SEROPREVALENCE OF HEPATITIS C AND HIV AMONG BLOOD DONORS IN KATHMANDU VALLEY

A

Dissertation

Submitted to the Central Department of Microbiology

Tribhuvan University

In Partial Fulfillment for the Award of the Degree of

Master of Science in Microbiology

(Medical)

By

Surendra Karki

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

2008

RECOMMENDATION

This is to certify that **Mr. Surendra Karki** has completed this dissertation work entitled "**Seroprevalence of Hepatitis C and HIV among Blood Donors in Kathmandu Valley**" as a partial fulfillment 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.

Dr. Prakash Ghimire	**Mr. Bishnu Raj Tiwari**	**Dr. Manita Rajkarnikar**
Associate Professor	Quality Officer/	Director
Central Department	Microbiologist	Nepal Red Cross Society
of Microbiology,	Nepal Red Cross Society,	Central Blood Transfusion,
Tribhuvan University	Central Blood Transfusion	Service, Kathmandu
Kirtipur, Kathmandu	Service, Kathmandu	
Date:……	Date:……..	Date:……..

CERTIFICATE OF APPORVAL

On the recommendation of **Dr. Prakash Ghimire, Dr. Manita Rajkarnikar and Mr. Bishnu Raj Tiwari**, this dissertation of **Mr. Surendra Karki** entitled "**Seroprevalence of Hepatitis C and HIV among Blood Donors in Kathmandu Valley**" has been approved for the examination and is submitted to Tribhuvan University in partial fulfillment of requirements for M.Sc degree in Microbiology.

_____

Dr. Anjana Singh
Head of Department
Central Department of Microbiology,
Tribhuvan University
Kirtipur, Kathmandu
Nepal

Date:……..

BOARD OF EXAMINERS

Recommended by:

Dr Prakash Ghimire, Ph.D.
Supervisor

Mr. Bishnu Raj Tiwari, M.Sc
Supervisor

Dr. Manita Rajkarnikar, MD
Supervisor

Approved by:

Dr. Anjana Singh, Ph.D.
Head of Department

Examined by:

Dr. Padam Bahadur Chand
Director, NCASC
External Examiner

Dr. Shrikant Adhikari, Ph.D.
Associate Prof., CDM, TU
Internal Examiner

Date:…….

ACKNOWLEDGEMENT

First of all, I would like to express my sincere and deepest gratitude to my respected Supervisors Dr. Prakash Ghimire, Associate Professor, Central Department of Microbiology, Tribhuvan University, Mr. Bishnu Raj Tiwari, Quality Control Officer, Dr. Manita Rajkarnikar, Director, Nepal Red Cross Society (NRCS), Central Blood Transfusion Service (CBTS), Kathmandu for their expert guidance, valuable suggestions and continuous support during my entire period of research work.

I am very much grateful to Dr. Manita Rajkarnikar, Director, NRCS, CBTS for cooperation in making Laboratory and data accessing facilities available throughout the study.

I am especially thankful to Mr. Anil Maharjan, Mr. Prem Man Byanjankar and Mr. Shrawan Shrestha for their support in Laboratory work and to all Staffs of NRCS, CBTS for their help during data collection.

I would like to express my sincere gratitude to Associate Prof. Dr. Dwij Raj Bhatta, Asst. Prof. Mr. Binod Lekhak, Asst. Prof. Ms. Saila Basnyat, and all the respected teachers and Staffs of Central Department of Microbiology for their helping hands in all possible ways.

I would like to express my sincere respect and many many thanks to all blood donors who were not only the part of this study but also the part of thousands of new lifes.

Finally, I would like to express my deepest gratitude to my family for their blessing, continious motivation, encouragement and support, which has brought me to this far.

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January, 2008 Surendra Karki

ABSTRACT

During the study period of December 1st 2006 to September 1st 2007, a total of 33,255 blood donors in Kathmandu Valley were screened for Anti HCV antibodies and Anti HIV-1 and 2 antibodies using third generation ELISA kits and automated ELISA Processor (BEP III) in Serology Laboratory of NRCS, CBTS.

The seroprevalence of hepatitis C was observed 0.66%, more in Volunteer donors (0.69%) than in Replacement donors (0.4%) ($P < 0.05$). In male donors the seroprevalence was 0.7% and in female donors it was 0.39% ($P < 0.05$). Surprisingly, the seroprevalence was almost similar in first time (0.65%) and repeat donors (0.67%) ($P > 0.05$). HCV was found to be most prevalent (0.82%) in age group of 21-30 years ($P < 0.05$), in males it was most prevalent (0.88%) in age group of 21-30 years ($P < 0.05$) and in females it was most prevalent (0.47%) in the age group of 41-50 years ($P > 0.05$). The seroprevalence of HIV was observed 0.19%, more in Volunteer donors (0.2%) than in Replacement donors (0.13%) ($P > 0.05$). In male donors the seroprevalence was 0.2% and in female donors it was 0.16% ($P > 0.05$). The seroprevalence was almost similar in first time (0.2%) and repeat donors (0.19%) ($P > 0.05$). The seroprevalence of HIV was found to be highest (0.24%) in age group of 21-30 years in male donors ($P > 0.05$) and in female donors it was highest (0.23%) in age group of 41-50 years ($P > 0.05$).

The coprevalence of HCV and HIV was observed 0.02%. HCV coprevalence was observed in 10.76% of HIV seropositive donors whereas HIV coprevalence was observed in 3.16% of HCV seropositive donors. HCV and HIV seropositivity was associated with each other ($P < 0.05$).

Hence, HCV and HIV seroprevalence among blood donors in Kathmandu Valley was 0.66% and 0.19% respectively.

Key words: HCV, HIV, Seroprevalence, Coprevalence, Blood donors

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AIDS:	Acquired Immunodeficiency Syndrome
ALT:	Alanine Amino Transferase
Anti HCV:	Antibodies to HCV
Anti HIV1/2:	Antibodies to HIV type 1or type 2or both
CBTS:	Central Blood Transfusion Service
CDC:	Center for Disease Control
EIA:	Enzyme Immuno Assay
FHI:	Family Health International
HAART:	Highly Active Anti-Retroviral Therapy
HCV:	Hepatitis C Virus
HIV-1:	Human Immunodeficiency Virus type 1
HIV-2:	Human Immunodeficiency Virus type 2
IDU:	Intravenous Drug Users
IF:	Immunofluroscence
LIA:	Line Immunoassay
NANB:	Non-A, Non-B
NCASC:	National Center for HIV/AIDS and STD Control
NRCS:	Nepal Red Cross Society
REDS:	Retrovirus Epidemiology Donor Study
RIBA:	Recombinant Immunoblot Assay
UNAIDS:	Joint United Nations Programme on HIV/AIDS
WB:	Western Blot
WHO:	World Health Organization

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CHAPTER -I

1. INTRODUCTION

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of viral hepatitis are caused by Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis A Virus (HAV), Hepatitis E Virus (HEV) and Hepatitis D Virus (HDV). HAV and HEV are transmitted via fecal oral route and they never lead to a chronic disease. HBV, HCV and HDV are transmitted via parenteral routes and they often lead to a chronic disease but development of chronic infection depends on various factors (Brooks et al., 2004, Fox et al., 2003). Hepatitis G Virus (HGV) infection, transmitted via parenteral routes is also prevalent worldwide but its clinical significance remains uncertain (Shuhart et al., 2003).

Hepatitis C is an acute or chronic necroinflammatory disease of the liver that is due to infection with Hepatitis C Virus (Thomas et al., 2000). It continues to be a major disease burden in the world. In 1999, WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people worldwide and 3 to 4 million people are newly infected each year. The prevalence rate of HCV in South-East Asia has been estimated to be 2.15% (WHO, 1999). Among the viral hepatitis, HCV is dreadful in the aspect that its morbidity rate is high as it establishes a state of chronic infection in as many as 85% of acutely infected patients whereas about 15% of acutely infected patients spontaneously clear the infection (Alter et al., 1992, Mattsson et al., 1993, Barrera et al., 1995, Villano et al., 1999).

In Nepal, Seroprevalence of Anti HCV antibodies among healthy looking general population has been reported ranging from 0.3-1.7% (Singh, 1992, Sawayama et al., 1996, Shrestha et al., 1998, Joshi, 1999, Shrestha, 2003, Shrestha, 2006). Shrestha et al. (1997) have also reported a HCV infection rate of 60% among illicit intravenous drug users and 5% among maintenance haemodialysis. In a recent study, the seroprevalence

of Anti HCV antibodies among healthy adults has been estimated to be 0.75% against the 85.5% seroprevalence among injecting drug users from Kathmandu (Shrestha, 2003).

HIV-1 discovered in 1983 (Barre-Sinoussi et al., 1983), has been shown to be virologically and serologically associated with early and late stages of AIDS (Gallo et al., 1984, Popovic et al., 1984) and has been described as more aggressive virus and responsible for global pandemic of AIDS (Schupbach, 2003). HIV-2 discovered in 1986 (Clavel et al., 1986), has been reported to be less pathogenic and rarely causing AIDS (Schim van der Loeff et al., 1999).

The number of people living with HIV in 2007 has been reported to be 33.2 million (30.6-36.1 million) (UNAIDS, 2007) which is about 6.3 million less than as estimated in 2006. A total of 39.5 million (34.1–47.1 million) people were living with HIV/AIDS in 2006, 2.6 million more than in 2004. In 2006 it had been reported that the number of people living with HIV has increased in every region of the world (UNAIDS, 2006). However, the recent AIDS Epidemic update of 2007 has shown a decrease in global HIV prevalence and it has been reported that the seroprevalence of HIV has been leveling off in many countries and is decreasing in Sub-Saharan Africa (AIDS Epidemic update, 2007). WHO has reported that the downward revision in prevalence of 6.3 million is largely due to improved and expanded surveillance, data collection and methodologies.

The first case of AIDS in Nepal was reported in 1988 (Gurubacharya et al., 1993). As of December 2007, National Center for AIDS and STD Control (NCASC) has officially confirmed 10,546 HIV positive cases and 1,610 confirmed cases of AIDS in Nepal. Among the total 10,546 HIV positive cases 24 cases (0.23%) are described to be associated with blood transfusion or organ transplantation (NCASC, 2007). UNAIDS has estimated the adult (15-49 years) HIV prevalence rate of 0.5% by the end of 2005 in general population whereas the number of people living with HIV in the same time has

been estimated to be 74,000 (UNAIDS, 2005). UN Nepal information platform has reported that over the last few years HIV/AIDS epidemic in Nepal has gained ground and Nepal has progressed from a low prevalence country to a country with 'concentrated epidemic'. Most of the HIV infections in Nepal have been caused by HIV-1 though recently seroevidence of HIV-2 has been reported from Bhairahava, Nepal (Chander et al., 2004). A situation analysis study of HIV/AIDS has reported that the young people, Mobile populations, Female Sex Workers, Men who have sex with men, Injecting drug users, and Children as the most vulnerable to HIV/AIDS in Nepal (Pokhrel et al., 2000). Around 10 years ago Nepal was described as a country having comparatively lower prevalence of HIV/AIDS compared to other countries in Southeast Asia. Seasonal migration to Indian Cities for seeking job and sexual trafficking across a porous Indian border (Seddon, 1998), fuelled by the bloody Maoist conflict, has raised Nepal's HIV prevalence second highest in the region after India (Singh et al., 2005).

Coinfection with HCV in HIV infected individuals is common, presumably due to the shared route of transmission of these viruses. Various studies suggest that in HIV as well as HCV positive individuals, progression to Cirrhosis and hepatocellular carcinoma is likely to occur more frequently and at a faster rate (Soto et al., 1997, Benhamou et al., 1999). Study on HCV/HIV coinfection is important for management of the Highly Active Antiretroviral Therapy (HAART) (Nelson et al., 2005).

Present study has been conducted among blood donors in Kathmandu Valley in order to study the HCV and HIV seroprevalence among different groups and subgroups of blood donors. Blood donors were chosen as study population because study on them would give at least the lowest range of HCV and HIV seroprevalence among the healthy looking general population who are not aware of their HCV or HIV seropositive status and might be actively involved in transmitting the infection to others. Thus, the data generated during the study would be helpful in formulating strategies for safe blood transfusion in Nepal.

CHAPTER-II

2. OBJECTIVES

2.1 General Objective

To study seroprevalence of HCV and HIV among blood donors.

2.2 Specific Objectives

- To study seroprevalence of HCV and HIV in male and female blood donors.
- To study seroprevalence of HCV in Volunteer and Replacement blood donors.
- To study seroprevalence of HIV in Volunteer and Replacement blood donors.
- To study seroprevalence of HCV in First time and Repeat blood donors.
- To study seroprevalence of HIV in First time and Repeat blood donors.
- To study coprevalence of HCV and HIV in blood donors.
- To compare the age wise trend of HCV and HIV seroprevalence in blood donors.

CHAPTER-III

3. LITERATURE REVIEW

3.1 Hepatitis C

Hepatitis C is an acute or chronic necroinflammatory disease of the liver that is due to infection with a unique hepatotropic virus, named as Hepatitis C virus (HCV). It is most efficiently transmitted via parenteral routes. The major clinical manifestation is progressive hepatic fibrosis, which leads to cirrhosis and increased risk of hepatocellular carcinoma (Thomas et al., 2000). It has been estimated that more than 170 million people (about 3% of world population) are infected with hepatitis C virus (WHO, 1999).

3.1.1 Discovery of HCV

The disease was first recognized in the early 1970s, when serologic test for hepatitis A virus (HAV) and hepatitis B virus (HBV) became generally available. It was noted at that time that most cases of transfusion associated hepatitis were not caused by either of these viruses, leading to the term non-A, non-B hepatitis (NANB) (Prince et al., 1974). Studies in chimpanzees confirmed the blood borne non-A, non-B hepatitis and called the agent as hepatitis C virus (Choo et al., 1989). This finding rapidly led to the molecular cloning of the complete viral genome and other major discoveries, including recognition of the proclivity of the virus to establish persistent infection and its strong association with chronic hepatitis, cirrhosis and hepatocellular carcinoma (Choo et al., 1991).

3.1.2 Classification.

The structure, genomic organization, and replication cycle of HCV are similar to those of members of the family flaviviridae, yet sufficiently distinct to merit classification within a separate, novel genus, Hepacivirus (Thomas et al., 2000). Thus, HCV is classified under family Flaviviridae, genus Hepacivirus. Various HCV viral genomes can be differentiated by RNA sequence analysis into at least six major genotypes (clades) and more than seventy subtypes (Brooks et al., 2004).

3.1.3 Virion properties

HCV is spheric, enveloped, single-stranded, positive sense RNA virus particle of 55-65 nm in diameter (Kaito et al., 1994). It has an internal nucleocapsid containing the RNA genome, closely surrounded by a lipid envelope containing the E1 and E2 glycoproteins. The envelope glycoproteins of HCV show several potential sites for N-linked glycosylation (4 sites in E1 and 11 sites in E2) and it is likely that the addition of relatively large carbohydrate groups is a major structural feature of the virus surface that may influence the ability of antibody to neutralize infectivity (Simmonds et al., 1998).

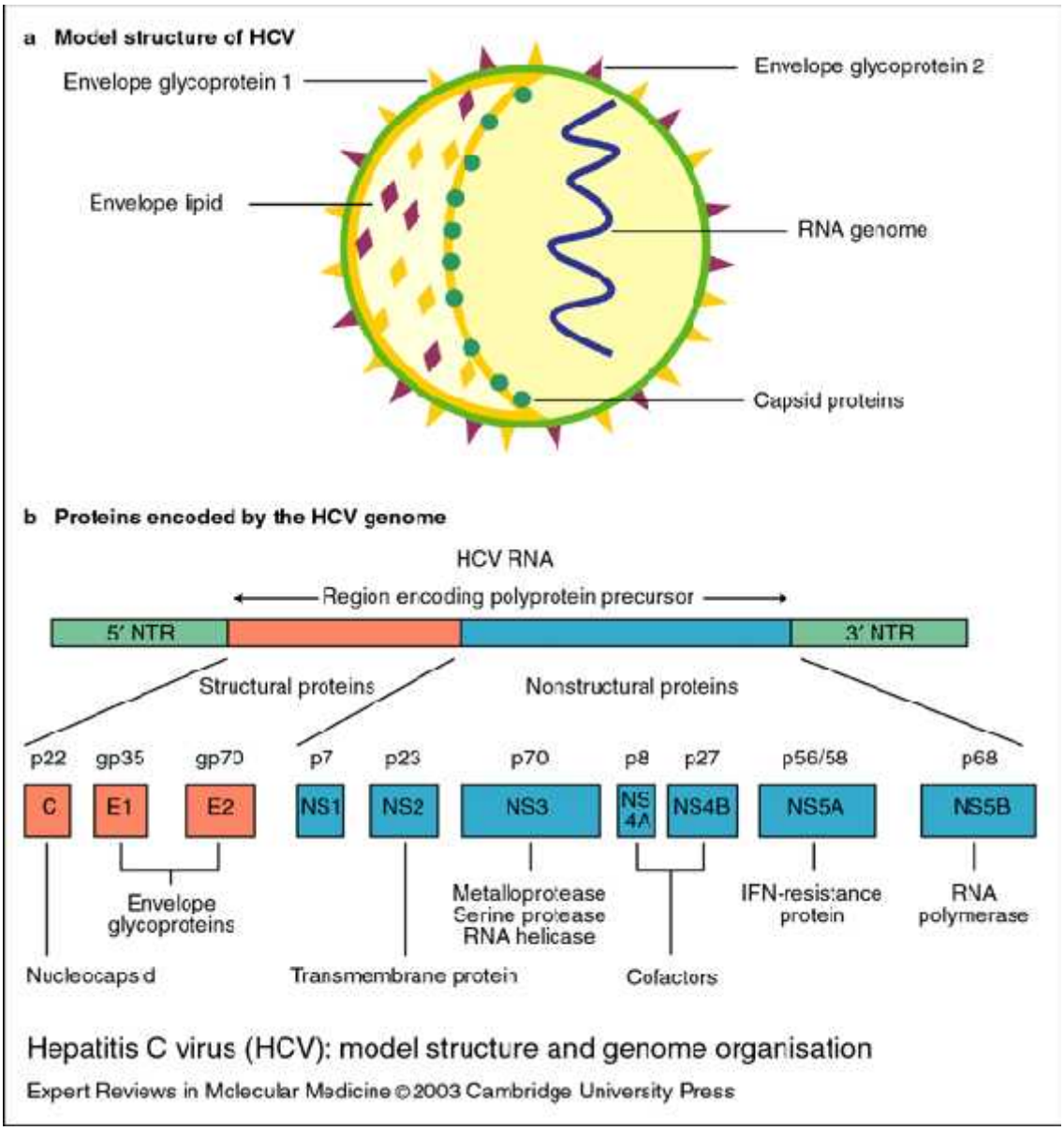
HCV is inactivated by exposure to chloroform, ether and other organic solvents and by detergents. The effect of heat and other inactivating procedures have been discovered by studies of the infectivity of products manufactured from plasma such as the factor VIII and IX concentrates used to treat clotting disorders. For example, dry heat treatment at 60^0 C, organic solvents (n-heptane) and detergents efficiently remove infectivity for HCV in recipients (Mannucci, 1993).

3.1.4 Organization of Hepatitis C Virus Genome

The genome of HCV is single stranded RNA of positive (protein coding) polarity. Although the genomic RNA of most positive strand RNA viruses is infectious, this has

not yet been unequivocally demonstrated for HCV. The putative genome of HCV is approximately 9.6 kb in length, as shown in figure 3.1b. The 5' untranslated region (UTR) is highly conserved with 92% homology among different HCV types. The 5'UTR and the initial part of core region form the HCV internal ribosome entry site (IRES), which is essential in initiating viral translation. Translation of the genome occurs along a single open reading frame, which encodes both structural and nonstructural proteins (Shuhart et al., 2003). The HCV 5' UTR is approximately 341 nucleotides in length. It demonstrates extensive secondary and tertiary RNA structure (Thomas et al., 2000).

Figure 3.1: Structure of Hepatitis C Virus and its genome



Source:www-ermm.cbcu.cam.ac.uk/03006938h.htm

The 3' UTR consists of a relatively variable 30 nucleotide segment downstream of the termination codon that is followed by a highly variable polyU (C) tract of approximately 100 nucleotides but apparently variable in length. Downstream of the poly U (C) tract, there is a highly conserved 98 base sequence (Tanaka et al., 1996). This highly structured 3' terminal 98-base sequence is the most conserved sequence of the HCV genome, and it is required for the viral replication (Thomas et al., 2000).

3.1.5 Proteins of HCV

The approximately 9 kilobase open reading frame encodes a polyprotein that is cotranslationally processed into at least 10 proteins. These include from the amino terminus, four structural proteins and six non-structural proteins that are involved in replication of the viral RNA. The structural proteins consists of the core protein (C, 16-21 kDa), envelope protein 1 (E1, 31-37 kDa), and envelope protein 2 (E2, 61-72 kDa). The amino terminus of E2 contains hypervariable regions (HVR1 and HVR2) that exhibit significant variation among HCV isolates. This variation allows the virus to escape neutralizing antibodies (Shimizu et al., 1994). There is a 7 kDa peptide (p7) located at the carboxyterminus of E2 and its function is unknown (Lin et al., 1994). The nonstructural proteins consists of NS2 (23 kDa), NS3 (70 kDa), NS4A (8 kDa), NS4B (27 kDa), NS5A (58 kDa), and NS5B (68 kDa). Two well conserved regions within NS4A have made it possible to design the enzyme linked immunosorbent assay (ELISA) that can detect HCV genotype-specific antibody.

3.1.6 Epidemiology

HCV is most often transmitted by percutaneous exposure to blood. However, the predominant modes of transmission may change over time and differ between and even within countries. In economically developed countries, most new HCV infections are related to illicit injection drug use, though blood transfusion were once important source of infection. HCV may also be transmitted between sexual partners and from a mother to her infant, though these are relatively uncommon compared with transmission of Hepatitis B Virus (Thomas et al., 2000).

3.1.6.1 Prevalence of HCV

HCV continues to be a major disease burden worldwide. In 1999, the WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people

worldwide and 3 to 4 million people are newly infected each year. The prevalence rate of HCV in South-East Asia has been estimated to be 2.15% (WHO, 1999).

Although, the prevalence of HCV is remarkably similar in many parts of the world, there are few distinct geographic regions in which infection is especially common. In Egypt, the seroprevalence of HCV is 10-30% in general population (Darwish et al., 1993, Hibbs et al., 1993, Abdel-Waha et al., 1994). Among the Asian countries, high seroprevalence rate have been found in certain geographic regions of Japan and Taiwan. In such areas HCV infection is more prevalent among persons older than 40 years and uncommon in those younger than 20 years (Nakashima et al., 1995). It has been suggested that such type of cohort effect is due to the mode of transmission that occurred through a practice that has been discontinued, such as traditional folk remedies (e.g. acupuncture) or reuse of needles for injection (Kiyosawa et al., 1994, Noguchi et al., 1997). Although not confirmed, it has been suspected that a national campaign to treat schistosomiasis infections was responsible for many infections in Egypt. Injection therapy for schistosomiasis was administered to entire villages until 1870s, and needles were frequently reused (Abdel-Waha et al., 1994). Seroprevalence of HCV among blood donors has been found to vary slightly in different parts of the world except in those parts where it has occurred in epidemic forms. Among the male blood donors in Karachi, Pakistan, the seroprevalence of HCV has been reported to be 1.8% with a trend of increasing proportion of donors from 1998-2002 (Aktar et al., 2004).

