TRANSFUSION TRANSMISSIBLE INFECTIONS AMONG BLOOD DONORS IN KATHMANDU, NEPAL

A

Dissertation

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This is to certify that Mr. Ashish Chandra Shrestha has completed this dissertation work entitled "TRANSFUSION TRANSMISSIBLE INFECTIONS AMONG BLOOD DONORS IN KATHMANDU, NEPAL" as a partial fulfilment of M.Sc. Degree in Microbiology under our supervision. To the best of our knowledge, this is an original research work of his and has not been submitted for any other degree.

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ABSTRACT

Screening of transfusion transmissible infections among blood donors can be a cost-effective approach not only to screen the blood before transfusion to avoid transfusion transmitted infections, but also to monitor the prevalence, distribution, and trends of the infections among healthy looking individuals. A cross-sectional research based at Nepal Red Cross Society, Central Blood Transfusion Service, Kathmandu, was carried out during March - September 2008 to investigate transfusion transmissible infections in blood donors. A total of 21,716 units of blood were tested for the presence of anti-HIV 1 and 2 IgG/IgM, HBsAg, anti-HCV IgG/IgM, anti-*Treponema pallidum* IgG/IgM/IgA using the commercial kits following standard protocols.

Seroprevalence of transfusion transmissible infections was observed to be 1.68% (HIV- 0.12%, HBV- 0.47%, HCV- 0.64%, Syphilis- 0.48%) with a male dominance of 1.76% compared to females with 1.18% ($P < 0.05$). Highest seroprevalence of TTIs (5.1%) was observed in the age group 51 to 60 years; with Syphilis (4.08%) as the most common infection. Higher prevalence of HIV (0.17%) in age groups 31-40 years, HBV (0.78%) in age groups 41 to 50 years, HCV (0.76%) in age groups 21-30 years was observed. Prevalence of TTIs was slightly higher (1.74%) among the repeated donors compared to the first time donors (1.61%) ($P > 0.05$). Co-prevalence of HIV and HCV was 0.02%, with statistically significant association. Co-prevalence of HCV and Syphilis was 0.009%, HIV and HBV co-prevalence was 0.004%, HBV and HCV co-prevalence was also 0.004%. The co-infection with HCV and HIV seropositive donors (18.51%) was higher compared to other coinfections.

Continuity of screening of donated blood with highly sensitive and specific tests and introduction of donor counselling which are positive to any of the above infections is the urgent need to avoid transmission of infection from the infected donors.

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ABBREVIATIONS

AIDS:	Acquired Immunodeficiency Virus
Anti-HCV:	Antibodies to HCV
Anti-HIV:	Antibodies to HIV
Anti-TP:	Antibodies to *Treponema Pallidum*
CBTS:	Central Blood Transfusion Service
CDC:	Centers for Disease Control and Prevention
EIA:	Enzyme Immuno Assay
ELISA:	Enzyme Linked Immunosorbent Assay
HBsAg:	Hepatitis B Surface Antigen
HBV:	Hepatitis B Virus
HCC:	Hepatocellular Carcinoma
HCV:	Hepatitis C Virus
HIV:	Human Immunodeficiency Virus
IDUs:	Injecting/Intravenous drug users
LIA:	Line Immunoassay
NCASC:	National Center for HIV/AIDS and STD Control
NRCS:	Nepal Red Cross Society
RIBA:	Recombinant Immunoblot assay
TTIs:	Transfusion Transmissible Infections
TPHA:	*Treponema pallidum* Haemagglutination Assay
UNAIDS:	Joint United Nations Programme on HIV/AIDS
VDRL:	Venereal Disease Research Laboratory
WHO:	World Health Organization

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CHAPTER – I

1. INTRODUCTION

Blood donation saves lives of millions. It's true that blood transfusion plays an important role in supportive care of medical and surgical patients. However, unsafe transfusion is also putting millions of people at risk of Transfusion Transmissible Infections (TTIs) (Diro et al., 2008). While in the past, the risk of transfusion-transmitted infections (TTIs) was accepted by patients and physicians as unavoidable, a low-risk blood supply is expected today (Bihl et al., 2007).

TTIs can exist as asymptomatic diseases in their hosts, so donors must be screened for high-risk behaviour (Barbara, 1993). Only continuous improvement and implementation of donor selection, sensitive screening tests and effective inactivation procedures can ensure the elimination, or at least reduction, of the risk of acquiring transfusion transmitted infections (Bihl et al., 2007). Advances in screening tests with better specificity and sensitivity have greatly helped reduce transfusion-associated risks.

The most common infections transmitted in blood transfusions are viral infections. Transfusion-transmitted viral infections are HIV-1 and -2, human T cell lymphotropic virus (HTLV) I and II, hepatitis C virus (HCV), hepatitis B virus (HBV), Cytomegalovirus (CMV), West Nile Virus (WNV). Other viruses which have been reported to be transmitted through transfusion include Hepatitis A virus (HAV) among haemophiliacs patient, Hepatitis E Virus in endemic areas, Hepatitis G virus, Epstein Barr Virus (EBV), Human Parvovirus B19 (HPV-B19), Human Herpesvirus 6, TT virus (TTV), SEN virus (SEN-V). Infections that can be transmitted through blood transfusion include malaria caused by *Plasmodium* species in malaria endemic areas, babesiosis caused by *Babesia microti*, Chagas disease caused by *Trypanosoma cruzi*, Toxoplasmosis caused by *Toxoplasma gondii*. Bacteria associated with TTIs are

Treponema pallidum causing Syphilis, bacterial contaminants such as normal flora of skin during blood collection (insufficient disinfection of venipuncture site), during handling of blood products (leaky seals) or from those that are likely to be derived from a donor bacteraemia. Human prion disease Variant CJD (vCJD), have been reported to be transmitted by blood transfusion (Kaur and Basu, 2005).

Donated blood is not routinely screened for all the above possible TTIs. Mandatory screening tests are carried out for HIV 1 & 2, HBV, HCV and Syphilis by blood transfusion centres as in Nepal Red Cross Society, Central Blood Transfusion Service. Other screening tests are performed depending upon the disease endemic areas and if cost effective laboratory testing is available.

Human Immunodeficiency Virus (HIV) has been of global concern since it has infected millions of people worldwide. In 1982 the first cases of AIDS obtained from blood or blood components were reported, but the aetiology of the infections was not known at that time. HIV transmission through blood transfusion include the window period (i.e. a short viraemic period in which the donor is infected with HIV at a very early stage and often tested negative in a donor screening test), HIV-antibody negative chronic carriers, HIV mutant infection, and laboratory error. The estimated risk for HIV transmission to date is between 0.14 – 1.1 per millions units transfused (Bihl et al., 2007).

Viral hepatitis is a systemic disease primarily involving the liver. The term 'viral hepatitis' refers to a primary infection of the liver by any one of a heterogeneous group of 'hepatitis viruses', which consists of types A, B, C, D and E. The only common feature of hepatitis viruses is their primary hepatotropism. Additional well-characterized viruses that can cause sporadic hepatitis are cytomegalovirus, yellow fever virus, Epstein-Barr virus, herpes simplex virus, rubella virus and the eneteroviruses (Hayces et al., 2002, Butel, 2007).

Hepatitis B has also been called type B hepatitis, serum hepatitis, homologous serum jaundice. The severe pathological consequences of persistent of HBV include development of chronic hepatic insufficiency, cirrhosis and hepatocellular carcinoma (HCC). In recent years, with the emergence of HBsAg escape mutants, the investigation of their implications for blood safety has become an important research priority (Zhang et al., 2001). HBV infection varies between 0.75 per million blood donations in Australia, 3.6 – 8.5 in the USA and Canada, 0.91 – 8.7 in Northern Europe, 7.5 – 13.9 in Southern Europe up to 200 per million donations in Hong Kong, largely reflecting the global epidemiology of HBV (Coste et al., 2005).

Hepatitis C is also called type C hepatitis, Parenterally transmitted non-A non-B hepatitis (PT-NANB), Non- B transfusion-associated hepatitis, Post transfusion non-A non-B hepatitis. HCV infections are common worldwide. It is estimated that about 3% of the world's population have HCV. Most persons (60%-80%) who have chronic hepatitis C have no symptoms. The risk of HCV transmission through blood transfusion was reduced remarkably after the implementation of blood donor serologic testing for HCV in 1990. The estimated risk for HIV transmission to date is between 0.10 – 2.33 per millions units transfused (Bihl et al., 2007).

Syphilis caused by *Treponema pallidum* can be classified into different clinical stages, including primary, secondary, early latent, late latent, and tertiary. One-third of untreated primary cases will progress to secondary syphilis. About one-third of untreated secondary cases will progress to latent infection. In general, latent syphilis is asymptomatic, and one-third of untreated latent cases will progress to tertiary syphilis. In addition, syphilis increases the risk of acquiring HIV infection (Brooks et al., 2007). Transmission of syphilis by blood transfusion has become extremely rare after implementation of the serologic test for antibodies to *T. pallidum.* It is not the transmission of syphilis that is worrisome, being a sexually transmitted disease, its presence points towards donor's indulgence in "high risk" behaviour and consequently higher risk of exposure to infections like HIV and hepatitis (Kaur and Basu, 2005).

In Nepal, as of 16 September, 2008, National Centre for AIDS and STD control (NCASC) has reported a total of 12,415 HIV Positives (including AIDS) cases of which 8,330 are male and 4,085 are females (NCASC, 2008). HIV seroprevalence among blood donors in different regions of Nepal and Kathmandu Valley has been reported to range from 0.019% to 0.41% (Chander et al., 2003, Thapa, 2004, Tiwari et al., 2008, Karki et al., 2008). HBV seroprevalence has been reported to range from 0.3% to 4.0% in general population by various studies conducted from 1990 to 2003 (Shrestha, 1990, Rai et al., 1994, Manandhar et al., 1998, Sawayama, 1999, Joshi et al., 2000, Bhatta et al., 2003, Nakashima et al., 2003). HBsAg seroprevalence among Nepalese blood donors has been reported to range from 0.45 to 1.26% (Joshi et al., 2002, Chander et al., 2003, Ghimire et al., 2006, Ghimire et al., 2007, Karki et al., 2008). HCV seroprevalence among Nepalese general population and blood donors has been reported to range from 0.3 to 1.7% (Shrestha et al., 1998, Singh, 1998, Sawayama et al., 1999, Shrestha, 2003, Shrestha, 2006, Karki et al., 2008). Very little information is available on the prevalence of Syphilis in general population, although one of the focussed study has reported it as 0.6% among Nepalese males (Joshi et al., 2007).

TTIs can pose a serious problem in transfusion medicine if timely analysis is not made on the prevalence of various possible infections. In our country, attempts have been made to study prevalence and coprevalence of HIV, HBV, and HCV in combination of only two of these infections. However, it seems to be necessary to know the prevalence of all these infections at the same time. The study is hence carried out to understand the current scenario of most prevalent infection and the likely coinfections that is seen. Syphilis being a sexually transmitted disease can serve the marker for HIV. Hence, study of Syphilis coinfections with the viral TTIs can be useful to know the possible correlations. Overall TTIs prevalence can reveal the problem of concerned infections in healthy looking part of general population.

Prevalence of any infection does vary with time and place of study. So, it is necessary to monitor the prevalence in given area in course of time to understand the current

scenario. The findings of present study not only update on the epidemic situation of the infections, but also give a clear picture of trends of that infection over period of time. It is not reasonable to study a large population to know the figures at extra cost of investigation. However, blood banks besides meeting the requirements of blood supply are important centres for investigations of some infections that are of concern to general public.

It is necessary to understand that blood donors represent the part of general population who are considered to be healthy looking and are ignorant of their infections. The infected individuals from such population are the potential source of infections in community. Study of TTIs among blood donors at the centre can be a cost-effective approach to monitor the prevalence, distribution, and trends of these infections among such healthy looking individuals. Similarly, the study on seroprevalence and coinfection rates of TTIs among the blood donors would provide data important in formulating the strategies for improving the management of safe blood supply in the country.

CHAPTER-II

2. OBJECTIVES

2.1. General Objective

To determine the seroprevalence of Transfusion Transmissible Infections (TTIs): (HIV, HBV, HCV and Syphilis) among blood donors in Kathmandu, Nepal.

2.2. Specific Objectives

- To estimate the seroprevalence of HIV, HBV, HCV and Syphilis in blood donors.
- To estimate the co-prevalence of HIV, HBV, HCV and Syphilis in blood donors.

CHAPTER-III

3. LITERATURE REVIEW

3.1. Transfusion Transmissible Infections

Blood transfusion, like other medical procedures, carries some risk of adverse events among recipients. These include both transmission of infectious diseases and non infectious adverse events. The latter may occur as a result of errors in handling or administering blood and blood products. Currently, as a result of donor screening and new test procedures, the risk of transmission of known infectious agents is extremely low; however, because blood is a biological product, unknown and emerging pathogens will always pose a threat to its safety (Public Health Agency of Canada, 2003).

