like viruses have been assigned to the family Hepadnaviridae (hepatitis DNA virus), for which (human) HBV is the prototype. This family contains two genera, the orthohepadnaviruses, infecting mammals, and the avihepadnaviruses, infecting birds. Amaximum sequence divergence of about 35% is found among the orthohepadnaviruses, compared to 25% among avihepadnaviruses. Designation of the Hepadnaviridae as a new family of viruses is based on the extremely small size of the viral genomes. Assignment to two genera is based, in addition to host range differences, on the strong DNA sequence similarities among all *orthohepadnaviruses* and all avihepadnaviruses, but an almost complete lack of homology between the two groups. Two species have been assigned in the avihepadnavirus group, with DHBV and heron hepatitis B virus (HHBV) as the prototypes, and some newer isolates that are as yet

unassigned. The *orthohepadnaviruses* have been divided into four distinct species, with HBV, WHV, GSHV, and WMHV as the prototypes (Knipe *et al.,* 2007).Hepatitis B virus can be further classified into eight major genotypes (A to H) based on nucleotide (nt) diversity. These genotypes have a distinct global geographical distribute (Stuyver *et al* 2000).

2.7. Stability

It is difficult to assess the stability of HBV due to the lack of a suitable laboratory culture system. 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 c° for 10 h in activates virus by a factor of 100–100-fold. Treatment with hypochlorite (10 1000 ppm available chlorine) or 2% glutaradehydrate for 10 min will inactivate virus 100 /1000-fold. Studies based on the survival of HBsAg show that this is much more resistant to destruction (Greenwood *et al*., 2002).

2.8. Transmission

The three main modes of transmission are via blood, during sexual intercourse, and perinatally from mother to newborn. The observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary. HBV infection is especially prevalent in addicts who use intravenous drugs. However, because blood transfusion is a modern procedure, there must be another, natural route of transmission. It is likely that sexual transmission and transmission from mother to child during birth or breast feeding are the natural routes (Levinson, 2010). HBV has been found in virtually all body secretions and excretions. However, only blood, body fluids containing visible blood, semen and vaginal secretions represent a

risk of transmission (CDC, 2001). The distinction is mad between three main modes of transmission: horizontal, vertical, and perinatal, that differs in the probability of inducing a chronic infection (Cacciola *et al.,* 2002). In developing countries the main routes of transmission are:

- Neonatal with an HBV carrier mother infecting her infant, usually during birth or soon after birth following close contact,
- transfer of HBV via open wounds, bites, cuts and grazes,
- sexual transmission,
- transfusion of infected blood or blood products when donor blood is not screened,
- needle stick injury and other sharp injuries,
- contamination of eye, and reuse of HBV contaminated needles, syringes, lancets, razors, and instruments including those used in tattooing ear piercing, acupuncture, and tribal ceremonies. (Cheesbrough, 2007).

2.9. Replication cycle

The infectious virion attaches to cells and becomes uncoated. In the nucleus, the partially double-stranded viral genome is converted to covalently closed circular double-stranded DNA (cccDNA) (Brooks *et al.*, 2010). Transcription is regulated by cell-type-specific factors and this is part of the basis for the tropism of this virus for the liver (Dimmock,*et al.,* 2007). The replication of HBV involves a reverse transcription step, and, as such, is unique among DNA viruses. HBV has a specific tropism for the liver. The attachment or adsorption of HBV to hepatocytes (liver cells) is mediated by the envelope protein (HBsAg) of the virus, probably by binding of HBsAg with

polymerized human serum albumin or other serum proteins. After viral entry, the partial double-stranded DNA (incomplete) is transported to the nucleus. The double-stranded DNA is organized as two strands. One, a short strand, is associated with the viral DNA polymerase and is of positive polarity. The complete or long strand is complementary and thus of negative polarity. The partial incomplete strand is formed into a complete double-stranded circular DNA, which is essential before transcription can take place. Host RNA polymerase directs the transcription of viral mRNAs to encode early proteins, including HBcAg, HBeAg, and viral DNA polymerase as well as full-length RNA (pre-genomic RNA). HBsAg is encoded later and associates with the membranes of endoplasmic reticulum or Golgi apparatus. HBcAg forms the core by enclosing the full-length positive-sense viral pre-genomic RNA along with viral DNA polymerase into maturing core particles late in the replication cycle. These full-length RNA strands form a template for a reverse transcription step in which negatively stranded DNA is synthesized. The RNA template strands are then degraded by ribonuclease H activity. A positive-stranded DNA is then synthesized, although this is not completed before virus maturation in which HBsAg-containing membranes of the endoplasmic reticulum or Golgi apparatus are wrapped over the nucleocapsid core, resulting in the variable-length, short, positive DNA strands found in the virions. The virions are released by exocytosis (Ryan *et al.*, 2010).