Table 1: Prevalence rate of HCV according to WHO Region

WHO Region	Total population (Millions)	Hepatitis C prevalence Rate %	Infected Population (Millions)	Number of Countries by WHO Region where data are not available
Africa	602	5.3	31.9	12

Americas	785	1.7	13.1	7
Eastern Mediterranean	466	4.6	21.3	7
Europe	858	1.03	8.9	19
South-East Asia	1500	2.15	32.3	3
Western Pacific	1600	3.9	62.2	11
Total	5811	3.1	169.7	57

Source: Weekly Epidemiological Record No 49, 1999, WHO

In Egypt, the HCV seroprevalence among blood donors has been found to vary from 14.4% to 26.6% (Darwish et al., 1992). Seroprevalence rates among blood donors in Saudi Arabia and Yemen have been reported to be 1.8 % and 2.1% respectively (El Guneid et al., 1993, Al Faleh et al., 1995). Intermediate rates of seroprevalence of HCV have been reported out of Asia. It has been reported that from 1995-2000, 0.49% HCV seropositive donors were detected among 3,485,648 blood donors in Japan (Tanaka et al., 2004), while seropositivity rate of 0.98% had been reported in 1992 among 10,905,489 blood donors (Yamaguchi et al., 1994). In China, prevalence rate of around 1% have been reported from Beizing and Wuhan (Zhang et al., 1992, Wang et al., 1994). Seroprevalence rate from Malaysia has been reported to be around 1.6% and in Singapore it has been reported to be around 0.54% (Duraisamy et al., 1993, Kuperan et al., 1993). Slightly higher rates of HCV, around 3.2% to 5.6% have been reported from Thailand (Songsivilai et al., 1997, Apichartpiyakul et al., 1999). In New Delhi of India, the seroprevalence rate has been reported to be 1.8% among blood donors (Panigrahi et al., 1997). In Africa, seroprevalence rate of 1.6% has been reported from Ethiopia and 0.9% from Kenya (Frommel et al., 1993, Ilako et al., 1995).

In developed nations, the HCV prevalence is typically 1-2% in the general population and less than 0.5% among blood donors. HCV seroprevalence of around 1.8% of the

general population with 2.7 millions having persistent infection has been reported in United States (Alter et al., 1999).

3.1.6.2 Prevalence of HCV in Nepal

In a study among general population, HCV seroprevalence has been reported to be 0.6%, in which second generation enzyme immunoassay and HCV RNA Polymerase Chain Reaction were used for screening a total of 2,860 healthy adults and 94% among IDUs (Shrestha et al., 1998). Similarly, in another study, HCV seroprevalence has been reported to be 1.7% in 458 sera tested from inhabitants of Bhadrakali (Suburban) and Villages of Khotang (Sawayama et al., 1999). In another study, the seroprevalence of HCV among blood donors was 0.3% among 1304 donors tested (Singh, 1992). Another study has reported a HCV infection rate of 60% among illicit intravenous drug users and 5% among maintenance haemodialysis (Shrestha et al., 1997). Similarly, according to a more recent study, the seroprevalene of HCV among injecting drug abusers from Kathamandu has been reported to be 85.5% against a 0.75% seropositivity rate of healthy adult controls (Shrestha, 2003). Similarly, a study conducted among, the healthy Nepalese males seeking jobs abroad, has shown a seropositivity rate of 0.35% (Shrestha, 2006).

3.1.6.3 Risk factors and Transmission of HCV

The major risk factors for transmission of HCV are intravenous drug abuse, blood transfusion, sexual activity and hemodialysis (Thodore et al., 2006). HCV transmission requires that infectious virions contact susceptible cells that are permissive for replication. HCV RNA can be detected in blood (including serum and plasma), saliva, tears, seminal fluid, ascetic fluid and cerebrospinal fluid (Liou et al., 1992, Chen et al., 1995, Fiore et al., 1995, Mendel et al., 1997). The magnitude of HCV viremia has been reported to be an important factor in determining the risk of transmission as indicated by a study including 2022 parenteral, sexual and perinatal HCV exposures, in which

HCV was transmitted by only those individuals having detectable viremia (Dore et al., 1997).

Percutaneous Transmission

It has been reported that more than 90% of seronegative recipients who are transfused with blood from HCV antibody positive donors undergo seroconversion (Vrielink et al., 1995). Thus, there is a high prevalence of HCV infection in multiple transfused thalasemic and hemophilic patients (Brettler et al., 1990, Eyster et al., 1993). With the introduction of EIA test, the risk of transfusion transmitted Hepatitis C has been substantially reduced (Donahue et al., 1992). Transmission may still occur rarely from donors with recent infections who have not developed detectable antibodies (Widell et al., 1996).

Studies have shown that HCV has also been transmitted by the administration of contaminated blood products. Large number of Hemophilic patients have been reported to be infected by contaminated clotting factor concentrates, and there have been several large outbreaks related to administration of contaminated intravenous immune globulin (Bjoro et al., 1994, Yap et al., 1994). Transplantation of organs from HCV infected donor almost always results in HCV infection in seronegative recipients (Terrault et al., 1995). Sharing of contaminated needles and other paraphernalia associated with illicit drug use account for majority of HCV infections among IDUs. Various studies have reported that, globally 50-95% of persons acknowledging drug use have HCV infection (Bell et al., 1990, Bolumar et al., 1992, Thomas et al., 1995). Some studies have shown that tattooing is associated with HCV infection (Ko et al., 1992, Sun et al., 1996). Similarly, folk remedies, such as acupuncture, scarification rituals and even human bites, may also be associated with HCV infection (Dusheiko et al., 1990).

Nosocomial Infection

Patient to patient transmission of HCV after a colonoscopic procedure and among the patient within hemodialysis units have been reported by some molecular epidemiological studies (Bronowicki et al., 1997, Allander et al., 1995, Schvarcz et al., 1997). Transmission of HCV has been reported to occur in healthcare workers after 2-8% of accidental needle-stick injuries to HCV infected patients (Kiyosawa et al., 1991, Ridzon et al., 1997). So, it has been estimated that the risk of transmission of HCV by accidental needle prick injuries is about 3% per documented exposure which is in between that of HIV(0.3% per documented exposure) and HBV (30% per documented exposure) (Kiyosawa et al., 1991). In contrast to the developed nations, in economically developing areas of the world the nosocomial HCV transmission has been thought to be common. Thus, it is likely that nosocomial are the leading source of HCV transmission worldwide (Thomas et al., 2000).

Sexual Transmission

The role of sexual activity in the transmission of HCV remains unclear. Although, not proved there are mounting circumstantial evidences that it occurs. HCV RNA has been detected in Semen and Saliva (Liou et al., 1992, Wang et al., 1992, Fiore et al., 1995) and studies have shown higher prevalence of HCV among commercial sex workers and persons with multiple sexual partners (Nakashima et al., 1992, Thomas et al., 1995, Utsumi et al., 1995). Acute HCV infection has also been reported in instances where sexual but not other, exposures were recognized (Capelli et al., 1997). However, the studies among long term sexual partners of HCV infected hemophiliac patients and transfusion recipients shows little or no evidence of HCV transmission, even if there has been unprotected sexual intercourse (Brettler et al., 1992, Gordon et al., 1992). HCV prevalence among homosexual men has been reported to be lower other infections such as HIV, HBV and Syphilis (Donahue et al., 1991). Thus, the available data suggests that HCV is transmitted less frequently as compared to HIV, HBV and Syphilis.

Perinatal Transmission

HCV is very rarely transmitted from mother to infant. Studies have reported variable frequency of perinatal transmission ranging from 0-8% (Reinus et al., 1992, Ohto et al., 1994, Thomas et al., 1998). Though HCV RNA has been detected in breast milk (Ogasawara et al., 1993), the risk of HCV transmission has reported to be similar in breast fed and bottle fed infants (Resti et al., 1995, Lin et al., 1995). The risk of mother to infant infection seems to be increased when the mother is HIV/HCV coinfected possibly due to higher viral load of HCV due to immunosuppression by HIV (Ohto et al., 1994).

3.1.6.4 Pathologic and Clinical features of HCV infection

HCV infection causes an indolent and slowly progressing liver disease that is asymptomatic until the development of decompensated liver disease and often, liver cancer.

Acute HCV infection

HCV transmitted by percutaneous exposure usually results in a non-icteric asymptomatic infection and most people become chronic carrier of the virus. HCV RNA can be detected in plasma within weeks of exposure in the same time or slightly earlier than the abnormal level of ALT (Alanine Aminotransferase) (Farci et al., 1991, Prince et al., 1993). Jaundice, fatigue, fever, nausea, vomiting and upper quadrant discomfort can occur, usually within 2-12 weeks of exposure and lasting from 2-12 weeks. Diagnosis requires PCR for RNA since acute infections may be seronegative for anti HCV (Fox et al., 2003).

Viremia persists in as many as 85% of acutely infected patients whereas about 15% of acutely infected patients spontaneously clear the infection and viremia is no longer

detected after 3-24 months of infection (Alter et al., 1992, Mattsson et al., 1993, Barrera et al., 1995, Villano et al., 1999). No correlation of antibody response detected in commercial diagnostic assays and viral clearance has been reported indicating that such assays are unlikely to measure neutralizing antibodies (Villano et al., 1999).

Chronic HCV infection

Chronic HCV is asymptomatic in majority of patients. However, compared with non hepatitis C control subjects, patient more often report fatigue and upper quadrant pain, hepatomegaly, tender liver, thrombocytopenia as well as elevated transaminases and bilirubin. ALT value fluctuates in most patients (Fox et al., 2003). A long term pattern of undetectable HCV RNA and normal serum ALT levels on multiple occasions are necessary to conclude that the HCV infection has terminated (Thomas et al., 2000).

3.1.7 Progression of disease

The major pathologic consequence of chronic HCV infection has been reported to be the development of hepatic fibrosis which may progress to life threatening cirrhosis and a greatly increased risk of hepatocellular carcinoma and these long term complications generally have been reported to occur more than 20 years after the onset of infection, though more rapid progression has been documented in some cases (Hopf et al., 1990, Kiyosawa et al., 1990, Tong et al., 1995). In various studies it has been estimated that the probability of cirrhosis occurring in 10-20 years after infection ranges from 5-25% (Di Bisceglie et al., 1991, Tremolada et al., 1992). Though excessive alcohol ingestion and HCV infection independently may cause cirrhosis, the combined exposure has been reported to induce a synergistic effect (Schiff et al., 1997, Corrao et al., 1998, Ostapowicz et al., 1998). HCV coinfection with HBV or HIV has been shown to be associated with increased level of HCV viremia and more rapid progression of liver disease (Sherman et al., 1993, Thomas et al., 1996, Bierhoff et al., 1997, Darby et al., 1997).

3.1.8 Diagnosis of Hepatitis C virus infection

3.1.8.1 Serologic Tests

The laboratory diagnosis of HCV infection is mainly based on the detection of antibodies to recombinant HCV peptides. The first generation enzyme immunoassays were based on c100-3 antigen (NS4 sequence fused to bacterial superoxide dismutase) (Kuo et al., 1989), and these assays have been reported to have limited sensitivity in both acute and chronic cases with a false positive rate of 50-70 % among low risk population like blood donors and in those with hypergammaglobulinemia (Gretch et al., 1992). The second generation EIA included additional synthetic antigens from the core (c22-3) and NS3 and NS4 regions (c33c and c100-3 combined to form c200) (McHutchinson et al., 1992, Nakatsuji et al., 1992) and these assays had been reported to have greater sensitivity and specificity in low prevalence population and highly sensitive as well as specific in high prevalence diagnostic setting (Aach et al., 1991, Gretch, 1997).

The third generation EIAs that is being used in this time includes an additional antigen from the NS5 region of the polyprotein and more importantly the reconfiguration of the core and NS3 antigens. The sensitivity of third generation assays have been reported to be around 97% and window period has been shortened to below 6-8 weeks of exposure (Gretch et al., 1997). The positive EIA results are generally evaluated by a so called confirmatory or supplemental test, Recombinant Immunoblot Assay (RIBA). The RIBA.1, RIBA.2 and RIBA.3 are the first, second and third generation variations, all of them employ plastic strips coated with individual bands of each recombinant antigens used in the corresponding EIA (Buffet et al., 1994, Chiaramonte et al., 1996). The RIBA is considered positive if two or more reactive bands are detected, indeterminate if single reactive band is detected or negative if no reactive band is detected. Generally, EIA and RIBA positive sera have been found to contain HCV RNA but the EIA

positive and RIBA indeterminate sera may also contain HCV RNA especially if the reactivity was to the core and NS3 antigens (Van Der Poel et al., 1991, McGuiness et al., 1993, Vrielink et al., 1995). The RIBA.3 has been approved for use by blood banks as a supplemental test to EIA.3. The EIA.3 has been reported to give fewer indeterminate results than RIBA.2 and better correlation with viremia when the specimens that are RIBA indeterminant are compared with those that test positive (Damen et al., 1995). It has been emphasized that the EIA has been configured to optimize sensitivity, as the primary use of assay is for screening for HCV infection. As with all tests, the predictive value of the EIA is directly related to the prevalence of the infection in the population screened. It has been reported that the specificity of EIA.3 while screening the IDU population is 98% as compared to about 50% while screening blood donors (Thomas et al., 2000).

3.1.8.2 Nucleic Acid Tests

For the diagnosis of active ongoing HCV infection, demonstration of viral RNA in serum or plasma is essential. Qualitative as well as quantitative RT-PCR has been developed and under ideal conditions, the sensitivity for this assay for HCV RNA has been advocated to be fewer than 100 copies per ml serum (Shuhart et al., 2003). In house or commercial RT-PCR assays can be used for qualitative detection of HCV RNA whereas branched-DNA methods are usually used for quantization.

3.1.8.3 Liver function Tests

An elevation of Aminotransferases is not required for diagnosis of HCV infection. Upto 30% of patients with chronic HCV have been reported to show normal ALT Level. (Thomas et al., 2000).

3.1.8.4 Liver Biopsy

Live biopsy has been described as the best method for assessing the severity of HCV infection and staging the disease (Goodman et al., 1995, Perrillo, 1997). Usually, the individuals undergoing for HCV testing include blood donors, and patients with known risk factors for HCV, chronic hepatitis (elevated ALT level for more than 6 months) and patients with acute symptomatic hepatitis (Shuhart et al., 2003). A single negative test for viral RNA does not exclude HCV infection in an EIA positive patient with an elevated ALT level and a risk factor whereas many blood donors with a positive EIA actually do not have HCV infection (Thomas et al., 2000).

The diagnosis of HCV infection by using gold standard serology for low prevalence population usually includes duplicate immunoassays (EIAs) with confirmation by third generation Recombinant Immunoblot assay (RIBA.3) (Thomas and Lemon, 2000). Due to economic constraints, RIBA has not been used in most of the seroprevalence studies in Nepal. Instead confirmation of the initial reactive result by a second ELISA test has been considered by various researchers viz. Shrestha (2003), Shrestha (2006) for serosurveillance study.

3.1.9 Treatment

Interferon alpha has been used as a backbone of treatment for chronic HCV. Rather than the monotherapy with Interferon alpha alone combination therapy with ribavirin has shown to be better than interferon monotherapy for both initial treatment and retreatment of patients who have relapsed after interferon therapy (Di Bisceglie et al., 1989, Neuman et al., 1998). The use of Pegylated interferon and ribavirin combination therapy has been shown to be superior to interferon plus ribivirin combination therapy (Mans et al., 2001).

3.1.10 Prevention

After introduction of EIA for screening blood and blood products for HCV the incidence of transfusion associated HCV infection has been reported to be substantially decreased (Donahue et al., 1992). Since, most of the new cases are associated with drug related high risk behaviors, this point has been described as the target for prevention (Thomas et al., 2000). The outcome of the needle exchange program for reducing HCV transmission among illicit drug users has been shown to be conflicting (Hagan et al., 1999). Currently, there is no vaccine available.

3.2 Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1 and HIV-2) have been described as the etiologic agents of Acquired immunodeficiency Syndrome (AIDS). AIDS is the end stage of virus mediated protracted pathogenic process in which the

immune system of an infected person and its ability to control infections or malignant progressive disorders are progressively destroyed (Schupbach, 2003). AIDS was first recognized in United States in 1981 with a sudden outbreak of opportunistic infections, *Pneumocystis carinii* pneumonia and Kaposi's sarcoma (Durack et al., 1981, Gottlieb et al., 1981).

3.2.1 Discovery and Origin of HIV

HIV-1 was the third retrovirus to be discovered. HIV-1 was discovered in 1983 (Barre-Sinoussi et al., 1983), and after one year it was proved to be virologically and serologically associated with early and latent stage of AIDS (Gallo et al., 1984, Popovic et al., 1984). HIV-2 was the fourth retrovirus to be discovered in 1986 from patients in Western Africa (Clavel et al., 1986). HIV-1 has been shown to be more aggressive virus and is mainly responsible for current AIDS pandemic whereas HIV-2 has been described as less pathogenic (Schupbach, 2003). The rates of heterosexual and mother to child transmission of HIV-2 has been reported to be low, and the infection seems to be more latent and this virus has been reported to cause AIDS very rarely (Schim van der Loeff et al., 1999).

Molecular epidemiological analysis has indicated that the HIV-1 epidemic is the result of cross species infection by Simian Immunodeficiency Virus from Chimpanzee to human in rural Africa (Gao et al., 1999) and the beginning of HIV-1 pandemic has been estimated to occur around 1930 (Korber et al., 2000). It has been concluded that the HIV-2 epidemic is also the result of multiple simian to human cross species transmission (Sharp et al., 1994).

3.2.2 Classification, HIV groups and subtypes

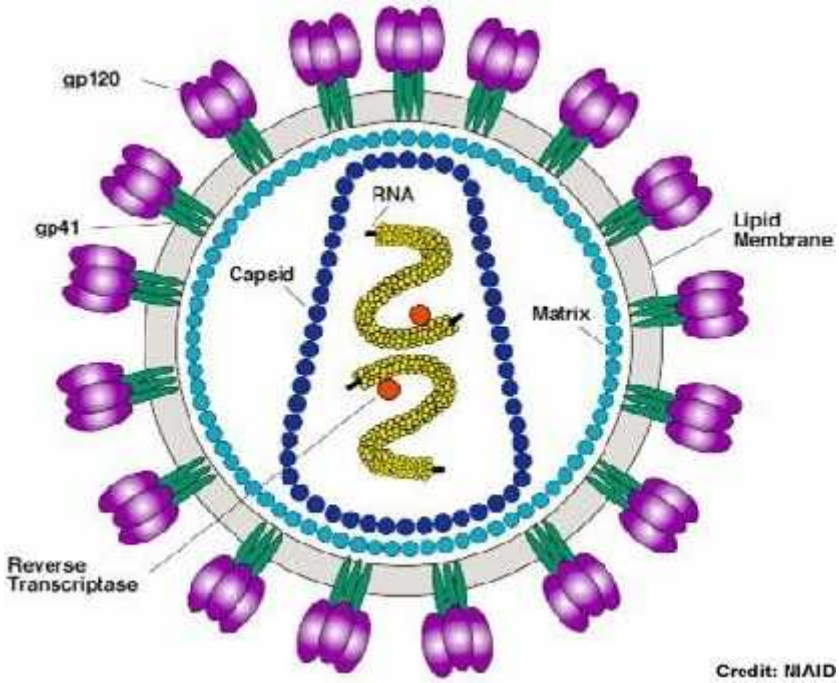
The two distinct types of AIDS viruses, HIV-1 and HIV-2 are the member of the genus Lentivirinae of the Retroviridae family. They are single stranded, positive sense, enveloped, icosahedral RNA viruses. The rapid mutation and recombination of the HIV

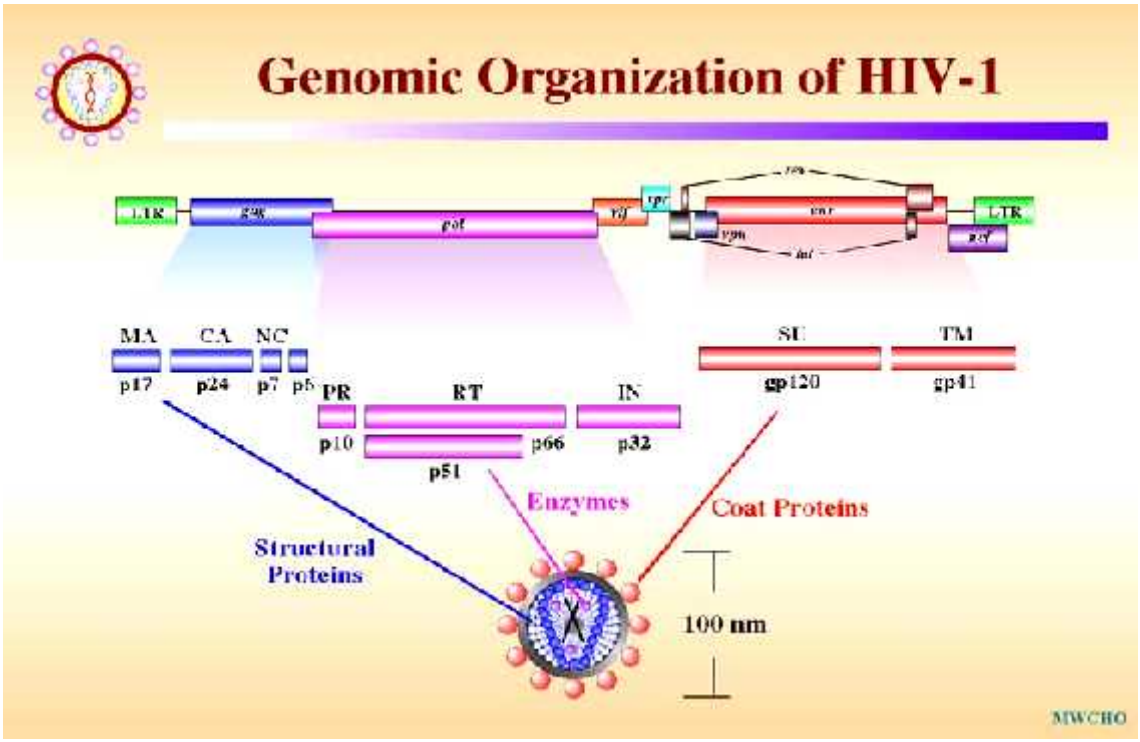
genome, has led to the development of various distinctive clades or subtypes of viruses (McCutchan et al., 2000) and virtually each isolate of HIV has been shown to be genetically unique (termed Quasispecies). Based on *env* gene sequences, HIV-1 has been characterized into three groups viz. M (Major), N (Non M non O) and O (Outlier). The group M has been reported to comprise about 95% of the global isolates and it has been divided into 9 subtypes (Clades or Clusters), A –K omitting E and I. Group N viruses have been isolated only two individuals from Cameroon (Simon et al., 1998). Group O have been predominantly reported from West Africa and these are reported to share less than 50% sequence homology with group M Strains. Similarly, six subtypes of HIV-2 (A to F) have been described. HIV-1 subtype C has been found to be most common in India whereas HIV-1 subtype B has been predominantly isolated from America, Australia and Europe. Subtype E has been shown to be most prevalent in Thailand and Southeast Asia and Subtype G in Russia. Almost all subtypes have been reported from Sub-Saharan Africa but the subtypes A, C and D have been shown to be most prevalent.