Although the risk of transfusion-transmitted infections today is lower than ever, the supply of safe blood products remains subject to contamination with known and yet to be identified human pathogens. Only continuous improvement and implementation of donor selection, sensitive screening tests and effective inactivation procedures can ensure the elimination, or at least reduction, of the risk of acquiring transfusion transmitted infections. In addition, ongoing education and up-to-date information regarding infectious agents that are potentially transmitted via blood components is necessary to promote the reporting of adverse events, an important component of transfusion transmitted disease surveillance. Thus, the collaboration of all parties involved in transfusion medicine, including national haemovigilance systems, is crucial for protecting a secure blood product supply from known and emerging blood-borne pathogens (Bihl et al., 2007).

The first step in reducing the risk of transmission of infectious diseases through blood is to select voluntary non-remunerated donors from low-risk populations who give blood

on a regular basis as these individuals are at a lower risk of transmitting transfusion-transmissible infections than are family/replacement donors, or paid donors. However, even with the most careful selection, some donors may be seropositive for HIV or other infectious agents. Therefore, rigorous screening of all donated blood is required to ensure the safety of the blood supply. The development and implementation of a national strategy for the screening of all donated blood for transfusion-transmissible infections, using the most appropriate and effective assays to test for HIV, hepatitis viruses, syphilis and other infectious agents is required to be implemented to ensure safety of the blood supply (WHO, 2008).

Infectious agents, including viruses, bacteria, and parasites, can be transmitted by human blood products. Of major importance are viruses such as human immunodeficiency virus types 1 and 2 (HIV-1/2), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell lymphotropic virus types I and II (HTLV-I/II). Also, other viruses such as cytomegalovirus, Epstein-Barr virus, human parvovirus B19, and hepatitis A and G viruses can be transmitted by blood products. Various methods are used to prevent transmission of blood-borne agents to recipients, such as donor selection, testing donated blood for various infectious agents, and viral inactivation of plasma derivatives (Vrielink and Reesink, 1998).

A spectrum of blood-borne infectious agents is transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors. Several strategies are implemented to reduce the risk of transmitting these infectious agents by donor exclusion for clinical history of risk factors, screening for the serological markers of infections, and nucleic acid testing (NAT) by viral gene amplification for direct and sensitive detection of the known infectious agents (Allain et al., 2009).

3.2. Human Immunodeficiency Virus

Human Immunodeficiency Virus (HIV) and its subtypes are retroviruses (members of the *Lentivirus* genus) and they are the etiological agents of Acquired Immunodeficiency Syndrome. Retroviruses infect a wide range of animal species and cause a variety of diseases including: tumours, wasting and auto-immune diseases, immunodeficiency syndromes and aplastic and haemolytic anaemias. The result of HIV infection is relentless destruction of the immune system leading to onset of the acquired immunodeficiency syndrome (AIDS). All HIV-infected persons are at risk for illness and death from opportunistic infectious and neoplastic complications as a consequence of the inevitable manifestations of AIDS (Moss et al., 1989).

3.2.1. Properties of HIV

HIV is roughly spherical enveloped virus, about 90-120 nm in size. Viral membrane is composed of two layers of fatty molecules called phospholipids derived from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are glycoprotein (gp) 120, and a stem consisting of gp41 molecules that anchor into the viral envelope. The virion has an outer icosahedral shell and an inner cone shaped core, enclosing the ribonucleoproteins (Simmonds, 2006, Butel, 2007). The genome is diploid, composed of two identical single stranded, positive sense RNA copies (Simmonds and Peutherer, 2006, Butel, 2007).

3.2.2. Epidemiology

3.2.2.1. Global Scenario

In 2007, between 30.6 and 36.1 million people were believed to live with HIV, and it killed an estimated 2.1 million people that year, including 330,000 children; there were 2.5 million new infections. (UNAIDS & WHO, 2007). Two thirds of the global burden

of disease occurs in sub-Saharan Africa, and approximately 25% of the global epidemic lies in Asia, particularly India, and, to a lesser extent, China (Tapper, 2007). UNAIDS and the WHO estimates, AIDS has killed more than 25 million people since it was first recognized in 1981, making it one of the most destructive pandemics in recorded history.

The epidemic varies by geographic region. The highest number of HIV infections is in sub-Saharan Africa with an estimated 21.6 to 27.4 million people currently living with HIV. Two million [1.5–3.0 million] of them are children younger than 15 years of age. More than 64% of all people living with HIV are in sub-Saharan Africa. South & South East Asia ranks second with 15% of the total HIV infected individuals. South Africa has the largest number of HIV patients in the world followed by Nigeria. India has an estimated 2.5 million infections (0.23% of population), making India the country with the third largest population of HIV patients. Many different cultural, social and behavioural aspects determine the regional characteristics of HIV disease. In the USA and the northern Europe, the epidemic has predominantly been in homosexual men, whereas in southern and Eastern Europe, Vietnam, Malaysia, North-east India and China the incidence has been greatest in injection drug users. In Africa, South Africa, South America and much of South-east Asia the dominant route of transmission is heterosexual and from mother to child (vertical). The economic and demographic impact of HIV infection in developing countries is profound as it affects the most economically productive and fertile ages (Todd et al., 2002).

3.2.2.2. HIV Prevalence in Nepal

Nepal reported its first cases of AIDS in July 1988 (Gurubacharya et al., 1994). Following this, a trend of gradual increase in the reported number of HIV infection is observed (Suvedi, 2006). As of 16 September, 2008, National Centre for AIDS and STD control (NCASC) has reported a total number of HIV Positives (including AIDS) to be 12,415 cases of which 8,330 are male and 4,085 are females. Cumulative death

among 2,037 AIDS cases (out of total HIV) is 505. HIV prevalence is highest among clients of sex workers followed by housewives and the overall prevalence was also higher among age groups 30-39 years (NCASC, 2008). The transition of HIV from high-risk behaviour group such as female sex workers to low risk behaviour group has been evident now (Suvedi, 2006). World Bank figures indicate that one-third of HIV infections nationwide are among Injecting Drug Users. At least 10 per cent of the 2 to 3 million Nepali migrant are infected with the virus (UNAIDS, 2007). Though there seems to be low prevalence of HIV in the general population, the prevalence highly differs among different groups. The overall seroprevalence among blood donors in the regional blood transfusion services in Nepal was reported to be 0.054%, in Morang the prevalence was 0.019%, in Banke was 0.095% and in Kaski was 0.05% (Tiwari et al., 2008).

3.2.3. Modes of Transmission

HIV is present in blood, semen and other body fluids such as breast milk and saliva. Exposure to infected fluid leads to a risk of contracting infection, which is dependent on the integrity of the exposed site, the type and volume of body fluid, and the viral load. HIV can enter either as free virus or within cells.

Sexual Transmission

HIV is primarily a sexually transmitted infection. Sexual transmission can occur when infected sexual secretions of one partner come into contact with the genital, oral, or rectal mucous membranes of another. Factors that have been considered to increase risk of acquisition of HIV by this mode are sexually transmitted infections (especially genital ulcers), cervical ectopy, non-circumcised, menstruation, sexual practice (receptive vs insertive anal sex, rectal or vaginal trauma, increased number of partners). Biological factors also seem to affect the efficiency of transmission; these factors include the level of viraemia, infectivity and virulence of a particular HIV strain, and presence of STDs such as genital ulcers (Plummer et al., 1991).

Vertical (Perinatal) Transmission

In prospective studies of infants born to HIV-infected women, transmission rates have ranged from 13% to 40%. In the absence of treatment, the transmission rate between the mother and child is around 25 percent (Coovadia, 2004). Virus has been isolated and genome demonstrated in the products of conception from as early as 8 weeks' gestation and in placental tissue infected in vivo and in vitro. Infection can occur during parturition as a result of transplacental bleeding or contact of abrasions with virus-containing fluids during passage along the birth canal. Mother to child transmission can occur via breast milk. Prospective cohort studies that compared breastfed and bottle-fed infants estimated the risk for transmission through breast milk to be 14-29%. Factors associated with increasing risk of acquisition of HIV by vertical transmission include older gestational age, lower birth weight, first born twin, vaginal vs elective caesarean delivery, prolonged rupture of membranes, chorioamnionitis, fetal trauma, no peripartum prophylaxis, longer duration breastfeeding (Folks and Khabbaz, 1998).

Transmission among Injecting Drug Users (IDUs)

Among IDUs, HIV is transmitted by parenteral exposure to HIV-infected blood through the use of contaminated needles or other injection equipment. Transmission risk after exposure for IDUs is 0.5-1.0%. Factors associated with HIV infection in IDUs include duration (years) of injection, frequency of needle sharing, number of needle-sharing partners, number of injections. In addition, unsafe sexual practices may contribute some infections among IDUs (Nelson et al., 1991).

Transmission by Blood and Blood Products

The transmission risk after exposure is > 90 % for blood or blood products. The virus is present both in white cells and in the plasma. A single infected unit included in a plasma pool (often contributed by 20,000 or more donors) has the potential to infect all recipients of the pool. Plasma products, such as immunoglobulins prepared by using one of the several fractionation processes, have not been associated with transmission of HIV. In countries where screening of blood for HIV has been instituted, the risk for

HIV transmission through screened blood has been estimated to be 1/36000 to 1/225000 per unit transfused. This residual risk is due to antibody-negative infected donors in the 'window' period prior to seroconversion. Transmission risk by tissue and organ donation such as semen, cornea, bone marrow, kidney, etc. to the recipient is 50 – 90% (Folks and Khabbaz, 1998).

Transmission in Health Care Settings

The use of unsterile syringes and needles by qualified health workers makes iatrogenic infection likely. The average risk of of seroconversion after a needlestick injury with HIV- positive blood is about 0.3% (Tokars et al., 1993). The risk after exposure of the eye, nose, or mouth to HIV- infected blood is estimated to be, on average, 0.1% (1 in 1,000). The risk after exposure of non-intact skin to HIV-infected blood is estimated to be less than 0.1% (CDC, 2003).

3.2.4. Serological Tests for HIV

3.2.4.1. Enzyme-Linked Immunosorbent Assays/Enzyme Immunoassays (ELISA)

ELISA is the most commonly used type of test to screen for HIV infection because of its relatively simple methodology, inherent high sensitivity, and suitability for testing large numbers of samples. It is the "gold standard" for testing used extensively in blood banking and patient screening in developed nations (Constantine et al., 1994).

a. Indirect ELISA

In this method, HIV antigen is attached to a well of a 96-well micro titre plate. After addition of serum suspected of containing antibodies to HIV in the well, other reagents are added which include enzyme conjugates that bind to specific HIV antibody and substrates/chromogens that produce colour in a reaction catalyzed by the bound enzyme conjugate (HIV InSite, 2006).

b. Competitive ELISA

In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen coated microtitre well. The more antigens present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well. The concentration of antigen is inversely proportional to the colour produced (Goldsby et al., 2003).

b. Double Antigen Sandwich Assay (DAGS)

It is the antigen sandwich method in which an enzyme (alkaline phosphatase/horse radish peroxidase) is conjugated to an HIV antigen. The antibody in the sample is "sandwiched" between 2 antigen molecules, 1 immobilized on the solid phase and 1 containing the enzyme. Subsequently, the addition of substrate results in colour development in proportion to antibody concentration. The antigen sandwich ELISA is considered the most sensitive screening method, given its ability to detect all isotypes of antibody, including IgM (Constantine et al., 1994).

c. Fourth-Generation Assays

Fourth generation screening tests detect both antibodies and antigen (p24 antigen). The average gain in time to detection compared with third generation kits is 3 to 5 days (Gurtler et al., 1998, Ly et al., 2001, Schupbach, 2003).

3.2.4.2. Rapid Tests

Rapid assays for detecting specific HIV antibody were developed in the late 1980s, and are defined as tests that can yield results in <30 minutes. Technical errors are common with these assays, however, because users become careless with these simple procedures (HIV InSite, 2006).

a. Dot Blot" or "Immunoblot"

They produce a well-circumscribed coloured dot on the solid phase surface if the test is positive. These rapid assays incorporate a built-in control to indicate that the test was performed correctly. This control is an antihuman immunoglobulin that binds any immunoglobulin in the sample and produces a separate indicator when all reagents are added. Most require drop-wise additions of reagents in the following sequence: buffer, sample, wash buffer, conjugate, wash buffer, substrate, and stop solution. Some assays substitute an IgG binding dye (protein A gold reagent) for the antiimmunoglobulin conjugate, thereby decreasing the procedure by a step (HIV InSite, 2006).

b. Immunochromatographic Assays

The newer 1-step rapid assays, also known as immunochromatographic assays, are convenient, self-contained tools for HIV serologic testing, consisting of a flat cartridge device, usually plastic or paper. Whole blood, oral fluid, or serum is placed at the tip of the device and allowed to diffuse along a strip that is impregnated with reagents (often protein A colloidal gold) that bind and permit visual detection of HIV antibodies; some use third-generation (antigen sandwich) technology. The test can also be performed on whole blood, or blood collected via fingerstick (HIV InSite, 2006).

c. Dipsticks

This is the other rapid test formats include, in which antigen is attached on the "teeth" of comb-like devices; several of these rapid tests have the ability to differentiate HIV-1 and HIV-2. Disadvantages include a subjective interpretation, difficulty in reading if the laboratorian is colour-blind, and a higher cost than that of the ELISA (HIV InSite, 2006).

d. Simple Tests

This type of HIV test requires longer than 30 minutes for results, but consists of procedures that can be performed easily without instrumentation. Within this class of tests are agglutination assays in which antigen-coated particles (red blood cells, latex

particles, or gelatin particles) are allowed to react with serum antibodies to form visible agglutination (HIV InSite, 2006).

