CHAPTER-I

1. INTRODUCTION

Everyday, over 6,800 person become infected with HIV and over 5,700 persons die from AIDS. Since the beginning of the epidemic, more than 25 million people have died of AIDS making it one of the most destructive pandemic in recorded history. Though promising development have seen in the recent years in the global effort to address the AIDS epidemic including increased access to effective treatment and prevention programme, the HIV pandemic remains the most serious of infectious disease challenges to public health.

First confirmed case of AIDS was identified in 1983 and by 1984 the Human Immunodeficiency Virus (HIV), subsequently named HIV-1 was isolated. HIV-1 has been described as more virulent than HIV-2 and responsible for global pandemic of full blown AIDS. AIDS is an immunodeficient condition which leads to disabling or life threatening opportunistic infection (Chakraborty, 2003). HIV is prevalent globally and transmitted in four principle ways: sexually, from mother to child, by injecting drug use, from infected blood and blood products.

Noting with deep concern that approximately 34 million people world wide are currently living with HIV/AIDS and 95% are in developing countries. It has been reported that in many parts of the world new HIV infection are heavily concentrated among young people (15-24 years of age) i.e. 40% of new infection. It has been estimated that 8.6 million people were living with HIV in Asia in 2006, out of which 2.2 million people were from south and south East Asia. India is the most popular country that is experiencing a highly varied HIV epidemic. Approximately 5.7 million people of whom 5.2 million were adults aged 15-49 years were living with HIV in 2005 (UNAIDS, 2006).

Since Nepal's first cases of HIV/AIDS were reported in 1988 the disease has primarily been transmitted by injecting drug use and unprotected sex. Over the last few years HIV/AIDS epidemic in Nepal has gained ground and Nepal has progressed from low prevalence country to one with so called concentrated epidemic in certain subgroups of the population (Gurubacharya et al., 2004).

As of December 2008, National Center for AIDS and STD Control (NCASC) has officially confirmed 12,933 HIV positive cases and 2,151 confirmed cases of AIDS in Nepal. Among the total 12,933 HIV positive cases 38 (0.29%) cases were described to be associated with blood transfusion or organ transplantation (NCASC, 2008). At the same time UNAIDS has estimated 75,000 people are living with HIV in Nepal (UNAIDS, 2008). Prostitution and other commercial sexual activities are not the legal activities in Nepal therefore; officially reported prevalence of HIV and AIDS in Nepal has been lowest among the other countries in South East Asia (Uribe et. al, 1997).

HIV prevalence among IDUs was found to be 6.8% to 34.7% depending on location. As of 2007 HIV prevalence among FSWs and their clients was less than 2% and 1% respectively and 3.3% among MSM (IBBS, 2007). According to IBBS (2007) 2.8% of migrants returning from Mumbai, India were HIV positive. Though the overall seroprevalence among the blood donors of Kathmandu valley was reported to be 0.19% (Karki et al., 2007), it varies in different regional blood transfusion services with overall seroprevalence of 0.054% and individual seroprevalence in Morang was 0.019%, in Banke was 0.095% and in Kaski was 0.05% (Tiwari et al., 2008).

Millions of lives are saved each year through blood transfusion. The emergence of HIV in the 1980s highlighted the importance of ensuring the safety, as well as the adequacy, of national blood supplies. The likely hood of HIV infection occurring in recipient of HIV positive blood is close to 100% and the risk of HIV transmission through screened blood has been estimated to be 1/36000-1/225000 units transfused. This residual risk is

due to antibody negative infected donors in the window period (Folks and Khabbaz, 1998).

AIDS has become a leading cause of illness and death among women of reproductive age in countries with high burden of HIV infection. Infants born to women living with HIV can become infected during pregnancy, labor and delivery or postpartum through breast feeding. An estimated 2.1 million children were living with HIV/AIDS at the end of 2007, two million of them in sub-Saharan Africa. Most of these children acquire HIV from their HIV infected mothers during pregnancy. With successful intervention the risk of mother to child HIV transmission can be reduced to 2%. However such intervention are still not widely accessible or available in the most resource limited countries where the burden of HIV is highest, and an estimated 1500 children get newly infected with HIV each day. Children account for more than 10% of all new HIV infections. Though most high income and developed countries has virtually eliminated new HIV infection from mother to child by the use of antiretroviral drugs, the avoidance of breastfeeding and elective caesarean section, global coverage of prevention of mother to child transmission (PMTCT) services is still low. In 2005, only about 11% of pregnant women living with HIV/AIDS gained access to HIV testing and counseling and antiretroviral prophylaxis intervention during pregnancy. In addition, most national programmes have paid little attention to primary prevention of HIV in women of childbearing age, preventing unintended pregnancies among women living with HIV and access to antiretroviral therapy for women and children (WHO, 2008).

Though in Nepal, few researches have been published regarding seroprevalence of HIV among pregnant and delivery cases NCASC has reported nearly 1% of pregnant/delivery cases in Nepal are HIV positive and it varies in the different PMTCT Sites 0.217% in Maternity Hospital, Thapathali, 0.438% in Bheri Zonal Hospital, Nepalgunj, 0.165% in BPKIHS, Dharan,0.073% in TUTH, Maharajgunj, 0.00% in Narayani Sub Regional Hospital, Birgunj, 0.19% in Western Regional Hospital, Pokhara, 0.484% in Mahakali Zonal Hospital, Mahendranagar, 1.10% in Achham

District Hospital, 0.00% in Koshi Zonal Hospital, Biratnagar, 0.04% in Bharatpur Hospital, Chitwan, 0.254% in Mechi Zonal Hospital, Jhapa, and 0.00% in Baglung District Hospital (NCASC, 2008). Among 4,307 HIV infected female in Nepal 3,043 were house wives (NCASC, 2008) which is second highest of the total HIV infection in Nepal. This indicates that HIV is also spreading to "low risk" women. It is more than likely that they contracted the virus from their husband who was earlier infected through casual sex or drug injecting patterns.

Damak Municipality is small well known town of Jhapa District, Mechi zone in far eastern region of Nepal covering 7,513 hectare area and total population of 58,590 with 29,684 male and 28,906 female. According to SPARSHA (2008), Damak report there are 348 (not a total) HIV positive in Jhapa, out of which, 46 are from Damak who are taking treatment and care services from SPARSHA, Damak and 19 are under ARV therapy till date.

Estimating the seroprevalence of HIV in a low risk population such as pregnant women and blood donors provides essential information for an effective implementation of AIDS control programmes, and also for the monitoring of HIV spread within a country. The present study was aimed to reveal the seroprevalence rate and compare the seroprevalence of HIV among blood donors and the delivery cases visiting the maternity ward of AMDA hospital Jhapa.

CHAPTER-II

2. OBJECTIVES

2.1 General Objective

To study HIV seroprevalence among blood donors and delivery cases.