3.2.3 Virion Properties

HIV is enveloped, positive sense, RNA virus with diameter of about 110nm (90-120 nm). The virions bud from the host cell membrane forming a sphere with an outer lipid bilayer and a nucleocapsid with a dense cone shaped core. It has been reported that the core appears to be attached with the viral outer envelope at its narrow end (Hoglund et al., 1992). The lipid envelope consists about 72 spiked knobs, which are assembled as trimers of the outer envelope protein gp120 bound to the transmembrane protein, gp 41.

Figure 3.2: HIV-1 Virion and genomic organization of HIV-1





Source: www.stanford.edu/.../2005gongishmail/HIV.html

The viral membrane has been found to be cholesterol rich and includes cellular proteins (Arthur et al., 1992). The nucleocapsid core is electron dense, cylindrical and bar shaped. The nucleocapsid is surrounded by matrix. The virion contains two identical copies of a single stranded positive sense genomic RNA of about 9-10 kilobases (kb) (Schupbach, 2003).

3.2.4 Bio-safety, Disinfection and Inactivation

HIV-1 and HIV-2 have been classified as biological safety hazard agents of moderate risk (Biosafety level 2). Strict adherence to safety precautions has been recommended. Needle stick or other puncture wounds, cuts, and skins contaminated by spills or splashes of specimen material should be thoroughly washed with soap and water and disinfected with non irritating disinfectant. Bleeding should be encouraged. An Antiretroviral post-exposure prophylaxis (PEP) has been recommended for percutaneous injury, contact of mucous membrane, or non-intact skin. The recommended PEP consists of a basic four week regimen of two drugs (Zidovudine and Lamivudine, Lamivudine and Stavudine or Didanosine and Stavudine (WHO, 1991, CDC, 2001).

HIV has been reported to be completely inactivated by treatment for 10 minutes at room temperature with any of the following: 10% household bleach, 50% ethanol, 35% isopropanol, 1% nonidet p40, 0.5% Lysol, 0.5% paraformaldehyde or 0.3% hydrogen peroxide. The virus has been reported to be inactivated by extremes of pH. HIV has been reported to be readily inactivated in liquids or 10%serum by heating at 56^{o}C for 10 minutes, but dried proteinaceous material has been reported to afford marked protection (Brooks et al., 2004).

3.2.5 HIV genome and Proteins

The two strands of positive sense RNA genome are linked near their 5’ ends. Each

strand of proviral genome consists 5'LTR (Long Terminal Repeat) and 3' LTR regions. HIV genome consists 9 genes or Open Reading Frames (ORFs).

3.2.5.1 Genes encoding structural proteins

1. *gag* gene

The *gag* gene encodes a precursor protein of 55 kd (kilodalton) called as p55 which is cleaved by the viral protease into the matrix protein p17, the capsid protein p24 and a C- terminal protein p15 which is subsequently cleaved into p7 and the nuclecapsid protein p9. The lack of protease function either through inhibition of drugs or following transfection of the p55 gene into cell that lacks protease results in the formation of noninfectious viral particles (Flexner, 1998). The capsid protein p24 has been described as the easiest antigen to be detected using sera from infected patients.

2. *env* gene

The *env* gene encodes a precursor protein gp160, which is glycosylated in the Golgi system, oligomerizes into dimers and trimers and is cleaved by the cellular protease into surface protein gp120 and the smaller transmembrane protein gp41. The genetic divergence among different strains of HIV resides mainly in the *env* gene sequence and enables the virus evade humoral response. So, it has been suggested that better understanding of the structure and function of *env* gene products might help in effective vaccine design (Kwong et al., 1998).

3.2.5.2 Genes encoding viral enzymes

The *gag-pol* ORF encodes a precursor protein of 160 kd called PR160gag-pol by ribosomal frameshifting at gag pol junction, which upon cleavage yields the three viral enzymes Protease, Reverse Transcriptase and Integrase. The protease is

autocatalytically cleaved from the precursor protein during the viral assembly process and is fully active as dimer. The similarity of viral proteases to other aspartyl proteases such as angiotensin converting enzyme has greatly facilitated the design of potent antiviral drugs, including inhibitors of dimerization and molecules that bind to active catalytic sites (Flexner, 1998).

The viral reverse transcriptase (RT) is an RNA dependent DNA polymerase. It serves three activities as RNA dependent DNA polymerase, DNA dependent DNA polymerase and RNAse H. The RT is first cleaved from precursor protein to form a p66 homodimer and after a second cleavage to form p66-p51 heterodimer. RT has the major role in the generation of diversity in retroviruses. During the replication the fidelity of the enzyme in case of HIV-1 has been found to range from 1/1700 to 1/4000 misincorporation per nucleotide per replication. So, for the 9.7 Kb HIV-1 proviral genome, the invivo error rate has been estimated to be one misincorporation per replication cycle (Varmus, 1988, Lukashov et al., 1998).

3.2.5.3 Regulatory genes

In addition to *gag*, *pol* and *env*, HIV has six accessory genes viz. *tat* (coding for trans activator of transcription), *rev* (encoding for regulator of viral expression), *vif* (encoding the virion infectivity factor), *vpr* (encoding the viral protein R), *vpu (*encoding the viral protein U in HIV-1) or *vpx* (encoding the viral protein X in HIV-2) and *nef* (encoding the negative regulatory factor). These viral regulatory genes and their products enable the virus to manipulate host cell processes and to achieve efficient replication under host selective pressure, thus contributing to disease progression (Emerman et al., 1998).

3.2.6 Epidemiology

3.2.6.1 Global prevalence

A total of 33.2 million (30.6–36.1 million) people were living with HIV in 2007, 6.3 million less than in 2006. The percentage of the world's adult population living with HIV has been leveling off and is declining in Sub-Saharan Africa. However, the sheer number of people living with HIV continues to increase, as there were only 29 million people living with HIV in 2001. WHO has reported that the downward revision in prevalence of 6.3 million is largely due to improved and expanded surveillance, data collection and methodologies. Roughly 70% of the difference is explained by reductions in HIV prevalence in India (which alone accounts for approximately half the revision) and several sub-Saharan African countries, including Nigeria, Mozambique, Zimbabwe, Kenya and Angola. The remaining 30% of revisions mostly occurred in a number of sub-Saharan African countries. Global HIV incidence (the number of new infections) decreased to 2.5 million [1.8-4.1 million] in 2007, down from 3.2 million [2.1-4.5 million] in 2001. HIV incidence declined the most in sub-Saharan Africa, where a total of 1.7 million [1.4- 2.4 million] people became infected with HIV in the past year, declining from 2.2 million [1.7–2.7 million] new infections in 2001. In addition to the declines in new infections in sub-Saharan Africa between 2001 and 2007, the estimated annual number of new HIV infections decreased clearly in South and South-East Asia (UNAIDS 2007).

A total of 39.5 million (34.1–47.1 million) people were living with HIV in 2006, 2.6 million more than in 2004. This figure includes the estimated 4.3 million (3.6–6.6 million) adults and children who were newly infected with HIV in 2006, which is about 400000 more than in 2004. In 2006 it has been reported that, in many part of the world, new HIV infections are heavily concentrated among young people (15-24 years of age). Among adults 15 years or above, young people accounted for 40% of new HIV infection in 2006. The burden of HIV and AIDS was highest in Sub-Saharan Africa,

where two thirds population (63%) of all adults and children with HIV globally were living. One third (32%) of all people with HIV globally live in Southern Africa and 34% of all death due to AIDS in 2006 occurred there (AIDS Epidemic update, 2006).

An estimate of global summary of HIV/AIDS has been shown below:

Number of people living with HIV in 2007	
Total	33.2 million (30.6-36.1 million)
Adults	30.8 million (28.2-38.6 million)
Women	15.4 million (13.9-16.6 million)
Children under 15 years	2.5 million (2.2-2.6 million)
People newly infected with HIV in 2007	
Total	2.5 million (1.8-4.1 million)
Adults	2.1 million (1.4-3.6 million)
Children under 15 years	420 000 (350 000-540 000)
AIDS death in 2007	
Total	2.1 million (1.9-2.4 million)
Adults	1.7 million (1.6-2.1 million)
Children under 15 years	330 000(310 000-380 000)

Source: AIDS epidemic update, 2007, UNAIDS

3.2.6.2 Epidemiological situation of HIV/AIDS in Nepal

The first case of AIDS in Nepal was reported in 1988 (Gurubacharya et al., 1994). As of December 2007, National Center for AIDS and STD Control (NCASC) has officially confirmed 10,546 HIV positive cases and 1,610 confirmed cases of AIDS among the total HIV in Nepal (NCASC, 2007). UNAIDS has estimated the adult (15-49 years) HIV prevalence rate of 0.5% by the end of 2005 in general population whereas the number of people living with HIV in the same time has been estimated to be 74,000 of which 16,000 are women. World Bank estimate has shown that one third of HIV

infection nationwide is among injecting drug users. About 5100 AIDS death have been reported by the end of 2005 (UNAIDS, 2006).

Over the last few years HIV/AIDS epidemic in Nepal has gained ground and Nepal has progressed from a low prevalence country to a country with 'concentrated epidemic'. A situation analysis study of HIV/AIDS conducted in 2000 has identified the young people, Mobile populations, Female Sex Workers, Men who have sex with men, Injecting drug users, and Children as the most vulnerable to HIV/AIDS in Nepal (Pokhrel et al., 2000). Most of the HIV infections in Nepal has been caused by HIV-1 though recently seroevidence of HIV-2 has been reported from Bhairahava, Nepal (Chander et al., 2004). Around 10 years ago Nepal was described as a country having comparatively lower prevalence of HIV/AIDS compared to other countries in Southeast Asia. Seasonal migration to Indian Cities for seeking job and sexual trafficking across a porous Indian border (Seddon, 1998), fuelled by the bloody Maoist conflict, has raised Nepal's HIV prevalence second highest in the region after India (Singh et al., 2005). About 40% of the an estimated 30,000 injecting drug users in Nepal have been reported to be infected with HIV (Karki, 2000) whereas about 68% of injecting drug users in Kathmandu Valley, 22% in Pokhara and 35% in Eastern Terai have been reported to be infected with HIV (New Era, 2002). A molecular Epidemiological study has shown that the epidemic among IDUs is exclusively caused by a subtype C of HIV of restricted genetic diversity and possibly of Indian origin (Oelrichis et al., 2000). The predominant mode of transmission of HIV has been described to be heterosexual contact with commercial sex workers. In Kathmandu valley about 17% of female commercial sex workers (FCSWs) have been reported to be infected with HIV (UNDP You and Aids, 2005) whereas only about 4% of Sex workers and 1.5% of their clients have been reported to be infected with HIV from Terai regions (FHI, 2000). About 50% of HIV cases in Nepal have been identified to be from the 29 Highway districts (FHI, 2002). UNAIDS has estimated that at least 10% of 2 to3 millions of Nepalese migrant workers in India have been infected with HIV (Irin News Network Reuters News Alert, 2005). Recently, it has been reported that 38% of repatriated sex trafficked Nepalese women

and girls have been tested positive for HIV (Silverman et al., 2007). HIV prevalence among migrants within Nepal and to Indian cities have been reported to be 2.3% and 8.9% respectively (Gurubacharya et al., 2004). Similarly, another study among male migrant returnees from Indian cities in Doti districts has reported a prevalence rate of 8% (Poudel et al., 2003).

3.2.6.3 Risk factors and Transmission of HIV

1. Sexual transmission

Globally, HIV is mostly transmitted through sexual contact. Both the fluid and cellular components of semen has been found to contain HIV, as do endocervical secretions. The rate of transmission of HIV depends upon the sexual behaviors and sexual practices vaginal intercourse in conditions of other sexually transmitted infections or during menses, sexual mixing patterns and level of condom use, have been recognized as factors affecting spread (Folks et al., 1998). Similarly, the efficiency of transmission is also dependant on level of viremia, infectivity and virulence of particular HIV strain, and the presence of STDs such as genital ulcers (Plummer et al., 1991).

2. Parenteral transmission

Parenteral route of transmission of HIV is common among IDUs due to exposure to HIV infected blood through the sharing and use of contaminated needles or other injections equipments. It has been reported that unsafe sexual practices may also have been contributing some infections among IDUs (Nelson et al., 1991).

3. Transmission via blood and blood products

The likelihood of HIV infection occurring in recipients of HIV positive blood is close to 100% and the prevalence of HIV among recipients of blood or blood products before

the screening of HIV in blood units is quite high. In countries where screening of blood for HIV has been instituted, the risk of HIV transmission through screened blood has been estimated to be 1/36000-1/225000 per unit transfused. This residual risk is reported to be due to antibody negative infected donors in the window period (Folks et al., 1997).

4. Perinatal transmission

Prospective studies of infants born to HIV infected women have shown the perinatal transmission rate to be ranging from 13-40%. Perinatal transmission can occur in utero, during birth and postnatally probably by breast feeding (Folks et al., 1997). The risk of perinatal transmission has been reported to vary with the disease state of the mother and is highest during acute primary infection and with advanced symptomatic disease (European Collaborative Study, 1992).

5. Transmission in Health care setting

The average risk of seroconversion after a needle-stick injury with HIV positive blood is about 0.3% (Tokars et al., 1993). Nosocomial transmission has been reported from different parts of world in various settings.

3.2.6.4 Pathogenesis and Clinical features

In HIV infection there are distinct three phases, Acute infection (4-8 weeks), Asymptomatic infection or clinical latency (10-11 yrs) and ultimately AIDS defining diseases and death (2-3 yrs). After the establishment of primary infection, the virus is disseminated to lymphoid organs where it persists with minimal expression for the time of clinical latency and finally a profound expression of HIV provirus occurs leading to immune suppression and onset of opportunistic infection as well as neoplasms and eventually, the death (Schupach, 2003).

Acute Retroviral Syndrome

It occurs in 50-70% of infected patients and is characterized by flu like or infectious mononucleosis like disease with fever, generalized lymphadenopathy, sore throat, arthralgia, myalgia, fatigue, rash, and/or weight loss. These symptoms typically resolve within 5-30 days (Schupbach, 2003).

Clinical Latency

The state of acute infection is followed by a long stage of disease free clinical latency. In most of the untreated adult patients, the median time to AIDS is estimated at 10-11 years. The incubation time may be as short as 2 to 3 years in 5-10% of patients who are rapid progressors (Schupbach, 2003).

AIDS

AIDS is the end stage clinical manifestation of HIV infection. Due to relentless production of HIV proteins, maintained by continuous viral replication in productively infected cells, and the ensuing elimination of host cells over many years finally lead to the destruction of immune system, which is clinically manifested by opportunistic infections and tumors. The infection in central nervous system (CNS) may lead to distinct HIV-associated disease, including the HIV associated dementia complex, vacuolar myelopathy, and sensory neuropathy (Price, 1996, Schupbach, 2003).

3.2.6.5 Laboratory diagnosis of HIV Infection

HIV is acquired most frequently through unprotected sexual contact, so a number of moral, ethical, legal, and psychological issues are related to HIV testing. Laboratory diagnosis is the only way to establish the HIV infection status of an individual.

The main purposes of HIV testing are:

1. to identify of asymptomatic and symptomatic HIV positive individuals.
2. to assure safety of blood and blood related products.
3. to motivate for behavior changes through counseling among those high risk behavior individuals who tested anti HIV negative.
4. to induce behavior change and prevent further HIV transmission by counseling in those individuals who tested anti HIV positive.
5. to monitor trends of HIV epidemic (Luft et al., 2004).

HIV infection can be detected by:

1. Indirect or serologic detection of HIV infection

i. Screening tests.
ii. Supplemental or Confirmatory tests.

2. Direct detection of HIV infection

i. Detection of p24 antigen
ii. Isolation of HIV
iii. Molecular amplification methods

i. Screening tests

a. Indirect binding assays

In this type of tests, the antigen bound to carrier serves as the target for the patient's antibody. Bound antibody is detected by second labeled antihuman antibody. This type of assay includes both the 'first generation tests' and 'second generation tests'. The first generation tests viz. EIA, IF, WB use antigen from viral lysate that contain full range of viral antigens as well as some cellular antigen. The second generation tests viz. EIA,

LIA use restricted number of recombinant or synthetic peptides as antigen (Schupbach, 2003).

b. Antibody capture assay

In this assay, capture agent (anti human Ig, protein A, G) specific for human Ig (frequently used for IgM, IgA, detection) is immobilized in carrier. Human immunoglobin of all specificities bound. Then specific labeled antigens bind to antibody of corresponding specificity. Labeled viral antigen may be lysate derived, recombinant or a synthetic peptide (Schupbach, 2003).

c. Double antigen sandwich assay (DAGS)

DAGS assay include the so called 'third generation test'. Antigen bound to carrier act as target for patient's corresponding antibody. Bound antibody is detected with, same labeled Antigen added in solution. The antigen used is recombinant or /and synthetic (Schupbach, 2003).

d. Fourth generation screening tests

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).

e. Rapid screening assays

Rapid tests can give results within minutes. These tests may be of different formats, including DAGS, indirect binding assays, antibody capture assays, agglutination or chromatographic assay. Some of the commercially available rapid screening tests are DIA Dot HIV1+2 assay, HIV TRI-DOT test, Oraquick Rapid HIV-1 antibody test,

Reveal Rapid HIV antibody test, UniGold Recombigen HIV antibody test etc. The diagnostic sensitivity of some of these tests has been reported to be somewhat inferior to third generation ELISA based antibody tests, especially in seroconversion panels (Kuun et al., 1997, Giles et al., 1999). However, others have been shown to give comparable diagnostic sensitivity and specificity, even during seroconversion and have been recommended for certain diagnostic settings (Zaw et al., 1999, Phillips et al., 2000).

ii. Supplemental or confirmatory tests

a. Western blot

In HIV testing western blot has remained principal confirmatory test worldwide (Mylonakis et al., 2000). The sensitivity of Western blot in seroconversion panels has been reported to be inferior to other third and fourth generation screening tests, and WB is prone to detect cross reactive antibodies, which results in high rate of indeterminate results (Pollet et al., 1991, Schupbach, 2003). The antigens in western blot are derived from the viral lysate or recombinant antigens, separated in polyacrylamide gel electrophoresis and blotted on nitrocellulose paper. The interpretation criteria for a positive western blot test are different as set by different authorities. The world health organization recommendation specifies any two of gp160, gp120, and gp41 (WHO, 1990). The American Red Cross specifies at least one band each from Env, Gag and Pol and the consortium for Retroviruses Serology Standardization specifies at least two bands, p24 or p31 plus gp41 or gp120/gp160 (Schupbach, 2003).

b.Immunoblot

In immunoblot recombinant or synthetic HIV proteins are mechanically applied onto the solid membrane and do not contain contaminating human cell proteins and are highly specific (Luft et al., 2004).

2. Direct detection of HIV infection

i. Detection of p24 antigen

The HIV *gag* gene encoded core protein (p24) antigen can be detected in serum or plasma during the acute phase of primary HIV infection (window period), during very late symptomatic stage of infection and in the newborns born to HIV infected mothers. Detection of p24 antigen is mostly performed in EIA format. The test principle consists of binding the p24 antigen present in a sample to anti-p24 specific, usually monoclonal, capture antibodies, which coat a solid support. Unbound sample components are washed away and bound antigen reacts with another p24 specific antibody conjugated with enzyme. For a confirmation of a reactive result, the sample must be subjected to an additional confirmatory neutralization assay (Schupbach, 2003).

The overall sensitivity of standard p24 antigen testing for detection of HIV infection in infants is 50 to 75% and specificity is greater than 95% (Borkowsky et al., 1989, Andiman et al., 1992).

ii. Isolation of HIV

HIV can be cultured from lymphocyte in peripheral blood. Leucocytes are separated from anticoagulated blood by Ficoll centrifugation and co-cultured with phytohemagglutinin stimulated leucocytes from healthy blood donors. In order to detect the viral growth, culture supernatant is periodically assayed for p24 antigen. Cultures usually become positive within 2 weeks, but culture times of up to 60 days have been reported. The procedure has been reported to be 90% sensitive at all stage of HIV infection, but the success rate is lower for asymptomatic patient (Burgard et al., 1992).

iii. Detection of viral nucleic acid

Amplification assays such as Reverse transcriptase PCR, DNA PCR and branched DNA tests are commonly used for detection and quantification of HIV in clinical specimens. With ultra sensitive protocols developed for all three methods the detection limit of 20-50 HIV-1 RNA copies per ml has been already reached (Yilmaz, 2001, Schupbach, 2003). PCR for viral DNA or RNA is particularly important for diagnosis of pediatric HIV-1 infection, and it has been reported to be clearly more sensitive than virus culture (Bremer et al., 1996, Nelson et al., 1996). Nucleic acid amplification methods are used for viral load determination, which is useful to monitor the progression of disease and antiretroviral therapy (Schupbach, 2003).

The diagnosis of HIV infection by using gold standard serology, usually duplicate immunoassays (EIAs) with confirmation by western blotting is neither feasible nor practical in many developing world locations due to technical and financial constraints (Anonymous, 1998). So, a strategy based on an alternative HIV testing strategy not requiring western blot proposed by Sato et al. (1994) has been recommended by WHO for resource limited settings.

3.2.6.6 Treatment

A large number of antiviral drugs are approved for treatment of HIV infections. Current drug regimens can prolong the survival of patient but not cure HIV. These include nucleoside analogue reverse transcriptase inhibitor (NRTIs), protease inhibitors (PIs), non nucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors. The fusion inhibitors are newest class of drugs that block virus entry into cell. Researches have shown that by taking three or more antiretroviral drugs at the same time, each attacking HIV in different points in its cycle of replication, then treatment is more effective than one or two drugs alone. So the common treatment is to use combination

of three or more drugs which include drugs from different classes. This is called HAART (highly active antiretroviral therapy). Such combination of drug has been shown to reduce the risk of developing resistance to any individual drug. HAART often can suppress viral replication below limits of detection in plasma, decrease viral load in lymphoid tissues, allow the recovery of immune responses to opportunistic infections, and prolong patient survival (Brooks et al., 2004).

3.2.6.7 Prevention

No vaccine is currently available for preventing HIV infection. Many candidate vaccines are under development and are at different stages of testing. Vaccine development is difficult because HIV mutates rapidly, is not expressed in all cells that are infected, is not completely cleared by host immune response after primary infection and lack of an appropriate animal model. The only way to avoid epidemic spread of HIV is to maintain a lifestyle that minimizes or eliminates the high risk factors (Brooks et al., 2004).

