

Chapter One

1.Introduction

1.1.Introduction

Blood transfusion, an integral part of medicine and surgery, also carries the risk of transfusion-transmissible infections like Hepatitis B and C, HIV and Syphilis, malaria and infrequently toxoplasmosis, brucellosis and viral infections like CMV, Epstein Barr Virus and Herpes (Widmann, 1985). Measuring their severity, WHO has recommended pre-transfusion blood test for HIV, HBV, HCV and Syphilis as mandatory(WHO, 2000). All these diseases are capable of causing significant mortality, morbidity along with a financial burden for both the affected person and the country.

Hepatitis B virus (HBV) is a member of the hepadnavirus family (Zuckerman, 1996). Causes hepatitis B infection which is a global health problem: an estimated two billion people(one-third of the global population) have been infected with HBV at some point in their life; of these, more than 350 million suffer from chronic HBV infection, resulting in over 600,000 deaths each year, mainly from cirrhosis or liver cancer (WHO, 2008).

Syphilis is a systemic, sexually transmitted disease caused by *Treponema pallidum* which can be spread by sexual contact(including genital , oral and/or anal contact) , blood transfusion, via vertical transmission (from mother to fetus)and via accidental inoculation from infectious material (Murray *et al.*, 2002). Approximately 10.6 million new cases reported in 2005 and 2008, according to the World Health Organization (WHO, 2012).

Primary, secondary, early latent syphilis: benzathine penicillin is first-line and oral azithromycin single dose is second-line (British Association for Sexual Health and HIV, 2008). Azithromycin has been shown to be as effective as benzathine penicillin in treating early syphilis. In case of late latent syphilis: benzathine penicillin is first-line. And in neurosyphilis: procaine penicillin with oral probenecid (Hook *et al.*, 2010).

After collection all samples were screened for Hepatitis B Virus and *Treponema Pallidum* (Detection of Treponemal Antibodies) by microwell ELISA.

1.2. Rationale

Hepatitis B is one of most common infectious diseases of the world infecting two billion people including an estimated 400 million chronically infected cases (Schreiber *et al.*, 1996) and is leading cause of liver cirrhosis and hepato cellular carcinoma.

Ulcerative sexually transmitted diseases (STDs) that cause sore, ulcer, or break in the skin or mucous membrane such as syphilis , disrupt barrier that provide protection against infections. The genital ulcer cause by syphilis can bleed easily and when they come in contact with oral and rectal mucosa during sexual contact, increase infectiousness and susceptibility to HIV and other STDs such as HBV.

Little is known about the detection of anti-treponemal antibodies among HBsAg positive carriers. So this study aimed to focus on the possibility of syphilis co-existence with HBsAg positive in blood donors.

1.3. Objectives

1.3.1. General objectives

To determine frequency of anti-treponemal antibodies among HBsAg positive blood donors.

1.3.2. Specific objectives

- 1- To detect anti-treponemal antibodies among HBsAg positive blood donors using ELISA technique.
- 2- To determine the risk factors of syphilis (age, marital status or history of previous blood transfusion) .

Chapter two

2. Literature Review

2.1. background

Sexually transmitted diseases (STDs) remain a public health problem of major significance in most parts of the world (Levine, 2003, Sellami *et al.*, 2003).

In Ethiopia the prevalence of HBV-syphilis co-infection was 0.11% (Tessema *et al.*, 2010). And in Koudougou (Burkina Faso) it was 0.66% among blood donors with multiples infections (Nagalo *et al.*, 2011) ..

In Tanzania the prevalence of HBV-syphilis co-infection was (2.2%) there were significant associations between syphilis and HBsAg, these could be due to the fact that these pathogens are sexually transmitted (Mecky *et al.*, 2006).

2.2. Hepatitis B Virus

2.2.1. Introduction

Hepatitis B virus (HBV) is a member of the hepadnavirus family (Zuckerman, 1996). The virus particle (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. These virions are 30-42 nm in diameter. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (Locarnini, 2004). The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses, and the 42 nm virions, which are capable of infecting hepatocytes, are referred to as "Dane particles" (Harrison, 2009). In addition to the Dane particles, filamentous and spherical bodies lacking a core can be found in the serum of infected individuals. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigens (HBsAg), and is produced in excess during the life cycle of the virus (Howard, 1986).

2.2.2 Transmission

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include sexual contact (Fairley and

Read, 2012), blood transfusions and transfusion with other human blood products (Buddeberg *et al.*, 2008), re-use of contaminated needles and syringes (Hughes, 2000) and vertical transmission from mother to child (MTCT) during childbirth. Without intervention, a mother who is positive for HBsAg has a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for HBeAg. HBV can be transmitted between family members within households, possibly by contact of nonintact skin or mucous membrane with secretions or saliva containing HBV (Buddeberg *et al.*, 2008). However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor (Shi *et al.*, 2011).

2.2.3. Pathogenesis

Viral hepatitis is a necroinflammatory liver disease of variable severity. Persistent infection by HBV is often associated with chronic liver disease that can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). Many studies suggest that HBV is not directly cytopathic for the infected hepatocyte (Chisari, 2000; Guidotti and Chisari, 2006). For example, during the early phase of HBV infection (i.e., before virus-specific T cells enter the liver), 100% of the hepatocytes may be infected without histological or biochemical evidence of liver disease (Guidotti *et al.*, 1999, Thimme *et al.*, 2003). Furthermore, when cellular immune responses are deficient or pharmacologically suppressed, HBV can replicate at high levels in the liver of patients [and in immunologically tolerant HBV] (Guidotti and Chisari, 2006) in the absence of cytological abnormalities or inflammation (Ferrari *et al.*, 2003, Ganem and Prince, 2004). Virus replication often results in the induction of an innate immune response which is preceded by rapid induction of interferon alpha/ beta ($IFN\alpha/\beta$) by the infected cell (Samuel, 1991). Production of $IFN\alpha/\beta$ induces the transcriptional expression of a large number of interferon inducible genes (ISGs) which in turn exert a variety of intracellular antiviral mechanisms that have the potential to minimize pathogenetic processes by limiting viral production and spread (Samuel, 1991; Wieland and Chisari, 2005).

2.2.3.1 The Antibody Response

The antibody response to the HBV envelope antigens is a T cell-dependent process (Tsui *et al.*, 1995). Because these anti-envelope antibodies are readily detectable in patients who clear the virus and recover from acute hepatitis, and they are usually undetectable in patients with chronic HBV infection, they are thought to play a critical role in viral clearance by complexing with free viral particles and removing them from circulation or by preventing their attachment and uptake by hepatocytes. This notion is supported by the observation that chimpanzees that resolved a previous infection are completely protected from rechallenge (Moss *et al.*, 1984). The appearance of neutralizing antibodies, however, occurs relatively late after HBV exposure and, thus, it is unlikely to contribute to the early phase of viral clearance during acute infection. Instead they probably prevent viral spread from rare cells that remain infected after resolution of HBV infection (Tsui *et al.*, 1995).

2.2.3.2 The CD4 T Cell Response

The peripheral blood CD4 T cell response to HBV is vigorous, and multispecific in patients with acute hepatitis who ultimately clear the virus, while it is relatively weak in persistently infected patients with chronic hepatitis (Ferrari *et al.*, 1990). Although the association between a strong CD4 T cell response, acute hepatitis, and viral clearance suggests that a relationship exists between these events (Tsui *et al.*, 1995; Ferrari *et al.*, 1990; Jung *et al.*, 1991) CD4 T cell depletion at the peak of HBV infection had no effect on viral clearance and liver disease in infected chimpanzees (Thimme *et al.*, 2003), suggesting that CD4 T cells do not directly participate in viral clearance and tissue damage.