2.2 Specific Objectives

- To study HIV seroprevalence among blood donors.
- To study HIV seroprevalence among delivery cases.
- To study HIV seroprevalence among male and female blood donors
- To study HIV seroprevalence in Volunteer and Replacement blood donors.
- To study HIV seroprevalence according to sex and age groups among the blood donors.
- To study HIV seroprevalence according to age groups among delivery cases.
- To compare the study of HIV seroprevalence among blood donors and delivery cases.

CHAPTER-III

3. LITERATURE REVIEW

3.1 Human Immunodeficiency Virus (HIV)

HIV is the human Retrovirus which is the etiologic agents of disabling or life threatening clinical condition called Acquired Immunodeficiency Syndrome (AIDS). It infects specific kind of T-lymphocyte (T-helper cell) in humans that is vital for proper functioning of the immune system. AIDS was first described in USA in 1981 amongst homosexuals, Haitians, Heroine addicts and hemophiliacs in whom the incidence of Kaposi's Sarcoma and Pneumocystis Carinii Pneumonia were alarmingly high (Chakraborty, 2003)

3.1.1 Discovery and Origin of HIV

HIV is thought to have originated in non-human primates in sub-Saharan Africa and transferred to humans early in the 20th century. Retrovirus was first described by Peyton Rous in 1911. In 1983, The French group, headed by Luc Montagnier, discovered the virus lymphadenopathy-associated virus (LAV), while in 1984 the American group, led by Robert Gallo, isolated the human T-cell lymphotropic virus type III (HTLV-III). Later on the International committee on virus nomenclature recommended generic name for this virus Human Immunodeficiency Virus (HIV) (Alcamo, 1997).

HIV-1 was the third Retrovirus to be discovered and termed LAV in 1983 (Barre-Sinoussi et al., 1983) where as HIV-2 was fourth Retrovirus discovered in 1986 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 is less virulent

and less transmittable and is largely confined to West Africa. 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 (Schim van der Loeff et al., 1999).

3.1.2 Classification of HIV groups and subtypes

HIV viruses (HIV 1 and HIV 2) belong to the Lentivirinae subfamilies of the family Retroviridae. Lentiviruses so named because they give rise to slowly developing disease in man. 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 isolates of HIV has been shown to be genetically unique.

There are two strains of HIV known to be exist, HIV-1 and HIV-2. 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 in only two individuals from Cameroon. Group O have been predominantly reported from West Africa (Simon et al., 1998).

Among 9 subtypes most prevalent sub types according to the worldwide distribution are subtypes B prevalent in North America and Europe subtype A and D mostly in Africa and subtype C most common in Asia. In 2000, the last year in which an analysis of global subtype prevalence was made, 47.2% of infections worldwide were of subtype C, 26.7% were of subtype A/CRF02 AG, 12.3 percent were of subtype B, 5.3% were of subtype D, 3.2% were of CRF AE, and the remaining 5.3% were composed of other subtypes and CRFs. Most HIV-1 research is focused on subtype B; few laboratories focus on the other subtypes (Osmanov et al., 2002). Similarly, six subtypes of HIV-2 (A to F) have been described.

3.1.3 General properties of HIV

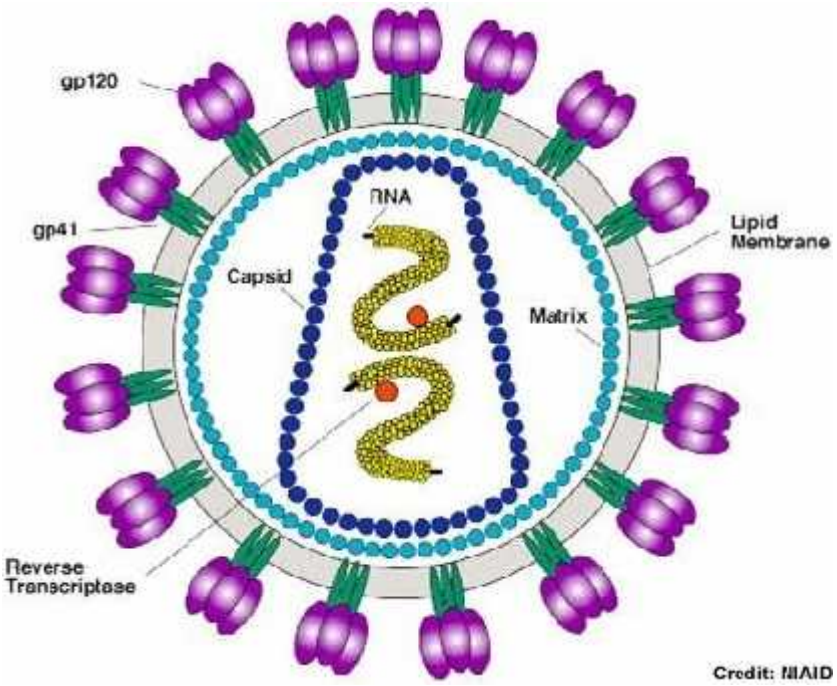
Structure and Morphology

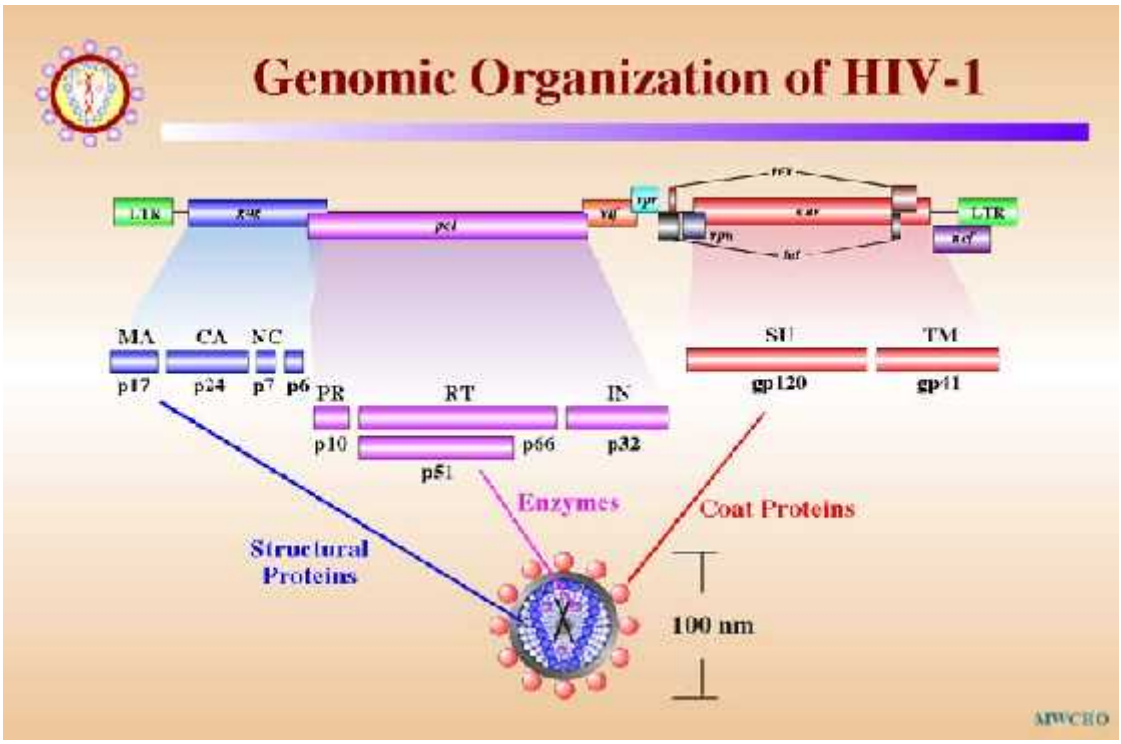
HIV is roughly spherical, enveloped, positive sense RNA virus with diameter of about 110nm (90-120 nm).