3.3 HCV/HIV Coinfection

Coinfection with HCV in HIV infected individuals is common, presumably due to the shared route of transmission of these viruses. The prevalence of HCV infection among HIV infected individuals varies substantially among different risk groups (Dieterich et al., 1999). The prevalence of HCV among injection drug users who are HIV infected is 50 to 90% (Huemer et al., 1990, Thomas et al., 1996). Among HIV positive hemophiliacs, coinfection with HCV has been reported upto 85 % (Zylbergberg et al., 1996, Dieterich et al., 1999). The rate of sexual transmission of HCV may be increased in the presence of HIV (Fiore et al., 1996, Wejstal, 1999). The rate of mother to infant transmission of HCV has been reported to increase in the presence of HIV, presumably due to high level of viremia observed in coinfected individuals (Thomas et al., 1998, Zanetti et al., 1999).

3.3.1 The impact of HIV on HCV infection

Only 20 -30% of immunocompetent individuals with HCV will progress to cirrhosis over an average of 15-30 years. Various studies suggest that in HIV positive individuals progression is likely to occur more frequently and at a faster rate (Soto et al., 1997, Benhamou et al., 1999). It has been found that coinfected patients have comparably higher levels of HCV viremia and HCV in other body fluids (Cribier et al., 1995). Although HIV/HCV coinfection results in a more rapid progression of liver disease ,its effect on mortality is controversial .Some studies have shown a higher rate of mortality from liver related disease in the coinfected patients (Di Martino et al., 2000), while others have not shown any effect on survival (Macias et al., 1998). Though false negative antibody tests are less common with newer assays in the setting of HIV infection and may relate to the degree of immunosuppression present (Berggren , 2001).

3.3.2 The impact of HCV on HIV infection

Although a matter of dispute, HCV may have a deleterious effect on HIV progression. The Swiss HIV cohort study and others have demonstrated that HCV infection was independently associated with an increased risk of progression to AIDS or death, despite a similar use of antiretroviral therapies within the coinfected groups as those with HIV alone (De Luca et al., 2002, Sulkowsky et al., 2002). Other studies have not shown any direct alteration of the course of HIV and progression to AIDS in the presence of HCV coinfection (Dorrucci et al., 1995, Bonacini et al., 2000).

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Equipments

Following equipments available at NRCS, CBTS were used during the entire period of study.

Centrifuge (Hettich, Rotina 35)

ELISA processor (BEP III ELISA Processor, Germany)

Refrigerator (White-Westinghouse, USA)

Incubator (Ambassador, India)

Micropipettes (Human, Germany)

4.1.2. Test kits and Reagents

HIV –Enzygnost Anti –HIV ½, Dade Behring, Germany

HIV TRI-DOT Kit (J. Mitra and Co)

Genedia anti –HCV ELISA kit, Green Cross Corporation, Korea

HCV TRI-DOT Kit (J. Mitra and Co)

4.1.3 Glasswares and others

Test tubes (Borosil, India)

Nonsterile Latex Disposable Gloves (Top Gloves, Malaysia)

Distilled water

Micropipette tips

4.2 Methodology

4.2.1 Study design

Present study was a descriptive cross-sectional study conducted in Nepal Red Cross Society, Central Blood Transfusion Service, Exhibition Road, Kathmandu, during a period of 9 months (1st December 2006 to 1st September 2007).

4.2.2 Study population

The study population was blood donors. Donors included in this study were donating blood in Blood Transfusion Center, Exhibition Road or in Mobile camps organized in Kathmandu Valley. The categories of donors that were present in this study were volunteers and replacement who may be first time or repeat donor. All the individuals selected by donor screening criteria as of NRCS, CBTS (Standard Operating Procedure, 2006) were requested to fill a donor questionnaire, also serving as a consent for HIV and HCV testing and each donor was given a donor number and for all later investigations, the donor number was used as identification.

Volunteer and Replacement Blood Donors

A volunteer (non-remunerative) donor is one who is not paid for the donated blood. A replacement donor is again a non remunerative donor who donated blood for a particular patient admitted in hospital in replacement.

First time and Repeat Donors

Donors who have made a recorded donation (by blood donor card/Register) or who

described themselves as repeat donors were defined as repeat donors. Donors who described themselves as first time donors were defined as first time donors.

4.2.3 Sample size and Inclusion criteria

Sample size for present seroprevalence study was estimated as per instructions of Health Research Methodology (2001) published by WHO. For the study on seroprevalence of HIV, a minimum sample size of 33,098 was estimated using the following parameters:

Confidence Interval- 95%
Assumed prevalence- 0.4% (Thapa, 2004)
Allowable error- 17%

Same sample size was used to study seroprevalence of HCV because the study was also aimed for overall coprevalence and coinfection rate.

Repeated samples from the same donor were not included in the study keeping in view of selection bias.

4.3 Collection of Blood sample

Blood samples were collected by medical professionals (Laboratory Technicians and Nurses) using aseptic technique. Before collection of sample each donor was requested to fill the donor form (Questionnaire).

With the help of sterile syringe, 350 ml blood was drawn in blood bag labeled with sample number. At the same time, about 5 ml of blood was drawn and dispensed in a test tube having labeled with corresponding sample number.

4.4 Sample processing

All serum samples were tested for presence of Anti HIV-1 and 2 antibodies and Anti HCV antibodies using the commercially available EIA based Test kits following the standard protocols recommended by the Kit manufacturers maintaining all the standard operating procedures developed by CBTS Laboratory.

4.4.1 Detection of anti HIV antibodies by Enzygnost Anti HIV ½ Test

The methodology used for the detection of anti HIV by ELISA was exactly followed as directed by the manufacturer's protocol (Dade Behring Marburg Gmbh, Anti HIV ½ plus, Germany).

i. All the reagents and serum were brought at room temperature
ii. The number of wells required for the assay was ascertained.
iii. 25 microliter /well sample buffer was filled in each well.
iv. 100 microliter/well of the negative control was pipetted into 2 wells and 100 microliter/well of the positive control in 2 wells.100 microliter/well of undiluted serum was dispensed into the following wells.
v. The test plate was then placed inside the Behring ELISA processor
vi. The remaining processing steps were then carried out fully automatically.

4.4.2 Detection of anti HIV antibodies by HIV TRI-DOT Test

The methodology used for the detection of anti HIV by HCV TRI-DOT was exactly followed as directed by the manufacturer's protocol (J. Mitra and Co. Pvt. Ltd).

i. All the reagents and serum were brought at room temperature
ii. 3 drops of buffer solution was added to the center of the test device.

iii. Using the sample dropper provided, a drop of serum to be tested was placed on the center of the device.
iv. 5 drops of buffer solution was added .
v. 2 drops of Protein A conjugate was added.
vi. 5 drops of buffer solution was added.
vii. Result was read immediately.

4.4.3 Detection of anti HCV antibodies by Genedia HCV ELISA Test

The methodology used for the detection of anti HCV by ELISA was exactly followed as directed by the manufacturer's protocol (Green Cross Corporation, Korea).

i. All the reagents and serum were brought at room temperature
ii The number of wells required for the assay was ascertained.
iii. 200 microliter/well Sample diluent was filled in each well.
iv. 10 microliter/well of the negative control was pipetted into 2 wells and 10 microliter/well of the positive control in to 2 wells.100 microliter/well of undiluted serum was dispensed into the following wells.
v. The remaining processing steps were then carried out fully automatically.

4.4.4 Detection of anti HCV antibodies by HCV TRI-DOT Test

The methodology used for the detection of anti HCV by HCV TRI-DOT was exactly followed as directed by the manufacturer's protocol (J. Mitra and Co. Pvt. Ltd).

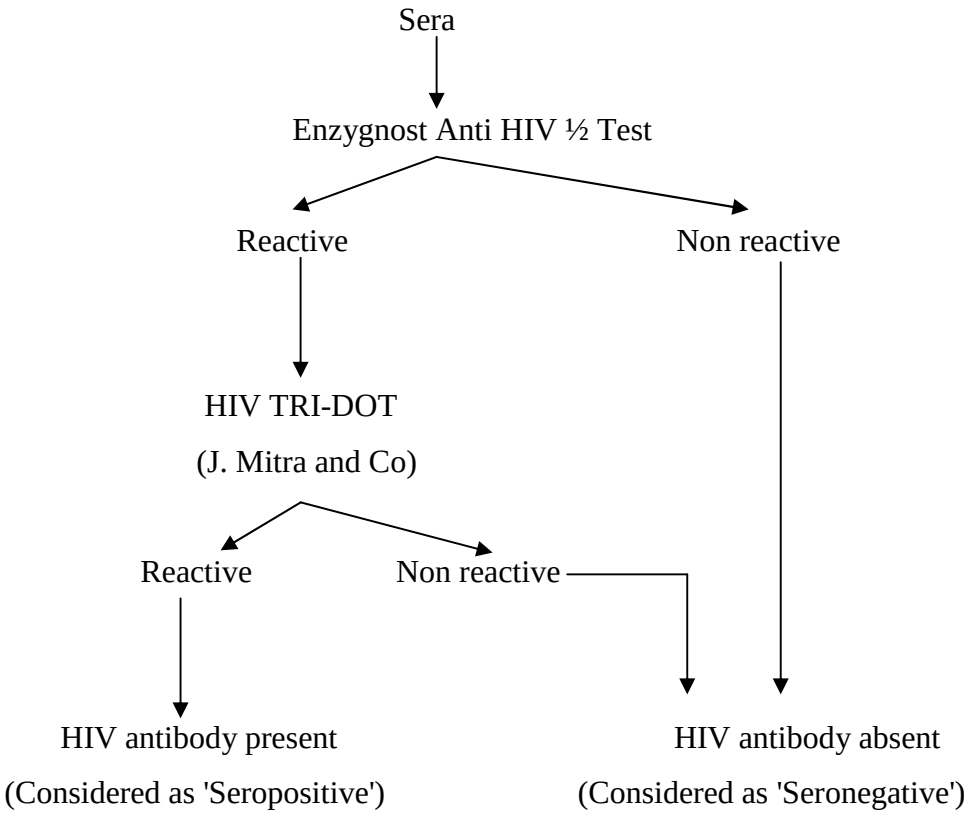
i. All the reagents and serum were brought at room temperature
ii. 3 drops of buffer solution was added to the center of the test device.
iii. Using the sample dropper provided, a drop of serum to be tested was placed on the center of the device.
iv. 5 drops of buffer solution was added .
v. 2 drops of Protein A conjugate was added.
vi. 5 drops of buffer solution was added.

vii. Result was read immediately.

4.4.5 Diagnostic strategy

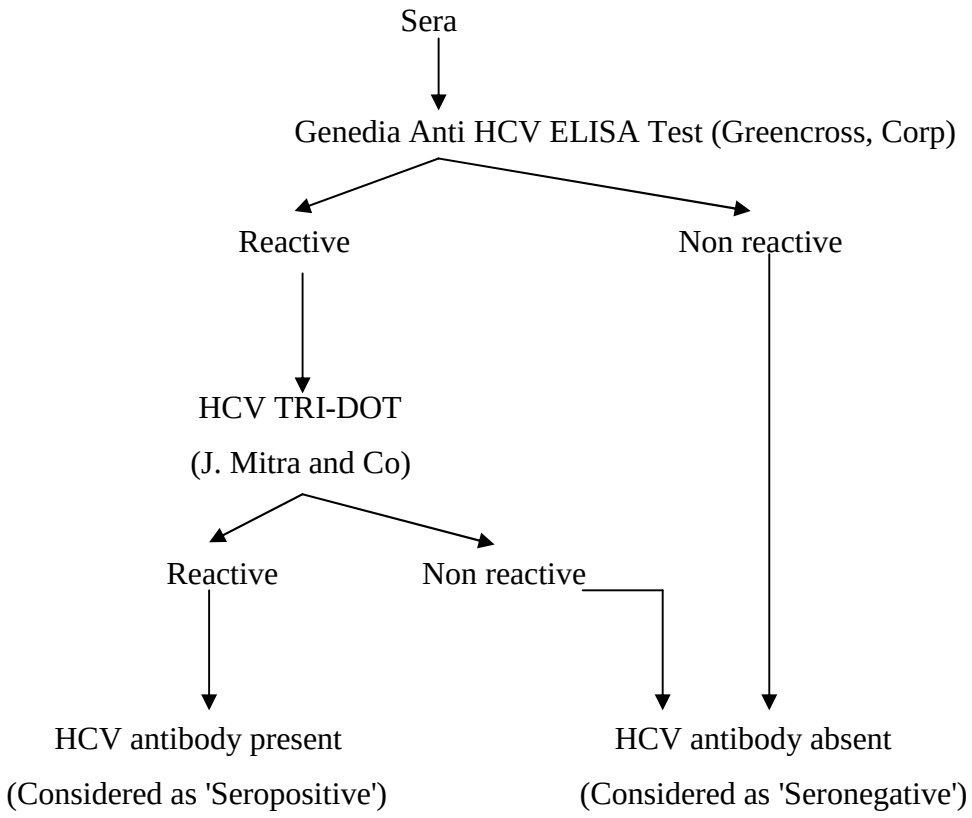
4.4.5.1 HIV testing strategy

HIV testing was done following the alternative HIV testing strategy II recommended by WHO.



4.4.5.2 HCV testing strategy

Anti HCV testing was done using following strategy that uses two different Enzyme Immunoassays.



4.5 Statistical Analysis

The statistical significance of the differences in seroprevalence was tested using Chi Square test and Pearson's correlation Test, whenever applicable. A detailed Statistical analysis is presented in Appendix-IV.

CHAPTER-V

5. RESULTS

During the study period, screening of antibodies to HCV (Anti HCV antibodies) and HIV (Anti HIV-1 and 2 antibodies) was done in sera of 33,255 blood donors in Serology Laboratory of CBTS, NRCS, Exhibition Road, Kathmandu using the third generation ELISA kits and an automated ELISA processor (BEP III).

5.1 Pattern of Study population

A total of 33,255 blood donors were included in the present study. Among the total, 28,989 (87.2%) were males, 4,266 (12.8%) were females, 29,552 (88.9%) were volunteer donors while 3,703 (11.1%) were replacement donors. Similarly, 16,476 (49.5%) donors were first time donors and 16,779 (50.5%) were repeat donors. The age of the blood donors ranged from 18-60 years, with the mean age of 28.9 years (Std. Dev = 8.4). The mean age of male donors was 29.04 years (Std. Dev = 8.4) and of female donor was 28.35 years (Std .Dev = 8.6).

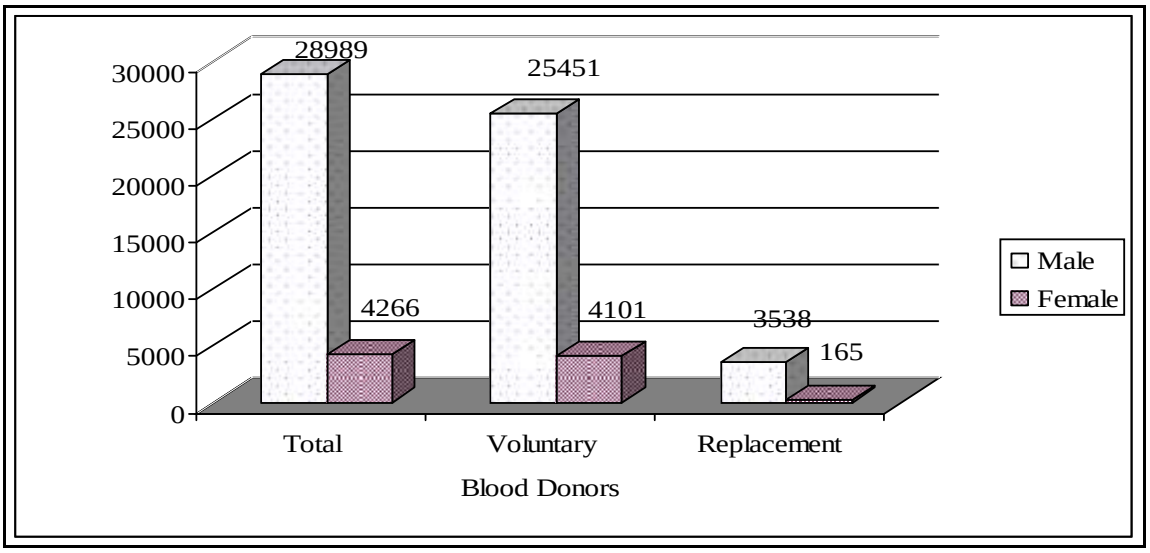


Figure 5.1: Type of donation and gender wise distribution of blood donors

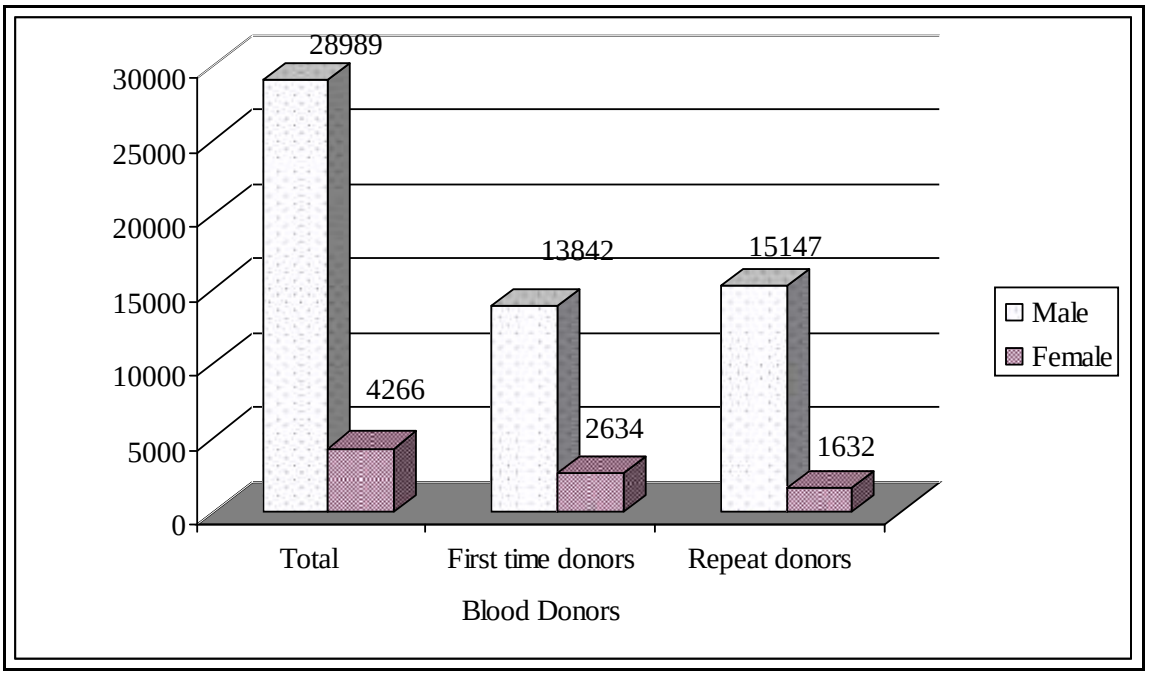


Figure 5.1.1: Times of donation and sex wise distribution of blood donors

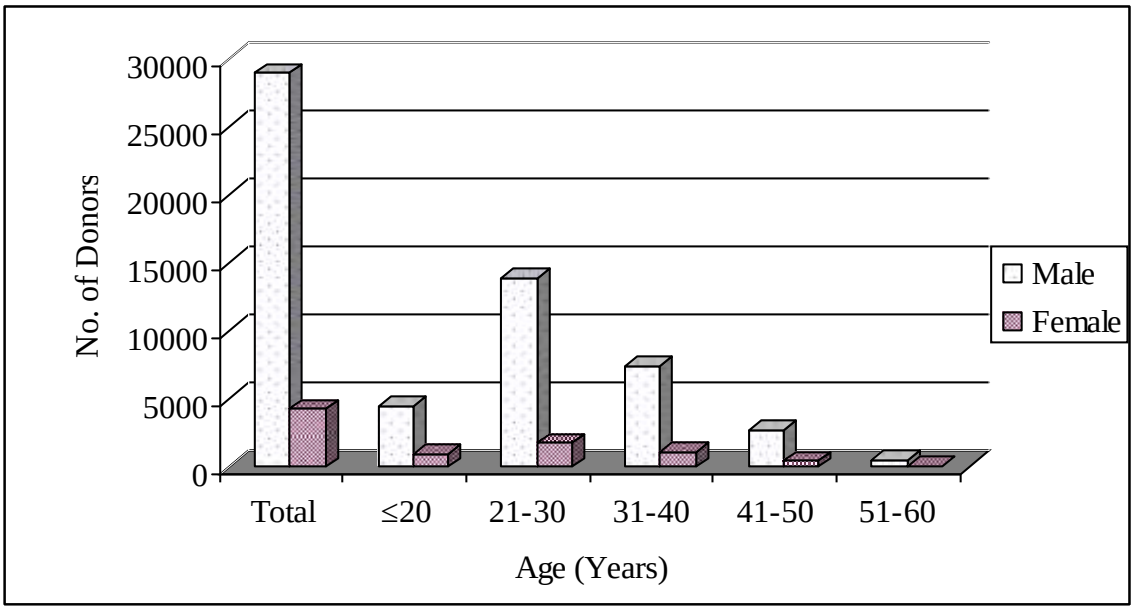


Figure 5.1.2: Age and sex wise distribution of blood donors

5.2 Seroprevalence of HCV

Among the total of 33,255 blood donors studied, 221 had positive serological test for Anti HCV antibodies. Thus, the overall seroprevalence of HCV among blood donors was found to be 0.66% (221/33,255). Higher seroprevalence was observed in male donors (0.7%) than in female donors (0.39%) and the difference observed in seroprevalence was statistically significant ($P < 0.05$) (Table 5.2).

S.N	Anti HCV Test	Male	Sero-Prevalence (%)	Female	Sero-Prevalence (%)	Total	Overall Sero-Prevalence (%)	P - value
1	Positive	204	0.7	17	0.39	221	0.66	P<0.05

2	Negative	28,785		4,249		33,034		
Total		28,989		4,266		33,255		

Table 5.2: Seroprevalence of HCV

5.3 Age and sex specific seroprevalence of HCV

The overall highest seroprevalence of HCV was observed in the age group 21-30 years followed by age group 31-40 years, whereas the mean age of the HCV seropositive donors was 28.16 years (Std. Dev = 6.8). The highest seroprevalence (0.82%), observed in age group 21-30 years, was significantly higher when tested with the other age groups as a whole ($P < 0.05$). Among the male donors, the highest seroprevalence (0.88%) was observed in the age group 21-30 years and the difference observed with other age groups as a whole was highly significant ($P < 0.05$). Among females, the highest seroprevalence (0.47%) was observed in the age group 41-50 years and the difference was not statistically significant when tested with other age groups as a whole ($P > 0.05$) (Table 5.3).

Age Group (Years)	Male	No. of Sero positive	Sero Prevalence (%)	Female	No. of Sero positive	Sero Prevalence (%)	Overall Sero prevalence (%)
≤20	4,462	21	0.47	972	4	0.41	0.46
21-30	13,931	123	0.88	1,773	6	0.33	0.82
31-40	7,388	50	0.67	1,067	5	0.46	0.65
41-50	2,742	9	0.32	419	2	0.47	0.34
51-60	466	1	0.21	35	0	0.00	0.19
Total	28,989	204		4,266	17		

Table 5.3: Distribution of HCV seropositive males and females according to age group

5.4 Pattern of HCV seroprevalence among volunteer and replacement Blood Donors

The overall seroprevalence of HCV was observed higher in volunteer blood donors than in replacement blood donors (0.69% vs. 0.4%) and the difference observed was statistically significant ($P < 0.05$). Higher seroprevalence was observed among male volunteer donors than among male replacement donors (0.74% vs. 0.39%) and the difference observed was statistically significant ($P < 0.05$). Contrastingly, higher seroprevalence was observed among female replacement donors than among female Volunteer donors (0.39% vs. 0.6%) but this result was not statistically significant ($P > 0.05$) (Table 5.4).