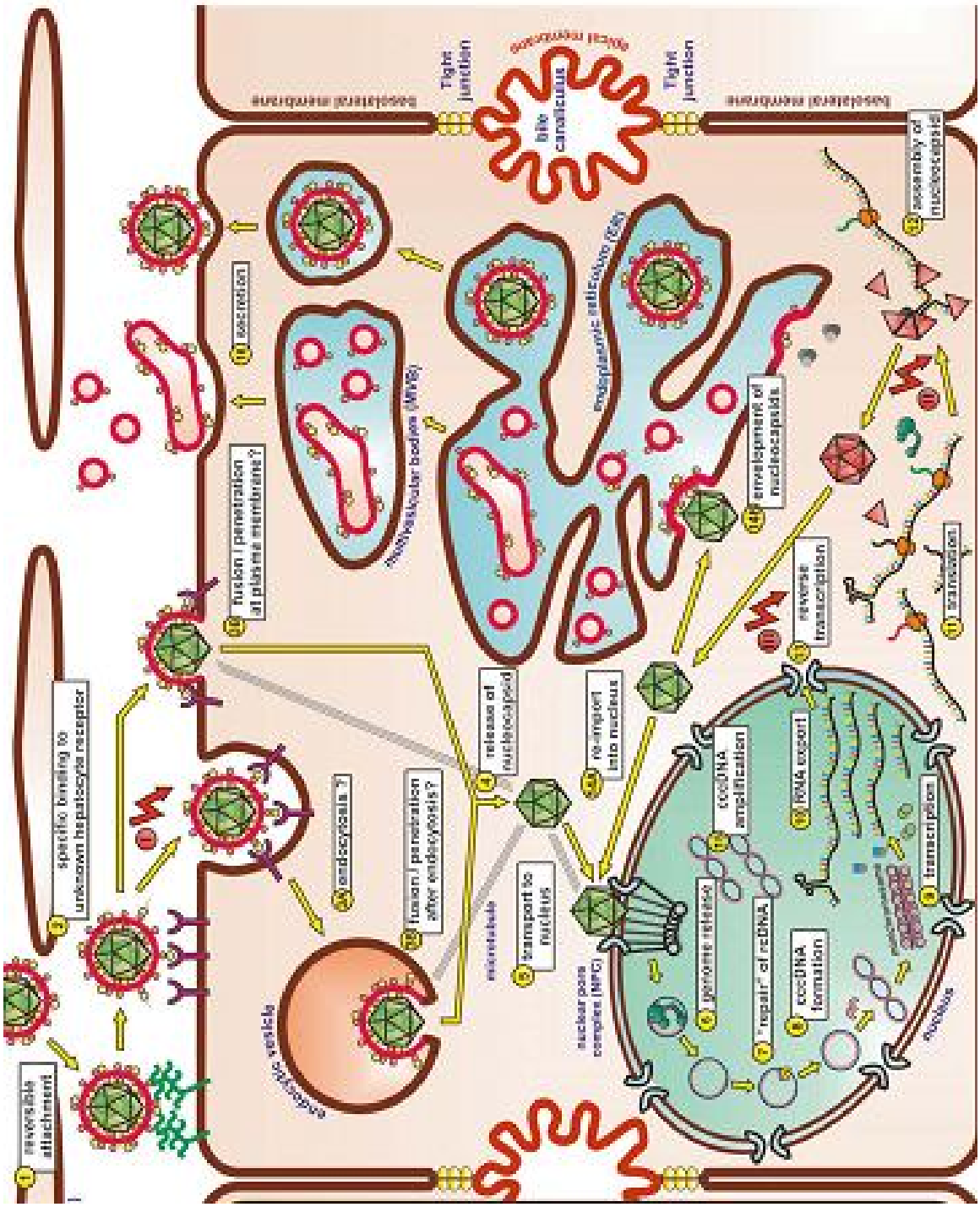

Figure 2.1: Life cycle of HBV (Stephan et al., 2010)

2.10. Clinical features

HBV is an important cause of acute liver disease which might deviate into a fatal fulminant form, or become chronic, persistent infection that can result in the eventual death of infected individuals from cirrhosis and liver cancer (Cornellissen *et al.*, 2012).

2.10.1. Acute infections

Acute type B hepatitis is usually a benign disease that spontaneously resolves in more than 90% of cases. Very infrequently it may evolve into chronic hepatitis, while in 0.1–1% of the patients develop a fulminant course (Decker, 1998). The percentage of patients exhibiting symptoms increases with age, with only 10% of infections in children being symptomatic as compared to about 50% of infections in young adults being symptomatic. Fulminant hepatitis and death may occur in <1 %. (Kudesia and Wreghitt, 2009). Acute HBV infections have an average incubation period of 90 days (range: 60 – 150 days) and can be defined as an abrupt clinical, biochemical, and /or histopathological manifestation of hepatic injury that occurs within six month of HBV exposure and that resolves spontaneously, in more than 90% of cases, within six month of onset of symptoms. Clinical symptoms include nausea, vomiting, abdominal pain, fever, jaundice, dark urine, change in stool colour, and hepatomegaly(Shepard *et al.*, 2006). Acute hepatitis may, however, produce clinically recognized symptoms during the resolution phase of the infection, including loss of appetite, nausea, vomiting, fatigue, and abdominal pain, conditions that may be due to the high production of IFNs, TNF and other inflammatory cytokines (Knipe *et al.*, 2007). HBV DNA can be normally detected one to two weeks after exposure, during which time

patients generally have no symptoms. The first serological HBV markers, namely HBsAg and hepatitis B e antigen (HBeAg) may be detected (Fattovich, 2003; Shepard *et al.*, 2006). Two to six weeks after exposure patients enter an immunoactive phase that is characterized by a decrease in serum HBV DNA and an increased in ALT levels and histological activity, indicating lysis of infected hepatocytes by the host's immune system.HBV antibodies, including antibodies to the hepatitis B core antigen (Anti-HBc), can be detected between four and eight weeks post exposure (Huang *et al*., 2006; Shepard *et al*., 2006). Patients are considered to have resolved the HBV infection when they become HBsAg negative and developed antibodies to HBsAb (anti-HBs), indicating clearance of the virus (Yim and Lok, 2006). A severe form of acute HBV infection is fulminant hepatitis in which the course of the infection is complicated by encephalopathy, with HBsAg often being undetectable at the time of diagnosis. The severity of liver injury in fulminant hepatitis indicates a vigorous immune response from the host and is associated with rapid viral clearance (Villeneuv, 2005). The appearance of the antibodies to HBsAg (anti-HBs) is the best serological indicator of recovery from the infection, and may take several months after HBsAg seroclearance. Persistence of high HBV-DNA levels and of HBeAg positivity predicts evolution of the infection (Whalley *et al.*, 2001).

2.10.2Chronic infections

Chronic HBV infections can be defined as the presence of HBsAg in the serum of an infected individual for at least six month or as the presence of HBsAg in a patient negative for immunoglobulin (IgM) antibodies to the hepatitis B core antigen (HBcAg) (Shepared *et al.*, 2006). Most chronically

infected individuals present with HBeAg - positive chronic hepatitis B ten to thirty years after their initial infections (Fattovich, 2003). Chronic HBV infections can be divided into four phases: immune tolerance; immune clearance (HBeAg positive); inactive carrier (HBeAg negative) and reactivation, although not all individuals pass through every phase (Yim and Lok , 2006). In the initial immune tolerance phase, analogous to the incubation period of an acute infection, infected individuals have high HBV loads and are positive for HBeAg (Villeneuv, 2005). The second phase, the immue clearance phase can be compared to symptomatic hepatitis in acutely infected individuals. HBeAg persist s along with high HBV viral loads and abnormal ALT levels (Villeneuv, 2005; Yim and Lok, 2006). The immunological response of the host results in active inflammation of the liver tissue, and hence progressive liver damage. There is an increased T cell response to HBcAg and HBeAg resulting in lysis of infected hepatocytes and flares of aminotransferase levels. The frequency and the severity of these flares increase the risk of cirrhosis and HCC developing. The immune clearance phase culminates in seroconversion from HBeAg to anti-HBe. This seroconversion occurs spontaneously at a rate of 5 – 10% per year, but may also result from treatment with interferon or other nucleoside analogs. The third phase of chronic HBV infection, the inactive carrier state, is characterized by the absence of HBeAg, the presence of HBsAg and anti-HBe, low or undetectable serum HBV DNA levels (<105 copies /ml), minimal to no liver inflammation and normal ALT levels. In some individuals, this state may persist indefinitely resulting in a sustained remission and a generally good prognosis. Approximately 1 – 5 % of chronically infected individuals progress from inactive carrier state to the fourth phase of infection, where there is a reactivation of HBV replication