Envelop is lipid bilayer with proteins spikes on the surface surrounding the capsid. It has been found to be cholesterol rich and includes cellular proteins (Arthur et al., 1992). It is derived from the membrane of a human cell when a newly formed virus particle buds from the infected cell. The lipid envelop consist about 72 spiked knobs, which are assembled as trimmers of the outer envelop protein gp 120 bound to the transmembrane protein gp 41 which helps the HIV to bind and fuse with a target cell.

Capsid is the protective protein (p17) coat which surrounds the core of virus. It has icosahedral symmetry.

Figure 3.1: HIV-1 Virion and genomic organization of HIV-1





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

Core is the electron dense inner part of the virion which looks like a truncated cone and encloses the genome, reverse transcriptase (RNA dependent DNA polymerase) integrase enzymes and two cellular transfer RNA (tRNAs) (Chakraborty, 2003).

3.2 Replication

The major receptor for HIV is the CD4 surface molecule which is marker found on T lymphocyte, B lymphocyte, NK cells, phagocytes etc. The co receptors like CCR5 and CXCR4 which are chemokines are also necessary for virus entry. CCR5 is widely expressed on lymphocytes, macrophages, dendritic cells of the rectal, vaginal and cervical mucosae.

Initially gp120 subunit of virus binds to the CD4 receptor and coreceptor on the host cells. The chemokine receptors produce a conformational change in the gp41 subunits which allows fusion of HIV (Dorms et al., 1997).

Once within the cell the viral particle uncoats to release the RNA. The Reverse Transcriptase enzyme enables the viral genome to copy itself from RNA to DNA and enter the nucleus of the host cell, becoming incorporated into the DNA of the host cell with the help of integrase enzyme.

Now the virus can replicate itself whenever the cell is stimulated to reproduce. The cell now acts as a factory for new viruses. The progeny virus buds out through the cell membrane and infects fresh cells (Kennedy, 2003).

HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of 10^9 to 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase. This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day (Robertson, 1995). This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV.

3.3 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 disinfectants: 10% household bleach, 50% ethanol, 35% isopropanol, 0.5% Lysol, 0.5% paraformaldehyde or 0.3% hydrogen peroxide and readily inactivated by the extremes of ph and 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).

3.4 Epidemiology

3.4.1 Global prevalence

HIV has had a world wide impact and there are virtually no areas that have not reported case of infection. But most people living with HIV/AIDS i.e. 95% resides in low and middle income countries where most new HIV infection and AIDS deaths occurs. It is estimated that about 0.6% of the world's population is infected with HIV. Since the beginning of the epidemic more than 25 million people have died of AIDS making it one of the most destructive pandemic in recorded history. Globally approximately 33.2 million people were living with HIV/AIDS in 2007. This includes 2.5 million children less than fifteen years of age and 15.4 million women (UNAIDS-WHO, 2008).

A total of 2.5 million people were believed to have been newly infected with HIV during 2007 including 420,000 children less than fifteen years of age. The total number of AIDS death in 2007 was 2.1 million including 1.7 million adults and around 330,000 children. However the number of people living with HIV continued to grow, the percentage of the world's population living with HIV is declining. According to recent epidemic update published by UNAIDS the total global epidemic of HIV/AIDS has declined in comparison to the last years data. 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 but the situation has changed. There was decreasement in HIV/AIDS population by 2007 by 6.3 million than in 2006. The global HIV incidence (the no of new infection in population per year) has been declined from 4.3 million in 2006 to 2.5 million in 2007 which may be due to the increased access to effective treatment and prevention by different national and international organization (UNAIDS, 2008).

The continent Sub Saharan Africa remains by far the most affected region where two thirds population (64%) of all adults and children with HIV globally were living and where more than three quarters (76%) of all AIDS death in 2007 occurred. Unlike other

regions, the majority of people living with HIV (61%) in Sub-Saharan Africa were women and two millions of them were children younger than 15 years of age (AIDS Epidemic update, 2006). In the 35 African nations with the highest prevalence, average life expectancy is 48.3 years 6.5 years less than it would be without the disease (UNAIDS, 2001).

HIV/AIDS in Asia was first detected in the early to mid 1980s. Thailand was the first Asian nation to report HIV infection followed by an explosive epidemic. South East Asia is the second worst affected area with 15% of the total infection in the world. Overall in Asia, an estimated 4.9 million (3.7-6.7 million) people were living with HIV in 2007, including the 440,000 people who became newly infected with HIV. Approximately, 300,000 died from AIDS in 2007 (UNAIDS, 2008).

By the mid to late 1980s it became evident that transmission of HIV was also increasing among other major HIV risk behavior groups within Asia. High HIV prevalence (up to 50% or more) was documented among female sex workers (FSW) in Thailand and in parts of India, notably Mumbai, during the mid to late 1980s. In addition, intense focal HIV epidemics were documented in Thailand, parts of north-east India, and the “golden triangle” area (where the borders of China, Myanmar and Thailand meet) in IDU populations beginning around the mid-to-late 1980s. 60% of all people with HIV in Asia are living in India (Stephen et al., 2006). India has estimated 2.5 million HIV infections in 2006 making India the third largest population of HIV infection with the national adult HIV prevalence of 0.36%. Among the SAARC countries India was the first one to have the case in 1986 and by 2002 had the highest estimated adult prevalence rate (0.8%) in SAARC regions. Being a neighbor country of Nepal, the incidence of HIV in china doesn’t significantly account for the increase in the incidence of HIV in Nepal. UNAIDS Global report 2006 has reported that 650,000 people are living with HIV cases in china of which 180,000 are women of age group 15-49 years.

It is estimated that 26,000 had reported death due to AIDS (UNAIDS, 2007). Since Nepal has open border with India it makes sound effect on the HIV epidemics of Nepal.

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

3.4.2 HIV/AIDS in Nepal

Although less than 1 percent of Nepal's adult population is estimated to be HIV positive, according to UNAIDS, the prevalence rate masks a concentrated epidemic among at-risk populations such as female sex workers (FSWs), injecting drug users (IDUs), men who have sex with men (MSM), and migrants. Since Nepal's first cases of HIV/AIDS were reported in 1988, the disease has primarily been transmitted by injecting drug use and unprotected sex (Gurubacharya et al., 1994). Available data indicate that there was a sharp increase in the number of new infections starting in 1996, coinciding with the outbreak of civil unrest. However, the incidence appears to be

leveling off with recent evidence of reduced prevalence and lower overall numbers (Poudel et al., 2003).