3.2.4.3. Confirmatory (supplemental) assays

a. Western Blot

The Western blot probably is the most widely accepted confirmatory assay for the detection of antibodies to the retroviruses. It is based on using an electrophoretic technique to separate HIV antigens derived from a lysate of virus grown in culture. This technique denatures the viral components, imparts a negative charge to the antigens, and separates them primarily on the basis of their molecular weights. The separation of antigens in the technique allows for the identification of specific antibodies to each of the viral antigens in a subsequent set of steps similar to the ELISA methodology. The Western blot technique can be utilized to distinguish HIV-1, HIV-2, HTLV-I, and HTLV-II infections (Constantine et al., 1994).

b. Indirect Immunofluorescent Antibody Assay

In this technique, cells (usually lymphocytes) are infected with HIV and are fixed to a microscope slide. Serum containing HIV antibodies is added and reacts with the intracellular HIV. The slide is washed and then allowed to react with anti immunoglobulin antibodies with a covalently bound fluorescence label attached. The reaction is visualized using a fluorescent microscope. This technique has the advantage of sometimes providing definitive diagnosis of samples that have yielded indeterminate results by Western blot analysis (HIV InSite, 2006).

c. Line Immunoassay

In this assay, recombinant or synthetic peptide antigens are applied on a nitrocellulose strip, rather than electrophoresed as in the Western blot. This use of "artificial" antigens decreases the presence of contaminating substances derived from cell culture that can

cause interference and sometimes false reactions. LIA use restricted number of recombinant or synthetic peptides as antigen (Schupbach, 2003).

3.2.5. Strategies for HIV Testing

The World Health Organization (WHO) has devised a testing approach that does not require use of the WB test. Strategy I is employed in places where the prevalence of HIV infection is 10% or greater, relies on a single rapid EIA test and is recommended for blood product screening and transplantation. Strategy II, recommended where the prevalence of HIV infection is 10% or greater, or where diagnosis of HIV-related diseases is required, calls for confirmation of initial positive EIA tests with a second EIA test. Strategy III, confirmation of two previous EIA positive tests with a third EIA test, is recommended when the prevalence of HIV is less than 10% in the population (Sato et al., 1994).

3.3. Hepatitis B Virus

Hepatitis B virus (HBV) is the most common form of parenterally transmitted viral hepatitis, and an important cause of acute and chronic infection of the liver. HBV is the only Hepadna virus causing infection in humans. It cannot yet be grown but can be transmitted to certain primates. Hepatitis B has also been called type B hepatitis, serum hepatitis, homologous serum hepatitis. Persistent HBV infection can lead to development of liver cirrhosis, hepatocellular carcinoma (HCC), chronic hepatic insufficiency (WHO, 2002, Butel, 2007).

3.3.1. Properties of HBV

Complete virions possess an isometric nucleocapsid or ‘core’ of 27 nm in diameter, surrounded by an outer coat approximately 4 nm thick. The protein of the virion coat is termed ‘surface antigen’ or HBsAg. It is sometimes extended as a tubular tail on one

side of the virus particle (Butel, 2007). Lipid in the outer protein shell or the HBs particles is derived from an intracellular compartment and not from the plasma membrane. The nucleocapsid of the virion consists of the viral genome surrounded by the core antigen (HBcAg) (Kann and Gerlich, 1998).

3.3.2. Epidemiology

3.3.2.1. Global Scenario

HBV has caused epidemics in parts of Asia and Africa, and is endemic in China and various other parts of Asia (Williams, 2006). More than 2,000 million people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus. Every year there are over 4 million acute cases of HBV, and about 25% of carriers, 1 million people a year die from chronic active hepatitis, cirrhosis or primary liver cancer (WHO, 2002).

The world can be divided into three areas where the prevalence of chronic HBV infection is high (>8%), intermediate (2-8%), and low (<2%). There is no seasonal trend for HBV infection and no high predilection for any age groups such as parenteral drug abusers, institutionalized persons, health care personnel, multiply transfused patients, organ transplant patients, hemodialysis patients and staff, highly promiscuous persons, and new born infants born to mothers with hepatitis B (Butel, 2007).

3.3.2.2. HBV Prevalence in Nepal

In Nepal hepatitis is a major public health problem with carrier rate of 200 thousand and accounts for 6% of acute hepatitis. 1% of the population is asymptomatic chronic hepatitis B surface antigen carriers, 39% of patients suffer from chronic liver disease and 37% with hepatocellular carcinoma are HBsAg seropositive (Shrestha, 1990). Seroprevalence of HBsAg in Nepal has been reported by various studies to range from

0.3 to 4% in the general population conducted from 1990 to 2003 (Shrestha, 1990, Rai et al., 1994, Manandhar et al., 1998, Sawayama et al., 1999, Joshi et al., 2003, Nakashima et al., 2003, Bhatta et al., 2003). Seroprevalence rate of HBV reported among blood donors were 0.82% nationwide and 0.92% in Kathmandu, over a period of 6 years (Karki et al, 2008).

3.3.3. Modes of Transmission

Sexual Transmission

Transmission has been very frequent between male homosexuals, depending on their sexual practices. Bodily secretions such as saliva, ejaculate, vaginal secretion or menstrual blood may also cause transmission, and titres of 10^6 may be present in these secretions (Kann and Gerlich, 1998).

Perinatal Transmission

Perinatal infection is uncertain, but it occurs probably during or shortly after birth as a result of a leak of maternal blood into the baby's circulation or of its ingestion or inadvertent inoculation. There is also a substantial risk of perinatal infection if the mother had acute hepatitis B in the second or third trimester of pregnancy or within 2 months of delivery. Although hepatitis B virus can infect the foetus in utero, this appears to be rare and is generally associated with ante-partum haemorrhage and tears in the placenta. It has been suggested that the soluble HBeAg may cross the placenta and tolerise the foetus in utero (Harrison et al., 2004).

Parenteral Transmission

Hepatitis B can be transmitted by infected blood and blood products through blood transfusion and injecting drug use. Transmission is most efficient by intravenous injection, 100 times less efficient by the intramuscular or percutaneous route and least efficient by mucosal contact (Kann and Gerlich, 1998). For a susceptible person, the risk from a single needlestick or cut exposure to HBV-infected blood ranges from 6-

30% and depends on the hepatitis B e antigen (HBeAg) status of the source of individual. Devices for ear or nose piercing or tattooing have been a source of transmission.

3.3.4. Serological Tests for HBV

Three clinical useful antigen-antibody systems have been identified for hepatitis B: hepatitis B surface antigen 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). The standard screening test is for HBsAg, which, if present in the serum, indicates that the patient is infected with HBV, either as a recent infection or as a carrier (Simmonds and Peutherer, 2006).

ELISA

ELISA technique detects various antibodies and antigens of HBV. **HBsA**g is detected qualitatively by solid phase EIA with anti-HBs coated in the microwell and addition of labelled anti-HBs. **Anti-HBs** is detected by a sandwich EIA with HBsAg at the solid phase and labelled HBsAg. **Anti-HBc** is determined by inhibition EIAs with recombinant HBcAg at the solid phase and labelled anti-HBc, the binding of which is inhibited by the addition of samples anti-HBc. **IgM anti-HBc** is detected by the anti- μ capture EIA. EIA binds via anti-μ antibodies IgM from the sample to the solid phase; thereafter labelled HBcAg is added. If the sample contains IgM anti-HBc, labelled HBcAg will be bound and quantification is necessary. HBeAg is determined in people with HBsAg (Hayces et al., 2002).

3.4. Hepatitis C

Hepatitis C is causes by Hepatitis C virus (HCV). The virus infects liver cells causing severe inflammation of the liver with long-term complications. Most new infections with HCV are subclinical. The majority (70-90%) of HCV patients develops chronic

hepatitis, and many are at risk of progressing to chronic active hepatitis and cirrhosis (10-20%). Hepatitis C is also called type C hepatitis, parenterally transmitted non-A non-B hepatitis (PT-NANB), Non-B transfusion-associated hepatitis, Post transfusion non-A non-B hepatitis. HCV infections are common world wide. It is estimated that about 3% of the world's population have HCV (WHO, 2002).

3.4.1. Properties of HCV

HCV is an enveloped virus with a diameter of about 50 nm. HCV has an internal nucleocapsid containing the RNA genome, closely surrounded by a lipid envelope containing the E1 and E2 glycoproteins. They closely resemble pestiviruses, particularly in the 6 nm spike-like projections from the virion surface that may correspond to the envelope glycoprotein (Simmonds et al., 1998).

3.4.2. Epidemiology

3.4.2.1. Global Scenario

HCV infection is common worldwide. WHO estimates that about 3% of the world's population has HCV and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer. As acute infection is generally asymptomatic, the incidence of HCV on a global scale is not well known. High rates of HCV antibody reactivity (>70%) have been reported in injecting drug users and in haemophiliacs (WHO, 2002). Intermediate prevalence of 20 to 30% has been observed in patients receiving haemodialysis. Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver damage, cirrhosis, and HCC. Approximately 20% of Egyptian blood donors are anti-HCV positive. The strong homogeneity of HCV subtypes found in Egypt (mostly 4a) suggests an epidemic spread of HCV. Since a history of injection treatment has been implicated as a risk factor for

HCV, a prime candidate to explain the high prevalence of HCV in Egypt is the past practice of parenteral therapy for schistosomiasis (WHO, 2002).

3.4.2.2. Prevalence in Nepal

Prevalence of Hepatitis C in general population determined by second generation ELISA and HCV RNA PCR was reported to be 0.6% (Shrestha et al., 1998). About 72% of the drug addicts were found to be intravenous drug abusers (IDA) and 94% of the IDA were anti-HCV positive (Shrestha et al., 1998). Reported range of HCV in general population of Nepal is 0.3 to 1.7% (Shrestha et al., 1998, Singh, 1998, Sawayama et al., 1999, Shrestha, 2003, and Shrestha, 2006). Seroprevalence of HCV among blood donors, nationwide and in Kathmandu was reported to be 0.47% and 0.71% respectively over a period of 6 years from 2001/2002 to 2006/2007 (Karki et al., 2008).

3.4.3. Modes of Transmission

Sexual Transmission

Although HCV is shed in semen and saliva, sexual transmission seems to be less efficient than in the case of HBV (Brink, 2006). Evidence suggesting that sexual transmission may occur includes the observation that HCV seems to be more common in people who have multiple sexual partners such as prostitutes and male homosexuals.

Perinatal Transmission

Mother-to-infant transmission has been observed, but appears to be unusual. Differences in the rates of maternal–infant transmission in different countries remain unexplained, and the importance of this route in perpetuating the reservoir of human infection is unknown, but could be relevant. Maternal–infant transmission is more likely in mothers with HCV RNA concentrations higher than 10^7 genomes/ml (Harrison et al., 2004).

Parenteral Transmission

Injecting drug users are at increased risk of hepatitis C infection because they may be sharing needles or other drug paraphernalia, which may be contaminated with HCV-infected blood.

Blood and Blood Products Transfusion

Repeated exposure to blood and blood products (in the absence of serological screening) is associated with a high risk of HCV exposure and infection. Thus, risk groups of HCV infection include transfusion recipients (especially recipients of pooled plasma products such as factor VIII, anti-D) (Power et al., 1995).

Other Modes of Transmission

Tattooing dyes, ink pots, stylets and piercing implements can transmit HCV-infected blood from one person to another if proper sterilization techniques are not followed. Personal care items such as razors, toothbrushes, cuticle scissors, and other manicuring or pedicuring equipment can easily be contaminated with blood. Sharing such items can potentially lead to exposure to HCV. Medical and dental personnel, first responders and military combat personnel can be exposed to HCV through accidental exposure to blood through accidental needle sticks or blood spatter to the eyes or open wounds. The average risk for infection after a needle sticks or cut exposure to HCV- infected blood is approximately 1.8% (CDC, 2003). People can be exposed to HCV via inadequately or improperly sterilized medical or dental equipment. Transplanted organs may also transmit HCV infection.

3.4.4. Serologic Tests for HCV

3.4.4.1. ELISA

The screening test for HCV is an enzyme-linked immunosorbent assay to detect specific HCV antibodies.

First Generation Tests

The first test to specifically detect antibody to HCV was based on the c100-3 antigen (Kuo et al., 1989). These tests were prone to non-specificity, especially when used to screen low risk populations or stored serum samples.

Second Generation Tests

The test included antigen to core region (c22) and one or more further non-structural regions NS3 (c33), NS4 (c100-3) or NS5 (Simmonds et al., 1998)

Third Generation Tests

The test includes the NS5 antigen. Third generation ELISAs are more sensitive and detect seroconversion earlier. Improvement of the test has been associated with improved reactivity of the c33 antigen component. This EIA can be used to detect antibodies within 4 to 10 weeks following infection (Uyttendaele et al., 1994).

False-negative results can occur in persons with compromised immune systems, such as people with HIV-1 infection, patients with renal failure, and patients with HCV associated essential mixed cryoglobulinemia. False-positive EIA results are more common in persons without risk factors and in other low-prevalence populations, including blood donors and health care workers.