leading to HBeAg –negative chronic hepatitis B. HBsAg, anti-HBe and HBV DNA are all detectable in the serum but HBeAg not. ALT levels are elevated and there is necro-inflammation of the liver tissue. Annually, approximately 8 – 10% of patients with HBeAg –negative chronic HBV infection develop cirrhosis compared to 2 – 5% in HBeAg-positive patients (Villeneuv, 2005; Yim and Lok, 2006).However, in 5-10 % of adult patients, the HBV infection will progress to chronic hepatitis B (CHB) which can lead to cirrhosis and hepatocellular carcinoma (HCC) which are life threatening. In contrast, in children, 90% HBV infection will progress to CHB and due to immunetolerance these children will not have active hepatitis at the early phase of infection. (Levinson, 2010).

2.11. Pathogenesis

HBV enters the blood stream and infects the cells of the liver by replicating within the cells. Symptoms may not be observed for 45 days or more, depending on the dose of HBV, the route of infection, and the individual. HBV genomes integrate into host chromosomes during replication and are the basis of chronic infections. Large amounts of HBV surface antigen (HBsAg) and virions are released in the blood. Immune complexes formed by HBsAg and antibody are responsible for hypersensitivity reactions that occur in some patients (e.g., arthritis, rash, liver damage, vasculitis, or arthral-gia). Liver parenchymal cell degeneration results from cellular swelling and necrosis; resolution of the infection allows the liver parenchyma to regenerate. Fulminant HBV infection, activation of chronic infection, or coinfection with the delta agent can lead to permanent liver damage or cirrhosis. Both cell-mediated immunity and inflammation are responsible for the resolution of HBV infection and its symptoms. Acute cases of HBV disease over time,

25% of persons who acquire HBV as children will develop cirrhosis or HCC as adults. Cirrhosis may develop as Consequence of repeated immune system attacks in which the normal hepatocytes are destroyed and replaced with fibrous tissue (Chamberlain, 2009). The pathogenesis of hepatitis B is due to the interaction of the virus and the host immune system in which the immune system attacks HBV and causes liver injury. Activated CD4+ and CD8+ lymphocytes recognise various HBV-derived peptides located on the surface of the hepatocytes, leading to immunologic reaction, impaired immune reactions (e.g. cytokine release, antibody production) or a relatively tolerant immune status, resulting in chronic hepatitis. In particular, a restricted T cell–mediated lymphocytic response occurs against the HBV-infected hepatocytes (Yang *et al.*, 2008). It is suggested that HBV is not directly cytopathic for infected hepatocyte. Rather, viral clearance and disease pathogenesis are largely mediated by adaptive immune response in HBV infection. it is widely believed that cytotoxic T-lymphocytes (CTLs) are the cells that clear viral infections by killing HBV-infected cells. This is likely to occur in adult patients, unlike in neonates, where neonatal tolerance to HBV is responsible for viral persistence following mother-infant transmission. This leads to high rates of chronic infections leading to liver cirrhosis and HCC. Most tumors in HBV-associated HCC result from random integration of HBV DNA in host cell DNA resulting in downregulation of cellular growth control mechanisms. Also, certain HBV protiens may directly participate in HCC development, for example, HBV X gene product transactivates cellular genes that control cellular growth (Chisari *et al*., 2009).

2.12. Cell and Tissue Tropism

The main cellular target of HBV is the hepatocyte, and in humans, these are the only cells convincingly shown to replicate the virus. However, the belief that other cells replicate the virus in humans has persisted based both on experimental evidence from human studies and research with animal models. The liver has a central role in synthesizing plasma proteins, storing and metabolizing glycogen as a source of energy, removing dead and dying cells from the bloodstream, and detoxifying harmful chemicals, among other things. Structurally, the liver is comprised of microscopic lobules into which blood enters from the hepatic artery and portal vein, which are situated in a region known as the portal triad, and exits via the hepatic vein. The lobule itself is not an anatomically defined structure but a region arbitrarily defined by the positioning of the portal tracts and central vein. Other cells include bile ductule epithelial cells, sinusoidal endothelial cells, hepatic stellate cells , and Kupffer cells, the resident liver macrophages. In addition to the portal vein and the hepatic artery, the triad also contains the bile duct, through which bile produced by hepatocytes during breakdown of bilirubin is exported to the gall bladder and small intestine, and lymphatics. Thus, in contrast to blood, which flows away from the portal tracts, lymph and bile flow toward the tracts (Knipe *et al*., 2007).

2.13. Occult hepatitis B infection in blood donors

Occult hepatitis B infection is defined as the presence of HBV-DNA without detectable HBsAg with or without anti-HBc or anti-HBs, outside the pre-seroconversion window period (Hu, 2002). This occult infection is mostly found in anti-HBc and/or anti-HBs positive individuals. It also occurs in individuals negative for all serum markers of HBV infection (Cacciola *et al*., 1999). These patients with occult infection may carry both integrated and free

HBV-DNA, in analogy to subjects belonging to the HBsAg positive categories considered above (Marusawa, 2000). In some cases, lack of HBsAg detection is due to rearrangements in the HBV genome that interfere with gene expression or lead to the production of an antigenically modified S protein that is not recognized by commercially available kits. However, in most cases HBV has no relevant genomic modification and the occult infection is a consequence of a profound suppression of viral replication and gene expression (Liang, 1991). Occult hepatitis B virus (HBV) infection is diagnosed when an HBV DNA test is positive but hepatitis B surface antigen (HBsAg) is undetectable. Occult HBV infection (OBI) may represent (i) acute infection in the window period, (ii) HBV tailend of chronic HBV infection, (iii) persistence of replication at low level after recovery in the presence of anti-HBs, or (iv)occurrence of an escape mutant in vaccinated or unvaccinated individuals not detected by current HBsAg assays(Biswas, 2003). Occult HBV infection has been reported as risk factor for hepatocellular carcinoma (Fabrizi *et al*., 2005). Occult HBV infection has also been reported in populations without symptomatic liver disease, such as blood donors, individuals with normal liver test or general populations (Henning *et al*., 2002).

2.14. Laboratory diagnosis of HBV

Diagnosis is based on clinical and epidemiological findings. It is impossible to differentiate HBV infection on clinical grounds alone; so definitve diagnosis should be established on the results of laboratory testing. Both serologic and molecular methods are available and used to distinguish between acute and chronic infections (CDC, 2012).