As of December 2007, the Government of Nepal reported 1,610 cases of AIDS and 10,546 HIV infections, which has grown to 13,000 infections by World AIDS Day 2008 out of which 4,300 are females and 3,040 are housewives (NCASC, 2008). Among the total HIV cases, 2,151 have full blown AIDS of which 509 died in December 2008 (NCASC, 2008). Whereas the number of people living with HIV in 2007 has been estimated to be 75,000 of which 16,000 are women (UNAIDS, 2008).

The epidemic in Nepal is driven by IDUs, migrants, sex workers and their clients, and MSM. Results from one study reveals that among IDUs in Kathmandu, Pokhara, and East and West Terai indicate that the highest prevalence rates have been found among urban IDUs, 6.8% to 34.7% of whom are HIV-positive, depending on location (IBBS, 2007). However, in terms of absolute numbers, Nepal's 1.5 million to 2 million labor migrants account for the majority of Nepal's HIV positive population. In one subgroup, 2.8% of migrants returning from Mumbai, India, were infected with HIV, according to the 2006 IBBS among migrants. Similarly, another study among male migrant returnees from Indian cities in Doti districts has reported a prevalence rate of 8% (Poudel et al., 2003).

As of 2007, HIV prevalence among FSWs and their clients was less than 2% and 1%, respectively and 3.3% among urban-based MSM. HIV and AIDS case reporting by the NCASC reports HIV infections to be more common among men than women, as well as in urban areas and the far western region of the country, where migrant labor is more common (NCASC, 2007). According to Nepal's 2007 United Nations General Assembly Special Session (UNGASS) report, labor migrants make up 41% of the total known HIV infections in the country, followed by clients of sex workers 15.5 % and IDUs 10.2 %. Street children are also one of the most vulnerable groups. The UNICEF report Increasing Vulnerability of Children in Nepal estimates the number of children

orphaned by HIV/AIDS to be more than 13,000. The national estimate of children 0 to 14 years of age infected by HIV is 2,500 (2007). About 50% of HIV cases in Nepal have been identified to be from the 29 Highway districts (FHI, 2002).

While the most recent data demonstrate a stabilizing of the epidemic and a downward trend in seroprevalence among several of the key high risk groups, a number of issues pose continued challenges for Nepal. Many sex workers are also IDUs, migrants, or both, increasing the spread of HIV among at-risk groups. A large portion of men who purchase sex are also married, making them potential conduits for HIV to bridge to the general population. Poverty, low levels of education, illiteracy, gender inequalities, marginalization of at-risk groups, and stigma and discrimination compound the epidemic's effects. Unsafe sex and drug injection practices, civil conflict, internal and external mobility, and limited adequate health care delivery multiply the difficulties of addressing HIV/AIDS. Moreover, existing care and support services are already overwhelmed as increasing numbers of HIV-infected individuals become sick with AIDS. Highest prevalence (0.5%) of HIV was among the people aged 15–49 years and 30% of were female (UNDP, 2005).

Number of people living with HIV/AIDS in Nepal in 2008 (December, 2008)

Condition	male	female	total
HIV positive (including AIDS)	8626	4307	12933
AIDS(Out of total HIV)	1529	622	2151

Cumulative HIV infection by sub-groups and sex

Sub-groups	Male	Female	Total
Sex worker (SW)	3	782	785
Clients of sex workers/STD	5710	104	5814
House wives	-	3043	3043
Blood or organ recipients	27	11	38
Injecting drug use	2313	45	2358
Men having sex with Men (MSM)	70	-	70
Children	450	298	748
Sub group not identified	53	24	77
Total	8626	4307	12933

Cumulative HIV infection by age group and sex

Age groups(years)	Male	Female	total
0-14	461	302	763
15-39	6883	3499	1778
> 40	1272	506	1778
Total	8626	4307	12933

Source: National Centre for AIDS and STD Control (Dec, 2008)

3.5 Risk factors and Transmission of HIV

The virus is primarily blood borne although it has been isolated in other body fluids including semen vaginal and cervical secretion, tears, saliva, cerebrospinal fluid and breast milk. Transmission of HIV depends on the infectiousness of the disease and the susceptibility of the uninfected partner. Infectivity seems to vary during the course of illness and is not constant between individuals. An undetectable plasma viral load does

not necessarily indicate a low viral load in the seminal liquid or genital secretions. However, each 10 fold increase in the level of HIV in the blood is associated with an 81% increased rate of HIV transmission (Laga et al, 1991; Tovanabutra et al., 2002). The main routes of transmission are as follows.

1. Sexual/horizontal transmission

Globally, unprotected sexual intercourse both (hetero and homosexual) is the main route of transmission and the groups most affected are sexually active men and women in their 20s to 40s. It is estimated that more than 80% of HIV infection are transmitted by the heterosexual intercourse and the infectivity rate per occurrence of sexual contact is approximately 0.3%. Anal intercourse between homosexual men is a common mode of transmission especially in western countries. The rate of transmission of HIV depends upon the sexual behaviors and sexual practices, vaginal intercourse in condition of other sexually transmitted infection or during menses, sexual practices and level of condom use, have been recognized as factors affecting spread (Folks and Khabbaz, 1998). Similarly the efficiency of transmission is also dependent on level of viraemia, infectivity and virulence of particular HIV strains and the presence of sexually transmitted disease (Plummer et al., 1991).

Early studies suggested that male to female transmission (15%) was more efficient than female to male (8%), but recent studies suggest that transmission may occur with equal frequency and depends on factors described above (Quinn et al., 2000).

2. Mother to child / vertical transmission

HIV infection from mother to baby can occur before (transplacental passage of virus in uterus), during (contact of abrasion with virus containing amniotic fluid), or after (postnatally via breast milk) birth. Prospectus study of infants born to HIV-infected women has shown the transmission rate ranging from 13-40%. It is believed that

approximately 30-50% of vertical transmission occurs in utero and 50-70% at or around the time of birth (Kuhn et al., 1997). Whilst the exact mechanism of vertical transmission is not certain chorioamnionitis, prolonged rupture of membrane, prematurity, intrauterine growth retardation and prolonged labor appear to play an important role in utero transmission (Tudor-Williams and lyall, 1999).

Factor affecting the vertical transmission

Viral factors: It has shown that HIV-1 has vertical transmission rate of 15-45%, while the rate for HIV-2 infection is significantly lower at 0-4% is rarely seen (Anderreasson et al., 1993) the risk of perinatal transmission appears to be very low in women with undetectable plasma viral loads through transmission of infection has been reported at all levels of maternal viral loads (Anderson, 2000; Ioannidis et al., 2001). Viral load in genital secretion is also important in vertical transmission (Kovacs et al., 2001).