3.4.4.2. RIBA

The recombinant immunoblot assay (RIBA) traditionally has been used to confirm HCV infection. The test uses a nitrocellulose strip containing bands of 2 HCV recombinant antigens (5-1-1 and c100-3). A positive immunoblot assay result is defined as the detection of antibodies against 2 or more HCV antigens, and an indeterminate assay result is defined as the detection of antibodies against a single antigen. The RIBA may be useful for the confirmation of positive EIA results in low-risk populations and has been shown to be specific for HCV infection. RIBA, as well as the EIA, is unable to

distinguish acute infection from chronic infection (Harris et al., 2002). The RIBA.1, RIBA.2 and RIBA.3 are the first, second and third generation variations of the immunoblot assay.

3.5. Syphilis

Syphilis is the best known of the disease caused by the members of the genus *Treponema* (Larsen et al., 1998). *Treponema pallidum* is the spirochaete which is capable of invading through intact skin and rapidly disseminate to all the organs of the body (Gillespie, 1994). Syphilis is transmitted primarily through sexual contact with an infected individual who is in the primary, secondary or early latent stage of the disease. *T. pallidum* can also be transmitted from mother to foetus and from an infected donor to a recipient through unscreened blood or direct blood transfusion (Public Health Agency of Canada, 2003).

3.5.1. Properties of *Treponema*

Treponema pallidum is a Gram-negative bacterium, 6-20 µm long, 0.1-0.2 µm wide and tightly coiled. The treponemes are motile by three flagella (axial filaments) that wrap around the surface of the organism and are covered by the outer membrane which contains lipopolysaccharide. They impart the characteristic rapid axial rotation and bending around the centre. There is a central peptidoglycan layer, and plasma membrane (Gillespie, 1994).

The spirochaete divides by binary fission approximately once every 30 hour. It is feebly viable outside its host, dies rapidly in water and is sensitive to drying. The only practicable method at present available for the growth of organisms as a source of diagnostic antigen for the laboratory research is infection of the rabbit testis. *T. pallidum* is microaerophilic; survives best in 1 to 4% oxygen. The saprophytic Reiter strain (Non-pathogenic Treponemes) grows on a defined medium of 11 amino acids, vitamins, salts,

minerals, and serum albumin. In proper suspending fluids and in the presence of reducing substrates, it may remain motile for 3 – 6 days at 25^0C. In whole blood or plasma stored at 4^0C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions (Brooks et al., 2007).

3.5.2. Epidemiology

3.5.2.1. Global Scenario

Venereal syphilis is worldwide in distribution. Between half a million and a million cases of congenital syphilis occur each year worldwide (Saloojee et al., 2004). Syphilis continues to be a major cause of pregnancy loss and adverse pregnancy outcome among women who do not receive antenatal syphilis screening and treatment (Watson-Jones et al., 2002). In 1993, the reported total rate of cases of syphilis in China was 0·2 cases per 100 000, whereas primary and secondary syphilis alone represented 5·7 cases per 100 000 persons in 2005. The rate of congenital syphilis increased greatly with an average yearly rise of 71·9%, from 0·01 cases per 100 000 livebirths in 1991 to 19·68 cases per 100 000 livebirths in 2005 (Chen et al., 2007).

3.5.2.2. Prevalence in Nepal

Syphilis seroprevalence in Nepal has been studied mostly among the high risk groups as Sexually Transmitted Infections. Very scarce information has been published on the prevalence among the healthy general population. Syphilis seroprevalence has been reported to be 0.6% among Nepalese males (Joshi et al., 2007). In a study conducted by Family Health International (FHI) and United States Agency for International Development (USAID) in 2000 among female sex workers and truckers, the most common Sexually Transmitted Infections (STIs) that affected the truckers were syphilis. Nearly half or 47.3 percent of the female sex workers (FSWs) had at least one STI and 12.4 percent had more than one. Seventy-seven percent had untreated syphilis. Syphilis

infection was documented 20.4% among sex trafficked women and girls in Nepal (Silverman et al., 2008).

3.5.3. Transmission

Sexual Transmission

Syphilis is a sexually transmitted infection, and the more sexual partners that individuals (or other members of their sexual network) have, the more likely they are to acquire syphilis. Syphilis is passed from person to person through direct contact with syphilis sore. Sores occur mainly on the external genitals, vagina, anus, or in the rectum. Sores also can occur on the lips and in the mouth. Transmission of the organism occurs during vaginal, anal, or oral sex. Approximately 30% of persons who have sex with an infected partner will develop syphilis (Sparling, 1990).

Vertical Transmission

The syphilis bacterium can infect the baby of a woman during her pregnancy. Depending on how long a pregnant woman has been infected, she may have a high risk of having a stillbirth or of giving birth to a baby who dies shortly after birth. An infected baby may be born without signs or symptoms of disease. Congenital syphilis remains a major cause of stillbirth, childhood morbidity, and mortality worldwide. More than 1 million infants are born with congenital syphilis each year (Saloojee et al., 2004).

Other Modes of Transmission

Transmission by kissing, blood transfusion and percutaneous injury are associated with syphilis transmission.

3.5.4. Serological tests for Syphilis

3.5.4.1. Nonspecific Tests Using the Cardiolipin Antigen

i. Venereal Disease Research Laboratory (VDRL) introduced in the year 1946, is performed by mixing heat–inactivate patient's serum with a freshly prepared suspension of cardiolipin-lecithin-cholesterol antigen and reading the resulting flocculation microscopically with a low-power objective (Young, 2006).

ii. Rapid Plasma Reagin (RPR) introduced in the year 1957, uses cardiolipin antigen with choline chloride (to block inhibitors in serum, eliminate the need for heat-inactivation and allow testing of plasma), EDTA (to stabilize the antigen and allow it to be used for up to 6 months when stored at 4-10^{0}C, and finely divided carbon particles (to enable the result to be read by eye) (Young, 2006).

3.5.4.2. Specific Tests

i. *T. pallidum* Immobilization (TPI) is performed by incubating the serum sample with complement and *T.pallidum* maintained in a complex medium anaerobically. If antibodies are present, the treponemes are immobilised, i.e., rendered nonmotile, when examined under dark ground illumination. The test is considered positive if the percentage of treponemes immobilised is 50 or more, negative if 20 or less, and inconclusive if in between (Ananthanarayan and Paniker, 1997).

ii. Fluorescent Treponemal Antibody-Absorption (FTA-Abs) The test is an indirect immunofluorescence assay in which *T.pallidum* is used as an antigen. Acetone-fixed treponemes are incubated with heat treated sera, and bound antibody is detected with a fluorescein-labelled conjugate and ultraviolet microscopy. The serum is first absorbed with a suspension of a non-pathogenic treponeme (sonicated Reiter's treponemes), which removes non-specific cross-reactive antibodies that may be directed against

commensal spirochaetes. The FTA-Abs test is positive in approximately 80, 100 and 95% of primary, secondary and late syphilitics, respectively, unlike the VDRL test, remains positive following successful therapy (Cockayne, 2006).

iii. *Treponema pallidum* Haemagglutination Assay (TPHA) was introduced in the year 1967. The test utilises extracts of *T.pallidum* antigen coated onto the surface of sheep or turkey red blood cells, and specific antibody in test sera causes causes haemagglutination. The test sera are pre absorbed with Reiter's treponemes to remove group specific antibody. The patient's serum is screened at an initial dilution of 1:80 and a positive test serum should be titrated (Gillespie, 1994). The TPHA is less sensitive than the FTA-Abs in primary syphilis (positive in 65%), but both give similar results for life following infection (Cockayne, 2006).

iv. Enzyme-Linked Immunoabsorbent Assay (ELISA)

ELISA test for syphilis allows rapid screening of large numbers of samples with potentially enhanced specificity. Assays that detect either IgM or IgG are available and are increasingly replacing the TPHA and VDRL tests for routine screening (Cockayne, 2006). In primary Syphilis, EIA for antitreponemal IgM becomes positive before the non-treponemal tests, and in early congenital syphilis is consistent with a positive diagnosis. All the positive results must be confirmed by repeat tests (Todd et al., 2002).

3.6. Coinfections

3.6.1. HIV and Viral Hepatitis Coinfection

Among the estimated 40 million persons infected with HIV worldwide, an estimated 2–4 million are chronically infected with HBV and an estimated 4–5 million are chronically infected with HCV. Several factors influenced these co-infection estimates, including geographic differences in the prevalence of chronic infection by age, the

efficiency of exposures that account for most transmission, and the prevalence of persons at high risk for infection (Alter, 2006).

3.6.2. HIV and HBV Coinfection

HBV and HIV share common routes of transmission. Therefore, markers of either active or past infection are present in many HIV-infected patients. Serological markers of past or present HBV infection have been reported in up to 90% of HIV-infected patients. It has been suggested that the HBVX-protein (HBx) super induces ongoing HIV-1 replication and HIV-1 long-term repeated transcription by synergizing with tat protein and with T-cell activation signals (Gómez-Gonzalo et al., 2001). These findings indicate that HBx could contribute to a faster progression to AIDS in HBV/HIV-co-infected individuals.

Sub-Saharan Africa accounts for most (65%) HIV infections worldwide and has a high prevalence of chronic HBV infection because of perinatal and early childhood transmission patterns. HBV infections acquired at young ages are more likely to progress to chronic infections, resulting in a high prevalence of chronic HBV infection among the general population of adolescents and adults at risk for sexually-acquired HIV. Sexual (and injection drug use) exposures account for most HBV and HIV infections in developed countries (Alter, 2006).

3.6.3. HIV and HCV Coinfection

For HCV infection, the geographic distribution of prevalence and primary mode of transmission relative to HIV are very different from those of HBV. HCV is not efficiently transmitted by perinatal or sexual exposures, which are important modes of transmission for HBV and HIV. HCV is predominantly found in persons who have had large or repeated percutaneous exposures to infectious blood, such as persons who received unscreened blood or untreated clotting factor products and injection drug users.

Although these persons are also at high risk for HIV, they account for a small proportion of the overall populations in most countries. Thus, in subpopulations of HIV-positive persons with history of injection drug use, 72–95% have been found to be co-infected with HCV; in HIV-positive persons who acquired their infection from sexual exposures, the prevalence of HCV coinfection has been found to be 8–35-fold lower, ranging from 1 to 12% among Men who have sex with men (MSM) and 9–27% among heterosexuals (Alter, 2006). It has been demonstrated in clinical studies that HIV infection causes a more rapid progression of chronic hepatitis C to cirrhosis and liver failure.

HCV is transmitted primarily by large or repeated direct exposures to contaminated blood. Therefore, coinfection with HIV and HCV infection is common (50%-90%) among HIV-infected injection drug users (IDUs).Coinfection is also common among persons with hemophilia who received clotting factor concentrates before con-centrates were effectively treated to in-activate both viruses (i.e.,products made before 1987). The risk for acquiring in-fection through perinatal or sexual expo-sures is much lower for HCV than for HIV. For persons infected with HIV through sexual exposure (eg, male-to-male sexual activity), coinfection with HCV is no more common than among similarly aged adults in the general population (3%-5%). Chronic HCV infection develops in 75%-85% of infected persons and leads to chronic liver disease in 70% of these chronically infected persons. HIV/HCV coinfection has been associated with higher titers of HCV, more rapid progression to HCV- related liver disease, and an increased risk for HCV-related cirrhosis (scarring) of the liver. Be-cause of this, HCV infection has been viewed as an opportunistic infection in HIV-positive people. It is not, however, considered an AIDS-defining illness (Hudson et al., 2007).

3.6.4. HBV and HCV Coinfection

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for a substantial proportion of liver diseases worldwide. Because the two hepatotropic viruses

share same modes of transmission, coinfection with the two viruses is not uncommon, especially in areas with a high prevalence of HBV infection and among people at high risk for parenteral infection. Patients with dual HBV and HCV infection have more severe liver disease, and are at an increased risk for progression to hepatocellular carcinoma (HCC). Treatment of viral hepatitis due to dual HBV/HCV infection represents a challenge (Liu and Hou, 2006).

3.6.5. HIV and Syphilis Coinfection

Persons with HIV infection have an increased incidence of neurosyphilis, reflecting the common risk factor of sexual transmission for both. There is a high coinfection rate with HIV, especially among men who have sex with men. In light of high coinfection rates, all HIV-infected patients should be tested for syphilis and vice versa. HIV can alter the clinical manifestations of syphilis and, in turn, syphilis has the potential to change the course and transmission of HIV (Stevenson et al., 2006). Presence of genital ulcer disease facilitates human immunodeficiency virus (HIV) transmission and their diagnosis is essential for the proper management (Turbadkar et al., 2007).

CHAPTER-IV

4. MATERIALS AND METHODOLOGY

4.1. Materials

During the study period, following materials were used as required in the procedures outlined in the methodology section.