2.14.1. Collection, transportation, and storage of samples

Serum or plasma can be used for the detection of serologic and molecular markers of HBV infection. HBV antigens and antibodies are stable at room temperature for several hours to days, can stored at 4°c for month, and can be frozen at – 20 to -70°c for many years. Repeated freezing and thawing can lead to degradation 0f HBV serologic markers. Furthermore, use of hemolyzed samples should be avoided as it interferes with the detection signals (Horvart and Tegtmeier, 2011).

2.14.2. Antigen detection

Several laboratory assays are available to detect HBV antigens in the patient's serum after infection. In general, HBV antigens are detected using highly sensitive techniques that use either solid phase immune assay or microparticles to capture the protein. The antigen is then detected with well-defined monoclonal antibodies specific for that antigen. A marker of active viral replication is the detection of HBsAg and /or HBeAg during primary infection and during chronic HBV infection (Hovart and Tegtmeier, 2011). By definition, HBV chronic carriers are those in whom HBsAg persists for more than 6 months in the presence of HBeAg or anti-HBe antibodies (Brooks *et al.,* 2010).

2.14.3. Serological tests

Several commercial assays are available to detect HBV-specific antibodies, which determine the stage of the disease and immunity due to vaccination. IgM antibody to HBcAg (IgM anti-HBc) persists for several weeks to months, and its presence indicates recent, acute infection of less than 6 months duration. Also its absence does not rule out chronic infection. After IgM anti-HBc disappears, total antibody to HBcAg remain positive indefinitely, and is the best serologic marker for documenting past infection with HBV. This

marker should not be present in vaccinated individuals unless they were infected with HBV prior to vaccination. A positive anti-HBc total may indicate an acute (HBsAg-positive,IgM anti-HBc positive), resolved(HBsAg-negative), or chronic (HBsAg-positive) infection. A positive anti-HBs result is indicative of immunity to HBV as a result of resolved infection, or from effective vaccination. Furthermore, this marker is used to monitor vaccine success. Both WHO and CDC recognize levels of 10 mlU/ml of anti-HBs as protective. Presences of anti-HB indicates the resolution of acute infection and is associated with a decrease in viral replication. Patient who have recovered from acute HBV infection will have detectable anti-HBe, and anti-HBs (Hovart and Tegtmeier, 2011).

2.14.4. Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA test, also known as enzyme immunoassay (EIA), contains an enzyme antibody complex that can be used as colour tracer for antigen antibody reactions. The enzymes used most often are horse radish peroxidase and alkaline phosphatase, both of which release a dye (chromogen) when exposed to their substrate (Cowan and Talaro, 2009). There are two basic methods. The direct ELISA detects antigens, and indirect ELISA detects antibodies. A microtiter plate with numerous shallow wells is used in both procedours. ELISA procedours are popular primarily because they require little interpretive skill to read the results tend to be clearly positive. Many ELISA tests are available for clinical use in the form of commercially kits. Procedures are often highly automated, with the results read by a scanner and printed out by computer (Tortora, *et al.,* 2010).

2.14. 5. Immunochromatographic Test (ICT)

Rapid diagnostic tests based on immunochromatographic principles are widely used in most developing countries, for the detection of HBsAg. These methods are considerably cheaper than EIA methods. (Ainsar *et al*., 2007). ICT or lateral flow assays require the addition of only one or no reagent and thus are extremely simple to perform. These tests use antibodies spotted onto nitrocellulose membranes with lateral or vertical flow of sample or reagent to interact with immobilized antibody. Specific antibody is absorbed onto a nitrocellulose membrane in the sample line, and control antibody is adsorbed onto the same membrane as second line. Both antibodies are conjugated to visualizing particles that are dried onto an inert fibrous support. Conjugate pad and striped membrane are combined to constract the test strip (Tang and Stratton, 2006).

2.14.6. Polymerase chain reaction (PCR)

PCR is an in vitro method for producing large amounts of specific DNA fragment of defined length and sequence from small amounts of complex template (Bhatia and Ichhpujani, 2008). Detection and /or quantitative of HBV DNA are useful in the initial characterization of HBV infection and monitoring of chronic infection, especially in patients on antiviral therapy. Many of assays that detect HBV/DNA use oligo primers that recognize a conserved sequence within the HBV percore/core gene. Conventional PCR and real-time PCR are commonly used to detect and quantify HBV DNA,respectively (Hovart and Tegtmeier, 2011).

2.15. Treatment

There is no specific treatment recommended for acute hepatitis B. Treatment should be considered for patients with rapid deterioration of liver function, cirrhosis or complications such as ascites, hepatic encephalopathy, or hemorrhage as well as those who are immunosuppressed (Ryan *et al*., 2010). Early antiviral treatment may be required in less than 1% of people, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy. Treatment lasts from six months to year, depending on medication and genotype. Although non of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. There are seven medications licensed for treatment of hepatitis B infection in the United States. These include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude), and the two immue system modulators interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys). The use of interferon which requires injections daily or thrice weekly has been supplanted by long-acting PEGylated interferon, which is injected only once weekly. The treatment reduces viral replication in the liver, thereby reducing the viral load (the amount of virus particles as measured in the blood). Response of treatment differs between the genotypes (Pramoolsinsup, 2002).

2.16. Prognosis

Hepatitis B virus infection may be either acute (self-limiting) or chronic (long-standing). Persons with self-limiting infection clear the infection spontaneously within weeks to months. Children are less likely than adults to clear the infection. More than 95 % of people who become infected as adults or older children will stage a full recovery and develop protective immunity to the virus. However, these drops to 30% for younger children and only 5% of newborns that acquire the infection from their mother at birth will clear the infection. This population has a 40% life time risk of death from cirrhosis or hepatocellular carcinoma. Of those infected between the ages of one to six, 70% will clear the infection. Hepatitis D (HDV) can occur only with a contaminant hepatitis B infection, because HDV uses the HBV surface antigen to form a capsid. Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer (Oliveri *et al.*, 1991).