S.N	Subjects	Male			Female			Overall Sero-prevalence (%)
		Anti HCV Neg	Anti HCV Pos	Sero-Prevalence (%)	Anti HCV Neg	Anti HCV Pos	Sero-Prevalence (%)	
1	Volunteer donors	25,261	190	0.74	4,085	16	0.39	0.69
2	Replacement donors	3,524	14	0.39	164	1	0.6	0.4
		P < 0.05			P > 0.05			P < 0.05

Table 5.4: Distribution of HCV seropositive males and females by type of donation

5.5 Pattern of HCV seroprevalence among first time and repeated donors

The overall seroprevalence of HCV among first time and repeat blood donors was almost similar (0.65% vs. 0.67% respectively). The HCV seroprevalence among male first time donors and among male repeat donors was almost similar (0.7% vs. 0.69% respectively) and the difference was not statistically significant (P > 0.05). Slightly higher seroprevalence was observed in female repeat donors than in female first time donors but the result was not statistically significant (0.42% vs. 0.37%) (P > 0.05) (Table 5.5).

5.6 Pattern of HCV seroprevalence among volunteer and replacement donors according to times of donation

The seroprevalence of HCV among first time volunteer donors was 0.71% and among first time replacement donors was 0.24% and the difference observed was statistically significant (P < 0.05).

S.N	Subjects	Male			Female			Overall Sero Prevalence (%)
		Anti HCV Neg	Anti HCV Pos	Sero Prevalence (%)	Anti HCV Neg	Anti HCV Pos	Sero Prevalence (%)	
1	First time donors	13,744	98	0.7	2,624	10	0.37	0.65
2	Repeat donors	15,041	106	0.69	1,625	7	0.42	0.67
		P > 0.05			P > 0.05			P > 0.05

Table 5.5: Distribution of HCV seropositive males and females according to times of donation.

S.N	Subjects	First time donors			Repeat donors		
		Anti HCV Neg	Anti HCV Pos	Sero-Prevalence (%)	Anti HCV Neg	Anti HCV Pos	Sero-Prevalence (%)
1	Volunteer donors	14,341	103	0.71	15,005	103	0.68
2	Replacement donors	2,027	5	0.24	1,661	10	0.59
		P < 0.05			P > 0.05		

Table 5.6: Distribution of HCV seropositive volunteer and replacement donors according to times of donation

The seroprevalence of HCV among repeat volunteer donors was 0.68% and among repeat replacement donor was 0.59%, the little difference observed was not statistically significant (P > 0.05) (Table 5.6).

5.7 Seroprevalence of HIV

Out of 33,255 blood donors studied, 65 had positive serologic test for Anti HIV antibodies. Thus, the seroprevalence of HIV among the blood donors was found to be 0.19%. No significant difference in seroprevalence was found between males and females, but the percentage of seropositive male donors (0.2%, 58/28,989) was found slightly higher than that of females (0.16%, 7/4,266) (P > 0.05).

S. N	Test	Male	Sero Prevalence (%)	Female	Sero Prevalence (%)	Total	Overall Sero Prevalence (%)	P-value

1	Anti HIV Positive	58	0.2	7	0.16	65	0.19	
2	Anti HIV Negative	28,931		4,259		33,190		P >0.05
Total		28,989		4,266		33,255		

Table 5.7: Seroprevalence of HIV

5.8 Age and sex specific seroprevalence of HIV

The highest percentage of blood donors were of the age group 21-30 years both in males and females. The overall highest seroprevalence i.e. 0.23% was observed in the age group 21-30 years. The highest seroprevalence among males i.e. 0.24% was observed in the age group 21-30 years followed by the age group 51-60 years and ≤ 20 years in which the seroprevalence was 0.21% and 0.20% respectively. The highest seroprevalence among female i.e. 0.23% was observed in age group 41-50 years followed by the age group 31-40 years in which the seroprevalence was 0.18% (Table 5.2 and Figure 5.11.4). The mean age of the HIV seropositive blood donors was 27.7 years (Std. Dev = 8). The difference observed in the highest age group was not also statistically significant when tested with other groups as a whole (P > 0.05).

Age Group (Years)	Male	No. of Sero positive	Sero Prevalence (%)	Female	No. of Sero positive	Sero Prevalence (%)	Overall Sero Prevalence(%)

≤20	4,462	9	0.20	972	1	0.10	0.18
21-30	13,931	34	0.24	1,773	3	0.16	0.23
31-40	7,388	9	0.12	1,067	2	0.18	0.13
41-50	2,742	5	0.18	419	1	0.23	0.18
51-60	466	1	0.21	35	0	0.00	0.19
Total	28,989	58		4,266	7		

Table 5.8: Distribution of HIV seropositive males and females according to age group

5.9 Pattern of HIV seroprevalence among volunteer and replacement Blood Donors

Among the total 29,552 volunteer donors, 25,451 were males whereas 4,101 were females and out of 3,703 replacement donors, 3,538 were males and 165 were females. The overall seroprevalence of HIV was observed higher in volunteer donors than in replacement donors (0.2% vs. 0.13%) but the difference was not statistically significant (P > 0.05). Higher seroprevalence was observed among male volunteer donors than among male replacement donors (0.2% vs. 0.14%) and this difference was not statistically significant (P > 0.05) (Table 5.9). Similarly, higher seroprevalence was observed among female volunteer donors than among female replacement donors (0.17% vs. 0.00%) but this difference was not statistically significant (P > 0.05).

S.N	Subjects	Male			Female			Overall
		Anti HIV Neg	Anti HIV Pos	Sero Prevalence (%)	Anti HIV Neg	Anti HIV Pos	Sero Prevalence (%)	Sero prevalence (%)

1	Volunteer Donors	25,398	53	0.2	4,094	7	0.17	0.2
2	Replacement Donors	3,533	5	0.14	165	0	0	0.13
		P > 0.05			P > 0.05			P > 0.05

Table 5.9: Distribution of HIV seropositive males and females according to type of donation

5.10 Pattern of HIV seroprevalence among first time and repeat blood donors

Among the total 16,476 first time blood donors, 13,842 were males and 2,634 were females and among 16,779 repeat blood donors, 15,147 were males and 1,632 were females. The overall seroprevalence of HIV among first time and repeat blood donors was almost same (0.20% vs. 0.19%). Slightly higher seroprevalence was observed among male first time donors than among male repeat donors (0.22% vs. 0.17%) but the difference observed was not statistically significant (P > 0.05). Considerably higher seroprevalence was observed among female repeat donors than among female first time donors (0.3% vs. 0.07%) but the difference observed was not statistically significant (P > 0.05) (Table 5.10).

S.N	Subjects	Male			Female			Overall
		Anti HIV Neg	Anti HIV Pos	Sero Prevalence (%)	Anti HIV Neg	Anti HIV Pos	Sero Prevalence (%)	Sero Prevalence (%)
1	First time donors	13,811	31	0.22	2,632	2	0.07	0.2
2	Repeat	15,120	27	0.17	1,627	5	0.3	0.19

	donors							
		P > 0.05			P > 0.05			P > 0.05

Table 5.10: Distribution of HIV seropositive males and females according to times of donation

5.11 Pattern of HIV seroprevalence among volunteer and replacement donors according to times of donation

Among the total 29,552 volunteer donors, 14,444 were first time donors and 15,108 were repeat donors and out of total 3,703 replacement donors, 2,032 were first time donors and 1,671 were repeat donors. The seroprevalence of HIV among first time volunteer donors was 0.21% and among first time replacement donors was 0.09% but the difference observed was not statistically significant (P > 0.05). The seroprevalence of HIV among repeat volunteer donors was 0.19% and among repeat replacement donor was 0.17%, the difference observed was not statistically significant (P > 0.05) (Table 5.11).

S.N	Subjects	First time donors			Repeat donors		
		Anti HIV Neg	Anti HIV Pos	Sero Prevalence (%)	Anti HIV Neg	Anti HIV Pos	Sero Prevalence (%)
1	Volunteer donors	14,413	31	0.21	15,079	29	0.19
2	Replacement donors	2,030	2	0.09	1,668	3	0.17
		P > 0.05			P > 0.05		

Table 5.11: Distribution of HIV seropositive volunteer and replacement donors according to times of donation

5.12 Coprevalence of HCV and HIV

Among the total 33,255 blood donors screened, 286 (0.86 %) donors were seropositive for HCV or HIV or both. Out of those 286 seropositive donors 262 (91.6 %) were males whereas 24 (8.4 %) (Figure 5.11.1) were females. Among the total of 33,255 blood donors, 7 donors were seropositive for HIV as well as HCV, thus giving the overall HCV/HIV coprevalence of 0.02 % (Table 5.11). Among the total of 65 HIV seropositive donors, 7 donors were seropositive for HCV, thus giving the HCV coprevalence of 10.76 % (7/65) among the HIV seropositive blood donors (Figure 5.11.2). Similarly, among the total of 221 HCV seropositive donors, 7 donors were seropositive for HIV, thus giving the HIV coprevalence of 3.16% (7/221) among the HCV seropositive donors (Figure 5.11.3). The association of the HCV and HIV seropositivity among the blood donors was found to be statistically significant ($P < 0.05$). All the seven HCV/HIV seropositive donors were male volunteer repeat blood donors except one of them who donated for first time and their age ranged from 24-28 years. Notably, the highest seroprevalence of HIV as well as HCV in females was

observed in the age group of above 30 years whereas the highest seroprevalence of HIV as well as HCV in males was observed in the age group of below 30 years (Figure 5.11.4 and Figure 5.11.5). The seroprevalence of HCV and HIV in overall blood donors according to different age groups is positively correlated (Pearson's correlation coefficient, r = + 0.97) and the correlation coefficient was statistically significant (P < 0.05) (Figure 5.11.6). The seroprevalence of HCV and HIV among female donors of different age groups was positively correlated (Pearson's correlation coefficient, r = +0.92) and the correlation coefficient was statistically significant (P < 0.05) (Figure 5.11.7). The seroprevalence of HIV among male and among female donors according to different age groups was strongly positively correlated (Pearson's correlation coefficient, r = +0.89) and the correlation coefficient was found to be statistically significant (P < 0.05) (Figure 5.11.4). Similarly, the seroprevalence of HCV among male and among female donors, according to different age groups was strongly positively correlated (Pearson's correlation coefficient, r = +0.83) and this correlation coefficient was also statistically significant (P < 0.05) (Figure 5.11.5).

S.N	Test	Anti HIV Positive	Anti HIV Negative	Total	P value
1	Anti HCV Positive	7(0.02%)	214	221	P < 0.05
2	Anti HCV Negative	58	32,976	33,034	
	Total	65	33,190	33,255	

Table 5.12: Coprevalence of HCV and HIV

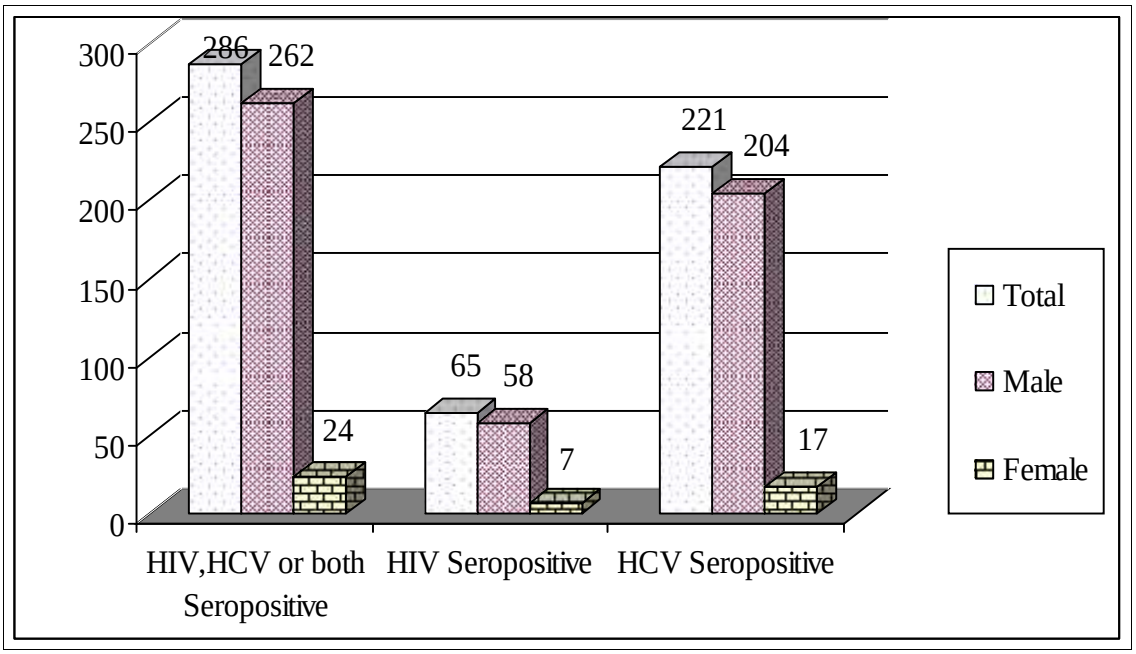


Figure 5.11.1: Sex wise distribution of HIV and HCV seropositive donors

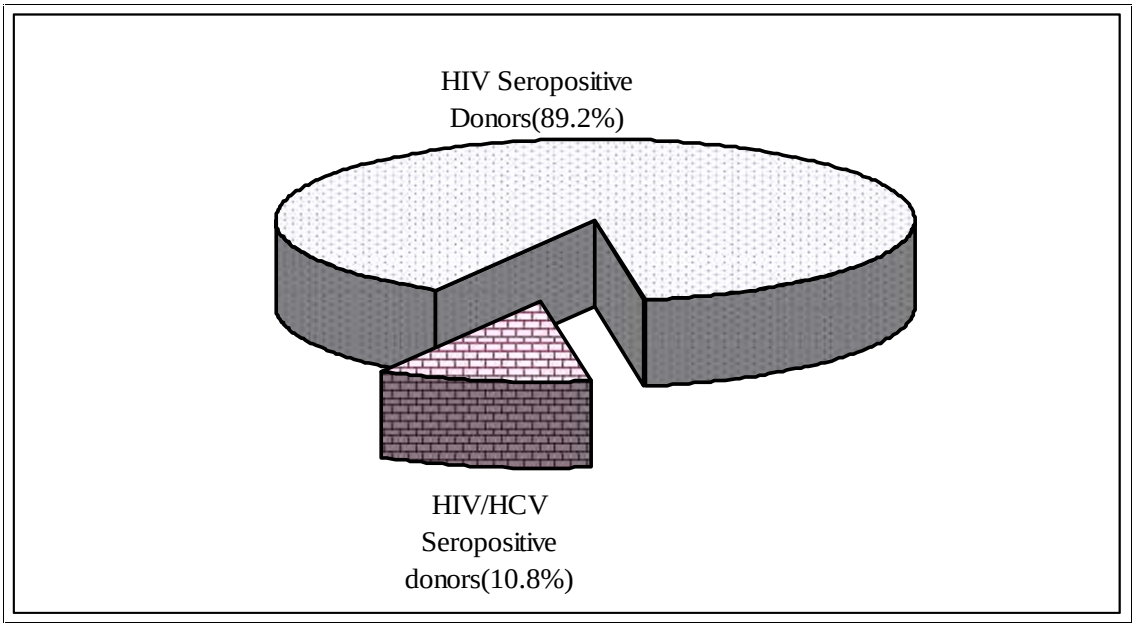


Figure 5.11.2: Coprevalence of HCV among HIV seropositive blood donors

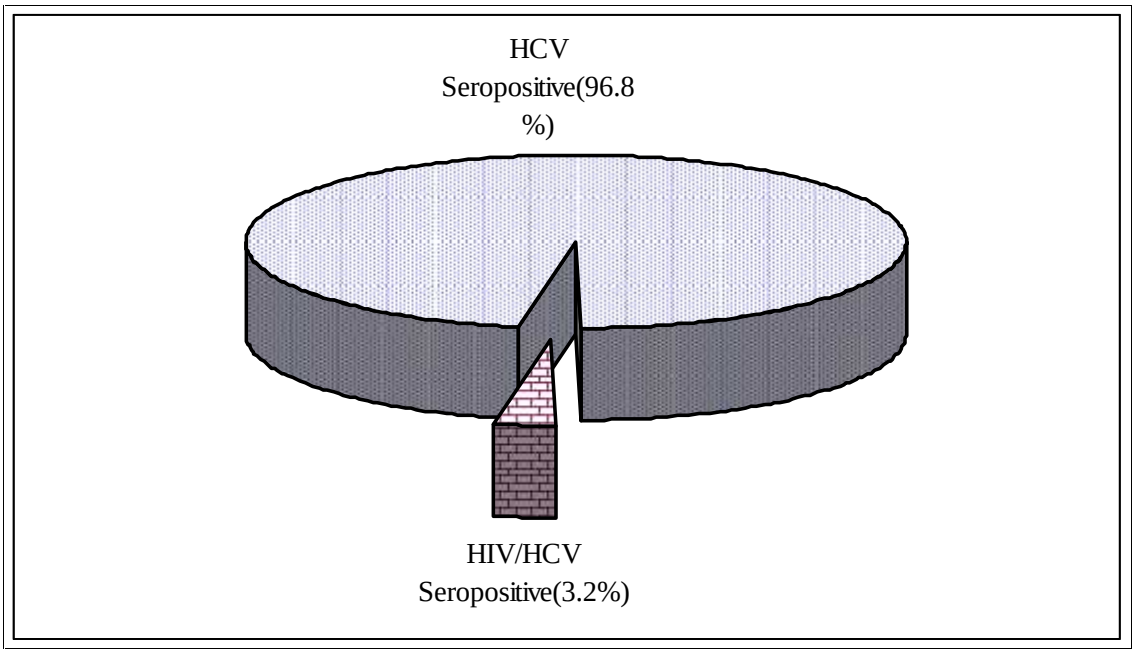


Figure 5.11.3: Coprevalence of HIV among HCV seropositive blood donors

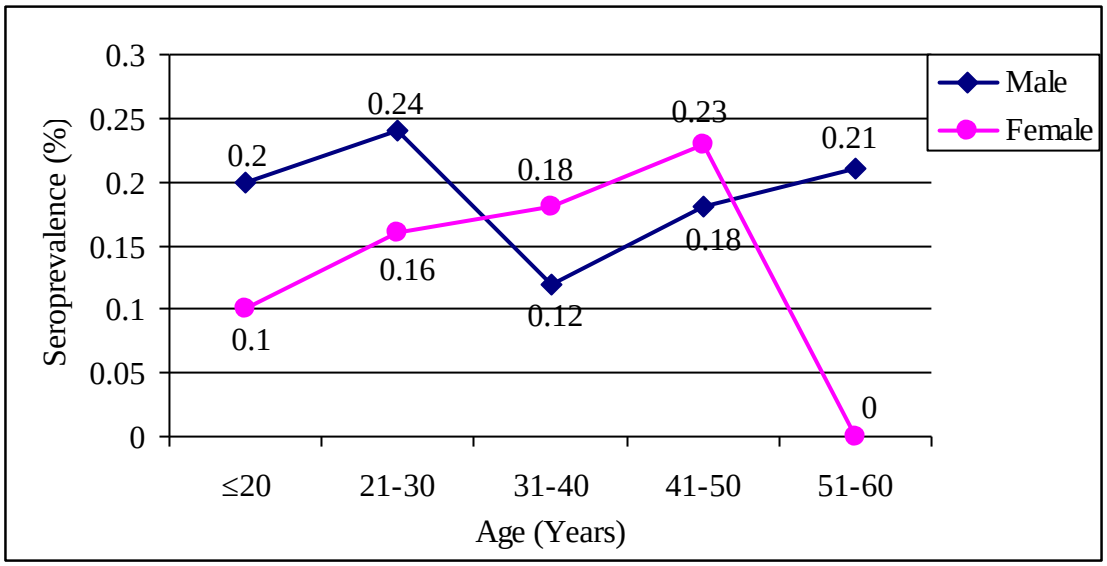


Figure 5.11.4: Age specific seroprevalence of HIV among male and female blood donors

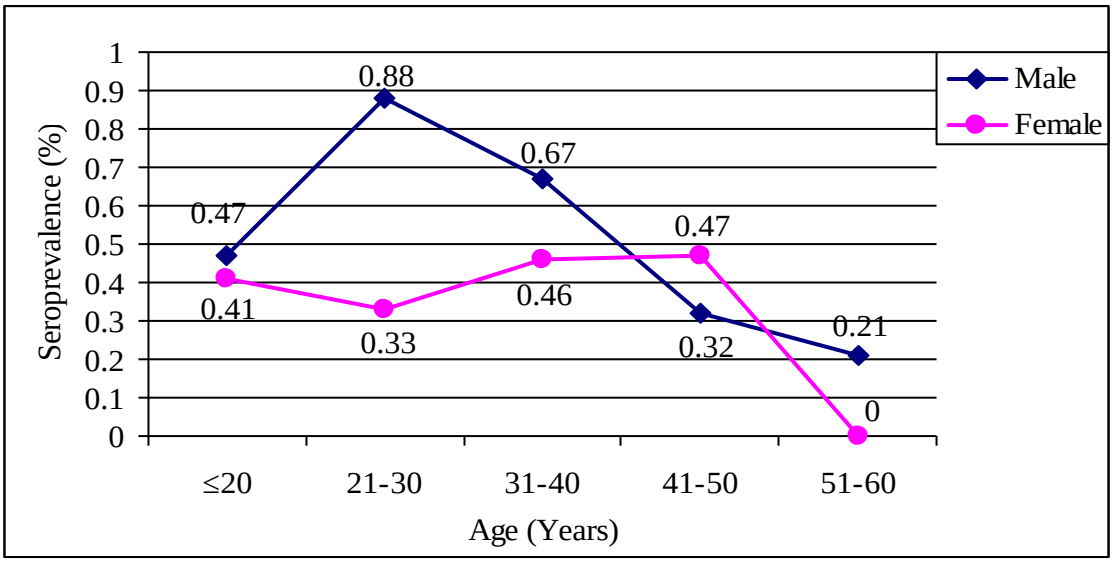


Figure 5.11.5: Age specific seroprevalence of HCV among male and female blood donors