Maternal health factors: The mother's stage of HIV infection as well as her general health can affect the rate of vertical transmission. It has shown that there is higher risk of transmission if there is low CD4 count, symptomatic disease or an AIDS defining illness (European collaborative study 1996). Mother with Hepatitis C infection also increases the perinatal HIV transmission as well as the risk of vertical transmission of Hepatitis C (Manzini et al., 1995).

Pregnancy and birth factors: In pregnancy, premature delivery at less than 34 weeks has been shown to increase the risk of vertical transmission (European collaborative study, 1992; Kuhn, 1997). Both prolonged rupture of membranes (more than four hours) and premature rupture of membrane have been associated with increased vertical transmission (Landesma et al., 1996).

Use of antiviral Therapy: Use of antiviral therapy for mother showed that maternal-infant HIV transmission was reduced by approximately from 25% to 8% (Connor et al., 1994).

Type of infant feeding: Postnatal transmission of HIV can occur through breast feeding also. Factors like cracked nipples and the duration of breast feeding considered to increase the risk of vertical transmission. It showed that the risk of transmission approximately doubles through breast feeding (Dunn et al., 1992).

3. Transmission via blood and blood products

Transmission via blood transfusion of infected blood products is less common now, due to the universal screening of donors and heat treatment of blood products. The likelihood of HIV infection occurring in recipients of HIV positive blood is close to 100% and 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 and Khabbaz, 1998).

4. Intravenous drug use

This mode of infection is prevalent among drug abusers sharing contaminated needles or injection as found in young generation. The risk of transmission among IDUs is 0.5 to 1.0%. It has been reported that unsafe sexual practices may also have been contributing some infections among IDUs (Nelson et al., 1996). Many sex workers are also IDUs, increasing the spread of HIV among at high-risk groups.

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). There is risk of needle-stick injury among medical and paramedical personnel of about 1%.

3.6 Multiple infection

Unlike some other viruses, infection with HIV does not provide immunity against additional infections, particularly in the case of more genetically distant viruses. Both inter and intra-clade multiple infections have been reported and even associated with more rapid disease progression (Gottlieb et al., 2004). Multiple infections are divided into two categories depending on the timing of the acquisition of the second strain. Coinfection refers to two strains that appear to have been acquired at the same time (or too close to distinguish). Reinfection (or superinfection) is infection with a second strain at a measurable time after the first. Both forms of dual infection have been reported for HIV in both acute and chronic infection around the world (Vanichseni et al., 2004; Smith et al., 2004).

3.7 Pathogenesis and Clinical features

There are four different stages of HIV disease in adults described by the centers of disease control (CDC) in 1992.

Group 1-Primary HIV infection (seroconversion)

This is the acute stage of infection with HIV following the seroconversion i.e. development of Anti HIV antibody within 4-6 weeks or 3 months. About 60-90% patient experience the flu like sign and symptoms at this stage like

- Glandular fever like illness
- Fever, malaise, diarrhoea, neuralgia
- Arthralgia, sore throat, headache
- Lymphadenopathy
- Maculopapular rash
- Ulceration of oropharynx etc.

Most patient recover spontaneously within 2-3 weeks except headache and lymphadenopathy which may persist.

Group-2 (Asymptomatic infection)

This stage is the clinically latent phase which may last for ten or more years even in the absence of antiviral therapy. During this period virus is replicating slowly in blood and lymphoid tissues.

Group-3 (Persistant Generalized lymphadenopathy)

The generalized lymphadenopathy appears to persist for at least 3 month in two or more noncontiguous extra inguinal sites. In this stage lymph nodes are enlarged, at least 1cm in diameter.

Group-4 (Symptomatic HIV infection)

About 80 to 100% patient develop symptomatic infection ranging from profound immunosuppression to full blown AIDS. At this stage patient begin experiencing symptoms from immunodeficiency due to gradual loss of helper T-lymphocytes. Replication of HIV in the cells may cause their destruction or immune system recognizes the virus infected CD4 T-cells as being abnormal and attacks this cell. In the laboratory, HIV infected CD4 T-cell form large syncytial mass which is eliminated by the immune system. Group-4 is further divided in 5 different sections in relation to the symptoms of immunodeficiency.

Stage 4A- Patient exhibits Constitutional disease (fever, weight loss, or unexplained diarrhoea) which was formerly termed as AIDS related complex (ARC)

Stage 4B- Patient exhibits HIV encephalopathy (AIDS) and neurological disease.

Stage 4C- Patient suffers from major and minor opportunistic infections specified as AIDS.

Major infections-Pnemocystis Carinii pneumonia, Toxoplasmosis, Cryptococcosis, Chronic Cryptosporidiosis, extra intestinal Strongyloidiasis, Candidiasis, (esophageal, bronchial, or pulmonary), Histoplasmosis, Mycobacterial infection with Mycobacterium avium-intracellulare complex, Cytomegalovirus infection, chronic mucocutaneous or disseminated Herpes simplex infection.

Minor infection- Multidermatomal Herpes zoster, nocardiosis, tuberculosis, recurrent salmonella bacteraemia, oral candidiasis.

Stage 4D- It includes the patient with secondary cancers, Kaposi's sarcoma (KS), non-Hodgkin's lymphoma, and primary central nervous system lymphoma.

Stage 4E- It comprises other condition attributable to immunosuppression by HIV. (Alcamo, 1997; Kennedy, 2003)

3.8 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 the infected person.
2. to identify carriers who may transmit HIV infection to other (specially blood or organ donors, sex partners, pregnant women).
3. to confirm the clinical diagnosis of AIDS.
4. to motivate for behavior changes through counseling among those high risk behavior individuals who tested anti HIV negative.
5. to induce behavior change and prevent further HIV transmission by counseling in those individuals who tested anti HIV positive.
6. to monitor trends of HIV epidemic (Luft et al., 2004).

Screening of blood and organ donors for HIV-1 and HIV-2 has been implemented since 1985 in U.S approved by FDA and in Nepal 1989. The most common screening assays detect anti-HIV antibodies in serum or plasma. The chronic nature of HIV infection allows the use of serologically test in its diagnosis. Unfortunately serology cannot detect recently infected individuals who are seronegative and potentially infectious i.e. in window period. HIV infection can be detected by:

1. Indirect or serologic test

i. Screening tests.
ii. Confirmatory tests (supplemental tests).

2. Direct detection of virus component

i. Detection of p24 antigen
ii. Isolation of HIV virus by culture
iii. Molecular detection methods i.e. by PCR

3. Non specific tests.

i. Blood count
ii. T-cell subset assay

i. Screening tests

a. ELISA

ELISAs are the first choice of screening test for detection of HIV antibodies. It is the “gold standard” test used extensively in blood bank and patient screening in developed nations (Constantine et al., 1994). Kits are available that can test for both HIV-1 and HIV-2. Sample is added to microtitre well plate that has been coated with HIV antigen(s). After a series of reagent addition, incubations and washings, the plate is placed in reading device. A specialised spectrophotometric plate reader measures the optical density of color that develops, if HIV antibody is present in the serum sample. 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' (Goldsby, 2003). Different types of ELISA that are in use are:

i. Indirect ELISA
ii. Competitive ELISA

b. Double antigen sandwich assay (DAGS)

DAGS assay include the so called 'third generation test'. It detects the antibody using an Antigen-Antibody-Antigen sandwich technique. The antigen used is recombinant or /and synthetic (Owen et al., 2008; Schupbach, 2003).

c. Fourth generation screening tests

Fourth generation screening tests combine detection of HIV antibody with detection of HIV antigen (p24 antigen). The average gain in time to detection compared with third generation kits is 3 to 5 days (Schupbach, 2003; Owen et al., 2008).