2.17. Prevention

Several vaccines have been developed to prevent infection by HBV. The first vaccine, which was licensed in 1981, was prepared from blood plasma from chronically infected individuals. It consisted of highly purified preparations of 20-nm particles that were treated to inactivate any residual virus infectivity (James and Ellen, 2008). A subunit vaccine, consisting solely of HBsAg, is effective in inducing protective immunity against HBV infection. The standard regimen consists of three doses, at 0, 1 and 6 months. The degree of protection induced by the vaccine is directly proportional to the level of anti-HBs achieved in the recipient's serum. The WHO recommends that HBV

vaccination be included as a routine universal vaccine of childhood (Will *et al*., 2005). The vaccine was originally prepared from plasma obtained from people who had long-standing hepatitis B virus infection. However, it is made using a synthetic recombinant DNA technology that does not contain blood products. One cannot be infected with hepatitis B from this vaccine. The risk of transmission from mother to newborn can be reduced from 20-90% to 5-10% by administrating to the newborn hepatitis B vaccine (HBV 1) and hepatitis B immune globulin (HBIG) within 12 hours of birth, followed by a second dose of hepatitis B vaccine (HBV 2) at 1-2 months and a third at no ealier than 6 months (24 weeks). Since 2% of infants vaccinated will not develop immunity after the first three dose series, infants born to hepatitis B-positive mothers are tested at 9 months for hepatitis B surface antigen (HBsAg) and to the hepatitis B surface antigen (anti-HBs). If post vaccination test results indicate that the child still susceptible. Following vaccination, hepatitis B surface antigen may be detected in serum for several days; this is known as vaccine antigenaemia(Martin *et al.*, 2004). The vaccine is administrated in either two – three or four - dose schedules into infants and adults, which provides protection for 85- 90% of individuals. Unlike hepatitis A, hepatitis B does not generally spread through water and food. Instead it is transmitted through body fluids thus; prevention is the avoidance of such transmission: unprotected sexual contact, blood transfusion, re-use of contaminated needles and syringes, and vertical transmission during child birth. Infants may be vac immunoprophylaxis starting from the newborn, multiple injections of small dose of hepatitis B immune globulin cinated at birth. Besides, the WHO-recommended joint, or oral lamivudine in HBV carrier mothers with a high degree of infectiousness ($>10^6$ copies/ml) in late pregnancy (the last three months of pregnancy), effectively and safely prevent

HBV intrauterine transmission, which provide new insight into prevention of HBV at the earliest stage (Shi *et al.*, 2010). Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb because it is prepared from sera of patients who have recovered from hepatitis B. It is used to provide immediate, passive protection to individuals known to be exposed to HBsAg-positive blood, e.g., after an accidental needle-stick injury. The recommendation regarding one common concern of medical students, the needle-stick injury from a patient with HBsAg-positive blood, is that both the vaccine and HBIG be given (at separate sites). This is true even if the patient's blood is HBeAb positive. Both the vaccine and HBIG should also be given to a newborn whose mother is HBsAg-positive. These are good examples of **passive–active** immunization, in which both immediate and long-term protections are provided (Levinson, 2010).

3. Materials and Methods

3.1. Study design

Descriptive cross sectional study.

3.2. Study area

This study was conducted in National Public Health Laboratory/ Kartoum State.

3.3.Study population

Blood donors attending blood bank department at National Public Health Laboratory.

3.4. Study duration

Study was carried out during 8 months period from March to October, 2014.

3.5. Sample size

A total of ninety one subjects (n=91) were enrolled in this study.

3.6. Data collection

Personal and clinical data were collected from blood donors by direct interviewing questionnaire from each subject (Appendix).

3.7.Ethical considerations

Permission to carry out the study was obtained from the College of Graduate Studies, Sudan University of Science and Technology. All blood donors examined were informed for the purpose of the study before collection of the samples and the verbal consent was taken from them.

3.8. Sample collection

Under aseptic condition after wearing the gloves, a local antiseptic (70% ethanol) was used to clean the skin. Venous blood (3mls) was obtained from blood donors. Samples were collected by vein puncture and haemolysis was avoided. Whole blood from vein puncture was collected into the collection containers. Serum was collected from whole blood in the collection tubes (did not contains anticoagulant) and left to settle for 30 minutes in the rack for blood coagulation and then was centrifuged at 3000 rpm for 5 minutes to get serum specimen supernatant. If blood specimen were not immediately tested they were kept at -20°C till used.

3.9. Laboratory Methods

The serological techniques ICT and ELISA were used for detection of HBsAg among blood donors attending The National Public Health Laboratory, blood bank department.

3.9.1. Immunochromatography test

3.9.1.1. Principle of the test

The hepatitis B surface antigen rapid test device is a qualitative, solid phase two-site sandwich immunoassay for detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the device. During testing, the serum specimen reacts with anti-HBsAg antibodies conjugated particles. The mixtures migrate upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this

colored line in the test line 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 has been added and membrane wicking has occurred.

3.9.1.2. Storage and stability

Stored as package in the sealed pounch. The kits were stored between 20 - 30°C. The test devices were kept in the sealed pounch until used. The expiry date was observed.

3.9.1.3. Procedure of the test

The test was conducted by removing the test device from foil pouch and placed it on a dry surface. Then 80µl were added from the serum specimen by using micropipette, into the sample window. Test results were interpreted within 10-30 minutes.

3.9.1.4. Interpretation of the results

Interpretations of the results were taken according to manufacture instructions as follow:

a) Positive results

If presence of two red lines as control line(C) and test line (T) within the result region indicates a positive result for HBV.

b) Negative results

The presence of only red control line (C) within the test result region indicates a negative result.

c) Invalid results

No presence of control line (C) within the result region indicates invalid results. The directions may not have been followed correctly or the test may have deteriorated. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. It is recommended that the specimen be retested.

3.9.1.5. Quality control

A procedural control is included in the test. A colored line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural techniques. Control standards are not supplied with this kit; however, it is recommended that a positive control (containing 10 ng/ml HBsAg) and a negative control (containing 0 ng/ml HBsAg) be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

3.9.2. Detection of HBsAg by capture Enzyme Linked ImmuboSorbant Assay (ELISA).

3.9.2.1.Principle

The Kit is an enzyme-immunoassay (AMS Labs Limited, United Kingdom) based on a “sandwich” principe; polysterene microtiter strip wells have been coated with monoclonal antibodies specific to HBsAg. Patient’s serum is

added to the microwells. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins, second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) and directed against a different epitope of HBsAg is added into the wells. During the second incubation step, these HRP-conjugated antibodies will be bound to any anti-HBs-HBsAg complex previously formed during the first incubation, and the unbound HRP-conjugate is then removed by washing. After washing to remove unbound HRP-conjugate, chromogen solutions containing tetramethyl-benzidine(TMB) and urea peroxide are added to the wells. In the presence of the antibody antigen-antibody (HRP) "sandwich" immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colorless.

3.9.2.2. Storage and stability of ELISA

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C. To assure maximum performance of this HBsAg ELISA Kit, during storage the reagents were protected from contamination with microorganisms or chemicals.