2.2.3.3 The CD8 T Cell Response

The HBV specific CD8 T cell response plays a fundamental role in viral clearance and the pathogenesis of liver disease. A vigorous polyclonal CD8 T cell response is readily detectable in the peripheral blood of patients with acute hepatitis who ultimately clear HBV. In contrast, the peripheral blood T cell response in chronically infected patients is

weak and narrowly focused (Penna *et al.*, 1991 ; Bertoletti *et al.*, 1991; Rehermann *et al.*, 1995 ; Tsui, *et al.*, 1995). The livers of these patients contain virus-specific T cells that likely contribute to disease pathogenesis, but for functional and/or quantitative reasons are unable to clear the infection. Interestingly, a recent study that examined a relationship between the number of intrahepatic HBV specific CD8 T cells, extent of liver disease, and levels of HBV replication in chronically infected patients indicated that inhibition of virus replication could be independent of liver damage, and that the functionality of HBV-specific CD8 T cells was more important than the number of T cells to control HBV replication (Maini *et al.*, 2000). Experiments in chimpanzees have shown that the viral clearance and the onset of liver disease coincide with the accumulation of virus-specific CD8 T cells and the induction of interferon gamma(IFN γ) and IFN γ -inducible genes in the liver (Guidotti *et al.*, 1999, Thimme *et al.*, 2003). Importantly, depletion of CD8 T cells at the peak of viremia delays viral clearance and onset of viral hepatitis until the T cells return, proving that the viral clearance and liver disease are mediated by virus specific CD8 T cells (Thimme *et al.*, 2003).

2.2.4 Signs and Symptoms

Acute infection with hepatitis B virus is associated with acute viral hepatitis – an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people.

A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result. The infection may be entirely asymptomatic and may go unrecognized (Terrault *et al.*, 2005).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Chronic carriers are encouraged to

avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of membranous glomerulonephritis (MGN) (Gan *et al.*, 2005). Symptoms outside of the liver are present in 1–10% of HBV-infected people and include serum-sickness–like syndrome, acute necrotizing vasculitis (polyarteritis nodosa), membranous glomerulonephritis, and papular acrodermatitis of childhood (Gianotti–Crosti syndrome) (Dienstag , 1981;Trepo and Guillevin , 2001). The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice (Alpert *et al.*, 1971). The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after the onset of jaundice, but can persist throughout the duration of acute hepatitis B (Liang, 2009). About 30–50% of people with acute necrotizing vasculitis (polyarteritis nodosa) are HBV carriers(Gocke *et al.*,1970). HBV-associated nephropathy has been described in adults but is more common in children(Lai *et al.*, 1991, Takekoshi *et al.*, 1978). Membranous glomerulonephritis is the most common form (Liang , 2009).

Other immune-mediated hematological disorders, such as essential mixed cryoglobulinemia and aplastic anemia (Liang, 2009).

2.2.5. Laboratory Finding

2.2.5.1 Large-scale screening for HBV infection

Diagnosis of hepatitis is made by biochemical assessment of liver function. Initial laboratory evaluation should include: total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, prothrombin time, total protein, albumin, serum globulin, complete blood count, and coagulation studies (Hollinger and Liang, 2001).

Diagnosis is confirmed by demonstration in sera of specific antigens and/or antibodies.

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

- hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs)
- antibody (anti-HBc IgM and anti-HBc IgG) to hepatitis B core antigen (HBcAg)
- hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe)

Tests specific for complete virus particles or DNA and DNA polymerase-containing virions, and for hepatitis Delta antigen (HDAg) and hepatitis Delta virus (HDV) RNA in liver and serum are available only in research laboratories (Thimme *et al.*, 2003).

HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious. (Mahoney and Kane, 1999; Hollinger and Liang, 2001).

Very early in the incubation period, pre-S1 and pre-S2 antigens are present. They are never detected in the absence of HBsAg. Hepatitis B virions, HBV DNA, DNA polymerase, and HBeAg are then also detected. The presence of HBeAg is associated with relatively high infectivity and severity of disease (Hollinger and Liang, 2001).

Anti-HBc is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis.

This test may therefore reliably diagnose acute HBV infection. IgG anti-HBc generally remains detectable for a lifetime (Mahoney and Kane, 1999 ; Hollinger and Liang, 2001).

Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity. Anti-HBe replaces HBeAg in the resolution of the disease (Mahoney and Kane, 1999 ; Hollinger and Liang, 2001).

Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity (Mahoney and Kane, 1999 ; Hollinger and Liang, 2001).

Acute hepatitis patients who maintain a constant serum HbsAg concentration, or whose serum HbeAg persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver disease (Hollinger and Liang, 2001).

2.2.5.2 Small-scale screening for HBV infection

Immunofluorescence studies, in situ hybridization, immunohistochemistry, and thin-section electron microscopy are used to examine pathological specimens for the presence

of HBV-associated antigens or particles, providing information about the relationship between HBV DNA replication and HBV gene expression (Hollinger and Liang, 2001). Within the hepatocyte, HBsAg localizes in the cytoplasm, and HBcAg is seen in the nucleus and/or the cytoplasm. Detection of complete virions in the liver is uncommon (Hollinger and Liang, 2001).

DNA hybridization techniques and RT-PCR assays have shown that almost all HBsAg/HBeAg-positive patients have detectable HBV DNA in their serum, whereas only about 65% of the HBsAg/anti-HBe-reactive patients are positive. All patients who recover from acute hepatitis B are negative for HBV DNA. On the other hand, some patients infected chronically who have lost their HBsAg remain HBV DNA positive (Hollinger and Liang, 2001).

2.2.6. Treatment

Antiviral medications for the management of chronic HBV infections currently available include alpha interferon (IFN- α) and three nucleoside analogs: lamivudine, adefovir and entecavir that inhibit viral nucleocapsid formation and block viral DNA synthesis by premature chain termination (Perrillo, 2005). The major determinant involved in the selection of drug-resistant mutation is the fitness of the mutants and the replication space available for the spread of mutants. In chronic hepatitis B, the replication space is provided by hepatocyte turnover, which allows the loss of HBV wild-type infected cells and the generation of noninfected hepatocytes that are susceptible to new HBV mutant infections. Long-term therapy of adefovir or entecavir mediates significant reduction in complementary DNA (cDNA), but still fails to eliminate chronic HBV infections (Zoulim, 2004 ; Sung, 2005) .

2.2.7. Vaccination

A program for universal vaccination of all newborns is a key step toward effective control of HBV infection throughout the world. Hepatitis B vaccination is highly cost-effective, in that it prevents infection with HBV and thus reduces the incidence of chronic hepatitis, cirrhosis, and HCC in the vaccinated population.

2.2.7.1 Active vaccination with hepatitis B vaccine

HBsAg is the antigen used in the formulation of the hepatitis B vaccine. It is produced from yeast through recombinant DNA technology. It is available as a single-agent preparation or as a fixed combination with other vaccines.

2.2.7.2 Passive vaccination with hepatitis B immunoglobulin (HBIG)

HBIG is prepared from plasma of individuals who have a high concentration of anti-HBs. The standard dose of HBIG is 0.06 mL/kg for all applications in adults. In standard doses, it provides temporary protection (i.e., for approximately 3–6 months) against HBV infection. HBIG is administered by intramuscular injection, preferably into the deltoid or gluteal muscle. If given with hepatitis B vaccine, administration of the HBIG vaccine should be in a different location.

Vaccination of unvaccinated adults exposed to risks of HBV infection (however, typically “high-risk” individuals frequently do not access or inform health-care facilities; hence the need for universal childhood vaccination).

2.3. *Treponema pallidum*

2.3.1. History of syphilis

The exact origin of syphilis is disputed (Kent and Romanelli, 2008). Two primary hypotheses have been proposed. One of the two primary hypotheses proposes that syphilis was carried from the Americas to Europe by the returning crewmen from Christopher Columbus's voyage to the Americas. The other hypothesis says that syphilis existed in Europe previously, but went unrecognized until shortly after Columbus' return. These are referred to as the Columbian and pre-Columbian hypotheses, respectively (Farhi and Dupin, 2010).

2.3.2. Structure and Biology

Treponema pallidum is a spirochete bacterium belonging to the Spirochaetaceae family. It has an approximate diameter of 0.18 µm and length of 6-20 µm. It cannot be cultured on bacteriological media (fastidious organism) that exhibits narrow optimal ranges of pH (7.2 to 7.4) and temperature "30 to 37°C" (Evans and Brachman, 1998). It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants. Traditionally this organism has been considered a strict anaerobe, but it is now known to be microaerophilic. Treponemes multiply by binary transverse fission.

The in vivo generation time is relatively long (30 hours). Despite intense efforts over the past 75 years, *T. pallidum* has not been successfully cultured in vitro. Viable organisms can be maintained for 18 to 21 days in complex media, while limited replication has been obtained by co-cultivation with tissue culture cells (Musher 1990).