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 (Giles et al., 1999; Kuhn et al., 1997). However, others have been shown to give comparable diagnostic sensitivity and specificity, even during seroconversion and have been recommended for certain diagnostic settings (Phillips et al., 2000, Zaw et al., 1999). HIV tests are performed on a wide range of body fluids viz: Serum, Plasma, whole blood and oral fluids. However, HIV rapid testing can be done using the whole blood drawn from client’s fingertips.

There are three formats of Rapid HIV tests:

1. Immuno-concentration (flow through device)
2. Immuno-chromatography (lateral flow)
3. Particle agglutination

Immunoconcentration format

HIV antibody links to bound HIV peptide antigen forming the flow through (or immunoconcentration) devices are usually cartridges, with HIV antigen attached to a membrane. The specimen and the individual reagents are each added to the cartridge in a series of steps. Presence of HIV antibody is indicated by the development of a colored spot or line (Toth et al., 2001). Some examples of flow through devices are HIV TRI-DOT and Genie II.

In HIV TRI-DOT antigens of HIV-1 (gp41) and HIV-2 (gp36) are immobilized on an immunofiltration membrane. As the serum sample passes through the membrane, HIV antibodies if present, bind to the immobilised antigens. Conjugate binds to the Fc portion of the HIV antibodies to give pinkish purple dots against a white background. If the result is non-reactive, we will see one visible dot in the control region. If the result is reactive, we will see either one or two visible dots. One dots for HIV-1 and another for HIV-2. At the control dot, Human IgG links to membrane bound antihuman IgG (Toth et al., 2001).

Immunochromatography format;

The Immunochromatography experiments are performed using lateral flow devices which are commercially available as test kits e.g. Hema-strip, Oraquick and Unigold etc. The samples from suspected of HIV infected person are applied to a pad (filter) where it is mixed with gold or selenium and colloid-antigen conjugate. When the current is applied the mixture migrates through the nitrocellulose strip to immobilized

recombinant antigens and synthetic peptides at the patient window. If HIV antibodies are present then a red line appears in the test area of the strip.

Capillary flow (lateral flow) devices resemble dipsticks. All of the necessary reagents are usually incorporated into the test strip embedded in the device. Specimen (and sometimes buffer or a reagent) added to the strip flows across the reagents, and a colored line develops in the presence of antibody. Most lateral flow devices also have an internal control that detects human IgG. This internal control indicates that specimen was added to the test strip. If no human IgG is detected, an internal control line doesn't develop indicating an invalid test (Guss, 1994). The reactive reaction shows two lines: one for the control band, and the other for the test. A band in the test area means a reactive result. A non-reactive reaction will show a control band only. The control band must always be present for the test result to be valid.

Particle agglutination format

Anti-HIV antibodies bind to the antigen-coated latex particles. Agglutination assays were among the first of the rapid tests developed. The round circles represent antigen-coated latex particles that bind to antibodies to HIV (represented by the "Y"). Agglutination or clumping occurs when the antibodies bind to the antigen-coated particles (Holmes et al., 2003). The test kits based on agglutination available in the market are from Capillus and Serodia Company.

ii. Supplemental or confirmatory tests

a. Western Blot (WB)

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 (Schupbach, 2003; Pollet et al., 1991). It identifies antibodies directed against individual polypeptides such as gp 120/41 and p24, which are coated onto nitrocellulose strips, which are then incubated with serum, enzymes labeled conjugate and substrate. Coloured bands indicate the presence of specific antibodies. Most institution now follow the CDC guidelines, which require reactive to at least two of the following antigens: p24, gp41, gp120/160 for a positive result that means require two bands.

Interpretation of HIV Antibody testing results

In interpreting antibody results it is important to remember that in the course of infection with HIV it may take up to three months for antibodies to develop. This period is called the seroconversion or "window" period. Thus using antibody assay alone may not detect those infected person at window period so other molecular technique will be required.

HIV antibody screening results are interpreted either as "Not Reactive" which is usually taken to indicate that the individual is not infected or as a "Reactive" results that indicates infection with HIV. With all reactive results, a second sample of blood will be required in order to repeat the test before confirmation (Kennedy, 2003).

2. Direct detection of HIV infection

Detection of p24 antigen

p24 antigen is present at high levels in serum both early (window period) and in the later stages of HIV infection and in the new born to HIV infected mothers. Detection of p24 antigen is mostly performed in EIA format. In this method, a solid phase is coated with anti-p24 antibody and serum, and enzyme conjugated with anti-p24 antibody and

substrate is added. For a confirmation of a reactive result, the sample must be subjected to an additional confirmatory neutralization assay (Scupbach, 2003; Kennedy, 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).

Isolation of HIV

This involves co-culture of patient's peripheral blood mononuclear cells (PBMC) or plasma with mitogen-stimulated PBMC from an HIV seronegative donor. In order to detect the viral growth culture supernatant is periodically assayed for p24 antigen. It is very labour intensive method and results may take up to a month, which is often an unacceptable length of time for clinicians and patients. 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).

Molecular techniques

a. Detection of HIV proviral DNA

This is useful molecular technique for diagnosis of pediatrics HIV-1 infection and patient during the window period. This highly sensitive method involves the use of PCR to detect DNA in peripheral blood mononuclear cells. PCR can detect one copy of viral DNA in one cell out of 100,000 to 1,000,000 cells present (Kennedy, 2003).

b. Detection of HIV viral load (quantification of HIV RNA)

Amplification assays such as Reverse transcriptase PCR, DNA PCR and branched DNA

tests are used for viral load determination, which is useful to monitor the progression of disease and antiretroviral therapy (Scupbach, 2003). It measures the amount of cell free HIV RNA present in the plasma and the result is given as HIV RNA copy number per milliliter. The lower detection limit of most assays is 50 HIV RNA copies per milliliter (Kennedy, 2003).

C. Nonspecific tests

1. Blood count: In a full blown AIDS, there is leucopenia with a lymphocyte count less than 400per cu mm, and thrombocytopenia.