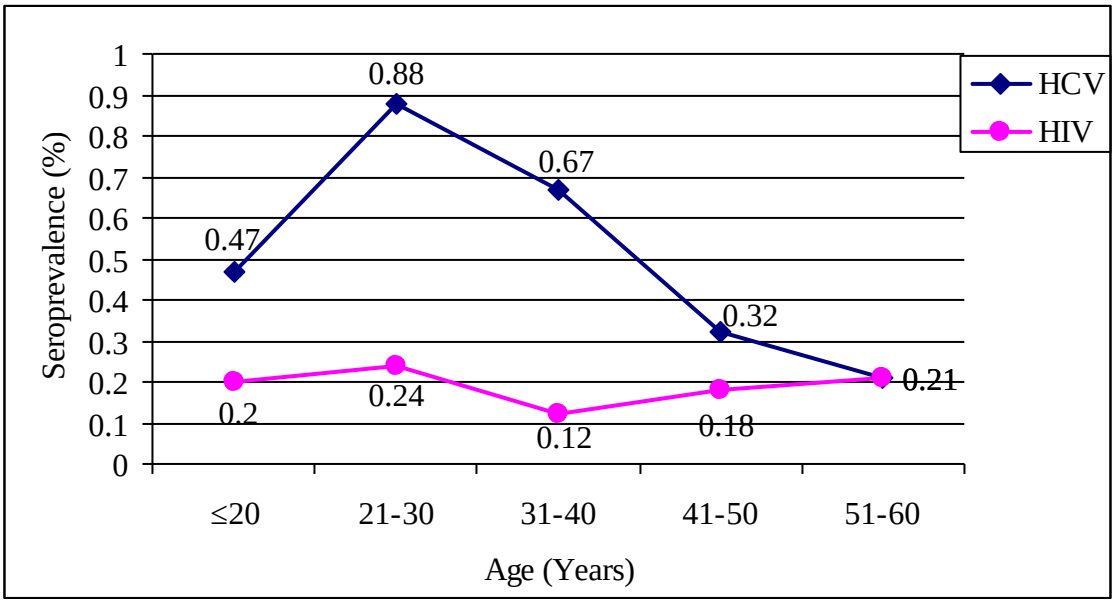


Figure 5.11.6: Age wise trend of HCV and HIV seroprevalence among male Blood Donor

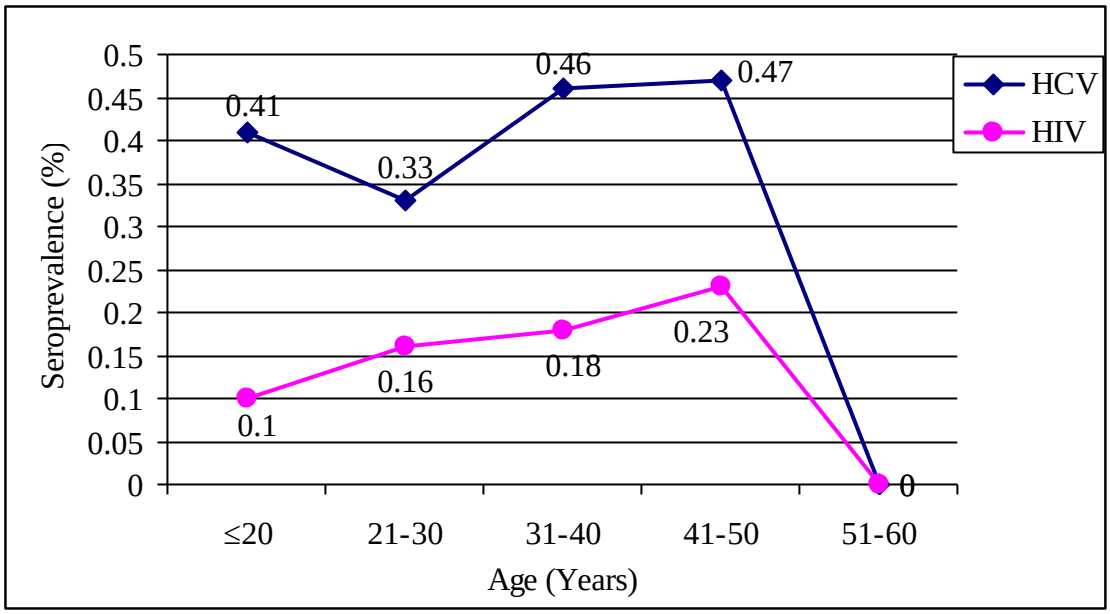


Figure 5.11.7: Age wise trend of HCV and HIV seroprevalence among female blood donors

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

A total of 33,255 blood donors were screened to detect the presence of Anti HIV-1 and/or 2 and Anti HCV antibodies. Repeatedly reactive test results in two different EIAs were considered as seropositive for HIV or HCV infection and these data were used in order to estimate the seroprevalence of respective infection as per NRCS, CBTS protocol. Blood donors were chosen as study population because it would give the most closer seroprevalence rate to healthy looking general population as well as it would highlight in different trends of seroprevalence according to age, gender, type of donor

and times of donation among donors that would ultimately give evidences to authorities for assessing their service and appropriately plan for assurance of blood safety.

Among the 33,255 blood donors screened, the overall seroprevalence of Anti HCV antibodies was observed to be 0.66% (221/33,255). Higher seroprevalence was observed among male donors (0.7%) than among female donors (0.39%) and the difference observed in seroprevalence was found to be statistically significant. The overall highest seroprevalence (0.82%) was observed among the age group 21-30 years and the difference observed with the other groups was statistically significant. Among the male donors, the highest seroprevalence (0.88%) was observed in the age group 21-30 years and the difference in seroprevalence observed with the other groups was statistically significant. Interestingly, among the females, the highest seroprevalence (0.47%) was observed in the age group 41-50 years but the difference in seroprevalence compared with other age groups was not statistically significant. This gender wise difference in the seroprevalence of Hepatitis C might be due to the difference in the risk factors and predominant modes of transmission among male and female donors.

Overall seroprevalence of HCV observed in this study was in accordance with the study of Shrestha (1998), in which a seroprevalence of 0.6% was observed among the general population. Notably, present seroprevalence was quite lower than as reported by Sawayama et al. (1996) among general population (1.7%) and by Joshi et al. (1999) among blood donors of Kathmandu valley (1.1%). Present seroprevalence was fairly higher than as reported by Singh (1992) among blood donors (0.3%) and by Shrestha (2006) among healthy males seeking jobs abroad (0.35%) but both of these studies had used very small sample size. Thus, there has been difficulty in compairing the results of this study with other studies done above as these studies have used very small sample size and different generation test kits.

Present seroprevalence of HCV among blood donors observed in this study was lower than as reported by Al Faleh et al. (1995) in Saudi Arabia (1.8%), Al Guneid et al.

(1993) in Yemen (2.1%), 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%), Panigrahi et al. (1997) in New Delhi of India (1.8%), Frommel et al. (1993) in Ethiopia (1.6%), Ilako et al. (1995) in Kenya (0.9%), Ampofo et al. (2002) in Ghana (8.4%), Carreto-Velez et al. (2003) in Irapuato of Mexico (1.14%), Gupta et al. (2004) in Ludhiana of India**.**

Studies from Northern India have reported HCV seroprevalence ranging from 0.53 - 5.1% among their blood donors (Choudhary et al., 1995, Makroo et al., 1999, Jain et al., 2003). Studies from Western India have reported HCV seroprevalence ranging from 0.34 - 2.5 % (Arankale et al., 1995, Deshpandey et al., 1998).

The seroprevalence of HCV observed in present study was relatively lower than as reported in different cities of Pakistan. In Pakistan, the seroprevalence of HCV has been reported 2.2% from Peshawar (Ahmad et al., 2004), 5.14% from Islamabad (Asif et al., 2004), 4 - 6.21% from Rawalpindi (Ryas et al., 2001, Khattak et al., 2002, Mumtaz et al., 2002, Chaudhary et al., 2007), 2.89-4.97% from Lahore (Ahmad et al., 2002, Rahman et al., 2002), 3.26% from Sialkot (Alam et al., 2001), 1.8-6.8% from Karachi (Ahmed et al., 2001, Akhtar et al., 2004), 1.87% from healthy blood donors of Queta (Ali et al., 2003) but Mahmood et al., (2004) have reported a lower seroprevalence (0.27%) in Multan.

Present seroprevalence of HCV among blood donors in Kathmandu Valley was higher than reported by Mutlu et al. (2004), in Turkey (0.37%), Yumiko et al. (2007), in Phillipines (0.3%), Tanaka et al. (2004) in Japan (0.49%), Thakral et al. (2006) in India (0.44%), Mehata et al. (2002) in Rajasthan of India (0.27%).

A thorough literature review suggests that seroprevalence of HCV among blood donors of Kathmandu valley is relatively lower than in other South Asian countries but fairly

higher than in developed countries. This might be probably due to geographical variations, education, testing facilities and strategies, risk behaviors etc.

In the present study, an overall trend of decreasing seroprevalence with increasing age except in very younger age (i.e. age group ≤ 20 years) was observed, which was similar to the data reported by Jain et al. (2003) from New Delhi of India. Such result might be possibly due to higher exposure rate to HCV in younger age. The maximum seroprevalence rate was observed at the age group 21-30 years which was also in accordance with the data reported by Jain et al. (2003). When the data was stratified and gender wise analysis was done this trend was found consistent with male donor population only and in case of female donors there was a decreasing trend from very younger age but there was a gradual but non significant increase in seroprevalence at the age of 31-40 and 41-50 years.

In the present study, a significant difference in seroprevalence of HCV among different sex was observed but no such significant difference had been reported by Matte et al. (2006) in Tanjania, Jain et al. (2003) in New Delhi of India whereas higher seroprevalence in male than in female donors (0.35% vs. 0.10%) had been reported from Philippines (Yumiko et al., 2007). However, a higher seroprevalence among female donors than in male donors (1.42% vs. 1.07%) had been reported from Irapuato of Mexico (Carreto-Velez et al., 2003). It has been suggested that such a gender wise difference of seroprevalence might be due to differences in the risk behavior, actual epidemic situation, education, average age of blood donors, donor selection criteria, laboratory diagnostic algorithms, risk factors and modes of transmission.

In the present study, higher seroprevalence of HCV was observed among volunteer donors than among replacement donors (0.69% vs. 0.4%) and the difference was statistically significant. The seroprevalence was higher among first time volunteer donors than among first time replacement donors (0.71% vs. 0.24%) and the difference was statistically significant. Contrastingly, higher seropositivity was reported among

first time replacement donors than in first time volunteer donors (0.60% vs. 0.27%) by Thakral et al. (2006) in India and similarly, higher seroprevalence among replacement donors has been reported by Nanu et al. (1997) from North India. The data in present study from Kathmandu valley does not support the assumption that, self motivated volunteer donors are the safe source of blood supply in the donor population studied.

In the present study, almost similar seroprevalence of HCV was observed among first time and repeat blood donors. This result was in accordance with that reported by Jain et al. (2003) in New Delhi of India and Chaudhary et al. (1995) from India. This result was also in accordance with the study by Retrovirus Epidemiology Donor Study (REDS) group (Schreiber et al., 2001) who also reported no difference in donor behavioural risk factors and incidence of HCV infection among frequent repeat whole blood donors than infrequent repeat whole blood donors. The seroprevalence of HCV among first time voluntary donors (0.71%) and among first time replacement donors (0.24%) observed in present study was significantly different and was totally in discordance with the study of Thakral et al. (2006). The seroprevalence among first time volunteer donors and among repeat volunteer donors was found to be almost similar (0.71% vs. 0.68%) which was against the assumption that the regular volunteer donors are the safest source of blood supply under the population studied. Such trend might either be due to the lack of awareness among both donor groups regarding minor modes of HCV transmission like tattooing, ear/nose piercing, use of contaminated Scissors and blades by Barbers and sharing of shaving kits which may be responsible for maintaining a relatively constant seroprevalence rate among the two groups or ineffectiveness of post donation education and counseling in the donor population studied.

Among the 33,255 blood donors screened, the overall seroprevalence of Anti HIV-1 and 2 was observed to be 0.19% (65/33,255). The seroprevalence rate observed in present study was significantly lower than reported by Thapa (2004) who reported a seroprevalence rate of 0.41% among the blood donors of Kathmandu. Relatively lower

seroprevalence rate was observed in present study than reported by Sharma et al., (2002) among donors in Tribhuvan University Teaching Hospital, Kathmandu (0.30%) and by UNAIDS/WHO (2002) who reported a quite higher seroprevalence rate (0.59%) (http://www.un.org.np/index.php). The decrease in the seroprevalence rate might be due to increased awareness to HIV/AIDS, self exclusion from donation by those who practice risk behavior, using of stringent donor selection criteria by the authorities, regarding seroprevalence of HIV.

Present seroprevalence of HIV among blood donors in Kathmandu Valley observed in this study was more or less similar to the data reported by Kaur et al (2001) in India (0.26%), by Mathai et al. (2002) in India (0.2%), by Dey et al. (2002) in India (0.32%), Carreto Velez et al. (2003) in Irapuato of Mexico (0.24%).

Higher seroprevalence of HIV among blood donors than observed in present study has been reported by Ampofo et al. (2002) in Ghana (3.8%), by Matte et al. (2006) in Dar Es Salaam of Tanzania (3.8%), by Mwangi (1999) in Nairobi of Kenya, 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%).

Present seroprevalence of HIV among blood donors in Kathmandu Valley observed in this study was quite higher than as reported in other studies among blood donors by Yumiko et al. (2007) in Phillipines (0.006%), by Gupta et al. (2004) in Ludhiana of India (0.084%), AyalaGaylan et al. (1997) in Mexico (0.02%), Rahaman et al. (2002) in Pakistan (0.001%), Kakepoto et al. (2002) in Pakistan (0.007%).

Such differences in seroprevalence might be due to geographical variation, actual epidemic situation, donor selection criteria, sensitivity and specificity of test kits as well as diagnostic algorithms used in each study.

In the present study, no significant difference in seroprevalence of HIV was observed between males and females (0.2% in male vs. 0.16% in female donors) which was in

accordance with the data reported by Thapa (2004). In contrast to this data Carreto Velez et al. (2003) from Mexico has reported a higher seroprevalence among male than among female donors (0.28% vs. 0.11%).

In the present study, the highest seroprevalence of HIV (0.23%) was observed in the age group 21-30 years and this result was consistent among male donors when stratification was done but among female donors the highest seroprevalence was observed in the age group 41-50 years whereas lowest seroprevalence among male donors was observed in the same age group (Figure 5.11.4). Such, interesting trend might indicate toward the differences in mode of acquisition of HIV, age during acquisition of HIV and other risk factors but this is very unlikely in our study population because the seroprevalence observed in females of age group 41-50 years was not significantly different with other age groups ($P > 0.05$) and there was a significant positive correlation of HIV seroprevalence between males and females of different age group ($P < 0.05$).

In the present study, no significant difference in seroprevalence of HIV was observed among voluntary and replacement donors though relatively higher seroprevalence was observed among volunteer donors than among replacement donors (0.2% vs. 0.13%) which was totally in accordance with the data reported by Thapa (0.43% vs. 0.26%). Contrastingly, other studies by Garg et al. (2001) in India, Sonwane et al. (2003) in India and Rukundo et al. (1997) in Uganda, Matte et al. (2006) in Tanzania have reported a lower seroprevalence of HIV among volunteer donors.

In the present study, almost same seroprevalence of HIV was observed among first time and repeat donors (0.20 % vs. 0.19% respectively). This result was totally in discordance with the data shown by Thapa (2004) who reported a significantly higher seroprevalence among first time donors than among repeat donors (0.53% vs. 0.25%). This might be due to the significant decrease in overall seroprevalence among blood donors in present study than reported by Thapa (2004) (0.19% vs. 0.41%) or might be due to ineffective post donation counselling.

Among the total 33,255 blood donors screened, the seroprevalence for either HCV or HIV or both was observed 0.86% (286/33,255) and 7 donors were seropositive for HIV as well as HCV, thus giving the overall HCV/HIV coprevalence rate of 0.02% (Table 5.11.1). The coprevalence of HCV among the HIV seropositive donors was 10.76 % (7/65) (Figure 5.11.2) and the coprevalence of HIV among the HCV seropositive donors was 3.16 % (7/221) (Figure 5.11.3).The association of the HIV and HCV seropositivity among the blood donors was found to be highly significant ($P < 0.05$).

Hempstead et al. (1991) has reported an overall 8% coprevalence of HCV among HIV seropositive blood donors from USA which is similar to the result of present study. Interestingly, in present study no coprevalence of HCV was observed among HIV seropositive female donors whereas Hempstead et al. (1991) has shown a 6% coprevalence of HCV among HIV seropositive female donors. Similarly, Mehata et al. (2002) from Rajasthan of India has reported a 1.6% coprevalence of HCV among HIV seropositive donors and 1.2% coprevalence of HIV among HCV seropositive donors. In comparison to above data, the coprevalence in both group of seropositive donors, observed in present study was alarmingly high.

Interestingly, in present study a significantly higher seroprevalence of HCV was observed among female donors of the age of 31-40 and 41-50 years, a trend seen in cases of sexually transmitted diseases whereas the same was observed to occur about the age of 21-30 years among male donors (Figure 5.11.4 and 5.11.5). Above data suggests that the predominant modes of transmission of HCV might be different in the different gender of donor population under study.

Among the seven HCV/HIV seropositive donors, six (85.7%) were volunteer repeat donors and all of them were in the age group of 21-30 years which suggests that the coinfection might be acquired due to practice of similar risk behavior.

In view of above situation, counselling is very important to lower the prevalence in repeat donors. Reporting of HCV and HIV status of all seropositive donors at the earliest point of time may also help in lowering the prevalence.

6.2 Conclusion

Present study revealed relatively lower seroprevalence of both HCV and HIV among blood donors compared to previous similar studies conducted in Nepal. HCV seroprevalence in blood donors was similar to the seroprevalence in general population when compared to other studies. Significantly higher seroprevalence of HCV was observed in male donors than in female donors but no significantly different seroprevalence of HIV was observed between male and female donors. Significantly higher seroprevalence of HCV was observed in volunteer donors than in replacement donors but no significantly different seroprevalence of HIV was observed between volunteer and replacement donors. Most importantly, no significant difference was observed in seroprevalence of HIV and HCV between the first time and repeat blood donors. Coprevalence of HCV and HIV was observed in blood donors and the coinfection rate of HCV in HIV seropositive donors and vice versa was alarmingly high.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

This study was conducted with the aim of studying seroprevalence of HCV and HIV among blood donors of Kathmandu Valley and compare the seroprevalence according to age, gender, type of donor and times of donation.

1. A total of 33,255 blood donors were included in the study. Among them, total males were 28,989 (87.2%) and females were 4,266 (12.8%). The donors age ranged from 18-60 years with the highest percentage of donors in the age group 21-30 years. 88.9% donors were volunteer doners whereas 11.1% doners were replacement donors. 49.5% donors were first time donors and 50.5 % were repeat donors.

2. The seroprevalence of HCV among total blood donors was 0.66%. Significantly, higher seroprevalence rate was observed among male donors than among female donors (0.7% vs. 0.39%) ($P < 0.05$).

3. The seroprevalence of HIV among total blood donors was 0.19%. No significant difference in seroprevalence was observed between male and female donors (0.2% vs. 0.16%) ($P > 0.05$).

4. The seroprevalence of HCV was highest (0.82%) in the age group of 21-30 years and the difference in seroprevalence with other age groups was statistically significant ($P < 0.05$).

5. The overall seroprevalence of HCV in male donors was observed in the age group of 21-30 years and the difference in seroprevalence with other age groups was statistically significant ($P < 0.05$) whereas the seroprevalence rate was highest in the age group 41-50 years in female donors but the difference with other age groups was not statistically significant ($P > 0.05$).

6. Decreasing seroprevalence of HCV was seen with increasing age except in very younger age (i.e. age group ≤20 years).

7. The seroprevalence of HIV was highest in the age group 21-30 years but the difference observed with other groups was not significant ($P > 0.05$).

8. The seroprevalence of HCV was signifcantly higher in Volunteer donors (0.69%) than in replacement donors (0.4%) ($P < 0.05$).

9. The seroprevalence of HIV was slightly higher among replacement donors (0.2%) than among volunteer donors (0.13%) but the difference was not statistically significant ($P > 0.05$).

10. Almost similar seroprevalence of HCV was observed among first time and among repeat blood donors (0.65% and 0.67%) ($P > 0.05$). Almost similar seroprevalence of HIV was observed among first time and among repeat blood donors (0.19% and 0.17%)($P > 0.05$).

12. The coprevalence of HCV/HIV among the total blood donor was 0.02% (7/33,255). The coprevalence of HCV among HIV seropositive donors was 10.76% (7/65). The coprevalence of HIV among HCV seropositive donor was 3.16% (7/221). 85.7% of coinfected donors were repeat volunteer donors and the age group of all the seven coinfected donors ranged from 24-28 years.

13. The HCV and HIV seropositivity was strongly associated with each other ($P < 0.05$).

7.2 Recommendations

Based on the findings of this study following recommendations have been made:

1. Effective post donation counselling and seroreactive donor notification, further testing for verification and counselling should be started to make regular repeat donors as the safe source of blood and blood products.

2. Mandatory screening of all donors for HIV and HCV with highly sensitive tools should be continued.

3. Adoption of WHO HIV testing algorithm in local context and strict follow up supervision is required to avoid discordant results, leading to quality test results.