The composition of *T. pallidum* (dry weight) is approximately 70 percent proteins, 20 percent lipids, and 5 percent carbohydrates. This lipid content is relatively high for bacteria. The lipid composition of *T. pallidum* is complex, consisting of several phospholipids, including cardiolipin, and a poorly characterized glycolipid which is biochemically and immunologically distinct from lipopolysaccharide. Although treponemes possess both outer and cytoplasmic membranes, they differ considerably in structure from enteric Gram-negative bacteria. Typically, three flagella originate from each end of the bacterium, and, winding about the bacterium within the periplasmic

space, overlap at the midpoint. The presence of peptidoglycan in the cell wall, originally surmised on the basis of the bacterium's exquisite sensitivity to penicillin, has been confirmed by biochemical analysis. Unlike Gram-negative bacteria in which the peptidoglycan underlies the outer membrane, in treponemes the murein layer overlies the cytoplasmic membrane. The cytoplasmic membrane covers the protoplasmic cylinder; this membrane contains the majority of the bacterium's integral membrane proteins and is particularly abundant in lipid-modified polypeptides “lipoproteins”(Musher, 1990).

2.3.3.Epidemiology

Humans are the only source of treponemal infection; there are no known nonhuman reservoirs. Venereal syphilis is distributed worldwide, and over the past several decades has become a significant public health problem in many underdeveloped countries. Infectivity rates correspond to the most sexually active age groups, being highest in the 20-to 24-year age group, slightly lower in the 15- to 19- year age group, and lower still in the 25- to 29- year age group. The peak incidence of syphilis was observed in 1946 to 1947. In the late 1940s, it was discovered that *T. pallidum* is exquisitely sensitive to penicillin G, and penicillin was found to be effective in eradicating syphilis of all clinical stages as well as the congenital infection. Following the adoption of penicillin as the mainstay of syphilotherapy, the number of new syphilis cases progressively decreased until 1958, after which the trend reversed and a steady increase has occurred. The late 1980's experienced a major increase in the incidence of early syphilis cases which was largely related to crack cocaine usage among inner city minorities. Improved surveillance methods have helped to control this syphilis epidemic(Chiu *et al.*, 1990). There are an estimated 12 million new cases per year; and 34,270 cases were reported in 2003 (Murray *et al.*, 2003).

2.3.4.Genome

The complete genome for *T. pallidum* was sequenced in July of 1998. The genome was sequenced using “the whole genome random sequencing method”. The genome consists

of a single double stranded circular DNA chromosome 1,138,006 base pairs long. It contains approximately 1,090 genes which encode approximately 1,041 proteins. These open reading frames account for 92.9% of the genomic DNA. 55% of genes were assigned defined roles and 17% were categorized based on similarities to other organisms. The average size of encoded proteins was estimated to range from 3235 to 172,869 daltons (Fraser *et al.*, 1998).

2.3.5. Transmission

Syphilis is transmitted primarily by sexual contact or during pregnancy from a mother to her fetus; the spirochaete is able to pass through intact mucous membranes or compromised skin. (Kent and Romanelli, 2004; Stamm 2010) It is thus transmissible by kissing near a lesion, as well as oral, vaginal, and anal sex (Kent and Romanelli, 2004). Approximately 30 to 60% of those exposed to primary or secondary syphilis will get the disease (Dylewski and Duong, 2007). Its infectivity is exemplified by the fact that an individual inoculated with only 57 organisms has a 50% chance of being infected (Eccleston *et al.*, 2008). It can be transmitted via blood products. However, it is tested for in many countries and thus the risk is low. The risk of transmission from sharing needles appears limited (Kent and Romanelli, 2004).

It is not generally possible to contract syphilis through toilet seats, daily activities, hot tubs, or sharing eating utensils or clothing (CDC, 2010). This is mainly because the bacteria die very quickly outside of the body, making transmission via objects extremely difficult (Csonka, 1994).

2.3.6. Pathology

T. pallidum is the causative agent of syphilis, a chronic infectious human disease transmitted between individuals via sexual intercourse or from mother to child in utero. *T. pallidum*'s virulence factor is still unknown. Untreated syphilis progresses in a series of distinct stages (primary, secondary, latent, and tertiary.) "Infection is initiated when *T. pallidum* penetrates dermal microabrasions or intact mucous membranes" resulting in primary syphilis.

Primary syphilis usually presents itself as a single chancre at the site of infection. Secondary syphilis occurs approximately 3 months after infection and presents itself with a variety of symptoms, most notably lesions of the skin and mucous membranes. These include a rash commonly on the palms of the hands, soles of the feet, face, and scalp. The breakdown of mucous membranes appears as patches on lips, inside the mouth, vulva, and vagina. Infected individuals may also experience fever, loss of appetite and weight loss during this stage. After several months, secondary symptoms will disappear; this is called the latent phase. Even though the infected individual is no longer showing symptoms, testing confirms that *T. pallidum* is still present. Transmission at this stage via sexual contact is rare. If untreated, latent phase may progress to tertiary phase. Tertiary syphilis doesn't manifest until years after initial infection (if it does at all) and can affect many different areas of the body. Tertiary syphilis can cause destructive lesions on skin and bones which are usually benign. The more deadly manifestations of late syphilis affect the cardiovascular system (especially the aorta) and the central nervous system causing infected individuals to experience insomnia and changes in personality (Rebecca *et al.*, 2006).

2.3.7. Clinical Manifestations

The clinical disease manifestations have been well characterized for over 100 years and are traditionally divided into five stages: incubating, primary, secondary, latent (early latent and late latent), and late or tertiary syphilis (neurosyphilis, cardiovascular syphilis and granulomatous syphilis) (Tramont, 2005).

2.3.7.1. Primary syphilis

Is the stage of infection which occurs 3 to 90 days (a median of 3 weeks) after infection. It is most commonly characterized by a single, painless chancre or ulcer that develops at the site of inoculation (Tramont, 2005). The chancre typically has a smooth base with raised and firm borders. However, in some persons, it does not develop at all; in other persons, it is so small that it may go completely unnoticed; and in others, especially those

with human immunodeficiency virus (HIV) infection, multiple ulcers may develop (Romanowski *et al.*, 1991; Rompalo *et al.*, 2001). The untreated lesion or lesions usually heal spontaneously in 2 to 8 weeks (range 1 to 12 weeks).

2.3.7.2.Secondary syphilis

The result of the interaction between a large spirochete load and the host's immune response, is the stage of infection in which there is widespread dissemination to various parts of the body. It becomes evident a mean of 6 weeks (range 2 to 16 weeks) after inoculation. In 90% of cases, there is a rash which most commonly is widely disseminated, maculopapular and involves the palms and soles but other dermatological manifestations are also common. In addition, over 50% of cases have fever, malaise, anorexia, weight loss, pharyngitis, laryngitis, and/or arthralgias. Other clinical manifestations include lesions in the mouth and oral cavity, lymphadenopathy, condyloma latum, glomerulonephritis, nephritic syndrome, hepatitis, arthritis, osteitis, and periosteitis (Rompalo *et al.*, 2001).

2.3.7.3. Latent syphilis

is the period of months to years post infection in which there are no outward clinical manifestations of disease despite viable organisms. Clinical relapses can occur during the first year of the latent stage (referred to as the early latent phase) and is felt to be the result of waning specific cellular immunity (Rompalo *et al.*, 2001).

2.3.7.4.Tertiary or late syphilis

occurs in up to 35% of untreated patients ten to twenty-five years after the initial infection. Late syphilis can be categorized into neurosyphilis, cardiovascular syphilis, and granulomatous syphilis. The latter two forms are uncommon in the antibiotic era due to the frequent exposure to antibiotics while today neurosyphilis is the most common manifestation of tertiary/late syphilis because of the generally poor penetration of antibiotics into the CNS (Tramont, 2005).

2.3.8.Laboratory Diagnosis

2.3.8.1.Direct Detection Methods

Direct detection methods are not widely available and require specialized laboratories with trained technicians. Hence, false negative tests are relatively common, about 30%.