3.9.2.3. Procedure of the test

The reagents and samples were allowed to reach room temperature (18-30°C) and the stock wash buffer was diluted 1 to 20. Then, 100µl of positive control, negative control, and specimen were added into their respective wells of the 96-well microtiterplate. 50 µl HRP-Congugate was added to each well except the blank, and mixed by tapping the plate gently after incubating the plate for 60 minutes at 37°C, the plate was washed 5 times with diluted wash buffer, after final wash cycle, the plate was blotted down on to paper. Then, 50 µl chromogen A and 50 µof chromogen B solution were dispensed into each well including the blank, and were mixed by tapping the plate gently. The plate was incubated at 37°C for 30 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP-Conjugate produced blue color in positive control and HBsAg positive sample wells. Finally 50 µ stop solution was added into each well and mixed gently. Intensive yellow color developed in positive control and HBsAg positive sample wells. Then, the plate reader was calibrated with the blank well and the absorbance was read at 450nm within 10 minutes after stopping the reaction.

3.9.2.4. Reading of the result

Finally the results were calculated by relating each samples optical density (OD) value to the cut-off value (C.O.) of the plate, samples giving an absorbance less than the cut-off value are considered negative which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit, samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surfaces antigen has probably been detected with this ELISA kit.

3.9.2.5. Calculation of cut-off value:

Cut-off value (C.O) = NC × 2.1

NC = the mean absorbance value for three negative controls

cut-off (C.O) = 0.05 × 2.1 = 0.105

3.9.2.6. Quality control range

The test results are valid if the quality control criteria are verified. The OD value of the blank well, which contains only chromogens and stop solution, is less than 0.080 at 450 nm. The OD value of positive control must be equal to or greater than 0.80 at 450 nm after blanking. The OD value of the negative control must be less than 0.100 at 450 nm after blanking.

3.9.2.7. Interpretation of the result

a) Negative results

Samples giving an optical density less than the cut-off value (C.O <0.1) are considered negative which indicates that no hepatitis B virus surface antigen has been detected with this HBsAg ELISA.

b) Positive result

Samples giving an optical density greater or equal to the cut-off value (C.O >0.1) are considered initially reactive, which indicates that HBV surface antigen has probably been detected.

c) Borderline

Samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

3.9.2.8. Performance characteristic

3.9.2.8.1. Diagnostic of specificity

Diagnostic of specificity was calculated as follows:

$$\text{Specificity} = \frac{\text{true}-\text{ve}}{\text{true}-\text{ve}+\text{false}+\text{ve}}$$

3.9.2.8.2. Diagnostic sensitivity

Diagnostic of sensitivity was calculated as follows:

$$\text{Sensitivity} = \frac{\text{true}+\text{ve}}{\text{true}+\text{ve}+\text{false}-\text{ve}}$$

3.10. Data analysis

Statistical Package of Social Sciences (SPSS version 11.5). Computer software was used for data analysis. Significant levels were set at ($P \leq 0.05$). Figures were performed by using Microsoft Office and Excel software program.

4. Results

A total of ninety one blood donors (n=91) were tested for HBsAg. The age range of the subjects used in this study was 18 to 50 years, with a mean age of 34 years, all of them were males. The seroprevalence of HBV revealed from ICT test (3.3%), where it was increased to (4.4%) by ELISA prouceder.

4.1. Distribution of blood donors according to age

Figure (4.1) shows that out of ninety one blood donors included in this study, 40% were among 18-30 year, 45% among 31-40 year and 15% among 41-50 year. Positive results for HBV infection was 2.2% among age group from 31-40 year and 1.1% among both age group18-30 year and 41-50 year respectivel, when tested by ELISA.

4.2. Distribution of blood donors according to marital status

Figure (4.2) revealed that the percentage of married blood donors 64% was higher than the single 36%. Positive cases of HBV infection was 3% among married and 1 % among single, were examined by ELISA.

4.3. The effect of previous blood transfusion on HBV infection

The result summarized in table (4.1) demonstrated that total of 13 donors were previous blood transfusion, only one of them (7.7%) who had positive for HBsAg when tested by ELISA, among age group 41-50.

4.4. ELISA Results

Figure (4.4) revealed that the same blood donors when tested by ELISA show 4 (4.4%) were positive HBsAg.

4.5. ICT Results

Figure (4.5) demonstrates that out of 91 examined blood donors 3 (3.3%) were found positive for HBsAg when screened by ICT.

4.6. Sensitivity and Specificity of ICT test

Table (4.4). illustrate the sensitivity and specificity.

4.7. Sensitivity and Specificity of ELISA test

Table (4.5). illustrate the sensitivity and specificity of ELISA test.