REFERENCES

Aach RD, Stevens CE, Hollinger FB, Mosley JW, Peterson DA, Taylor PE, Johnson RG, Barbosa LH, Nemo GJ (1991) Hepatitis C virus infection in post-transfusion

Hepatitis. An analysis with first and second generation assays. N Engl J Med 325: 1325-1329

Abdel-Wahab MF, Zakaria S, Kamel M, Abdel-Khaliq MK, Mabrouk MA, Salama H, Esmat G, Thomas DL, Strickland GT (1994) High seroprevalence of hepatitis C infection among risk groups in Egypt. Am J Trop Med Hyg 51: 563-567

Abe K, Inchauspe G, Shikata T, Prince AM (1992) Three different patterns of hepatitis virus infection in Chimpanzees. Hepatology 15: 690-695

Ahmad J, Taj AS, Rahim A, Shah A, Rehman M (2004) Frequency of Hepatitis B and Hepatitis C in healthy blood donors of NWFP: a single center experience. J Postgrad Med Inst 18: 343-352

Ahmad S, Gull J, Bano KA, Aftab M, Kokhar MS (2002) Prevalence of anti Hepatitis C antibodies in healthy blood donors at Services Hospital Lahore. Pakistan Postgrad Med J 13: 18-20

Aktar S, Younus M, Adil S, Jafri SH, Hassan F (2004) Hepatitis C virus infection in asymptomatic male volunteer blood donors in Karachi, Pakistan. J Viral Hepat 11: 527–535

Alam M, Ahmed D (2001) Prevalence of antibodies to Hepatitis C virus in blood donors in Sialkot. J Coll Physicians Surg Pak 11: 783

Al-Faleh FZ, Ramia S, Arif M, Ayoola EA, al-Rashed RS, al-Jeffry M, Hossain A, el-Hazmi M (1995) Profile of hepatitis C virus and the possible modes of transmission of the virus in the Gizan area of Saudi Arabia: a community-based study. Ann Trop Med Parasitol 89: 431–437

Ali N, Nadeem M, Qamar A, Qureshi AH, Ejaz A (2003) Frequency of Hepatitis-C virus antibodies in blood donors in Combined Military Hospital, Quetta. Pak J Med Sci 19: 41-44

Allander T, Gruber A, Naghavi M, Beyene A, Soderstrom T, Bjorkholm M, Grillner L, Persson MA (1995) Frequent patient to patient transmission of hepatitis C virus in haematology ward. Lancet 345: 603-607

Alter MJ, Krusjon Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslov RA, Margolis HS (1999) The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 341: 556-562

Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE (1992) The natural history of community acquired hepatitis C in the United States. N Engl J Med 327: 1899-1905

Ampofo W, Nii-Treb N, Ansah J, Abe K, Naito H, Aidoo S, Nuvor V, Brandful J, Yamamoto N, Ofori-Adjei D, Ishikawq K (2002) Prevalence of blood-borne infectious diseases in blood donors in Ghana. J Clin Microbiol 40: 3523–3525

Andiman WA, Silva TJ, Shapiro TD, O'Connor T, Olson B (1992) Predictive value of the human immunodeficiency virus 1 antigen test in children born to infected mothers. Pediatr Infect Dis J 1: 436-40

Anonymous (1998) The importance of simple/rapid assays in HIV testing. Wkly Epidemiol Rec 73: 321-326

Apichartpiyakul C, Apichartpiyakul N, Urwijitaroon Y, Gray J, Natpratan C, Katayama Y, Fujii M, Hotta H (1999) Seroprevalence and subtype distribution of hepatitis

C virus among blood donors and intravenous drug users in northern/ northeastern Thailand. Jpn J Infect Dis 52: 121–123

Arankalle VA, Chadha MS, Jha J, Amrapurkar DN, Banerjee K (1995) Prevalence of anti HCV antibodies in Western India. Indian J Med Res l01: 91-93

Arthur LO, Bess JW Jr, Sowder RC, Benveniste RE, Mann DL, Chermann JC, Henderson LE (1992) Cellular proteins bound to immunodeficiency viruses: Implications for pathogenesis and vaccines. Science 258: 1935-1938

Arthur RR, Hassan NF, Abdallah MY, el-Sharkawy MS, Saad MD, Hackbart BG, Imam IZ (1997) Hepatitis C antibody prevalence in blood donors in different governorates in Egypt. Trans R Soc Trop Med Hyg 91: 271-274

Asif N, Kokhar N, Ilahi F (2004) Seroprevalence of HBV, HCV and HIV infection among voluntary non remunerated and replacement donors in Northern Pakistan. Pak J Med Sci 20: 24-28

Ayala GJJ, Guerra AFJ, Avalos FJ, Mora BP, Casillas RA (1997) Prevalence of viral markers for hepatitis B, C and human immunodeficiency virus in volunteer blood donors in northeast Mexico. Rev Gastroenterol Mex 62: 250-253

Barrera JM, Bruguera M, Ercilla MG, Gil C, Celis R, Gil MP, del Valle Onorato M, Rodes J, Ordinas A (1995) Persistent hepatitis C viremia after acute self limiting post-transfusion hepatitis. Hepatology 21: 639-644

Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler- Blin C, Veginet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency Syndrome (AIDS). Science 220: 868-871

Bassily S, Hyams KC, Fouad RA, Samaan MD, Hibbs RG (1995) A high risk of hepatitis C infection among Egyptian blood donors: the role of parenteral drug abuse. Am J Trop Med Hyg 52: 503-505

Bell J, Batty RJ, Farrell GC, Crewe EB, Cunningham AL, Byth K (1990) Hepatitis C virus in intravenous drug users. Med J Aust 153: 274-276

Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, Vidaud M, Bricaire F, Opolon P, Katlama C, Poynard T (1999) Liver fibrosis progression in human immunodeficiency virus and hepatitis C co-infected patients. Hepatology 30: 1054-1058

Berggren R (2001) False negative hepatitis C is associated with low CD4 counts in HIV/HCV coinfected patients. 8th Conference on Retroviruses and opportunistic infections: 212 (Abstract 561)

Bierhoff E, Fischer HP, Willsch E, Rockstroch J, Spengler U, Brackmann HH, Oldenburg J (1997) Liver histopathology in patients with concurrent chronic hepatitis C and HIV infection. Virchows Arch Int J Pathol 430: 271-277

Bjoro K, Froland SS, Yun Z, Samdal HH, Haaland T (1994) Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. N Engl J Med 331: 1607-1611

Bolumar F, Hernandez-Aguado I, Ferrer L, Ruiz I, Avino MJ, Rebagliato M (1996) Prevalence of antibodies to hepatitis C in a population of intravenous drug users in Valencia Spain, 1990-1992. Int J Epidemiol 25: 204-209

Bonacini M, Puoti M (2000) Hepatitis C in patient with human immunodeficiency virus infection: diagnosis, natural history, meta-analysis of sexual and vertical transmission and therapeutic issues. Arch Int Med 160: 3365-3373

Borkowsky W, Krasinski K, Paul D, Holzman R, Moore T, Bebenroth D, Lawrence R, Chandwani S (1989) Human immunodeficiency virus type 1 antigenemia in children. J Pediatr 114: 940-945

Bremer JW, Lew JF, Copper E, Hillyer GV, Pitt J, Handelsman E, Brambilla D, Moye J, Hoff R (1996) Diagnosis of infection with human immunodeficiency virus type 1 by a DNA polymerase chain reaction assay among infants enrolled in the Women and Infants Transmission Study. J Padiatr 129: 198-207

Brettler DB, Alter HJ, Dienstag JL, Forsberg AD, Levine PH (1990) Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients. Blood 76: 254-256

Bronowicki JP, Venard V, Botte C, Monhoven N, Gastin I, Chone L, Hudziak H, Rihn B, Delanoe C, LeFaou A, Bigard MA, Gaucher P (1997) Patient to patient transmission of hepatitis C virus during colonoscopy. N Engl J Med 337: 237-240

Brooks GF, Butel JS, Morse SA (2004) Jawez, Melnick, and Adelberg's Medical Microbiology, 23rd edn. McGraw Hill Companies

Buffet C, Charnaux N, Laurent-Puig P, Chopineau S, Quichon JP, Briantias MJ, Dussaix E (1994) Enhanced detection of antibodies to hepatitis C virus by use of a third generation recombinant immunoblot assay. J Med Virol 43: 259-261

Burgard M, Mayaux MJ, Blanche S, Ferroni A, Guihard-Moscato ML, Allemon MC, Ciraru-Vigneron, Firtion G, Floch C, Guillot F, The HIV infection in newborns French collaborative study group (1992) The use of viral culture and p24 antigen testing to diagnose human immunodeficiency virus infection in neonates. N Engl J Med 327: 1192-1197

Capelli C, Prati D, Bosoni P, Zanuso F, Pappalettera M, Mozzi F, De Mattei C, Zanella A, Sirchia G (1997) Sexual transmission of hepatitis C virus to a repeat blood donor. Transfusion 37: 436-440

Carreto-Vélez MA, Carrada-Bravo T, Martínez-Magdaleno A (2003) Seroprevalence of HBV, HCV and HIV in blood donors in Irapuato. Salud Pública de México 45: S690-S693

CDC (2001) Updated U.S Public Health Guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. Morb Mort Wkly Rep 50: 1-52

Chander A, Pawaha VK (2004) Seroprevalence of HIV-1/HIV-2 Infection in Bhairahawa, Western Nepal - A Hospital Based Study. J Nep Health Research Council 2: 1-4

Chaudhary IA, Samiullah, Khan SS, Masod R, Sardar MA, Mallhi AA (2007) Seroprevalence of Hepatitis B and C among the healthy blood donors at Fauji Foundation Hospital, Rawalpindi. Pak J Med Sci 23: 64-67

Chen M, Yun Z-B, Sallberg M, Schvarcz R, Bergquist I, Berglund HB, Sonnerborg A (1995) Detection of Hepatitis C virus RNA in the cell fraction of saliva before and after oral surgery. J Med Virol 45: 223-226

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M (1989) Isolation of a cDNA clone derived from a blood borne non A, non B viral hepatitis genome. Science 244: 359-362

Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina-Selby R, Barr PJ, Weiner AJ, Bradley DW, Kuo G, Houghton M (1991) Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci USA 88: 2451-2455

Choudhury N, Ramesh V, Saraswat S, Naik S (1995) Effectiveness of mandatory transmissible diseases screening in Indian blood donors. Indian J Med Res 101: 229-232

Clavel F, Guetard D, Brun-vezinet F, Chamaret S, Rey MA, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C , Rouzioux C (1986) Isolation of a new human retrovirus from West African patients with AIDS. Science 223: 343-346

Corrao G, Arico S (1998) Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. Hepatology 27: 914-919

Cribier B, Rey D, Schmitt C ,Lang JM, Kirn A, Stoll-Keller F (1995) High hepatitis C viremia and impaired antibody response in patients coinfected with HIV. AIDS 9: 1131-1136

Damen M, Zaaijer HL, Cuypers HT, Vrielink H, van der Poel CL, Reesink HW, Lelie PN (1995) Reliability of third generation recombinant immunoblot assay for hepatitis C virus. Transfusion 35: 745-749

Darby SC, Ewart DW, Giangrande PL, Spooner RJ, Rizza CR, Dusheilko GM, Lee CA, Ludlam CA, Preston FE (1997) Mortality from liver cancer and liver disease in hemophilic men and boys in UK given blood products contaminated with hepatitis C. Lancet 350: 1425-1431

Darwish MA, Raouf TA, Rushdy P, Constantine NT, Rao MR, Edelman R (1993) Risk factors associated with a high seroprevalence of hepatitis C virus infection in Egyptian blood donors. Am J Trop Med Hyg 49: 440-447

Darwish NM, Abbas MO, Hady SI, Mohammed TA (1992) Hepatitis C virus infection in blood donors in Egypt. J Egypt Pub Health Assoc 67: 223-236

De Luca A, Bugarini R, Cozzi Lepri AC, Puoti M, Girardi E, Antinori A, Poggio A, Pagano G, Tositti G, Cadeo G, Macor A, Toti M, D'Arminio Monforte A, Italian Cohort Naïve Antiretrovirals Study Group (2002) Coinfection with hepatitis C viruses and outcome of initial antiretroviral regimens in previously naïve HIV-infecdted subjects. Arch Int Med 162: 2125-2132

Deshpande A, Kumar A, Khodaiji S, Gupta AD (1998) Prevalence of hepatitis C virus antibody in healthy blood donors. Indian J Hemat Blood Transf 16: 71-72

Di Bisceglie A, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ (1991) Long term clinical and histopathological follow up of chronic post transfusion hepatitis. Hepatology 14: 969-974

Di Martino V, Esteban JI, Tainturier M-H, Benhamou Y, Bochet M, Opolon P, Poynard T (2000) Impact of HIV Coinfection on the Long-Term Follow-Up of HCV-Related Cirrhosis. Hepatology 32: A203

Dieterich DT, Purow JM, Rajapaksa R (1999) Activity of combination therapy with interferon alfa-2b plus ribavirin in chronic hepatitis C patient coinfected with HIV. Semin Liv Dis 19: 87-94

Donahue JG, Munoz A, Ness PM, Brown DE Jr, Yawn DH, McAllister HA Jr, Reitz BA, Nelson KE (1992) The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med 327: 369–373

Donahue JG, Nelson KE, Munoz A (1991) Antibody to hepatitis C virus among cardiac surgery patients, homosexual men and intravenous drug users in Baltimore, Maryland. Am J Epidemiol 134: 1206-1211

Dore GJ, Kaldor JM, McCaughan GW (1997) Systematic review of role of polymerase chain reaction in defining infectiousness among people infected with hepatitis C virus. BMJ 315: 333-337

Dorrucci M, Pezzotti P, Phillips AN, Lepri AC, Rezza G (1995) Coinfection of hepatitis C virus with human immunodeficiency virus and progression to AIDS, Italian Seroconversion study. J Infect Dis 172: 1503-1508

Durack DT (1981) Opportunistic infections and Kaposi's sarcoma in homosexual men. N Engl J Med 305: 1465-1467

Duraisamy G, Zuridah H, Ariffin MY (1993) Prevalence of hepatitis C virus antibodies in blood donors in Malaysia. Med J Mal 48: 313–316

Dusheiko GM, Smith M, Scheuer PJ (1990) Hepatitis C virus transmission by human bite. Lancet 336: 503-504

El Guneid AM, Gunaid AA, O'Neill AM, Zureikat NI, Coleman JC, Murray-Lyon IM (1993) Prevalence of hepatitis B, C and D virus markers in Yemeni patients with chronic liver disease. J Med Virol 40: 330–333

Emerman M, Malim MH (1998) HIV-1 regulatory /accessory genes: Keys to unraveling viral and host cell biology. Science 280: 1880-1884

European Collaborative Study (1992) Risk factors for mother-to-child transmission of HIV-1. Lancet 339: 1007-1012

Eyster ME, Diamondstone LS, Lien JM, Ehmann WC, Quan S, Goedert JJ (1993) Natural history of hepatitis C virus infection in multitransfused hemophiliacs: Effects of coinfection with human immunodeficiency virus. The multicenter hemophilia cohort study. J Acquir Immune Defic Syndr 6: 602-610

FHI (2000) STD and HIV prevalence survey among female sex workers and truckers on highway routes in the Terai http://www.fhi.org/en/HIVAIDS/pub/survreports/nepalstdprev.htm

FHI (2002) Behavioral surveillance survey in the highway route of Nepal http://www.fhi.org/en/HIVAIDS/pub/survreports/sexworkersnepal.htm

Farci P, Alter HJ, Wong D, Miller RH, Shih JW, Jett B, Purcell RH (1991) A long term study of Hepatitis C virus replication in non-A, non-B hepatitis. N Engl J Med 325: 98-104

Fiore RJ, Potenza D, Monno L, Appice A, DiStefano M, Giannelli A, LaGrasta L, Romanelli C, DiBari C, Pastore G (1995) Detection of HCV RNA in serum and seminal fluid from HIV-1 coinfected intravenous drug addicts. J Med Virol 46: 364-367

Flexner C (1998) HIV protease inhibitors. N Engl J Med 338: 1281-1292

Folks TM, Khabbaz RF (1998) Retroviruses and associated diseases in humans. In: Coller L, Balows A, Sussman M (eds), Topley and Wilson's Microbiology and Microbial Infections. Virology, 9th edn. Vol 2. Arnold

Fox RK, Wright TL (2003) Viral Hepatitis. In: Friedman SL, McQuaid KR, Grendell JH (eds), Current Diagnosis and Treatment in Gastroenterology. 2nd edition. LANGE

Frommel D, Tekle-Haimanot R, Berhe N, Aussel L, Verdier M, Preux PM, Denis F (1993) A survey of antibodies to hepatitis C virus in Ethiopia. Am J Trop Med Hyg 49: 435–439

Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224: 500-503

Gao F, Bailes E, Robertson DL, Chen YL, Rodenburg CM, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, Sharp PM, Hahn BH (1999) Origin of HIV-1 in the Chimpanzee Pan troglodytes. Nature 397: 436-441

Garg S, Mathur DR, Garg DK (2001) Comparision of seropositivity of HIV, HBV, HCV and Syphilis in replacement and voluntary blood donors in Western India. Ind J Pathol Microbiol 44: 409-412

Giles RE, Perry KR, Parry JV (1999) Simple/rapid test devices for anti HIV screening: do they come up to the mark? J Med Virol 59: 104-109

Girardi E, Zaccarelli M, Tossini G, Puro V, Narciso P, Visco G (1990) Hepatitis C virus infection in intravenous drug users: Prevalence and risk factors. Scand J Infect Dis 22: 751-752

Goodman ZD, Ishak KG (1995) Histopathology of Hepatitis C virus infection. Semin Liv Dis 15: 70-81

Gordon SC, Patel AH, Kulesza GW, Barnes RE, Silverman AL (1992) Lack of evidence for the heterosexual transmission of hepatitis C. Am J Gastroenterol 87: 1849-1851

Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A (1981) Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 305: 1425-1431

Gretch DR (1997) Diagnostic tests for hepatitis C. Hepatoloy 26: 43S-47S

Gupta N, Kumar V, Kaur A (2004) Seroprevalence of HIV, HBV, HCV and syphilis in voluntary blood donors. Indian J Med Sci 58: 255-257

Gurubacharya VL, Rana T, Subedi BK (1994) Profile of AIDS cases in Nepal. J Nep Med Assoc 31: 337-339

Gurtler L, Muhlbacher A, Michl U, Hofmann H, Paggi GG, Bossi V, Thorstensson R, Villaescusa G, Eiras B, Hernandez JM, Melchior W, Donie F, Weber B (1998) Reduction of the diagnostic window with a new combined p24 antigen and human immunodeficiency virus screening assay. J Virol Methods 75: 27-38

Haghan H, McGough JP, Thiede H, Weiss NS, Hopkins S, Alexander ER (1999) Syringe exchange and risk of infection with hepatitis B and C viruses. Am J Epidemiol 149: 203-213

Hempstead M, Cross R, DelValie C, Kessler D, Pace L, Roldan M, Ruschmann D, Uehlinger J (1991) Antibody to hepatitis C virus (HCV) in blood donors positive for HIV. Int Conf AIDS 7: 331 (abstract no. M.C.3132)

Hibbs RG, Corwin AL, Hassan NF, Kamel M, Darwish M, Edelman R, Constantine NT, Rao MR, Khalifa AS, Mokhtar S (1993) The epidemiology of antibody to hepatitis C in Egypt. J Infect Dis 168: 789-790

Hopf U, Moller B, Kuther D, Stemerowicz R, Lobeck H, Ludtke-Handjery A, Walter E, Blum HE, Roggendorf M, Deinhardt F (1990) Long term follow up of post transfusion and sporadic chronic hepatitis non A non B and frequency of circulating antibodies to hepatitis C virus. J Hepatol 10: 69-76

Huemer HP, Prodinger WM, Larcher C, Most L, Dierich MP (1990) Correlation of hepatitis C virus antibodies with HIV-1 seropositivity in intravenous drug addicts. Infection 18: 122-123

Huglund S, Ofverstedt LG, Nilsson A, Lundquist P, Gelderblom H, Ozel M, Skoglund U (1992) Spatial visualization of the maturing HIV-1 core and its linkage to the envelope. AIDS Res Hum Retroviruses 8: 1-7

Ilako FM, McLigeyo SO, Riyat MS, Lule GN, Okoth FA, Kaptich D (1995) The prevalence of hepatitis C virus antibodies in renal patients, blood donors and patients with chronic liver disease in Kenya. East Afr Med J 72: 362–364

Irin News Network Reuters News alert (2005) Nepal: Focus on the plight of rural people living with HIV/AIDS http://www.irinnews.org/report.asp?ReportID=45194&SelectRegion=Central_Asia

Jain A, Rana SS, Chakravarty P, Gupta RK, Murthy NS, Nath MC, Gururaja S, Chaturvedi N, Verma U, Kar P (2003) The prevalence of hepatitis C virus antibodies among the voluntary blood donors of New Dehli, India. Eur J Epidemiology 18: 695-697

Joshi M, Manandhar SP, Ghimire P (1999) Seroprevalence of Hepatitis B and Hepatitis C Infection among Blood Donors of kathmandu Valley. Journal of Institute of Science and Technology 12: 43-50

Kaito M, Watanabe S, Tsukiyama-Kohara K, Yamaguchi K, Kobayashi Y, Konishi M, Yokoi M, Ishida S, Suzuki S, Kohara M (1994) Hepatitis C virus particle detected by immunoelectron microscopic study. J Gen Virol 75: 1755-1760

Karki BB (2000) Rapid assessment among drug users in Nepal. Aids Watch 5: 3–5

Kaur H, Dhanoa J, Pawar G (2001) Hepatitis C infection amongst blood donors in Punjab- a six year study. Indian J Hematol Blood Transfn 19: 21-22

Khattak MF, Salamat N, Bhatti FA, Qureshi TZ (2002) Seroprevalence of Hepatitis B, C and HIV in blood donors in Northern Pakistan. J Pak Med Assoc 52: 398-402

Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akhahane Y, Nishioka K, Purcell RH (1990) Interrelationship of blood transfusion, non-A non-B hepatitis and hepatocellular carcinoma: Analysis by detection of Antibody to hepatitis C virus. Hepatology 12: 671-675

Kiyosawa K, Sodeyama T, Tanaka E, Nakano Y, Furuta S, Nishioka K, Purcell RH, Alter HJ (1991) Hepatitis C in Hospital employees with needlestick injuries. Ann Intern Med 115: 367-369

Kiyosawa K, Tanaka E, Sodeyama T, Yoshizawa K, Yabu K, Furuta K, Imai H, Nakano Y, Usuda S, Uemura K (1994) Transmission of hepatitis C in an isolated area in Japan: Community-acquired infection. Gastroenterology 106: 1596-1602

Ko YC, Ho MS, Chiang TA, Chang SJ, Chang PY (1992) Tattooing as a risk of hepatitis C virus infection. J Med Virol 38: 288-291

Kolykhalov AA, Feinstone SM, Rice CM (1996) Identification of the highly conserved sequence element at the 3' terminus of hepatitis C virus genome RNA. J Virol 70: 3363-3371

Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, Hahn BH, Wolinsky S, Bhattacharya T (2000) Timing the ancestor of the HIV-1 pandemic strains. Science 288: 1789-1796

Kumar RM, Shahul S (1998) Role of breast feeding in transmission of hepatitis C virus to infants of HCV infected mothers. J Hepatol 29: 191-197

Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevans CE (1989) An assay for circulating antibodies to a major etiologic virus of human non-A non-B hepatitis. Science 244: 362-364

Kuperan P, Choon AT, Ding SH, Lee G (1993) Prevalence of antibodies to hepatitis C virus in relation to surrogate markers in a blood donor population in Singapore. Southeast Asian J Trop Med Pub Health 24: 127–129

Kuun E, Brashaw M, Heyns ADP (1997) Sensitivity and specificity of standard and rapid HIV antibody test evaluated by seroconversion and non-seroconversion low titre panels. Vox Sang 72: 11-15

Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA (1998) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393: 648-659

Lam JPH, McOmish F, Burns SM, Yap PL, Mok JY, Simmonds P (1993) Infrequent vertical transmission of hepatitis C virus. J Infect Dis 167: 572-576

Lin H-H, Kao J-H, Hsu H-Y, Ni YH, Chang MH, Huang SC, Hwang LH, Chen PJ, Chen DS (1995) Absence of infection in breast fed infants born to hepatitis C virus-infected mothers. J Pediatr 126: 589-591

Liou TC, Chang TT, Young KC, Lin XZ, Lin CY, Wu HL (1992) Detection of HCV RNA in saliva, urine, seminal fluid and ascites. J Med Virol 37: 197-202

Luft S, Seme K, Poljak M (2004) Laboratory diagnosis of human immunodeficiency virus infection. Acta Dermatoven APA 13: 43-49

Lukashov VV, Goudsmit J (1998) HIV heterogeneity and disease progression in AIDS: A model of continuous virus adaptation. AIDS 12: 43S-52S

Ly TD, Laperche S, Courouce AM (2001) Early detection of human immunodeficiency virus infection using third and fourth generation assays. Eur J Clin Microbiol Infect Dis 20: 104-110

Macias J, Pineda JA, Leal M, Abad MA, Garcia-Pesquera F, Delgado J, Gallardo JA, Sanchez –Quijano A, Lissen A (1998) Influence of hepatitis C virus infection on

the mortality of antiretroviral treated patients with HIV disease. Eur J Clin Mic Infect Dis 17: 167-170

Mahmood MA, Khawar S, Anjum AH, Ahmed SM, Rafiq S, Nazir I (2004) Prevalence of Hepatitis B, C and HIV infection in blood donors of Multan region. Ann King Edward Med Coll 10: 459-461