4.1.1. Equipments

Automated ELISA processor (BEP III ELISA Processor, Germany)
Microplate reader (Platos R496, AMD Diagnostic, Austria)
Microplate washer (Platos W96, AMD Diagnostic, Austria)
Centrifuge (Rotina 35, Hettich Zentrifugen, Germany)
Refrigerator (White Westinghouse, USA)
Deep Refrigerator (White Westinghouse, USA)
Micropipettes (Human, Germany)
Incubator (Ambassador, India)

4.1.2. Test kits and Reagents

Enzygnost Anti * HIV ½ Plus, Dade Behring, Germany
Enzygnost HBsAg 5.0, Dade Behring, Germany
EIAgen HCV Ab kit, Adaltis, Italy
SD Syphilis ELISA 3.0, Standard Diagnostics, Inc., Korea
SD Bioline HIV – ½ 3.0, Standard Diagnostics, Inc., Korea
Virucheck HBsAg, Orchid Biomedical Systems, India
SD Bioline HCV, Standard Diagnostics, Inc., Korea

4.1.3. Glassware and Others

Test tubes (Borosil, India)
Disposable examination gloves (Powdered latex examination gloves, Handsafe products, Malaysia)
Micropipette tips (Human, Germany)
Sodium hypochlorite solution (Biolab diagnostics, India)
Distilled water (prepared in the laboratory)
Test tube rack, tissue paper, marker, bar codes for microplate, adhesive seals, watch, mixing vessels, bottles (locally produced)

4.2. Methodology

4.2.1. Type of Study

The study is a descriptive cross-sectional study, carried out from March 2008 to September 2008 at NRCS, CBTS.

4.2.2. Study Population Selection

Prior to blood collection, the donors were requested to fill up questionnaire to evaluate if they are eligible for donation as per the criteria set by NRCS, CBTS (Standard Operating Procedure, 2006). Donors might be new first time donors or repeated donors; they might be volunteers or replacement donors.

Central Blood Transfusion Centre is major blood collection centre in Nepal where blood collection occurs at CBTS or blood is collected in the donation camps organized within Kathmandu; reach for processing within 3 hours of collection.

4.2.3. Sample Size

Sample size for the study was estimated using the software WinPepi Ver.3.8 (Appendix III). Sample number included the number of blood donors donating blood only once during the study period. Thus attempts were made to determine the true seroprevalence number excluding the possibility of sampling bias.

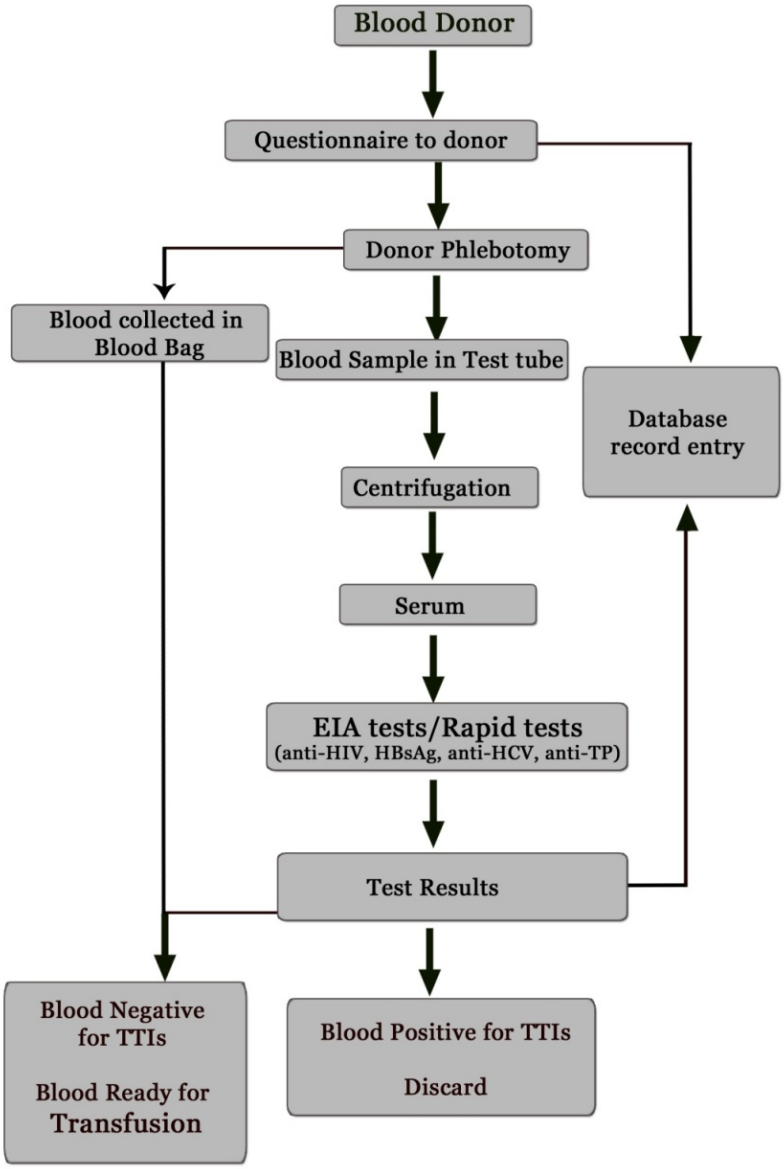

Figure 4.1: Flow Diagram of Blood Collection and Testing

4.2.4. Collection of Samples

Blood samples were collected by authorized medical professionals (nurses and laboratory technicians) using aseptic technique. Using a sterile syringe, 350 ml blood was drawn in blood bag labelled with a unique sample number. 5 ml of the same blood was dispensed in a small clean test tube labelled with the corresponding sample number for mandatory screening of the TTIs.

4.2.5. Separation of Serum/Plasma

Blood samples collected in small test tubes were centrifuged at 2000 rpm for 4 minutes to separate serum/plasma required for screening tests as below.

4.2.6. Testing for Transfusion Transmissible Infections

Serum samples separated as above were used to test for anti-HIV IgG and IgM, HBsAg, anti-HCV IgG and IgM and anti-*Treponema pallidum* IgG, IgM and IgA using the kits mentioned above. The tests were performed using semi-automatic or automated ELISA processor depending upon the load of samples collected each day.

Performance of the ELISA Test

The microwell holder with microwells (loaded with samples and controls in respective wells) of the test kit was labelled with a unique bar code number and placed in the ELISA processor for further automatic processing set manually beforehand.

4.2.6.1. Testing for Anti HIV 1 and 2 Antibodies

Anti HIV 1 and 2 testing was done using serum samples separated as above, following standard protocol as per instruction of the kit manufacturer. Testing was done following WHO strategy 2 for surveillance/diagnosis (WHO, 2004). First assay used was

Enzygnost Anti-HIV ½ Plus for the detection of anti- HIV ½ IgG and IgM and the second assay used was SD Bioline HIV-½, Immunochromatographic test kit - detecting anti HIV 1 & 2 IgG, IgM and IgA antibodies in the test serum sample.

a. Anti-HIV 1 and 2 Antibodies Test Using Enzygnost Anti-HIV ½

Presence of anti-HIV 1 and 2 antibodies in the separated serum samples were detected following standard protocols (Standard Operating Procedure, 2006). Test serum samples (100 µl) were loaded in each well of the pre-coated ELISA plates and incubated at 37^0C for 30 minutes. After washing the plates, the reaction was further developed with the addition of 100 µl conjugate (recombinant HIV protein and synthetic HIV peptides, peroxidase-conjugated). The reaction was further developed with addition of the 100 µl chromogen Tetramethylbenzidine dihydrochloride (TMB), which was stopped with the addition of 0.5 N H_2SO_4. Colour developed was detected using ELISA reader at 450 nm. Test samples were considered reactive or non-reactive based on the Optical Densities (ODs) in comparison with the Positive Control (PC)/ Negative Control (NC) and cut-off ODs were noted for record (Photograph 1).

b. Anti-HIV 1 and 2 Antibodies Test Using SD Bioline HIV – ½ 3.0

Test serum sample with positive result by Assay 1 was further tested for confirmation using SD Bioline HIV-½ 3.0 test kit. 10 µl of serum sample was added in the well of the kit. 120 µl of sample diluents provided with the kit was added and result of the test interpreted after 5 minutes for coloured band formation in test window with the development of control line, as per kit manufacturer's instruction leaflet (Photograph 5).

4.2.6.2. Testing for Hepatitis B Surface Antigen

Similar to HIV testing, Hepatitis B surface antigen was also detected first using Enzygnost HBsAg test kit. All the positive samples with first test were further

confirmed with second assay Virucheck HBsAg Immunochromatographic test kit manufactured by Orchid Biomedical Systems.

a. Hepatitis B Surface Antigen Test Using Enzygnost HBsAg 5.0

Presence of HBsAg in the separated serum samples were tested following standard protocols developed by CBTS incorporating the critical steps mentioned in the kit inserts. 100 µl of serum samples and controls were loaded in the pre-coated wells of the commercial ELISA plate. 25 µl Conjugate 1 (Anti-HBs/Biotin) was added and incubated at 37^0C for 60 minutes. Plates were washed to remove unbound reactants, and then 100 µl of Conjugate 2 (Streptavidin/Peroxidase) was added to each well and incubated at 37^0C for 30 minutes. The plates were again washed and the reaction was further developed with the addition of 75 µl chromogen solution (TMB) in each well. The reaction was terminated by the addition of 0.5 N H_2SO_4 and OD of the developed colour was measured using ELISA reader at 450 nm. The results were correlated with the quality control standards (Positive Control and Negative Controls) and were noted for records. Test samples were considered reactive or non-reactive based on corresponding ODs of positive controls and negative controls (Photograph 2).

b. Hepatitis B Surface Antigen Test Using Virucheck HBsAg

Test samples positive by Enzygnost HBsAg 5.0 were further tested by Viru-check HBsAg - one step test for HBsAg. 50 µl of serum sample was dispensed in the sample well of the kit. Result (development of pink-purple band at the Control region and test region) was read after 15 minutes and recorded (Photograph 6).

4.2.6.3. Testing for Anti HCV Antibodies

Strategy used for the diagnosis of anti HCV antibodies in the serum samples is outlined in the figure 4.2. Assay 1 used was Adaltis, EIAgen HCV Ab kit and Assay 2 was SD Bioline HCV.

a. Anti-HCV Antibodies Test Using Adaltis, EIAgen HCV Ab Kit

Anti-HCV antibodies in the separated serum samples were tested following standard protocols (Standard Operating Procedure, 2006). 200 µl of negative and positive controls were loaded in the respective wells (HCV antigens coated) of ELISA plate. 200 µl of sample diluent was dispensed in the sample wells (HCV antigen coated) and 10 µl serum sample was added in the sample well. 50 µl assay diluent was dispensed in all the wells. After incubation for 45 minutes at 37^0C and washing, 100 µl Enzyme Conjugate (horseradish peroxidase conjugated goat polyclonal antibodies) was added in the wells. The micro-plate after incubation for 45 minutes at 37^0C was washed and reaction was further developed with the addition of 100 µl chromogen (TMB) solution. With the incubation for 15 minutes, the reaction was stopped with addition of 0.3 M Sulphuric acid. Colour intensity of the product in the wells was measured at 450 nm and the results noted. Test samples were considered reactive or non-reactive based on the correlation with PC/NC control well readings (Photograph 3).

b. Anti-HCV Antibodies Test Using SD Bioline HCV

Serum sample detected positive by Adaltis, EIAgen HCV Ab kit was further tested with SD Bioline HCV. 10 µl of suspected sample was added in the sample well of the kit. 4 drops of assay diluents provided with the kit was dispensed and allowed to pass in room temperature. Result was read after 5 minutes for the coloured band in the test and control line of the result window (Photograph 7).

4.2.6.4. Testing for Anti-*Treponema pallidum antibodies* using SD Syphilis ELISA 3.0

Anti-*Treponema pallidum* antibodies were detected in the sample following the instruction provided by the kit manufacturer (SD Syphilis ELISA 3.0). For the positive samples the test was repeated once for reproducibility/reconfirmation of the test results,

as there was only one kit available for detection of anti-Treponemal antibody. 100 µl samples/controls were dispensed in wells coated with *Treponema pallidum* antigens (47, 17, 15KDa). 25 µl of conjugate (antigen conjugated to horseradish peroxidase) was added to all the wells. It was further incubated at 37^0C for 90 minutes. After washing, reaction was further developed with the addition of 100 µl substrate (TMB). The reaction was stopped after 30 minutes of incubation with addition of 1 N H_2SO_4. Intensity of coloured product was measured at 450 nm and results recorded (Photograph 4).

4.2.7. Recording and Reporting of Test Results

All the test results were recorded in Microsoft Access 2007 suitable for further analysis using available tools.

Test result was provided to only those blood donors who were interested in knowing their sero status to the TTIs.

4.2.8. Statistical Analysis

WinPepi Ver 3.8 was used for sample size estimation and Chi Square tests. SPSS 11.5 was used for calculation of standard deviations.