2.3.8.1.1.Dark Field Microscopy

Dark field microscopy remains a sensitive, direct, and quick method for diagnosing syphilis in active lesions (Tramont, 2005). However, it requires a special dark field equipped microscope and skilled technicians that are not usually available at most medical facilities today. Specimens for microscopic examination are best obtained from serous transudate of moist lesions such as a primary chancre, condyloma latum (wart like lesions, sometimes extensive), or mucous patches (shallow ulcers on mucous membranes, non-painful unless secondarily infected). However, they also can be collected from dry skin or lymph nodes by non-bactericidal saline aspiration. When obtaining a sample, the surface should be cleaned with non-bactericidal saline and gently abraded with dry gauze, just enough so that a few scattered red blood cells are seen on the slide.

The cleaning should be performed without soap, a topical antiseptic or bactericidal saline because dead and non-motile organisms are difficult to visualize. The specimen should be placed on a glass slide with a cover slip placed on top. When using serous exudates, a drop of non-bactericidal saline may be added if the serous preparation is too thick. Under dark field microscopy, *T. pallidum* will appear as corkscrew shape in spiraling motion with a 90° undulation about its midpoint. At least three specimens should be examined before deciding that a lesion is non-syphilitic (Tramont, 2005).

2.3.8.1.2.Polymerase chain reaction (PCR)

PCR can also be used to detect *T. pallidum* genetic material, most often the polymerase A (PolA) gene (Genest *et al.*, 1996; Grange *et al.*, 2012; Hazlett *et al.*, 2005; Heymans *et al.*, 2010; Jethwa *et al.*, 1995; Koek *et al.*, 2006; Liu *et al.*, 2001; Palmer *et al.*, 2003). To date, it is most commonly used to diagnose congenital syphilis. However, it has also been shown to be effective at diagnosing primary syphilis with sensitivities between 73% and 95% and specificities 95% (Grange *et al.*, 2012; Heymans *et al.*, 2010; Palmer *et al.*,

2003). for the diagnosis of primary syphilis, swabs of the ulcer can be submitted to the CDC for processing with prior approval from the local health department (CDC, 2013). Other specimen types are acceptable but have less diagnostic value. Sensitivities of blood samples in primary syphilis are as low as 18% (Grange *et al.*, 2012).

2.3.8.1.3. Immunofluorescent and Immunoperoxidase Antibody Staining

Specific immunofluorescent or immunoperoxidase antibody staining can be used to visualize organisms, including nonviable spirochetes, from mucocutaneous lesions, lymph nodes, or dry skin (Cummings *et al.*, 1996; Tramont, 2005). In addition, it can be used for examining non-frozen biopsy material (Tramont *et al.*, 1995).

2.3.8.2. Serological Tests

2.3.8.2.1 Nontreponemal Reaginic Antibody Tests

Syphilis reaginic antibodies are IgG and IgM antibodies directed against a lipoidal antigen resulting from the interaction of host tissues with *T. pallidum* or from *T. pallidum* itself (Tramont, 2005). The earliest cardiolipin antigens used to measure reaginic antibody

were crude extracts from beef livers or hearts and false-positive tests were common. However, today's preparation, the cardiolipin-cholesterol-lecithin, is much purer and hence there are less false-positive reactions. The nontreponemal test first developed was the Venereal Disease Research Laboratory (VDRL) slide test (Tramont *et al.*, 1995). In this test, serum is heated to 56° C and tested to see if it can flocculate a suspension of a cardiolipin-cholesterol-lecithin antigen. Except for its use in diagnosing neurosyphilis, it has now been largely replaced by the modifications such as the rapid plasma reagin (RPR) card test, automated reagin test (ART), and the toluidine red unheated test (TRUST). In addition, there is also now a modified RPR test that can be done without requiring a laboratory at the point of care (U.S. Preventive Services Task Force, 2004).

Nontreponemal tests become positive shortly after initial infection, peak during the secondary or early latent stage, and then decline with time (Hart, 1986). In primary syphilis, an antibody response may not yet have been generated so nontreponemal tests may be negative. In secondary syphilis, virtually 100% of infected persons have positive

serological tests for syphilis but in some patients the titers are so high that the test is reported as negative due to the prozone phenomenon (Tramont, 2005). If the clinical index of suspicion is high, appropriate serum dilutions should be performed. In latent and late syphilis, titers decline, usually to $< 1:4$, and actually become negative in 25% of untreated persons (Hart, 1986).

On the other hand, whenever there is a strong immunologic stimulus (e.g., acute bacterial or viral infection, vaccination, HIV infection), a "false" positive occurs (Joyanes *et al.*, 1998; Tramont, 2005). In addition, persons who use injection drugs, have autoimmune or connective tissue diseases (especially systemic lupus erythematosus), or hypergammaglobulinemic states may have "false" positive results. These persons often also have blood tests positive for other factors frequently associated with autoimmune disease such as antinuclear, antithyroid, or antimitochondrial antibodies; rheumatoid factor; and cryoglobulins. A negative specific treponemal test will confirm that the test is a false positive and that syphilis can be excluded (Tramont, 2005).

2.3.8.2.2. Fluorescent Treponemal Antibody-Absorption (FTA-abs)

The FTA-abs uses *T. pallidum* harvested from rabbit testes as the antigen in a standard indirect immunofluorescent antibody test. The first step is to remove so called "natural" cross-reacting antibody that may have been raised against saprophytic treponemes of the oral cavity or genital tract by absorbing the patient's serum with nonpathogenic treponemal antigen (referred to as "sorbent"). The next step is to place the patient's "absorbed" serum on a slide which contains that has fixed *T. pallidum* as the antigen. If specific antibody to *T. pallidum* is in the patient's serum, then it is detected when fluorescein-labeled antihuman gamma globulin is added to the slide and examined under a fluorescence microscope. Its interpretation can be quite subjective.

This test is used to confirm or refute a positive nontreponemal test. If there is a high index of suspicion, it also is used to make a diagnosis of syphilis even when a nontreponemal test is negative (Tramont, 2005).

2.3.8.2.3.*T.pallidum* Haemagglutination Assay (TPHA) and Microhemagglutination Assay for Antibodies to *T. pallidum* (MHATP)

The TPHA also measures specific treponemal antibody. It is easier to perform than the FTA-abs and is as specific but not as sensitive, especially in early disease. The MHATP test is similar to the TPHA test except it uses a microtiter plate. "Sorbent" is always used to increase its specificity (Joyanes *et al.*, 1998).

2.3.8.2.4.Enzyme-linked Immunosorbent Assay (ELISA)

The syphilis ELISAs are automated tests that use a technique called a qualitative sandwich immunoassay to detect *T. pallidum* specific antibodies. An ELISA may detect only IgG or IgM but most assays are polyvalent. As with all ELISAs, an enzyme is conjugated with anti-human antibodies and only those wells that contain *T. pallidum* specific antibodies conjugated to the enzyme will exhibit a color change. The sensitivity and specificity of ELISAs are similar to TPHAs and FTA-Abs (CDC, 2013). When compared with the sensitivities of the RPR test and the MHA-TP, ELISA is more sensitive in all stages of syphilis except in secondary syphilis when all tests show 100% sensitivity (CDC, 2013).

2.3.8.2.5.Immunochromatographic Membrane Test (ICT)

This is a rapid test that detects *T. pallidum* antibodies employing anti-human immunoglobulins gold conjugate and highly purified TP recombinant proteins bound to a membrane. The sensitivity and specificity are both greater than 98% .It has an advantageous over an ELISA in that it is visually read and can be performed at the point of care. With minimum training, health care professionals and other staff can give patients test results in 10-15 minutes at a physician's office, emergency room, or clinic. In 2104, the United State Food and Drug Administration (FDA) granted a Clinical Laboratory Improvement Amendments (CLIA) waiver so that one such test, the Syphilis Health Check, can be done at the point of care (U.S. Food and Drug Administration, 2014) Other point-of-care tests will likely be approved.

2.3.8.2.6.Immunochromatographic Strip (ICS)

The ICS test is "lateral flow" test in which antibodies in a specimen are detected by becoming bound to antigens, marked with dye, on a cellulose strip. Sensitivity and specificity are both 85% to 98% (Herring *et al.*,2006). Like ICTs, it requires no special training to read, laboratory equipment to run, or refrigeration of reagents or samples. Results can be given in less than 30 minutes at the point of care.