Makroo RN, Raina V, Kaushik V (1999) Prevalence of hepatitis C virus antibody in healthy blood donors. Indian J Med Res 110: 123-125

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollor R, Goodman ZD, Koury K, Ling M, Albrecht JK (2001) Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 358: 958-965

Mannuci PM (1993) Clinical evaluation of viral safety of coagulation factor VIII and IX concentrates. Transfusion 64: 197-203

Matee MI, Lyamuya EF, Mbena EC, Magessa PM, Sufi J, Marwa GJ, Mwasulama OJ, Mbwana J (1999) Prevalence of transfusion-associated viral infections and syphilis among blood donors in Muhimbili Medical Centre in Dar es Salaam, Tanzania. East Afr Med J 76: 167–171

Mathai J, Sulochana PV, Satyabhama S, Nair PK, Sivakumar S (2002) Profile of Transfusion Transmissible Infections and associated risk factors among blood donors of Kerala. Ind J Pathol Microbiol 45: 319-322

Mattsson L, Sonnerborg A, Weiland O (1993) Outcome of acute symptomatic non-A non-B hepatitis: A 13-year follow up study of hepatitis C virus markers. Liver 13: 274-278

Mayumi M (1997) Infection with GB virus C and hepatitis C virus in drug addicts, patients on maintenance hemodialysis, or with chronic liver disease in Nepal. J Med Virol 53: 157-161

McCutchan FE (2000) Understanding the genetic diversity of HIV-1. AIDS 14: 31S-44S

McGuiness PH, Bishop GA, Lien A, Wiley B, Parsons C, McCaughan GW (1993) Detection of serum hepatitis C virus RNA in HCV antibody seropositive volunteer blood donors. Hepatology 18: 485-490

McHutchinson JG, Person JL, Govindarajan S, Valinluck B, Gore T, Lee SR, Nelles M, Polito A, Chien D (1992) Improved detection of hepatitis C virus antibodies in high risk populations. Hepatology 15: 19-25

Mehta NM, Purohit A, Haag A, Mathur A, Joshi KC, Joshi R, Behra R (2002) Coinfection rate of HIV and Hepatitis C Virus (HCV) among blood Donors in Rajasthan, India. Int Conf AIDS 12: 14

Mendel I, Muraine M, Riachi G, el Forzli F, Bertin C, Colin R, Brasseur G, Buffet-Janvresse C (1997) Detection and Genotyping of the hepatitis C RNA in tear fluid from patients with chronic hepatitis C. J Med Virol 51: 231-233

Mumtaz S, Rehman MU, Muzaffar M, Hasan M, Iqbal M (2002) Frequency of seropositive blood donors for hepatitis B, C and HIV viruses in railway hospital Rawalpindi. Pak J Med Res 41: 51-53

Mutlu B, Meric M, Willke A (2004) Seroprevalence of hepatitis B and C virus, human immunodeficiency virus and syphilis in the blood donors. Mikrobiyol Bul 38: 445-448

Mwangi JW (1999) Viral markers in blood donor population. East Afr Med J 76: 35-37

Mylonakis E, Paliou M, Lally M, Flanigan TP, Rich JD (2000) Laboratory testing for infection with the human immunodeficiency virus: established and novel approaches. Am J Med 109: 565-576

Nakashima K, Ikematsu H, Hayashi J, Kishihara Y, Mutsutake A, Kashiwagi S (1995) Intrafamilial transmission of hepatitis C virus among the population of an endemic area of Japan. JAMA 274: 1459-1461

Nakatsuji Y, Matsumoto A, Tanaka E, Ogata H, Kiyosawa K (1992) Detection of chronic hepatitis C virus infection by four diagnostic systems: first generation and second generation enzyme linked immunosorbent assay, second generation recombinant immunoblot assay and nested polymerase chain reaction analysis. Hepatology 16: 300-305

Nanu A, Sharma SP, Chatterjee K, Jyoti P (1997) Markers for transfusion transmissible infections in north Indian voluntary and replacement blood donors: Prevalence and trends 1989-1996. Vox Sang 73: 70-73

NCASC (2007) Cumulative HIV and AIDS situation of Nepal. Final report

Nelson KE, Vlahov D (1991) Sexually transmitted diseases in a population of intravenous drug users: association of seropositivity to HIV 1. J Infect Dis 164: 457-463

Nelson M, Matthews G, Brook MG, Main J and BHIVA coinfection guideline committee on behalf of British HIV Association (2005) BHIVA guidelines on

HIV and chronic hepatitis: coinfection with HIV and hepatitis C virus infection. HIV Medicine 6: 96-106

Nelson RP, Price LR Jr, Halsey AB, Graven SN, Resnick L, Day NK, Lockey RF, Good RA (1996) Diagnosis of Pediatric Human immunodeficiency virus infection by means of a commercially available polymerase chain reaction gene amplicfication. Arch Pediatr Adolesc Med 150: L40-L45

Neuman AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS (1998) Hepatitis C viral Dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 282: 103-107

New Era (2002) Behavioral and sero prevalence survey among IDUs in Kathmandu http://www.un.org.np/uploads/reports/UNAIDS/2002/behavioral_sero_ktm.pdf

Noguchi S, Sata M, Suzuki H, Mizokami M, Tanikawa K (1997) Routes of transmission of hepatitis C virus in an endemic rural area of Japan-Molecular epidemiologic study of hepatitis C virus infection. Scand J Infect Dis 29: 23-28

Oelrichs RB, Shrestha IL, Anderson DA, Deacon NJ (2000) The explosive human immunodeficiency virus type 1 epidemic among injecting drug users of Kathmandu, Nepal, is caused by a subtype C virus of restricted genetic diversity. J Virol 74: 1149–1157

Ogasawara S, Kage M, Kosai K, Shimamatsu K, Kojiro M (1993) Hepatitis C virus RNA in saliva and breast milk of hepatitis C carrier mothers. Lancet 341: 561-561

Ohto H, Terazawa S, Sasaki N, Sasaki N, Hino K, Ishiwata C, Kako U, Ujiie N, Endo C, Matsui A (1994) Transmission of Hepatitis virus from mothers to infants. N Engl J Med 330: 744-750

Osella AR, Misciagna G, Leone A, DiLeo A, Fiore G (1997) Epidemiology of hepatitis C virus infection in an area of Southern Italy. J Hepatol 27: 30-35

Ostapowicz G, Watson KJR, Locarnini SA, Desmond PV (1998) Role of alcohol in the progression of liver disease caused by hepatitis C virus infection. Hepatology 27: 1730-1735

Panigrahi AK, Panda SK, Dixit RK, Rao KV, Acharya SK, Dasarathy S, Nanu A (1997) Magnitude of hepatitis C virus infection in India: prevalence in healthy blood donors, acute and chronic liver diseases. J Med Virol 51: 167–174

Perrillo RP (1997) The role of liver biopsy in hepatitis C. Hepatology 26: 57S-61S

Phillips S, Grande TC, Pau CP, Candal D, Hu DJ, Parekh BS (2000) Diagnosis of human immunodeficiency virus type 1 infection with different subtypes using rapid tests. Clin Diagn Lab Immunol 7: 698-699

Plummer FA, Simonsen JN, Cameron DW, Ndinya-Achola JO, Kreiss JK, Gakinya MN, Waiyaki P, Cheang M, Piot P, Ronald AR (1991) Cofactors in male female sexual transmission of HIV type1. J Infect Dis 163: 233-239

Pokharel BR, Aryal S, Bhattarai (2000) Situation Analysis of HIV/AIDS in Nepal,National Center for AIDS and STD control, Teku, Kathmandu, December, Final draft, Report

Pollet DE, Saman EL, Peeters DC , Warmenbol HM, Heyndrickx LM, Wouters CJ, Beelaert G, van der Groen G, Van Heuverswyn H (1991) Confirmation and differentiation of antibodies to human immunodeficiency virus 1 and 2 with a strip-based assay including recombinant antigens and synthetic peptides. Clin Chem 37: 1700-1707

Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224: 497-500

Poudel KC, Okumura J, Sherchand JB, Jimba M, Murakami I, Wakai S (2003) Mumbai disease in far western Nepal: HIV infection and Syphilis among male migrant returnees and non migrants. Trop Med Int Health 8: 933-939

Price RW (1996) Neurological complications of HIV infection. Lancet 348: 445-452

Prince AM, Brotman B, Grady GF, Kuhns WJ, Hazzi C, Levine RW, Millian SJ (1974) Long incubation post –transfusion hepatitis without serological evidence of exposure to hepatitis B virus. Lancet 2: 241-246

Prince AM, Brotman B, Inchauspe G, Pascual D, Nasoff M, Hosein B, Wang CY (1993) Patterns and prevalence of hepatitis C virus infection in post transfusion non-A, non-B hepatitis. J Infect Dis 167: 1296-1301

Rahman M, Akhtar G, Lodhi Y (2002) Seroprevalence of Hepatitis C antibodies in blood donors. Pak J Med Sci 18: 193-196

Reinus JF, Leikin EL, Alter HJ, Cheung L, Shindo M, Jett B, Piazza S, Shih JW (1992) Failure to detect vertical transmission of hepatitis C virus. Ann Intern Med 117: 881-886

Resti M, Azzari C, Lega L, Rossi ME, Zammarchi E, Novembre E, Vierucci A (1995) Mother to infant transmission of hepatitis C virus. Acta Paediatr 84: 251-255

Ridzon R, Gallagher K, Ciesielski C, Ginsberg MB, Robertson BJ, Luo CC, DeMaria A Jr (1997) Simultaneous transmission of human immunodeficiency virus and hepatitis C from a needle-stick injury. N Engl J Med 336: 919-922

Rukundo H, Tumwesigye N, Wakwe VC (1997) Screening for HIV1 through the regional blood transfusion service in Southwest Uganda: the Mbarara experience. Health Trasit Rev 7: 101-104

Ryas M, Hussain T, Bhatti FA, Ahmed A, Tariq WUZ (2001) Epidemiology of Hepatitis C virus infection in blood donors in Northern Pakistan. J Rawal Med Coll 5: 56-59

Sarkodie F, Adarkwa M, Adu-Sarkodie Y, Candotti D, Acheampong JW, Allain JP (2001) Screening for viral markers in Volunteer and Replacement blood donors in West Africa. Vox Sang 80: 142-147

Sato PA, Maskill WJ, Tamashiro H, Heymann DL (1994) Strategies for laboratory HIV testing: an examination of alternative approaches not requiring western blot. W.H.O Bulletin 72: 129-134

Sawayama Y, Hayashi J, Ariama I, Furusyo N, Kawasaki T, Kawasaki M, Itoh K, Acharya GP, Kashiwaqi S (1999) A ten years serological survey of hepatitis A,B and C viruses infections in Nepal. J Epidemiol 9: 350-354

Schiff ER (1997) Hepatitis C and Alcohol. Hepatology 26: 39S-42S

Schim van der Loeff MF, Aaby P (1999) Towards a better understanding of the epidemiology of HIV-2. AIDS 13: 69S-84S

Schreiber GB, Glynn SA, Busch MP, Sharma UK, Wright DJ, Kleinman SH (2001) Incidence rates of viral infection among repeat donors: Are frequent donor safer; Retrovirus Epidemiology Donor Study. Transfusion 41: 730-735

Schupbach J (2003) Human Immunodeficiency Viruses. In Murray PR, Baron EJ, Jorgensen JH, Pfaller M, Yolken RH (eds) Manual of Clinical Microbiology, 8th edn. Vol 2. ASM Press

Schvarcz R, Johansson B, Nystrom B, Sonnerborg A (1997) Nosocomial transmission of hepatitis C virus. Infection 25: 74-77

Seddon D (1998) HIV-AIDS in Nepal: The coming crisis. Bull Concern Asian Sch 30: 35-45

Seeff LB (1997) Natural history of hepatitis C. Hepatology 26: 21S-28S

Sharp PM, Robertson DL, Gao DL, Hahn BH (1994) Origins and diversity of human immunodeficiency viruses. AIDS 8: 27S-42S

Sherman KE, O'Brein J, Gutierrez AG, Harrison S, Urdea M, Neuwald P, Wilber J (1993) Quantitative evaluation of hepatitis C virus RNA in patients with concurrent human immunodeficiency virus infections. J Clin Microbiol 31: 2679-2682

Shimizu YK, Hijikata M, Iwamoto A, Alter HJ, Purcell RH, and Yoshikura H (1994) Neutralizing antibodies against hepatitis C virus and the emergence of neutralization escape mutant viruses. J Virol 68: 1494-1500

Shrarma C, Acharya B, Pradhan S, Shakya S, Tuladhar NR (2001) Retrospective study of donors screening at Tribhuvan University Teaching Hospital, Kathmandu, Nepal in the past three years period. Journal Nep Assoc Med Lab Sci 4: 21-22

Shrestha B (2006) Serological surveillance of Anti HCV antibody among Nepalese males. J Nepal Health Research Council 4: 7-11

Shrestha IL (2003) Seroprevalence of antibodies to hepatitis C virus among injecting drug users from Kathmandu. KUMJ 1: 101-103

Shrestha SM, Shrestha DM, Gafney TE, Maharjan KG, Tsuda F, Okamoto H (1996) Hepatitis B and C infection among drug abusers in Nepal. Trop Gastroenterol 17: 212-213

Shrestha SM, Shrestha S, Tsuda F, Sawada N, Tanaka T, Okamoto H, Miyakawa Y, Shrestha SM, Subedi NB, Shrestha S, Maharjan KG, Tsuda F, Okamoto H (1998) Epidemiology of hepatitis C virus infection in Nepal. Trop Gastroenterol 19: 102-104

Shuhart MC, Gretch DR (2003) Hepatitis C and G Viruses. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller M, Yolken RH (eds) Manual of Clinical Microbiology, 8th edn. Vol 2. ASM Press

Silverman JG, Decker MR, Gupta J, Maheswari A, Willis BM, Raj A (2007) HIV Prevalence and Predictors of Infection in Sex-Trafficked Nepalese Girls and Women. JAMA 298: 536-542

Simmonds P, Mutimer D, Follet EAC (1998) Hepatitis C. In: Collier L, Balows A and Sussman M(eds) Topley and Wilson's Microbiology and Microbial infection, Virology, 9th edn. Vol 1. Edward Arnold, London, United Kingdom

Simon F, Mauclere P, Roques P, Loussertajaka I, Mullertrutwin MC, Saragosti S, Georgescourbot MC, Barresinoussi F, Brunvezinet F (1998) Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med 4: 1032-1037

Singh R (1992) Prevalence of hepatitis C in blood donors-A pilot study. JNMA 30: 1-6

Singh S, Mills E, Honeyman S, Suvedi BK, Pant NP (2005) HIV in Nepal: Is the Violent Conflict Fuelling the Epidemic? PLoS Med 2:e216

Songsivilai S, Jinathongthai S, Wongsena W, Tiangpitayakorn C, Dharakul T (1997) High prevalence of hepatitis C infection among blood donors in northeastern Thailand. Am J Trop Med Hyg 57: 66–69

Sonwane BR, Birare SD, Kulkarni PV (2003) Prevalence of Seroreactivity among blood donors in rural population. Ind J Med Sci 57: 405-407

Soto B, Sanchez-Quizano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, Rey C, Abad MA, Rodriguez M, Sales Gilabert M, Gonzalez F, Miron P, Caruz A, Relimpio F, Torranteras R, Leal M, Lissen E (1997) Human immunodeficiency virus modifies the natural history of chronic parenterally acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol 26: 1-5

Standard Operating Procedures (2006) Nepal Red Cross Society, Central Blood Transfusion Service, Exhibition Road

Sulkowsky MS, Moore RD, Mehta SH, Chaisson RE, Thomas DL (2002) Hepatitis C and progression of HIV disease. JAMA 288: 199-206

Sun DX, Ghang FG, Geng YQ, Xi DS (1996) Hepatitis C transmission by cosmetic tattooing in women. Lancet 347: 541-541

Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H (2004) Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,748 first-time blood donors during 1995- 2000. Inter J virology 47: 32-40

Tanaka T, Kato N, Cho MJ, Sugiyama K, Shimotohno K (1996) Structure of the 3' terminus of the hepatitis C virus genome. J Virol 70: 3307-3312

Terrault NA, Wright TL (1995) Hepatitis C virus in setting of transplantation. Semin Liver Dis 15: 92-100

Thakral B, Marwaha M, Chawla YK, Saluja K, Sharma A, Sharma RR, Minz RW, Agnihotri SK (2006) Prevalence and Significance of hepatitis C virus (HCV) seropositivity in blood donors. Ind J Med Res 124: 431-438

Thapa D (2004) Seroprevalence of hepatitis B and HIV among volunteer blood donors of Kathmandu. M.Sc Thesis, Central Department of Microbiology, IOST, TU, Kathmandu, Nepal

Theodore SY, Jamal MM (2006) Epidemiology of Hepatitis C virus (HCV) infection. Int J Med Sci 3: 41-46

Thomas DL, Lemon SM (2000) Hepatitis C. In Mandell GL, Bennett JE, Dolin R (eds) Mandell, Douglas and Bennett's Principle and practice of Infectious diseases, 5th edn. Vol 2. Churchill Livingstone

Thomas DL, Shih JW, Alter HJ, Vlahov D, Cohn S, Hoover DR, Cheung L, Nelson KE (1996) Effect of human immunodeficiency virus on hepatitis C virus infection among injection drug users. J Infect Dis 174: 690-695

Thomas DL, Villano SA, Reister KA, Hershow R, Mofenson LM, Landesman SH, Hollinger FB, Davenny K, Riley L, Diaz C, Tang HB, Quinn TC (1998) Perinatal transmission of hepatitis C virus from human immunodeficiency virus type-1 infected mothers. J Infect Dis 177: 1480-1488

Thomas DL, Zenilman JZ, Alter HJ, Shih JW, Galai N, Carella AV, Quinn TC (1995) Sexual transmission of hepatitis C virus among patients attending Baltimore Sexually transmitted diseases clinics-an analysis of 309 sexual partnerships. J Infect Dis 171: 768-775

Tokars JI, Marcus R, Culver DH, Schable CA, McKibben PS,Bandea CI, Bell DM (1993) Surveillance of HIV infection and zidovudine use among health care workers after occupational exposure to HIV 1 infected blood. The CDC Coopeative Needlestick Surveillance Group. Ann Intern Med 118: 913-919

Tong MJ, El-Farra NS, Reikes AR, Co RL (1995) Clinical outcome after transfusion associated hepatitis C. N Engl J Med 322: 1463-1466

Tremolada F, Casarin C, Alberti A, Drago C, Tagger A, Ribero ML, Realdi G (1992) Long term follow up of non A, non B (type C) post transfusion hepatitis. J Hepatol 16: 273-281

UNAIDS (2006) AIDS epidemic update
http://www.unaids.org/wad/2006/Epiupdate2006_

UNAIDS (2007).AIDS epidemic update

http://www.unaids.org/wad/2007/Epiupdate2007_

UNDP You and Aids (2005) The HIV portal for South Asia-Nepal at a glance http://www.youandaids.org/Asia%20Pacific%20at%20a%20Glance/Nepal/

Utsumi T, Hashimoto E, Okumura Y, Takayanagi M, Nishikawa H, Kigawa M, Kumakura N, Toyokawa H (1995) Heterosexual activity as a risk factor for the transmission of Hepatitis C virus. J Med Virol 46: 122-125

Van der Poel CL, Cuypers HTM, Reesink HW, Weiner AJ, Quan S, Di Nello R, Van Broven JJ, Winkel I, Mulder-Folkerts D, Exel-Oehlers PJ (1991) Confirmation of hepatitis C virus infection by new four antigen recombinant immunoblot assay. Lancet 337: 317-319

Varmus H (1988) Retroviruses. Science 240: 1427-1435

Villano SA, Vlahov D, Nelson KE, Cohn S, Thomas DL (1999) Persistence of viremia and the importance of long term follow up after acute hepatitis C infection. Hepatology 29: 908-914

Vrielink H, Van der Poel CL, Reesink HW, Zaaijer HL, Scholten E, Kremer LC, Cuypers HT, Lelie PN, van Oers MH (1995) Look back study of infectivity of anti HCV ELISA positive blood components. Lancet 345: 95-96

Wang JT, Wang TH, Sheu JC, Lin JT, Chen DS (1992) Hepatitis C virus RNA in saliva of patients with post transfusion hepatitis and low efficiency of transmission among spouses. J Med Virol 36: 28-31

Wang Y, Tao QM, Zhao HY, Tsuda F, Nagayama R, Yamamoto K, Tanaka T, Tokita H, Okamoto H, Miyakawa Y (1994) Hepatitis C virus RNA and antibodies among blood donors in Beijing. J Hepatol 21: 634–640

Wezstal R (1999) Sexual transmission of hepatitis C virus. Hepatology 31: 92-95

WHO (1990) Proposed WHO criteria for interpreting results from western blot assay for HIV-1, HIV-2 and HTLV-I/HTLV-II. Wkly Epidemiol Rec 65: 281-283

WHO (1991) Biosafety guidelines for diagnostic and research laboratories working with HIV, Vol 9. World Health Organization, Geneva, Switzerland

WHO (1997) Hepatitis C: Global prevalence. Wkly Epidemiol Rec 72: 341-348

WHO (1999) Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepatol 6: 35–47

WHO (2001) Health Research Methodology: A guide for training in Research Methods. Second edition

Widell A, Elmud H, Persson MH, Jonsson M (1996) Transmisson hepatitis C via both erythrocyte and platelet transfusion from a single donor in serological window-phase of hepatitis C. Vox Sang 71: 55-57

Yamada N, Tanihara K, Takada A, Yorihuzi T, Tsutsumi M, Shimomura H, Tsuji T, Date T (1996) Genetic organization and diversity of the 3' noncoding region of the hepatitis C virus genome. Virology 223: 255-261

Yap PL, McOmish F, Webster ADB, Hammarstrom L, Smith CI, Bjorkander J, Ochs HD, Fischer SH, Quinti I, Simmonds P (1994) Hepatitis C virus transmission by intravenous immunoglobulin. J Hepatol 21: 455-460

Yilmaz G (2001) Diagnosis of HIV infection and laboratory monitoring of its therapy. J Clin Virol 21: 187-196

Yumiko Y, Takashi O, Yoshitaka K, Dorothy MDA, Prisca SAL, Christopher JG (2007) The prevalence of HIV, HBV and HCV among Filipino blood donors and overseas work visa applicant. Bulletin WHO 85: 131-137

Zanetti AR, Tanzi E, Newell ML (1999) Mother to infant transmission of hepatitis C virus. J Hepatol 31: 96-100

Zaw M, Frerichs RR, Ol KY, Eskes N (1999) Local evaluation of a rapid HIV assay for use in developing countries. Trop Med Int Health 4: 216-221

Zhang YY, Hansson BG, Widell A, Nordenfelt E (1992) Hepatitis C virus antibodies and hepatitis C virus RNA in Chinese blood donors determined by ELISA, recombinant immunoblot assay and polymerase chain reaction. APMIS 100: 851–855

Zylberberg H, Pol S (1996) Reciprocal interactions between human immunodeficiency virus and hepatitis C virus infections. Clin Infect Dis 23: 1117-1125