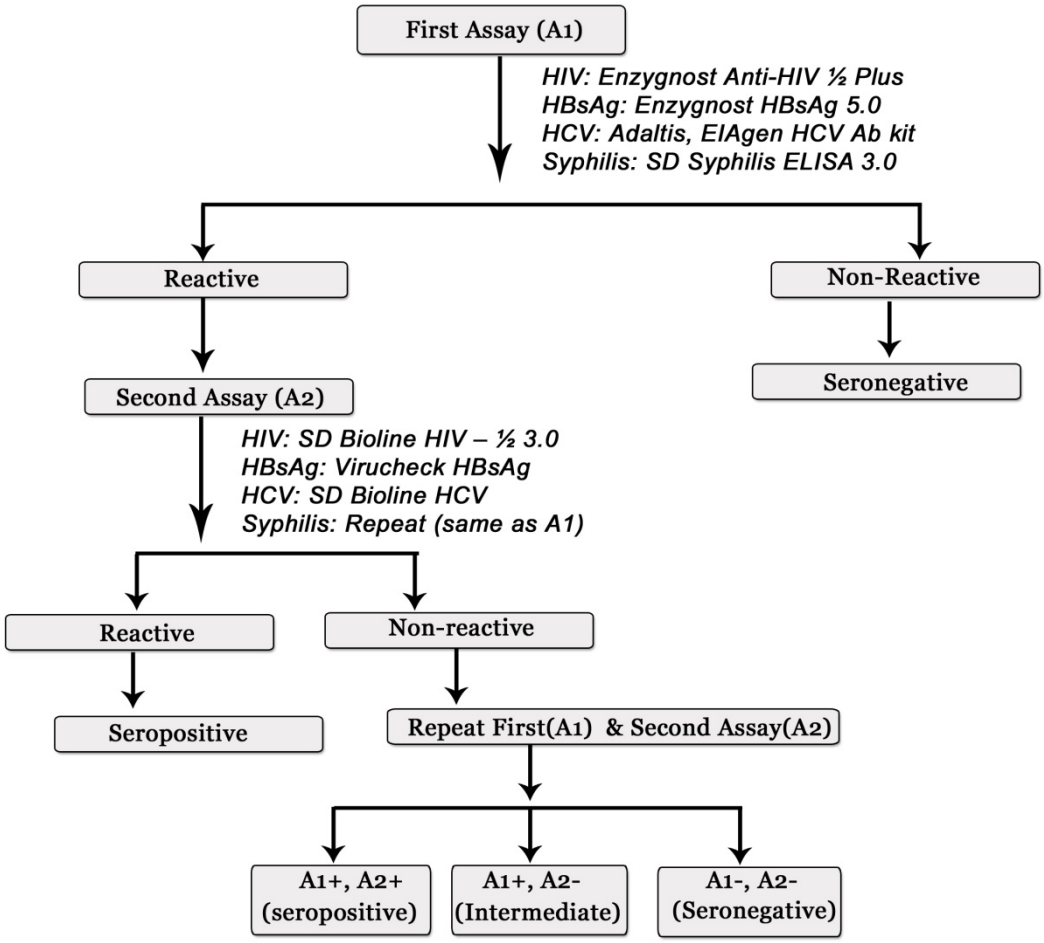

Figure 4.2: Modified Flow Chart Showing the Strategy Used in the Study for TTIs Testing (WHO - HIV assays: operational characteristics (Phase 1): report 15 antigen/antibody ELISAs, 2004)

CHAPTER – V

5. RESULTS

During the study period from March 2008 to September 2008 at NRCS, CBTS, a total number of 21,716 sera samples from blood donors were subjected to anti-HIV, HBsAg, anti-HCV and anti-*Treponema pallidum* (Syphilis) serological tests. Of these, numbers of male donors were 18,434 (84.9%) and the numbers of female donors were 3,282 (15.1%). All these donors were included in the determination of seroprevalence of respective Transfusion transmissible infections (TTIs).

5.1. Seroprevalence of TTIs (HIV, HBV, HCV & Syphilis)

A total of 365 blood donors among 21,716 (1.68%) donors were found infected with the TTIs under consideration. Prevalence of TTIs among males (1.76%) was slightly higher than females (1.18%). Higher seroprevalence of TTIs among male donors compared to female donors was statistically significant.

Table 5.1: Distribution of TTIs Seroprevalence

S.N.	TTIs	Male		Female		Total		P- Value
		No.	%	No.	%	No.	%	
1.	Yes	326	1.76	39	1.18	365	1.68	P < 0.05
2.	No	18,108		3,243				
Total		18,434		3,282		21,716		

5.2. Distribution of TTIs Seroprevalence and Age Group

Highest (5.1%) and statistically significant seroprevalence of TTIs was observed in the age group 51 to 60 years. In the same age group prevalence among males was highest

(5.33%) and statistically significant compared to male donors of other age group taken together. The mean age of donors with TTIs was 32.81 years (SD=9.39).

Table 5.2: Distribution of TTIs Seroprevalence and Age Group

Age Group (Years)	No. of Donors	Male			Female			Overall (%)
		No.	TTIs positive		No.	TTIs positive		
			No.	%		No.	%	
≤ 20	3,310	2,623	21	0.80	687	5	0.72	0.78
21- 30	9,818	8,565	132	1.54	1,253	15	1.19	1.49
31- 40	5,763	4,845	102	2.10	918	12	1.3	1.97
41- 50	2,433	2,045	52	2.54	388	6	1.54	2.38
51- 60	392	356	19	5.33	36	1	2.7	5.10
Total	21,716	18,434	326		3,282	39	1.18	

5.3. Distribution of TTIs Seroprevalence and First Time Vs Repeated Donors

Among the 9,993 first time donors, 1.61% (161) was found infected with TTIs under investigation. Among 11,723 repeated donors, 1.74% (204) of them was found infected with TTIs. However, the difference between first and repeated donors was statistically insignificant.

Table 5.3: Distribution of TTIs Seroprevalence and First Time Vs Repeated Donors

S.N	Donation Times	Male		Female		Total	
		No.	TTIs positive	No.	TTIs positive	No.	TTIs positive
1.	**First Time**	8,076	139 (1.72%)	1,917	22 (1.14%)	9,993	161 (1.61%)
2.	**Repeated**	10,358	187 (1.80%)	1,365	17 (1.24%)	11,723	204 (1.74%)
Total		18,434	326 (1.76%)	3,282	39 (1.18%)	21,716	365 (1.68%)
P - Value		P > 0.05		P > 0.05		P > 0.05	

5.4. Seroprevalence of HIV

Of the 21,716 blood donors screened during the study period, 0.12% (27/21716) was found positive to HIV antibodies. Higher seroprevalence of 0.13% was observed in the male blood donors in comparison to female blood donors with 0.06% seroprevalence. However, the difference was statistically insignificant.

Table 5.4: Distribution of HIV Seroprevalence

S.N.	HIV test	Male		Female		Total		P- Value
		No.	%	No.	%	No.	%	
1.	**Positive**	25	0.13	2	0.06	27	0.12	P > 0.05
2.	**Negative**	18,409		3,280		21,699		
Total		18,434		3,282		21,716		

5.5. Distribution of HIV Seropositive Donors and Age Group

Highest but statistically insignificant seroprevalence of 0.17% was observed in donors of age group 31 to 40 years compared to other age groups as a whole. Mean age of HIV positive donors was 30.70 years (SD=7.86).

Table 5.5: Distribution of HIV Seropositive Donors and Age group

Age Group (Years)	No. of Donors	Male			Female			Overall (%)
		Total No.	HIV positive		Total No.	HIV positive		
			No.	%		No.	%	
≤ 20	3,310	2,623	2	0.07	687	-	-	0.06
21- 30	9,818	8,565	11	0.12	1,253	1	0.08	0.12
31- 40	5,763	4,845	9	0.18	918	1	0.10	0.17
41- 50	2,433	2,045	3	0.14	388	-	-	0.12
51- 60	392	356	0	0.00	36	-	-	0.00
Total	21,716	18,434	25		3,282	2		

5.6. Distribution of HIV Seroprevalence and First Time Vs Repeated Donors

Among the total donation during the period of study, 9,993 were first time and 11,723 were repeated donors. Seroprevalence of HIV in first time donors was 0.14% (0.16% in male and 0.05% in female) and in repeated donors was 0.11% (0.11% in male and 0.07% in female). Though the seroprevalence was slightly higher in first time donors than repeated donors, difference was statistically insignificant.

Table 5.6: Distribution of HIV Seroprevalence and First Time Vs Repeated Donors

S.N	Donation Times	Male		Female		Total	
		No.	HIV positive	No.	HIV positive	No.	HIV positive
1.	First Time	8,076	13 (0.16%)	1,917	1 (0.05%)	9,993	14 (0.14%)
2.	Repeated	10,358	12 (0.11%)	1,365	1 (0.07%)	11,723	13 (0.11%)
Total		18,434	25	3,282	2	21,716	27
P - Value		P > 0.05		P > 0.05		P > 0.05	

5.7. Seroprevalence of Hepatitis B surface Antigen (HBsAg)

Out of the total 21,716 donated blood screened, 0.47% (102/21,716) was positive for hepatitis B surface antigen (HBsAg). Prevalence in male (0.50%) was higher compared to female (0.30%), but the difference was statistically insignificant.

Table 5.7: Distribution of HBsAg Seroprevalence

S.N.	HBsAg test	Male		Female		Total		P- Value
		No.	%	No.	%	No.	%	
1.	Positive	92	0.50	10	0.30	102	0.47	P > 0.05
2.	Negative	18,342		3,272		21,614		
Total		18,434		3,282		21,716		

5.8. Distribution of HBsAg Seropositive Donors and Age Group

Seroprevalence of HBsAg was highest and statistically significant in the age group 41 to 50 years (0.78%). Mean age of HBsAg seropositive donors was 32.27 years (SD=8.71).

Table 5.8: Distribution of HBsAg Seropositive Donors and Age Group

Age Group (Years)	No. of Donors	Male			Female			Overall (%)
		No.	HBsAg positive		No.	HBsAg positive		
			No.	%		No.	%	
≤ 20	3,310	2,623	7	0.26	687	-	-	0.21
21- 30	9,818	8,565	41	0.47	1,253	4	0.31	0.45
31- 40	5,763	4,845	25	0.51	918	4	0.43	0.50
41- 50	2,433	2,045	17	0.83	388	2	0.51	0.78
51- 60	392	356	2	0.78	36	-	-	0.51
Total	21,716	18,434	92		3,282	10		0.47

5.9. Distribution of HBsAg Seroprevalence and First Time Vs Repeated Donors

Seroprevalence of HBsAg in first time donors was 0.51% (0.55% in male and 0.31% in female) which was higher than in repeated donors with seroprevalence of 0.43% (0.45% in male and 0.29% in female). Difference observed was statistically insignificant.

Table 5.9: Distribution of HBsAg Seroprevalence and First Time Vs Repeated Donors

S.N	Donation Times	Male		Female		Total	
		No.	HBsAg positive	No.	HBsAg positive	No.	HBsAg positive
1.	**First Time**	8,076	45 (0.55%)	1,917	6 (0.31%)	9,993	51 (0.51%)
2.	**Repeated**	10,358	47 (0.45%)	1,365	4 (0.29%)	11,723	51 (0.43%)
Total		18,434	92	3,282	10	21,716	102
P - Value		P > 0.05		P > 0.05		P > 0.05	

5.10. Seroprevalence of Hepatitis C Virus

Among the total 21,716 units of donated blood screened, the overall HCV seroprevalence was 0.64% (0.69% in male and 0.33% in female), with statistically significant difference among males and females.

Table 5.10: Distribution of HCV Seroprevalence

S.N.	HCV test	Male		Female		Total		P- Value
		No.	%	No.	%	No.	%	
1.	Positive	128	0.69	11	0.33	139	0.64	P < 0.05
2.	Negative	18,306		3,271		21,577		
Total		18,434		3,282		21,716		

5.11. Distribution of HCV Seroprevalence and Age Group

Seroprevalence of HCV among blood donors of age group 21 to 30 years was 0.75%, higher and statistically significant than other age groups as a whole. Mean age of HCV seropositive donors was 30.28 years (SD=7.57).

Table 5.11: Distribution of HCV Seroprevalence and Age Group

Age Group (Years)	No. of Donors	Male			Female			Overall (%)
		No.	HCV positive		No.	HCV positive		
			No.	%		No.	%	
≤ 20	3,310	2,623	5	0.19	687	2	0.29	0.26
21- 30	9,818	8,565	69	0.80	1,253	6	0.47	0.76
31- 40	5,763	4,845	40	0.82	918	2	0.21	0.72
41- 50	2,433	2,045	12	0.58	388	1	0.25	0.53
51- 60	392	356	2	0.56	36	-	-	0.51
Total	21,716	18,434	128	0.69	3,282	11	0.33	0.64

5.12. Distribution of HCV Seroprevalence and First Time Vs Repeated Donors

Out of 9,993 first time donors, 0.58% (58/9993) was positive to HCV. Among 11,723 repeated donors 0.69% (81/11723) was positive to HCV. Seroprevalence was also higher in male repeated donors (0.71%) compared to male first time donors (0.66%). Likewise seroprevalence was higher among female repeated donors (0.51%) in comparison to female first time donors (0.20%). Difference of seroprevalence between first time and repeated donors was statistically insignificant.