2.3.8.2.7.Line immunoassay (LIA)

The LIA uses recombinant and synthetic polypeptide antigens derived from *T. pallidum* proteins to determine if a clinical specimen has treponemal antibodies (Hagedorn *et al.*, 2002). In a recent study, the sensitivity and specificity of LIA were 100 % and 99 %, respectively. Like ICTs and the ICS test, it is inexpensive, rapid, and requires no special laboratory equipment or highly trained personnel so it is well suited for use in developing countries (Herring *et al.*,2006).

2.3.9.Treatment

2.3.9.1 Early infections

The first-choice treatment for uncomplicated syphilis remains a single dose of intramuscular benzathine penicillin G (CDC, 2015). Doxycycline and tetracycline are alternative choices for those allergic to penicillin; however, due to the risk of birth defects these are not recommended for pregnant women (CDC, 2015). Resistance to macrolides, rifampin, and clindamycin is often present. Ceftriaxone, a third-generation cephalosporin antibiotic, may be as effective as penicillin-based treatment. It is recommended that a treated person avoid sex until the sores are healed (CDC, 2015).

2.3.9.2 Late infections

For neurosyphilis, due to the poor penetration of penicillin G into the central nervous system, those affected are recommended to be given large doses of intravenous penicillin for a minimum of 10 days (Kent and Romanelli, 2008; Stamm, 2010). If a person is allergic, ceftriaxone may be used or penicillin desensitization attempted. Other late presentations may be treated with once-weekly intramuscular penicillin G for three weeks. If allergic, as in the case of early disease, doxycycline or tetracycline may be

used, albeit for a longer duration. Treatment at this stage limits further progression, but has only slight effect on damage which has already occurred (Kent and Romanelli, 2008).

Jarisch-Herxheimer reaction

One of the potential side effects of treatment is the Jarisch-Herxheimer reaction. It frequently starts within one hour and lasts for 24 hours, with symptoms of fever, muscles pains, headache, and a fast heart rate (Kent and Romanelli, 2008). It is caused by cytokines released by the immune system in response to lipoproteins released from rupturing syphilis bacteria (Radolf and Lukehart, 2006).

2.3.10. Prevention

Obviously, the most effective prophylaxis against contracting syphilis is avoidance of sexual contact with persons who harbor the spirochete. Condoms use during sexual intercourse can also be inferred to be protective from studies of HIV infection. Currently, prophylactic drugs are not recommendations in any group of patients with syphilis except those who have been exposed to a person with syphilis and in this population, it is unclear whether medicines prevent infection or treat very early disease, e.g. "incubating" syphilis (Tramont, 2005).

We recommend that persons sexually exposed to primary, secondary, or latent syphilis of less than 1- year's duration within the previous 90 days be treated for syphilis even if they have negative serological tests for syphilis because there is data to suggest that therapy is most successful when initiated early and the treponemal burden is relatively low (Hardy *et al.*, 1970). Treatment with 2.4 million units of benzathine penicillin is recommended.

Transmission occurs only when mucocutaneous lesions are present, which is usually within one year after initial infection (Tramont, 2005), so contact with persons with syphilis greater than one year in duration is not an immediate indication for treatment (CDC, 2010). Instead, it is recommended that these persons be followed clinically and serologically for syphilis. Persons with exposure greater than 90 days prior to evaluation need not have immediate treatment, unless they have positive serological test, serological results will be delayed or follow up is uncertain. Blood transmission is rare today because of the low incidence of disease and blood storage procedures.

3.Materials and Methods

3.1Study Design

This was prospective and descriptive cross- sectional study.

3.2 Study Area and duration

This study was carried out in Khartoum state. Blood samples were collected from donors attending Khartoum Teaching Hospital and Ibn Seena Hospital -blood bank from February to June 2015 .

3.3Study Population

Blood donors who attending Khartoum Teaching Hospital and Ibn Seena Hospital -blood bank.

3.4 Inclusion Criteria

Physically fit people (all males) aged 18-60 years.

3.5Exclusion Criteria

Potential donors were excluded if they were below 18 years old, weighed less than 50 kg, had anemia and a history of jaundice within the past six months, engaged in high-risk behavior and donated blood within the past three months.

3.6 Sample size and sampling technique

One hundred thirty six blood samples having HBV(n=136) were collected as randomized sampling from blood donors.

3.7 Data Collection

Data were collected by direct interviewing questionnaire (Appendix 1).

3.8 Ethical Consideration

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

3.9 Methods

3.9.1. Specimens Collection

Under sterile condition , 3ml of venous blood were collected in sterile plain containers and allowed to clot at room temperature. The sera were obtained by centrifugation of the blood at 3000 rpm for 5 minutes. Then the sera were separated from the clot and transferred into new sterile labeled plain containers and stored at -20°C until used.

3.9.2 Processing of Specimens

Hepatitis B surface antigen and anti-treponemal antibodies were detected by RPR and 4th generation ELISA.

3.9.3 ELISA for detection of anti- treponemal antibodies in HBsAg positive donors

Biorex (Biorex Diagnostics Limited ,U K) is enzyme linked immunosorbent assay for qualitative detection of anti-Treponema antibodies in human serum or plasma . It was intended for screening of blood donors and as aid for diagnosis and management of clinical conditions of syphilis .

3.9.3.1 Principle

The detection of IgG anti-TP antibodies is achieved by antigen sandwich enzyme linked immunosorbent assay, serum antibodies combine with purified and inactivated antigen coated on the polystyrene surface of the microwell test strip (assay plate). Residual serum removed from the assay plate by washing and HRP- conjugated anti-human IgG added. After incubation, the microwells are washed and a colorless substrate system, tetramethylbenzidine \hydrogen peroxide(TMB\H₂O₂) added. The substrate is hydrolysed by the HRP, if present, and the chromogen changes to a blue color. After stopping the reaction with acid (H₂SO₄) 2M, the TMB become yellow. Color development is indicative of the presence of anti- treponemal antibodies in the test sample.

3.9.3.2 Assay Procedure

3.9.3.2.1 Reagent Preparation

Reagents and samples were allowed to reach the room temperature for at least 15-30 minutes.

3.9.3.2.2 Numbering wells

Needed strips were set in the strips holder (Appendix 4) and sufficient number of wells was numbered including 3 for negative controls (Appendix 4) , 2 for positives controls (Appendix 4) and one for blank and other wells for specimens.

3.9.3.2.3 Adding Horseradish Peroxidase (HRP) Conjugates

About 100 µl HRP- conjugates for anti-treponemal antibodies were added into each well except the blank well.

3.9.3.2.4 Adding Sample

Twenty µl of positive controls , negative controls and specimens were added into their respective wells.

3.9.3.2.5 Incubation

Plates were covered with plate cover(Appendix 4) and incubated for 60 minutes at 37 °C in dry incubator.

3.9.3.2.6 Washing

At the end of incubation , the plate's covers were removed and the contents were discarded, each well was washed 5 times with diluted wash buffer (Tween 20) (Appendix 4). After final washing cycle , the plate was turned down onto blotting paper and taped gently to remove any remaining liquid.

3.9.3.2.7 Coloring

Then 50 µl of chromogen A (Appendix 4) and 50 µl of chromogen B (Appendix 4) were dispensed into each well including the blank . The strips were covered with the plate cover and incubated for 15 minutes at 37 °C in dark incubator . The enzymatic reaction between chromogenic solution and HRP-conjugate produced a blue color in positive Controls and anti-TP positive sample wells .

3.9.3.2.8 Stopping Reaction

Plate cover was removed and 50 µl stop solution (2M Sulphuric acid) (Appendix 4) was added into each well , intensive yellow color develops in positive controls and positive sample wells.

3.9.3.2.9 Measuring the Absorbance

The plate reader was calibrated with the blank well and the absorbance was read at 450nm .Cut off value was calculated and the results were evaluated.

3.9.3.2.10 Interpretation of results and quality control

The results were interpreted according to the manufacture instructions . Each micro plate has been considered separately when calculating and interpreting results of the assay , regardless of the number of plates concurrently processed.

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

3.9.3.2.11 Calculation of the cut-off value

$C.O = *Nc + 0.18$.

*Nc = the mean absorbance value for the three negative controls

If one of the negative control values does not meet the quality control range specification, it should be discarded and the mean value calculated again using the remaining two values . If more than one negative control does not meet the range specification, the test is invalid and must be repeated .