2. T-cell enumeration: In AIDS, the count of CD4T lymphocytes falls below 200/cu mm. The normal CD4:CD8 cell ratio of 2:1, is reversed to 0.5:1 (Chakraborty, 2003)

3.9 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. Azidodideoxythymidine**,** also known as zydovudine, is till now the drug of choice. There is currently no vaccine or cure for HIV or AIDS. The only known method of prevention is avoiding exposure to the virus. However, a course of antiretroviral treatment administered immediately after exposure, referred to as post-exposure prophylaxis, is believed to reduce the risk of infection if begun as quickly as possible (Conner, 2005). Current treatment for HIV infection consists of highly active antiretroviral therapy, or HAART. This has been highly beneficial to many HIV-infected individuals since its introduction in 1996, when the protease inhibitor-based HAART initially became available. Current HAART options are combinations (or "cocktails") consisting of at least three drugs belonging to at least two types, or "classes" of antiretroviral agents. Typically, these classes are two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a

non-nucleoside reverse transcriptase inhibitor (NNRTI). New classes of drugs such as Entry Inhibitors provide treatment options for patients who are infected with viruses already resistant to common therapies, although they are not widely available and not typically accessible in resource-limited settings (DoHS, 2002).

3.10 Prevention

No effective vaccine has yet been found out. Extremely high rate of mutation of envelope glycoprotein of the virus and lack of suitable animal models for HIV infection have made difficulty in development of vaccine. Preventive measure recommended by WHO includes health education program and public awareness campaigns, safe sex practices, screening of blood and blood products, avoiding sharing of needles among IDUs, identification of high risk group isolation and treatment of HIV infected and AIDS patient (Kennedy, 2003).

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Equipments

Centrifuge (Alpine, India)
Refrigerator (Videocon, India)
Micropipette (Borosil, India)

4.1.2. Test kits

HIV TRI-DOT Kit (Biomed Industries, India)
Determine HIV1/2 Kit (Abbott Japan Co, Ltd)
Capillus HIV1/2 Kit (Trinity Biotech PLC, Ireland)

4.1.3 Glasswares and others

Test tubes (Borosil, India)
Disposable latex gloves
Distilled water
Test tubes rack
Marker, adhesive seals, micro droppers

4.2 Methodology

4.2.1 Study site and study period

This study was a descriptive cross sectional study conducted, in Nepal Red Cross Society, NRCS sub chapter Blood transfusion service and AMDA hospital situated in Damak, Jhapa District over a period of five month from July 2008 – November 2008.

4.2.2 Study population

The study population was blood donors and delivery cases (womens) admitted for child birth at AMDA hospital in the maternity ward. Donors might be volunteers or replacement donors. All volunteers blood was collected either from the mobile camps organized by the Damak BTS or different social organization.

Delivery cases, those who are going to give a birth to a child with 35-36 weeks of pregnancy.

Population selection

All the donors were selected by filling questionnaires form and each donor was given number for the identification of the samples whereas all the delivery cases admitted in the hospital ward were selected for HIV test.

4.3 Collection of Blood sample

Blood sample were collected using aseptic technique. With the help of sterile syringe 5 ml blood was collected and dispensed in a clean test tube labeled with corresponding sample number.

4.3.1 Separation of serum /plasma

After dispensing the blood sample in the labeled tubes, it was allowed to clot without disturbing the tubes for 30 minutes. Serum was separated as supernatant by centrifuging blood at 3000 rpm for 5 minutes and the separated serum was transferred carefully to labeled clean screw capped vials with the help of sterile aspirator.

4.4 LABORATORY TESTING:

4.4.1 Detection of anti HIV-1 and 2 antibodies by HIV TRI -DOT Test Kit

The methodology used for the detection of anti HIV by HIV TRI DOT was exactly followed as directed by the manufacturer's protocol (Annex-II)

4.4.2 Detection of anti HIV-1 and 2 antibodies by Determine HIV1/2 Test Kit

The methodology used for the detection of anti HIV by Determine HIV1/2 was exactly followed as directed by the manufacturer's protocol (Annex-III)

4.4.3 Detection of anti HIV-1 and 2 antibodies by Capillus HIV1/2 Test Kit

The methodology used for the detection of anti HIV by Capillus HIV1/2 was exactly followed as directed by the manufacturer's protocol (Annex-IV)

4.5 Analysis

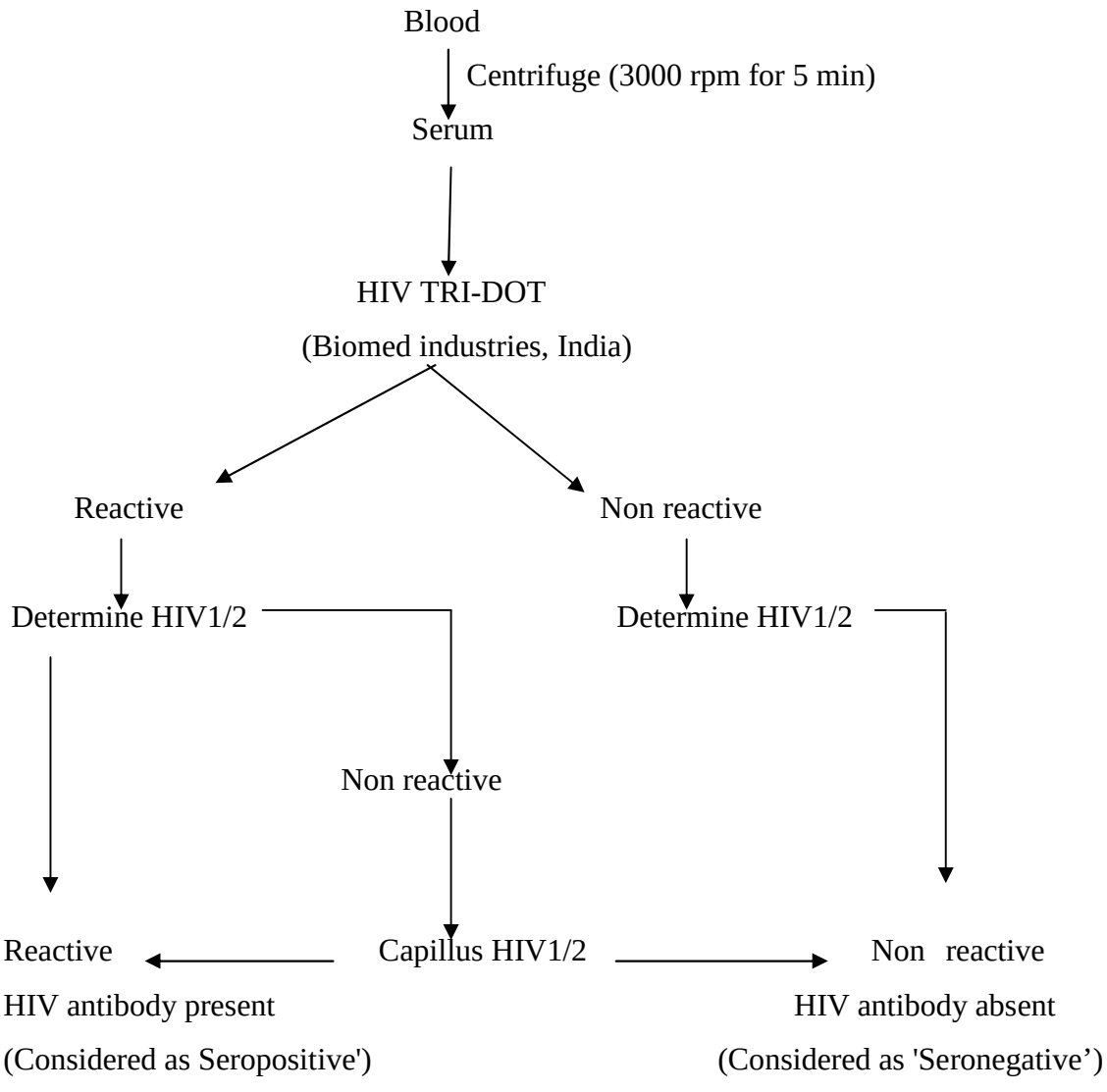
The significance of difference in seroprevalence of HIV among the donors and delivery case was tested by using the software "Winpepi ver 7.9".