Table 5.12: Distribution of HCV Seroprevalence and First Time Vs Repeated Donors

S.N	Donation Times	Male		Female		Total	
		No.	HCV positive	No.	HCV positive	No.	HCV positive
1.	First Time	8,076	54 (0.66%)	1,917	4 (0.20%)	9,993	58 (0.58%)
2.	Repeated	10,358	74 (0.71%)	1,365	7 (0.51%)	11,723	81 (0.69%)
Total		18,434	128	3,282	11	21,716	139
P - Value		P > 0.05		P > 0.05		P > 0.05	

5.13. Seroprevalence of Syphilis

Among the 21,716 units of donated blood tested for presence of anti-*Treponema pallidum* antibodies, 0.48% (106/21716) was found positive. Difference observed in syphilis prevalence in male and female donors was statistically insignificant.

Table 5.13: Distribution of Syphilis Seroprevalence

S.N.	Syphilis test	Male		Female		Total		P- Value
		No.	%	No.	%	No.	%	
1.	Positive	90	0.48	16	0.48	106	0.48	P > 0.05
2.	Negative	18,344		3,266		21,610		
Total		18,434		3,282		21,716		

5.14. Distribution of Syphilis Seroprevalence and Age Group

Syphilis seroprevalence was 4.08% in the age group 51 to 60 years, higher and statistically significant compared to prevalence of other age groups taken together. Mean age of Syphilis seropositive donors was 37.23 years (SD=10.76).

Table 5.14: Distribution of Syphilis Seropositive Donors and Age Group

Age Group (Years)	No. of Donors	Male			Female			Overall Sero Prevalence (%)
		No.	Syphilis positive		No.	Syphilis positive		
			No.	%		No.	%	
≤ 20	3,310	2,623	7	0.26	687	3	0.43	0.30
21- 30	9,818	8,565	15	0.17	1,253	4	0.31	0.19
31- 40	5,763	4,845	32	0.66	918	5	0.54	0.64
41- 50	2,433	2,045	21	1.02	388	3	0.77	0.98
51- 60	392	356	15	4.21	36	1	2.77	4.08
Total	21,716	18,434	90	0.48	3,282	16	0.48	

5.15. Distribution of Syphilis Seroprevalence and First Time Vs Repeated Donors

Overall seroprevalence of syphilis was slightly higher in repeated donors (0.54%) compared to first time donors (0.42%). The difference was statistically insignificant.

Table 5.15: Distribution of Syphilis Seroprevalence & First Time Vs Repeated Donors

S.N	Donation Times	Male		Female		Total	
		No.	Syphilis positive	No.	Syphilis positive	No.	Syphilis positive
1.	**First Time**	8,076	31 (0.38%)	1,917	11 (0.57%)	9,993	42 (0.42%)
2.	**Repeated**	10,358	59 (0.56%)	1,365	5 (0.36%)	11,723	64 (0.54%)
Total		18,434	90	3,282	16	21,716	106
P - Value		P > 0.05		P > 0.05		P > 0.05	

5.16. HIV and HBV Co-infection

Only one of the donors was seropositive to both HBV (HBsAg) and HIV. Thus, co-prevalence of HIV and HBV among the blood donors was 0.004% (1/21,716). Total number of HBsAg seropositive donors was 102 (0.47%) and the number of HIV seropositive donors was 27 (0.12%). Among 27 HIV seropositive donors, 1 was also positive to HBV, thus HBV and HIV co-infection was 3.70% (1/27) among HIV seropositive donors. Similarly, among 102 HBV seropositive donors only one was HIV positive with co-infection rate of 0.98% (1/102) among HBV seropositive donors. . The association of the HIV and HBV seropositivity among the blood donors was found to be statistically insignificant. Donor seropositive to both HIV and HBV was a repeated male donor, 40 years of age.

Table 5.16: Distribution of HIV and HBV Co-prevalence

S.N	Particulars	HBsAg positive	HBsAg negative	Total	P-value
1.	**HIV positive**	1 (0.004%)	26	27	P > 0.05
2.	**HIV negative**	101	21,588	21,689	
Total		102	21,614	21,716	

5.17. HIV and HCV Co-infection

Co-prevalence of HIV and HCV was 0.02% (5/21,716) among the blood donors. Among the donors, 5 of them were seropositive to both HIV and HCV. Total number of HCV seropositive donors was 139 (0.64%) and the number of HIV seropositive donors was 27 (0.12%). Among 27 HIV seropositive donors, 5 were also positive to HCV with co-infection of 18.51% (5/27) among HIV seropositive donors. Similarly, among 102 HCV seropositive donors, 5 of them were seropositive to HIV with co-infection of 3.59% (5/139) among HCV seropositive donors. The association of the HIV and HCV seropositivity among the blood donors was found to be statistically significant. Out of

the 5 HIV and HCV seropositive donors, 2 of them were repeated donors and 3 of them were first time donors. All of these donors were male, aged 21 to 50 years.

Table 5.17: Distribution of HIV and HCV Co-prevalence

S.N	Particulars	HCV positive	HCV negative	Total	P-value
1.	**HIV positive**	5 (0.02%)	22	27	P < 0.05
2.	**HIV negative**	134	21,555	21,689	
Total		139	21,577	21,716	

5.18. HIV and Syphilis Co-infection

About 106 donors were seropositive to Syphilis and 27 donors were seropositive to HIV only; but there were no donors seropositive to both HIV and Syphilis. Hence, co-infection of HIV and Syphilis was not observed during the study.

5.19. HBV and HCV Co-infection

A total number of 139 donors were HCV seropositive and 102 donors were HBsAg seropositive. Co-prevalence of HCV and HBV observed was 0.004% (1/21,716). Among 139 HCV seropositive donors, only one was positive to HBV giving HBV co-infection of 0.71% (1/139) among HCV seropositive donors. Among 102 HBV seropositive donors, only was positive to HCV, giving HCV co-infection of 0.98% (1/102) among HBV seropositive donors. Statistical analysis showed that there was no significant association between HBV seropositivity and HCV seropositivity.

Table 5.19: Distribution of HBV and HCV Co-prevalence

S.N	Particulars	HCV positive	HCV negative	Total	P-value
1.	**HBsAg positive**	1 (0.004%)	101	102	P > 0.05
2.	**HBsAg negative**	138	21,476	21,614	
Total		139	21,577	21,716	

5.20. HBV and Syphilis Co-infection

There was no any case of HBV and syphilis co-infection. However, 106 donors were positive to Syphilis and 102 donors were positive to HBsAg individually.

5.21. HCV and Syphilis Co-infection

HCV and Syphilis co-prevalence was observed as 0.009% (2/21,716). Co-infection of Syphilis among HCV seropositive donors was 1.43% (2/139) and co-infection of HCV among Syphilis seropositive donors was 1.88% (2/106). It was found that HCV seropositivity and Syphilis seropositivity was not associated among blood donors.

Table 5.21: Distribution of HCV and Syphilis Co-prevalence

S.N	**Particulars**	**Syphilis positive**	**Syphilis negative**	**Total**	**P-value**
1.	**HCV positive**	2 (0.009%)	137	139	P > 0.05
2.	**HCV negative**	104	21,473	21,577	
Total		106	21,610	21,716	

5.22. Distribution of Proportion of TTIs

Determination of seroprevalence of each infection and the co-infections revealed that of the 365 TTIs infected donors, 131 blood donors were HCV seropositive, 104 donors were Syphilis seropositive, 102 were HBV seropositive, 21 were HIV seropositive and 9 of them were co-infected with either of the two infections.

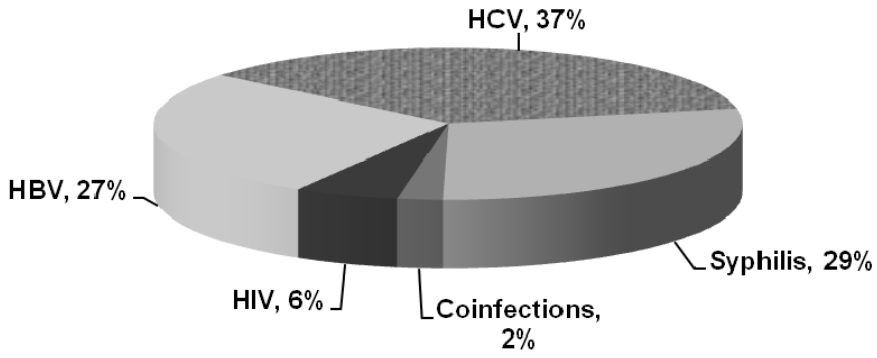

Figure 5.22: Distribution of Transfusion Transmissible Infections

5.23. Distribution of Co-infections

Among 9 of the co-infected donors, 5 of them were co-infected with HIV and HCV, 2 of them were HCV and Syphilis positive, 1 of them was HIV and HBV positive and 1 of them was HBV and HCV seropositive.

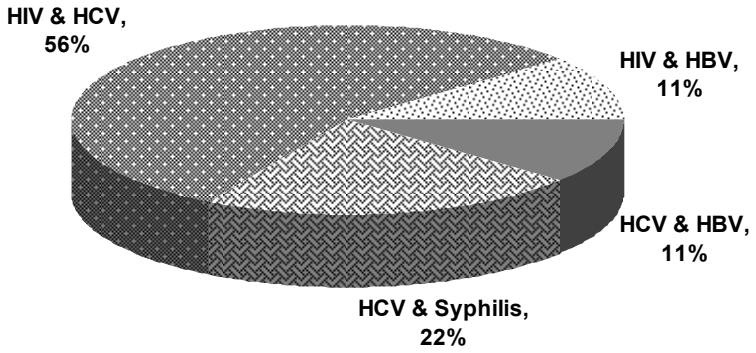

Figure 5.23: Distribution of Proportions of Different TTIs Co-infections

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1. Discussion

During the study period from March to September 2008, 21,716 units of the donated blood were screened for presence of anti-HIV, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg as per Nepal Red Cross Society, Central Blood Transfusion Service routine practice protocol. This study has been carried out based at NRCS/CBTS with the objectives to better understand the current scenario of TTIs in healthy looking blood donors.

Overall TTIs seroprevalence in the current study was 1.68% which means about 17 individuals in 1000 blood donors or healthy looking individuals was infected with either of the TTIs, which is lower than even one of the infection reported by most of the investigators :- Mathai et al., (2002) in Kerala, India (TTIs-3.1%, HBV-1.3%, HCV-1.4%, HIV-0.2%, Syphilis-0.2%), Diro et al., (2008) in Ethiopia (HIV-4.5%, HBV-8.2%, HCV-5.8%), Matee et al., (1999) in Tanzania (HIV-8.7%, HBV-11%, HCV-8%, Syphilis-12.7%), Luksamijarulkul et al., (2002) in Thailand (HIV-0.69%, HBV-4.61%, HCV-2.90%), Chaudhary et al., (2007) in Rawalpindi (HBV-2.45%, HCV-2.52%). Seroprevalence of TTIs observed in the study was lower compared to prevalence in the above countries, which may be attributed to lower infection rates of TTIs in Nepal.

Seroprevalence of TTIs was significantly higher among male donors (1.76%) compared to female donors (1.18%). TTIs considered for the study can be all transmitted by sexual transmission. The findings could indicate some risk behaviours of male such as outside socialization, multiple sex relationships etc and may also be due to less no of females coming for blood donation.

It has been observed that TTIs seroprevalence in overall was found significantly increasing with increasing age groups. Higher prevalence in higher age groups can be due to general understanding that as the age increases, the individuals gets more chances of exposure and hence are more susceptible to various sexually transmitted infections.

Seroprevalence of TTIs was relatively higher among repeated blood donors (1.74%) than among first time blood donors (1.61%). This is an alarming situation requiring immediate action in appropriate counselling of donors before and after the testing and need of communication of test results to the donors, not only to let the donor know but also to prevent him/her from donating again with the infected blood which requires testing and also proper disposal of the infected blood and product, lowering unnecessary expenditure of the NRCS, CBTS.

Overall HIV seroprevalence was found to be 0.12% (27/21,716) with higher prevalence of 0.13% among males compared to 0.06% among females, indicating high risk behaviours of males. The difference was however not statistically significant. Overall seroprevalence of HIV was relatively higher among donors of age group 31 to 40 years. Prevalence among males and females was also higher in the same age group with 0.18% and 0.1% respectively. Higher prevalence can denote higher risk behaviours such as unsafe sexual activities in this age group. Overall prevalence rate of 0.14% observed among the first time donors was higher compared to repeated donors with prevalence of 0.11%. Though prevalence was also higher among first time male donor (0.16%) compared to repeated male donors (0.11%), prevalence among repeated female donor (0.07%) was higher than among first time female donors (0.05%) ($P > 0.05$).

HIV seroprevalence observed (0.12%) was lower than that reported as 0.19% by Karki (2008) and 0.41% by Thapa (2004) in Kathmandu Valley. Prevalence was similar to the findings of Chander and Pahwa (2003) in Bhairahawa, Nepal (0.13%), but higher than in Morang (0.019%), Banke (0.095%), and Kaski (0.05%) as reported by Tiwari et al., (2008).

The lower prevalence may be due to stringent donor selection criteria, self exclusion of high risk associated groups from blood donation and increased public awareness.

The present study findings is lower than the similar other studies carried out in rest of the world: Diro et al., (2008) in Ethiopia (4.5%), Matee et al., (1999) in Tanzania (8.7%), Ogunkolo et al., (2006) in Nigeria (0.87%), Luksamijarulkul et al., (2002) in Thailand (0.69%), Sarkodie et al., (2001) in Ghana (2.4%), by Sonwane et al., (2003) in India (1.9%), Rukundo et al., (1997) in Uganda (3.9%).