3.9.3.2.12 Quality Control Range

The test results were considered valid and the quality control criteria are verified as follows:

- The OD value of the blank well was less than 0.080 at 450 nm.
- The OD value of the positive controls was equal to or greater than 0.80 at 450/630nm or at 450 nm after blanking .
- The OD value of the negative controls was less than 0.100 at 450/630 nm or at 450 nm after blanking .

3.9.4 RPR for detection of anti- treponemal antibodies in HBsAg positive donors

3.9.4.1 Principle

The rapid plasma regain or RPR card test is non-treponemal method for the serological detection of syphilis. The antigen- a particulate carbon suspension coated with lipid complexes – agglutinate in the presence of serum regain. Regains are antibodies present in the sera of syphilitic patients. Visible agglutination in the form of black clumps which can be viewed macroscopically, indicate the presence of such antibodies in sample tested.

3.9.4.2 Assay procedure

3.9.4.2.1 Reagent preparation

Reagents and samples were allowed to reach the room temperature.

3.9.4.2.2 Adding samples

About 50µl of the sample and 1 drop of the control was added into separate circle on the card, then antigen resuspended gently.

3.9.4.2.3 Adding antigen

About 1 drop of free falling antigen was added to each test circle, then mixed with disposable pipette/stirrer and spread over the entire area enclosed by ring. After that cards rotated at 100r.p.m. for 8minutes.

3.9.4.2.4 Reading and interpretation

The results were read macroscopically for the presence or absence of clumps within a minute of removing the card from rotator and interpreted as reactive, weakly reactive or non reactive.

3.9.4.2.5 Quality control

Each run of tests should be validated with positive and negative control.

3.10 Data Analysis

Collected data were analyzed by a computer system using Statistical Package for Social Science (SPSS) program ,version 18.

4. Results

4. Results:

One hundred thirty six samples (100%) were found positive for anti-treponemal antibodies by RPR and 6 (4.4%) were found to be positive by ELISA.

The distribution of population (volunteers) according to age and marital status are shown in tables 1 and 2, and figures 1 and 2.

Table 1 and figure 1 showed that the highest frequency of population range between 26-33 years.

Table 1: Distribution of population according to the age group (n=136)

| Age(years) | Frequency | Percentage |
|------------|-----------|------------|
| 18-25 | 35 | 25.7% |
| 26-33 | 57 | 41.9% |
| 34-41 | 34 | 25.1% |
| 42-49 | 9 | 6.6% |
| Above 50 | 1 | 0.7% |
| Total | 136 | 100.0% |

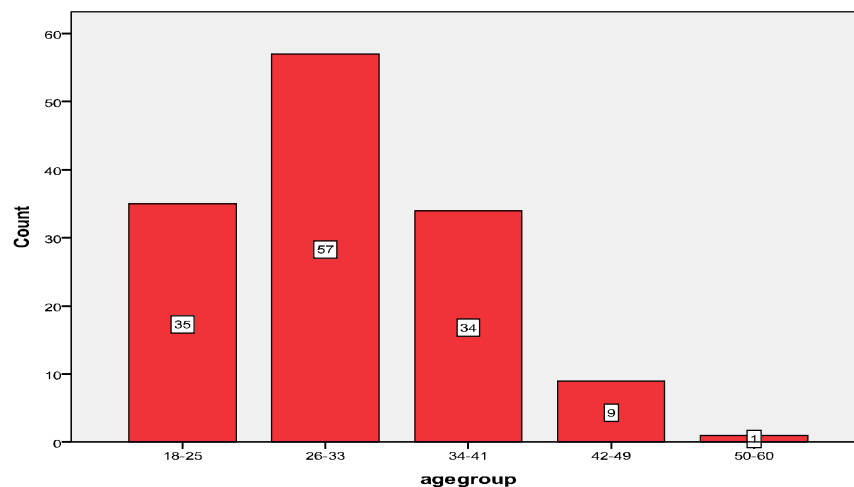


Fig1: Distribution of population according to the age group (year)

Table2 and figure 2 showed that the highest frequency of population were unmarried.

Table2:Distribution of population according to marital status(n=136)

| Marital status | Frequency | Percentage |
|----------------|-----------|------------|
| Married | 58 | 42.6% |
| Unmarried | 78 | 57.4% |
| Total | 136 | 100% |

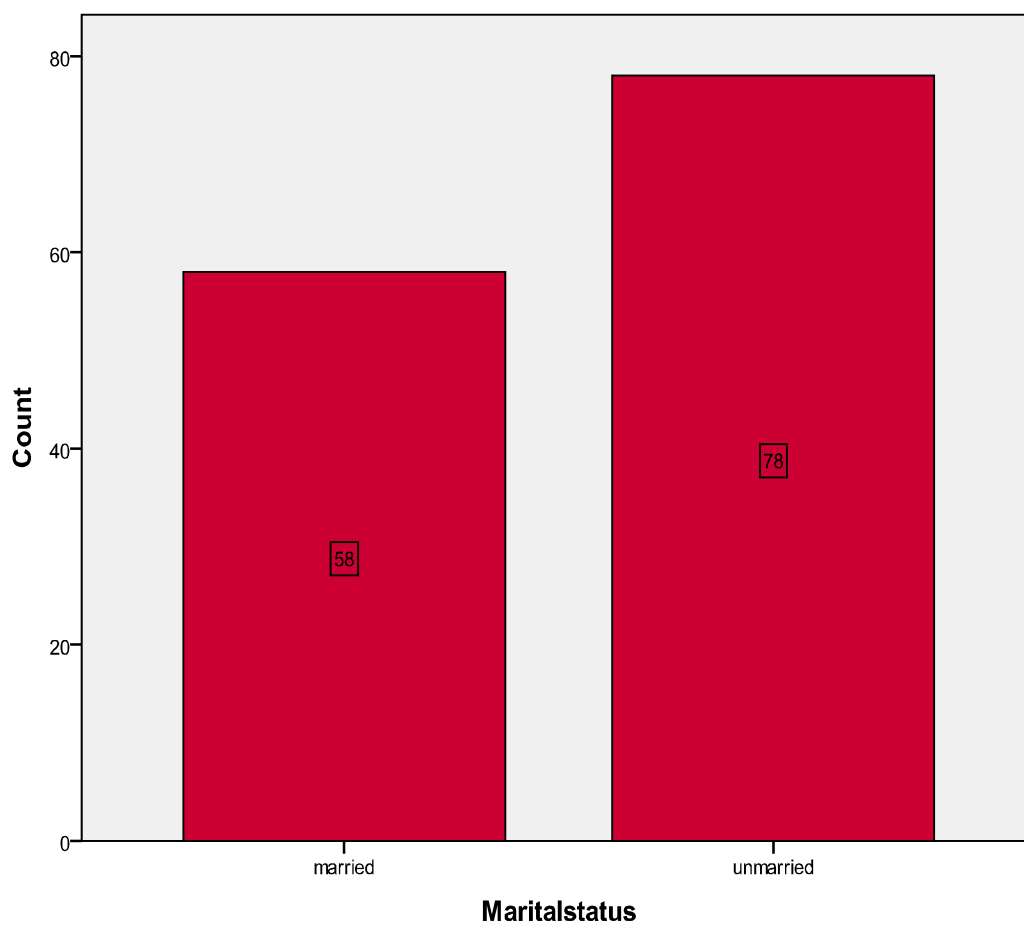


Fig 2: Distribution of population according to the marital status

The results revealed that 136 (100%) and 6 (4.4%) of the donors were positive for syphilis by RPR and ELISA respectively as shown in table 3, 4 and figure 3,4.

Table3 showed that the highest frequency of syphilis positive cases were found in age group (34-41) year .