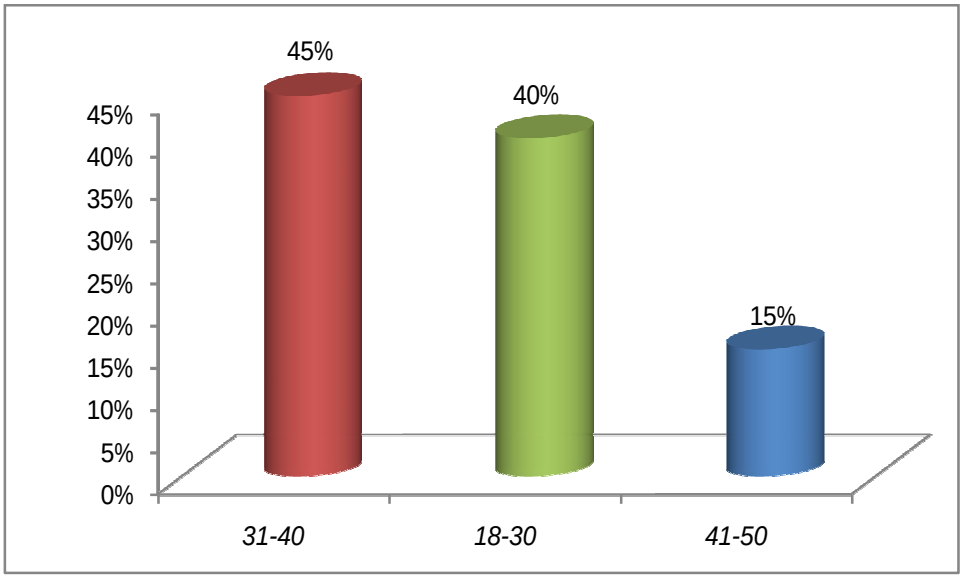

Fig 4.1. Distribution of blood donors according to age

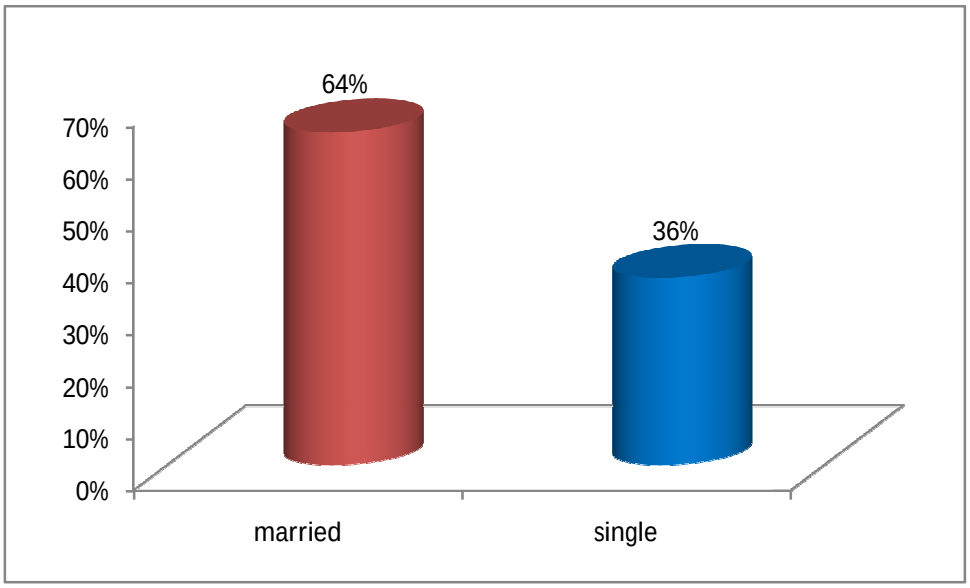

Fig 4.2 Distribution of blood donors according to marital status

Table 4.1 The effect of previous blood transfusion on HBV infection

			HBsAg		Total
			Yes	No	
Blood transfusion		Count	1	12	13
	% of blood transfusion		7.7%	92.3%	100%
		Count	3	75	78
	% of non blood transfusion		3.3%	82.4%	85.7%
Total		Count	4	87	91
		% of Total	4.4%	95.6%	100.0%

Chi- square = 0.53 ; P value ($p > 0.05$)

Table 4.2.prevalence of HBsAg using ELISA test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	**positive**	4	4.4	4.4	4.4
	negative	87	95.6	95.6	100.0
	Total	91	100.0	100.0	

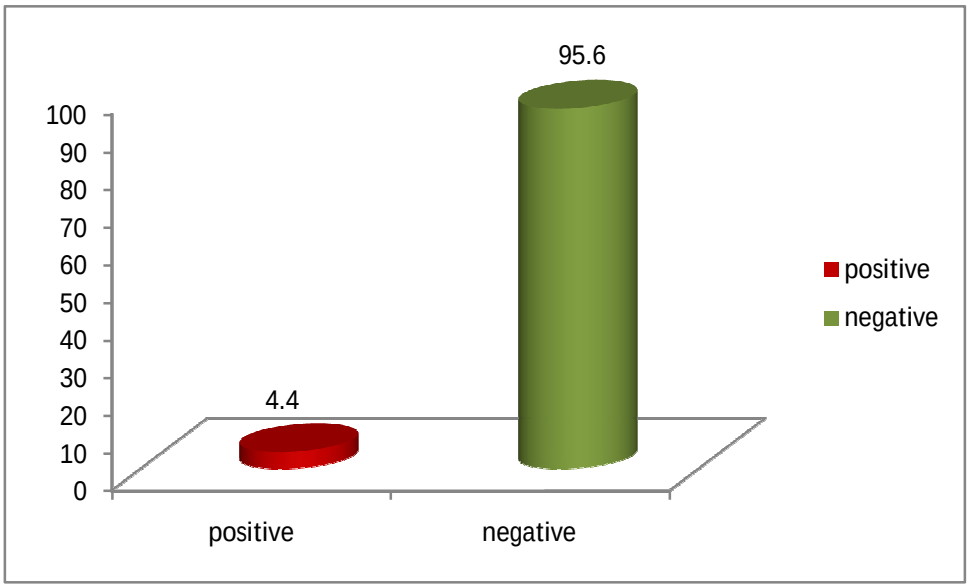

Fig 4.3. Percentage of HBsAg tested by ELISA

Table 4.3. prevalence of HBsAg usingI CT test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	**positive**	3	3.3	3.3	3.3
	negative	88	96.7	96.7	100.0
	Total	91	100.0	100.0	

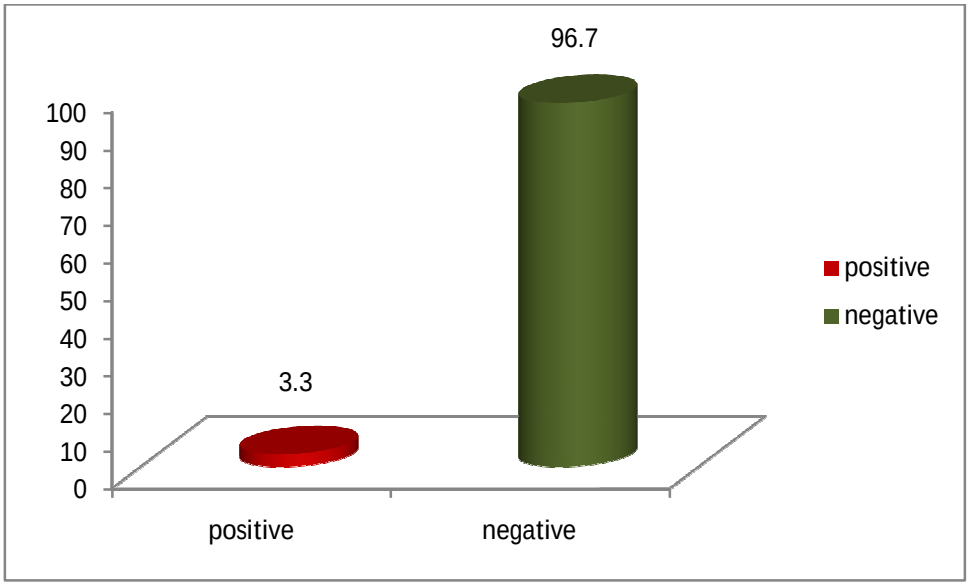

Fig 4.4. Prevalence of HBsAg using ICT test

Table 4.4. Sensitivity and Specificity of ICT test

ICT	Disease present	Disease absent
Test positive	3	0
Test negative	0	88

$$\text{Sensitivity} = \frac{\text{true+ve}}{\text{true+ve+false−ve}}$$

$$= \frac{3}{3+1} = 0.75 \times 100 = 75\%$$

$$\text{Specificity} = \frac{\text{true−ve}}{\text{true−ve+false+ve}}$$

$$= \frac{87}{87+0} = 1 \times 100 = 100\%$$

Table 4.5 Sensitivity and Specificity of ELISA test

ELISA	Disease present	Disease absent
Test positive	4	0
Test negative	0	87

$$\text{Sensitivity} = \frac{\text{true+ve}}{\text{true+ve+false−ve}}$$

$$= \frac{4}{4+0} = 1 \times 100 = 100\%$$

$$\text{Specificity} = \frac{\text{true−ve}}{\text{true−ve+false+ve}}$$

$$= \frac{87}{87+0} = 1 \times 100 = 100\%$$

Fig 4.5. ICT Rapid test device, two colored lines on left strip indicates positive for HBsAg while right strip appear one red line in the (C) region indicates negative.