However, in the present study HIV seroprevalence was higher than that reported by El-Hazmi (2004) in Central region, Saudi Arabia (0.00%), Gupta et al., (2004) in Ludhiana, India (0.084%), Yumiko et al., (2007) in Phillipines (0.006%), Ayala et al., (1997) in Mexico (0.02%), Tserenpuntsag et al., (2008) in Mongolia with none of the seropositive donors.

Current HIV seroprevalence was similar to those reported by Chattoraj et al., (2008) in Calcutta, India (0.13%), Andrade Neto et al., (2002) Brazil (0.14%).

Differences in seroprevalence can be attributed to lifestyle of general population, donor selection criteria, sensitivity and specificity of test kits used along with preference of diagnostic algorithms.

The present study results showed that 0.47% of blood donors were found to be HBsAg seropositive with male dominance of 0.5% over 0.3% prevalence in females; however the difference was not statistically significant. Overall seroprevalence of HBsAg was highest among the donors of age group 41 to 50 (0.78%). The finding can only refer to factors such as infections acquired in later years of life which could not be prevented without hepatitis B vaccination in early years. Requirement of vaccination is thus recommended even for adults. Prevalence among male and female donors in this age group was 0.83% and 0.78% respectively. HBsAg seroprevalence rate among the first

time donors (0.51%) was more than among repeated donors (0.43%). Pattern of prevalence among first time male donor (0.55%) and repeated male donor (0.45%), first time female donor (0.31%) and repeated female donor (0.29%) also indicated that seroprevalence of HBsAg among first time donors was higher compared to the repeated donors ($P > 0.05$).

Prevalence of HBV determined (0.46%) was less marked than the findings in the Kathmandu valley by Joshi et al., (2002) as 1.26%, Ghimire et al., (2006) as 0.88%. Current prevalence was similar to the reporting of Chander and Pahwa (2003) in Bhairahawa, Nepal (0.45%) and Karki et al., (2008) in Kathmandu (0.53%). Comparing this study with similar investigation conducted among blood donors in Kathmandu Valley indicate that HBV prevalence seems to be decreasing. The decreasing trend can be attributed to various factors such as availability of vaccine and mass vaccination programmes, increasing awareness of HBV as well as self exclusion of high risk groups from blood donation, strict donor selection by health officers or even specificity of the test kit used.

HBV seroprevalence in the present study (0.46%) was lower than the prevalence reported by Luksamijarulkul et al., (2002) in Thailand (4.61%), Chattoraj et al., (2008) in Calcutta, India (0.99%), Tserenpuntsag et al., (2008) in Mongolia (8.1%), Chaudhary et al., (2007) in Rawalpindi (2.45%), Bhattacharya et al., (2007) in West Bengal (1.28% -1.66%), Matee et al., (1999) in Tanzania (11%), El-Hazmi (2004) in Central region, Saudi Arabia (1.5%).

Overview of the similar investigation shows that the HBV prevalence in Kathmandu is lower than the other countries. These variations might be due to geographical variations, risk behaviours of the population under study, kits and strategy used for tests, period of study and donor selection criteria or even awareness status of the people in particular area. The figure determined here is the prevalence among healthy donors and the findings do differ from the observations of Shrestha (2002) as 0.93%, Manandhar et al.,

(1998) as 4.0%, Sawayama et al., (1999) as 1.1%, Shrestha (1990) as 0.9%, due to differences in population selected for study.

HCV seroprevalence during the study period was found to be 0.64% with high prevalence of 0.69% among male donors compared to 0.33% female donors. The difference of prevalence in males and females was statistically significant. Results indicate that prevalence of HCV is similar in age groups 21 to 30 years (0.76%) and 31 to 40 years (0.72%). Infections in these age groups can be referred to both the established factors unsafe sexual practices and IDUs. Among males, prevalence in age group 21 to 30 years was 0.80% and 31 to 40 years was 0.82%, which was similar. Whereas among females, HCV prevalence observed in age group 21 to 30 years was 0.47%, which was higher compared to 0.21% in 31- 40 years age group. HCV seroprevalence among repeated donors was 0.69% and thus higher than among first time donors with prevalence of 0.58%. Similar pattern of higher prevalence among repeated male donors (0.71%) compared to first time male donor (0.66%) and among repeated female donor (0.51%) compared to first time female donor (0.2%) ($P > 0.05$). This is also another alarming situation outlining needs for improvement in counselling and need for strategies in reporting the test results to the donors by NRCS, CBTS.

HCV seroprevalence determined in the current study (0.64%) was lower than reported as 1.10% by Joshi et al., (2002) in blood donors and as 1.7% by Sawayama et al., (1996) among general population. However, the prevalence is higher than in Bhairahawa, Nepal reported as 0.13% by Chander and Pahwa, (2003). Current figure was similar to prevalence of 0.71% reported by Karki et al., (2008). Present seroprevalence was fairly higher than as reported by Singh (1998) among blood donors (0.3%) and by Shrestha (2006) among healthy males seeking jobs abroad (0.35%).

Present HCV seroprevalence was lower than the investigations of Chaudhary et al., (2007) in Rawalpindi, Pakistan (2.52%), Diro et al., (2008) in Ethiopia (5.8%), Gupta et al., (2004) in Ludhiana, India (1.09%), Matee et al., (1999) in Tanzania (8%),

Tserenpuntsag et al., (2008) in Mongolia(8.7%), Darwish et al., (1992) and Bassily et al., (1995) in Egypt (14.4-26.6%), Wang et al., (1994) and Zhang et al., (1992) in Beizing and Wuhan of China (1%), Duraisamy et al., (1993) in Malaysia (1.6%), Apichartpiyakul et al., (1999) and Songsivilai et al., (1997) in Thailand (3.2-5.6%).

Current HCV prevalence was higher than as reported by El-Hazmi (2004) in Central region, Saudi Arabia (0.4%), Bhattacharya et al., (2007) in West Bengal (0.28-0.35%), Chattoraj et al., (2008) in Calcutta (0.19%).

Differences in HCV seroprevalence must have been due to variation in geographical distribution, population differences in terms of lifestyle, awareness, sensitivity and specificity of tests, donor selection criteria, etc.

Overall seroprevalence of syphilis was observed to be 0.48% in both male and female. Similar prevalence was observed among male and female donors. Seroprevalence of syphilis among donors increased with the age. A prevalence of 4.08% was among the donors of age group 51 to 60 years. As the detection was both IgG and IgM antibodies specific to *Treponema Pallidum,* even the treated cases, with the past infection beside the primary stage cases could have been detected. Seroprevalence of syphilis was higher in repeated donors (0.54%) compared to first time donors (0.42%). However prevalence among first time female donors was 0.57%, thus higher than among repeated female donors with 0.36%. In males, repeated donors showed higher prevalence of 0.56% compared to first time donors with prevalence of 0.38% ($P > 0.05$).

Syphilis seroprevalence observed in the present study (0.48%) was lower than reported by Gupta et al., (2004) in Ludhiana, India (0.85%), Matee et al., (1999) in Tanzania (12.7%), Tserenpuntsag et al., (2008) in Mongolia (2%).Chattoraj et al.(2008) in Calcutta, India (0.62%) and Bhattacharya et al., (2007) in West Bengal, India (0.68% - 0.8%). Finding was comparable to the investigations of Chander and Pahwa, (2003) in

Bhairahawa (0.39%). The difference may be attributed to test kit, geography, inclusion criteria etc.

Although present study revealed very small number of TTIs co-infections, donated blood should be continuously screened for others when one of them is found positive to benefit the prevention of further transmission and also for appropriate treatment, if any.

6.2. Conclusion

The finding of this study was that TTIs prevalence among blood donors in Kathmandu is low in comparison to the study conducted elsewhere. The prevalence of HIV, HCV, HBV and syphilis also seems to be decreasing compared to previous studies. TTIs prevalence was dominant among the males than females. Both the first time and repeated donors were equally likely to be infected with the TTIs, indicating the need of immediate action for strengthened counselling of the donors before donation, reporting the results of the tests after donation and follow up counselling for prevention of further transmission of the infection. Blood donors of higher age groups revealed higher seroprevalence. The most prevalent infection was HCV followed by Syphilis, HBV and then HIV. Higher co-infection was observed as HIV and HCV co-infection. Other co-infections observed were HCV and Syphilis, HIV and HBV, and HBV and HCV co-infections. No Syphilis and HIV co-infection, Syphilis and HBV co-infection were observed in the study population.

CHAPTER VII

7. SUMMARY AND RECOMMENDATION

7.1. Summary

The study was carried out to determine the overall magnitude of Transfusion Transmissible Infections in blood donors at NRCS, CBTS. The infections under study were HIV, hepatitis B, hepatitis C and Syphilis. Seroprevalence of each infection were determined and TTIs seroprevalence was estimated. The prevalence was studied on the basis of sex, age groups and times of donation. Co-infections prevalent were also determined. Major findings of the study can be summarized as follows:

1. The study included a total of 21,716 blood donors, donating blood only once during the 6 months period. Of these, the numbers of male blood donors were 18,434 (84.9%) and the numbers of female donors were 3,282 (15.1%). Numbers of repeated donors were 11,723 (53.98%) and the first time donors were 9,993 (46.02%). Age of the blood donors was between 18 to 60 years with the mean age of 29.56 years (SD = 8.74).

2. Overall seroprevalence of the TTIs was 1.68% (365/21,716) with seroprevalence of 1.76% (326/18,434) among male donors and 1.18% (39/3,282) among female donors ($P < 0.05$).

3. Highest overall TTIs seroprevalence of 5.1% (males 5.33%, females 2.7%) was observed in the age group 51 to 60 years, compared to other groups. ($P < 0.05$).

4. Seroprevalence of TTIs among repeated donors was 1.74% (male 1.80%, female 1.24%), which was higher than among first time donors with prevalence of 1.61% (male 1.72% female 1.14%) ($P > 0.05$).

5. Seroprevalence of HIV among the blood donors was 0.12%. Higher seroprevalence of 0.13% was observed in the male blood donors compared to female blood donors with seroprevalence of 0.06% ($P > 0.05$).

6. Highest HIV seroprevalence of 0.17 % (male 0.18%, female 0.10 %) was seen in donors of age group 31 to 40 years, compared to other groups ($P > 0.05$).

7. Overall seroprevalence of HIV among first time donor was 0.14% (male 0.16%, female 0.05%) and among repeated donors was 0.11% (male 0.11% female 0.07%) ($P > 0.05$).

8. Overall seroprevalence of HBsAg was 0.47%, with 0.5% prevalence among male donors and 0.30% among female donors ($P > 0.05$).

9. Highest HBsAg seroprevalence of 0.78% (male 0.83%, female 0.51%) was observed in the age group 41 to 50 years, compared to other groups ($P < 0.05$).

10. Seroprevalence of HBsAg in the first time donors was 0.51% (male 0.55%, female 0.31%), which was higher than in the repeated donors with 0.43% (male 0.45%, female 0.29%) ($P > 0.05$).

11. HCV seroprevalence was 0.64% with male dominance of 0.69% in comparison to female donors with 0.33% ($P < 0.05$).

12. Highest HCV seroprevalence of 0.76% (male 0.80%, female 0.47%) was observed in the age group 41 to 50 years, compared to other groups ($P < 0.05$).

13. HCV seroprevalence among repeated donors was 0.69% (male 0.71%, female 0.51%) which was higher than among the first time donors with prevalence of 0.58% (male 0.66%, female 0.20%) ($P < 0.05$).

14. Syphilis prevalence was 0.48% with prevalence of 0.48% among male and also 0.48% among female donors ($P > 0.05$).

15. A very high syphilis seroprevalence of 4.08% was observed among donors of age group 51 to 60 years. In this age group, prevalence among males was 4.21% and among females was 2.77% ($P < 0.05$).

16. Syphilis seroprevalence was higher in repeated donors with 0.54% (male 0.56%, female 0.36%) compared to first time donors with 0.42% (male 0.38%, female 0.57%).

17. HIV/HCV coprevalence was 0.02%, relatively higher to co-prevalence of HCV/Syphilis (0.09%), HCV/HBV (0.004%) and HIV/HBV (0.004%). The co-infection with HCV and HIV seropositive donors (18.51%) was higher compared to other co-infection observed.

18. Proportion of HCV seroprevalence was 35.89%, Syphilis seroprevalence was 28.49%, HBV seroprevalence was 27.94%, HIV seroprevalence was 5.75% and co-infection was 2.46% among the total TTIs observed.

19. Of the 2.46% co-infection observed in the total TTIs, proportion of HIV/HCV co-infection was 55.55%, HCV/Syphilis co-infection was 22.22%, HIV/HBV co-infection was 11.11% and HCV/HBV co-infection was 11.11%.

7.2. Recommendation

Based on the findings of the study, following recommendations are put forward for concern:

1. Mandatory screening of all the TTIs concerned should be continued following standard algorithms developed by WHO/Government.

2. TTIs seroprevalence in first time and repeated was found similar, so donor notification and counselling should be immediately implemented to make the regular blood donors as the safe source of blood.

3. Younger donors may be encouraged for decreasing the chances of transmission of TTIs.

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