Table3:Syphilis positive cases detected by ELISA according to age group (n=136)

| Age(year) | Frequency | Percentage |
|-----------|-----------|------------|
| 18-25 | 1 | 0.7% |
| 26-33 | 1 | 0.7% |
| 34-41 | 3 | 2.3% |
| 42-49 | 1 | 0.7% |
| Above 50 | 0 | 0% |
| Total | 6 | 4.4% |

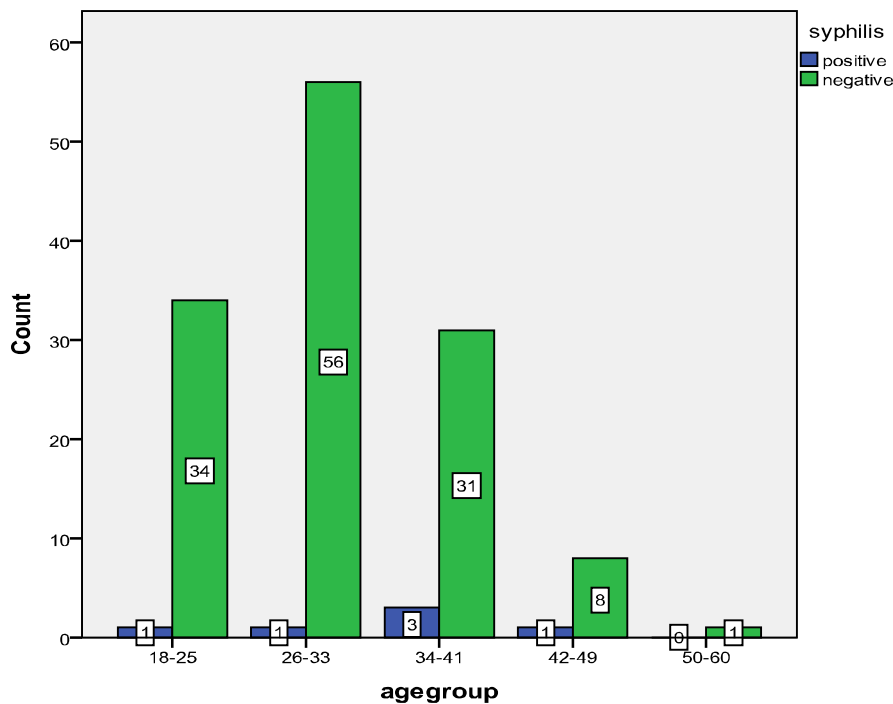


Fig3: Syphilis positive and negative cases according to age group

Table4:Syphilis positive cases detected by RPR according to age group (n=136)

| Age(year) | Frequency | Percentage |
|-----------|-----------|------------|
| 18-25 | 35 | 25.7% |
| 26-33 | 57 | 41.9% |
| 34-41 | 34 | 25.1% |
| 42-49 | 9 | 6.6% |
| Above 50 | 1 | 0.7% |
| Total | 136 | 100.0% |

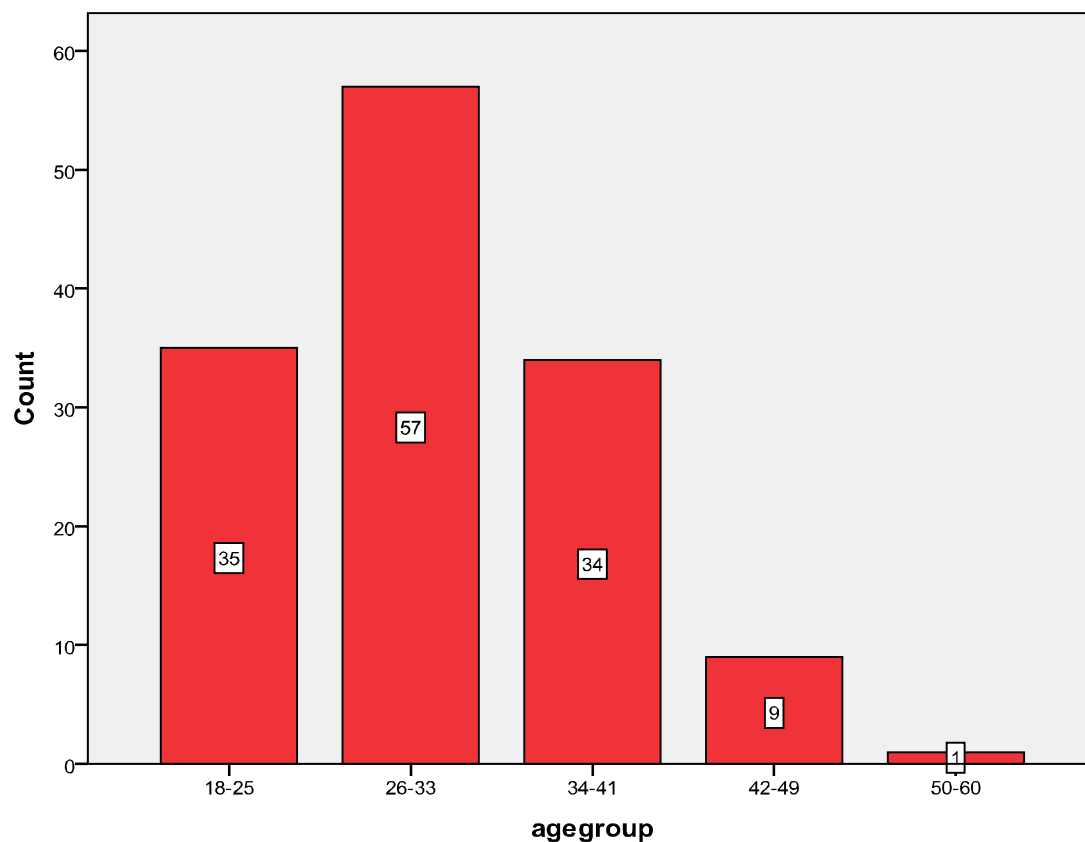


Fig4: Syphilis positive and negative cases by RPR according to age group

Table5 showed that the highest frequency of syphilis positive cases were among married donors.

Table5:Syphilis positive cases detected by ELISA according to marital status

| Marital status | Frequency | Percent% |
|----------------|-----------|----------|
| Married | 4 | 2.9% |
| Unmarried | 2 | 1.5% |
| Total | 6 | 4.4% |

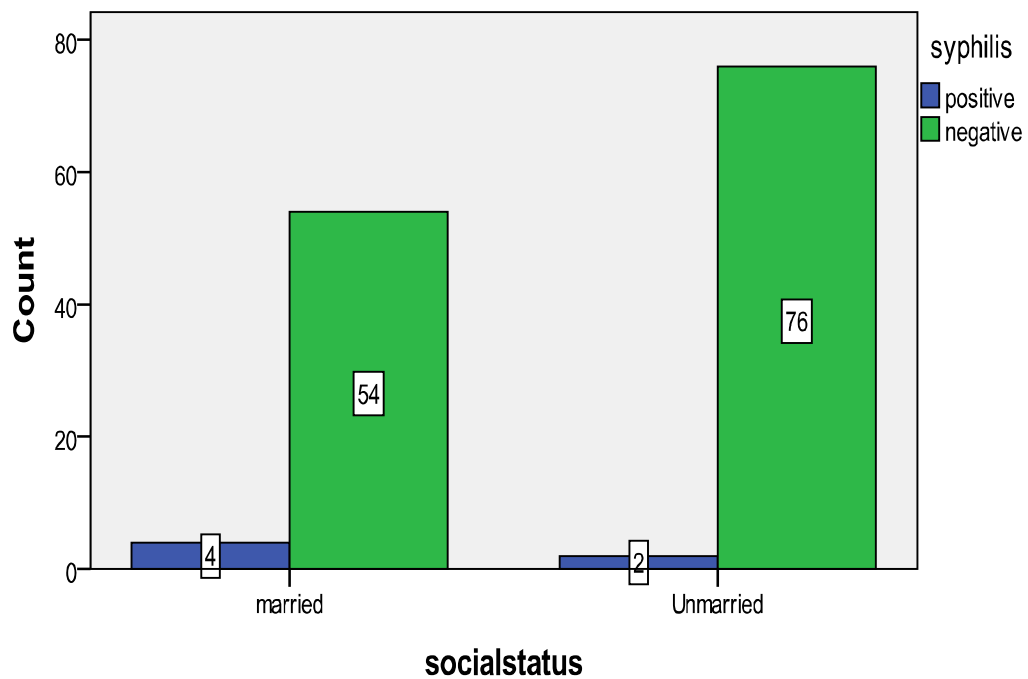


Fig5: Syphilis positive and negative cases according to marital status

Table6: Syphilis positive cases detected by RPR according to marital status

| Marital status | Frequency | Percentage |
|----------------|-----------|------------|
| Married | 58 | 42.6% |
| Unmarried | 78 | 57.4% |
| Total | 136 | 100% |