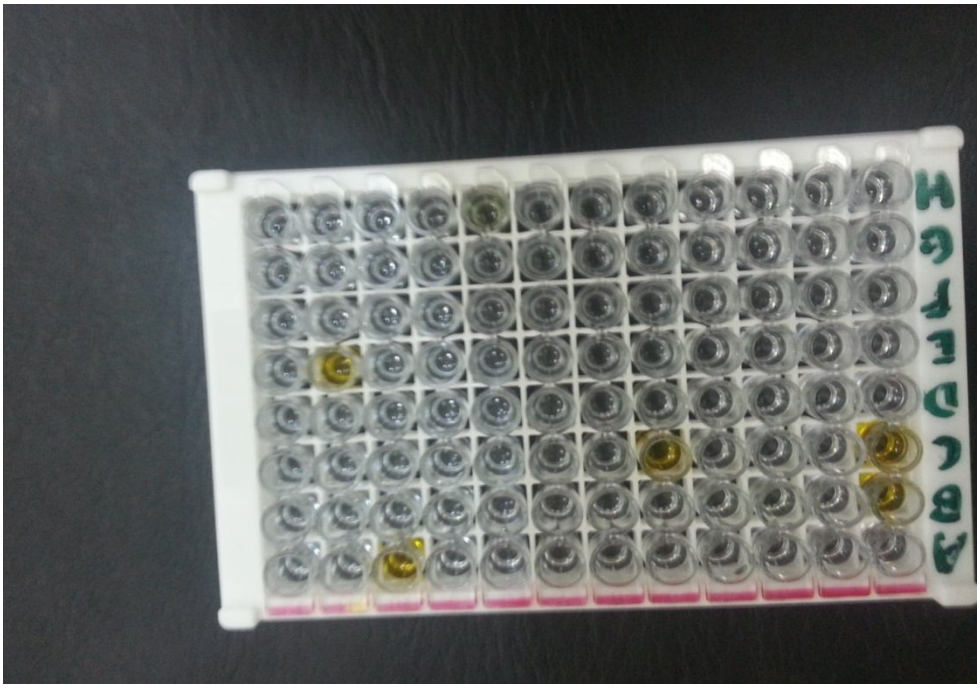

Fig 4.7. ELISA microtiter plate yellow wells indicates positive HBsAg

5. Discussion

Viral hepatitis is among the major blood transfusion transmissible diseases. In blood banking, a false negative result may lead to transfusion of infected blood to an uninfected individual; this can be disastrous to the community, so a very sensitive method is required to detect the most hidden blood infection. This study was carried out in blood bank of National Public Health Laboratory on a total of ninety one blood donors to assess the sensitivity and specificity of immune chromatography test (ICT) and ELISA in HBsAg infection. All subjects examined in this study were males. Out of these 3 blood donors (3.3 %) were positive for HBsAg, when tested by ICT, whereas it was increased to 4 (4.4%) by ELISA procedure ran on the same serum samples, there is one false negative result tested by ICT. Our study showed that ICT technique 75% sensitivity and 100 % specificity for detecting HBsAg, while ELISA revealed 100% sensetivity and 100% specificity. The Rapid tests that are currently available for the detection of HBsAg range in sensitivity from 50-94% relative to ELISA results depending on the technology applied (Yu, 2008).

In another study ICT and ELISA were compared for detection of HBsAg in healthy individuals from Karachi and showed comparable sensitivity and specificity of ICT kits with ELISA technique (Qasmi *et al.,* 2000).

The results showed that the ICT used in this study for HBsAg screening was less sensitive when compared with ELISA since additional positive sample were detected by ELISA for HBV. This study agrees with previous studies in other countries, which have stated that rapid test kits are not sensitive enough to be used solely for the detection of HBsAg (Khan *et al*., 2010).

The mean seroprevalence of HBV calculated from both tests was (3.9%). Similar results were obtained in an Indian study which showed that there was HBsAg prevalence of 3.43% during the year 2008 (Nilima *et al.*, 2010). Also, in another study conducted among the blood donors in tertiary hospital in Tabuk, North Western Saudi Arabia showed a prevalence of about 3.0% (El Beltagy *et al*., 2008) which was similar to our results. These findings were slightly higher than those reported by Olokoba *et al.*, 2009; Bhatti *et al*., 2007 in Pakistani donors (2.2%). However, our results were lower than those observed by Nagi *et al* (2007) in Northern Sudan (5.1%), McCarthy *et al* (1994) Southern Sudan (26%) and Nwankwo *et al* (2012) in Kano, Nigeria (11.1%) among blood donors.

As obtained in this study, previous blood transfusion (one donor 1.1% positive HBsAg) had no statistical significance effect ($p > 0.05$) on HBV infection among blood donors examined. The small sample size used in this study could be a possible reason for these findings, as blood transfusion are still risk factors for HBV transmission among individuals. However, sexual activities, which were not tested in this study, are still known to be major routes for HBV transmission worldwide.

5.1. Conclusion

It was concluded that ELISA was more sensitive than ICT, but both tests had 100% specificity.

In this study, it was concluded that rapid test kits are not sensitive enough to confirm the hepatitis status of a donor in blood transfusion.

5.2. Recommendations

1. This subject requires more studies, using large sample size at different hospitals to acquire more accurate results.
2. ELISA technique could be used as screening test to detect presence of HBsAg among blood donors but the positive results should be confirmed by performing PCR to detect HBV-DNA.
3. Employing more confirmatory technique like RT-PCR to compare between the sensitivity and specificity.
4. General surveillance through mass screening to identify those with infection and instituting appropriate treatments, mass immunization of the uninfected population against the virus and public health education to enlighten blood donors of the possible risk factors and routes of infection are indeed advocated.

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