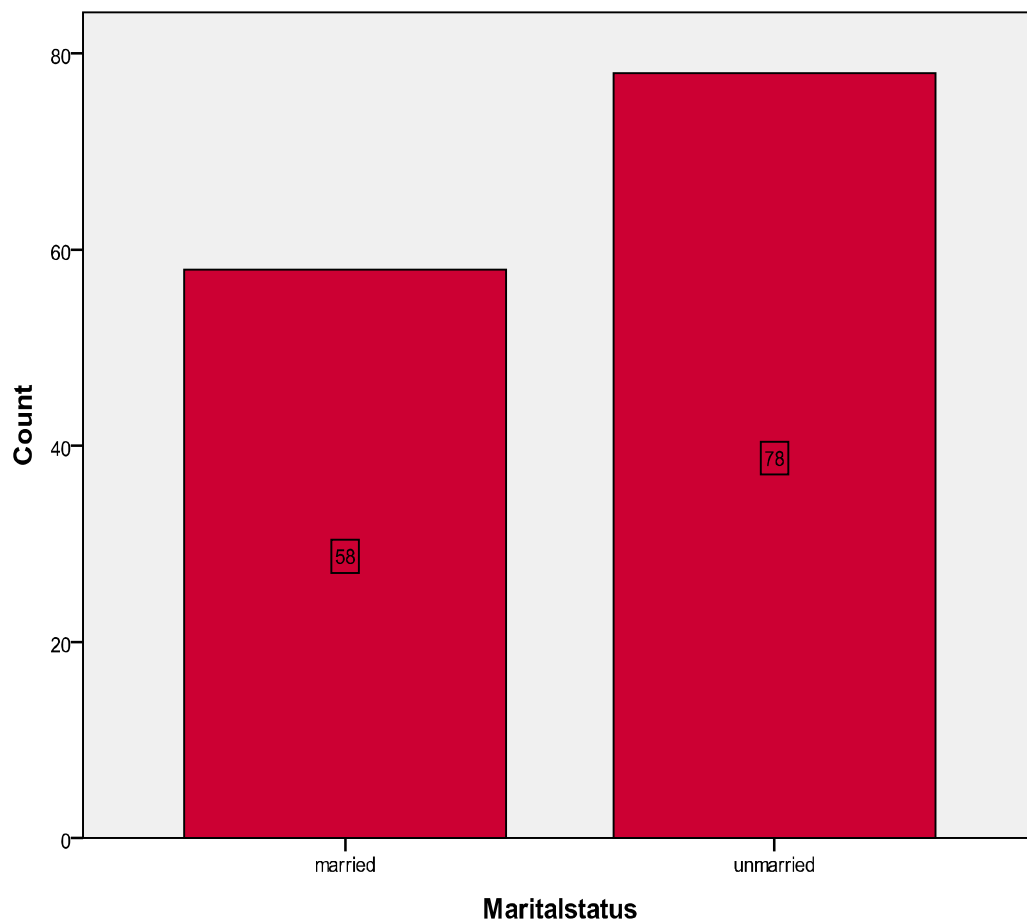


Fig6: Syphilis positive and negative cases according to marital status

5. Discussion

5.1 Discussion

HBV infection is considered as a major infectious disease due to the broad range of clinical spectrum and the progressive complications displayed by the infected individuals. Syphilis continues to be a major problem in the tropics causing genital ulcers and systemic manifestations.

This study investigated the seroprevalence of syphilis among HBsAg positive blood donors in Khartoum state by Rapid plasma regain (RPR) and Enzyme Linked Immunosorbent Assay (ELISA). 136 hepatitis B positive sample were collected from donors with different ages (18-60), all of them were male and have no history of previous blood transfusion.

The prevalence of syphilis - HBsAg co-infection among donors in this study was (4.4%) this results relatively, high in comparison to the result reported for seroprevalence of human immunodeficiency virus, hepatitis B and C viruses and syphilis among blood donors in Koudougou (Burkina Faso) in 2009 (0.66%) (Nagalo, 2011). This result is also high in comparison with result for seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors at Gondar University Teaching Hospital, Northwest Ethiopia (0.11%) (Tessema *et al.*, 2010). These variations in results were possible due to large sample size used by previous investigators 4,520 donors used by Nagalo *et al.*, 2011 and 1,240 used by Tessema *et al.*, 2010. Furthermore these considerable differences could be complex multifactorial due to possible ethnic background, socioeconomic status, geographical location and health care service provided. This difference could not be explained, but it is possible that it is due to geographical differences.

This results also high in comparison to result reported in In Tanzania which found that the prevalence of HBV-syphilis co-infection was (2.2%) (Mecky *et al.*, 2006).

With regard to the co-infections, there was an association between syphilis and HBV. This association could be due to the fact that these infections share similar modes of transmission (predominantly blood and high-risk sexual behaviors). Screening the high-risk population for this viral infection would aid early detection of co-infections and hence early treatment, which, if initiated, would help to decrease the further spread of these blood-borne infections.

5.2 Conclusion:

Seroprevalence of syphilis among HBsAg positive blood donors ((4.4%) is alarming and indicates that blood safety remains an issue of major concern.

High prevalence of syphilis among age group (34-41)year 3 (2.3%),other age groups (18-25), (26-33) and (42-49) 1 positive cases in each group(0.7%).

Also prevalence of syphilis high among married 4 (2.9%) in comparison with unmarried 2(1.5%).

Analysis of our results revealed that history of blood transfusion not influenced on the rate of syphilis positivity among the donors.

5.3 Recommendation:

- 1- Regular education programs for school students about the danger of this disease, transmission and prevention.
- 2- Formulation of safe blood transfusion policies and implementation of standard laboratory screening procedure at all level is strongly recommended.
- 3- Further studies aimed at determining the epidemiology of transfusion-transmissible infections among the general population will be of value in determining the population prevalence.
- 4-Employing recent confirmatory immunological technique like western blot, southern blot and polymerase chain reaction(PCR).
- 5- A strict selection criterion of donors, with emphasis on getting voluntary donors is also highly recommended.

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Appendix 1
Sudan university of science and technology
College of graduate studies
Questionnaire

Index.....

Name.....

Age..... Sex.....

Marital status:

Married..... Unmarried.....

Did you have history of previous blood transfusion:

Yes..... No.....

Did you have any symptom or complain:

Yes..... No.....

If yes specify.....

Sample collected :

Blood..... other.....

Result of HBsAg:

Positive..... Negative.....

Surgical operation:

Yes No.....

If yes how many :.....

Appendix 2

1.1 Equipment's:

| | |
|-----------------------------------|-----------------------|
| Centrifuge | Hettich-Germany |
| Automatic micropipette | Axiom-Germany |
| Alarm clock | Quartz-Japan |
| Micro well reader(450nm+/-2nm) | Stat-fax-USA |
| Micro well washer system | Stat-fax-USA |
| Automatic pipette (multi channel) | Axiom-Germany |
| Dry incubator(37+/-2c) | Storr picenardi-italy |

Appendix 3:

2.1 Reagent:

- ✓ Deionized or distilled water
- ✓ Alcohol
- ✓ ELISA kit
- ✓ Concentrated washing solution
- ✓ Negative control(human)yellow
- ✓ Positive control(human)red
- ✓ Conjugate ready to use blue
- ✓ Substrate pink
- ✓ Stop solution H₂ So₄-0.5M

Appendix 3:

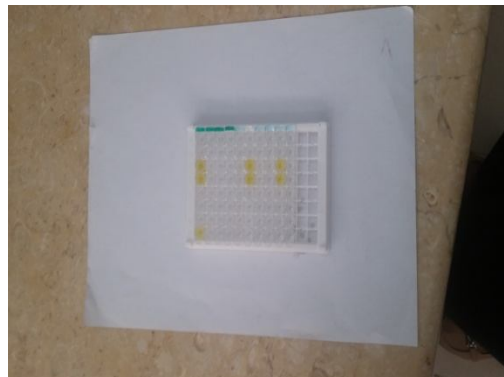
3.1 Other materials:

- ✓ Cotton
- ✓ Disposable pipette tips
- ✓ Vacuum tubes
- ✓ Syringes
- ✓ Alcohol swap
- ✓ Microplates :12strip of 8wells coated with T. Pallidum rAg

- ✓ Automatic microtiter plate washer or squirt bottle
- ✓ Bag for storing unused wells
- ✓ Test tube and racks
- ✓ Plane container



ELISA reader



Microwells plate of ELISA show positive (yellow) and negative (colorless) results.