4.6 Diagnostic strategy

4.6.1 HIV testing strategy

The process of screening of HIV for the detection of HIV antibody was performed as recommended by the WHO. Two Rapid test kit HIV TRI-DOT and Determine test were used for the screening and Capillus test kit was used for the confirmation.

Flow chart as reference to the protocol of WHO, BTS guideline



Source: Midified WHO BTS 99.1

CHAPTER-V

5. RESULTS

During the study period from July 2008 to November 2008 screening of anti HIV antibodies (HIV-1 and HIV-2) were done in altogether 2005 sera samples 795 in blood donors of Damak BTS, and 1210 in delivery cases admitted at AMDA hospital in maternity ward using rapid test kits.

Pattern of Study Population

A total of 795 blood donors and 1210 delivery cases were included in the present study. Among the total blood donors 638 (80.25%) were males 157 (19.74%) were females, 533 (67.04%) were volunteer donors while 262 (32.95%) were replacement donors.

Age of the blood donors ranged from 18-60 years, with the mean age of 29.92 years (Std. Dev=8.4).The mean age of male donors was 30.42 years (Std. Dev=12.95) and of female donors was 27.86 (Std. Dev=9.38). The mean age of delivery case was 24.11 years (Std. Dev=5.29).

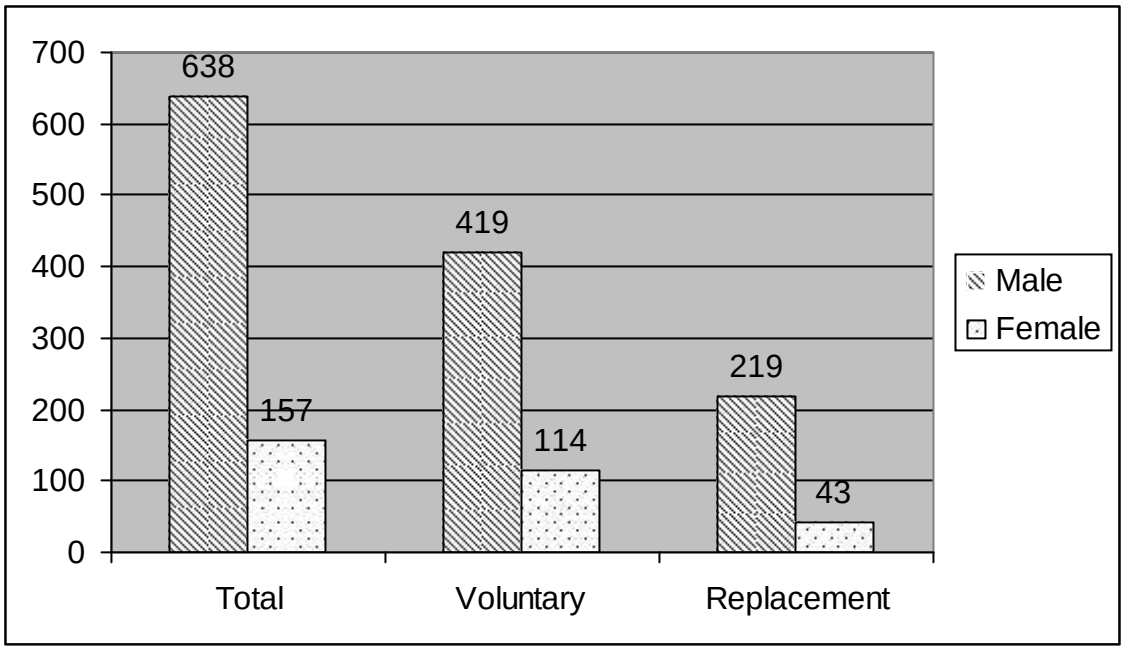


Figure No. 5.1.1: Type of Donation and Gender Wise Distribution of Blood Donors.

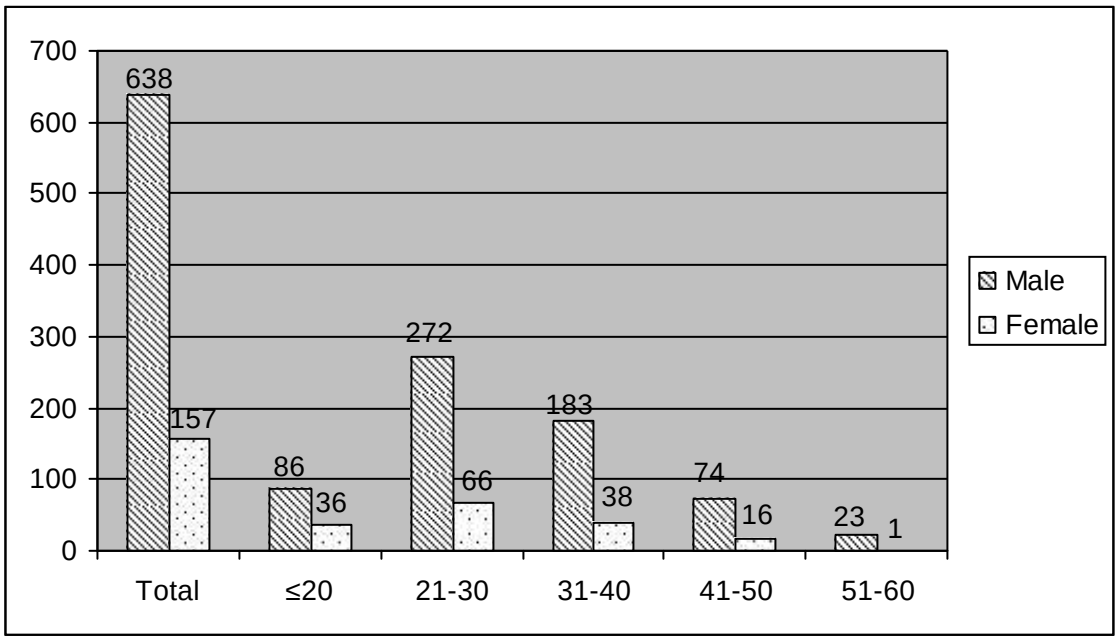


Figure No. 5.1.2: Age and Sex Wise Distribution of Blood Donors

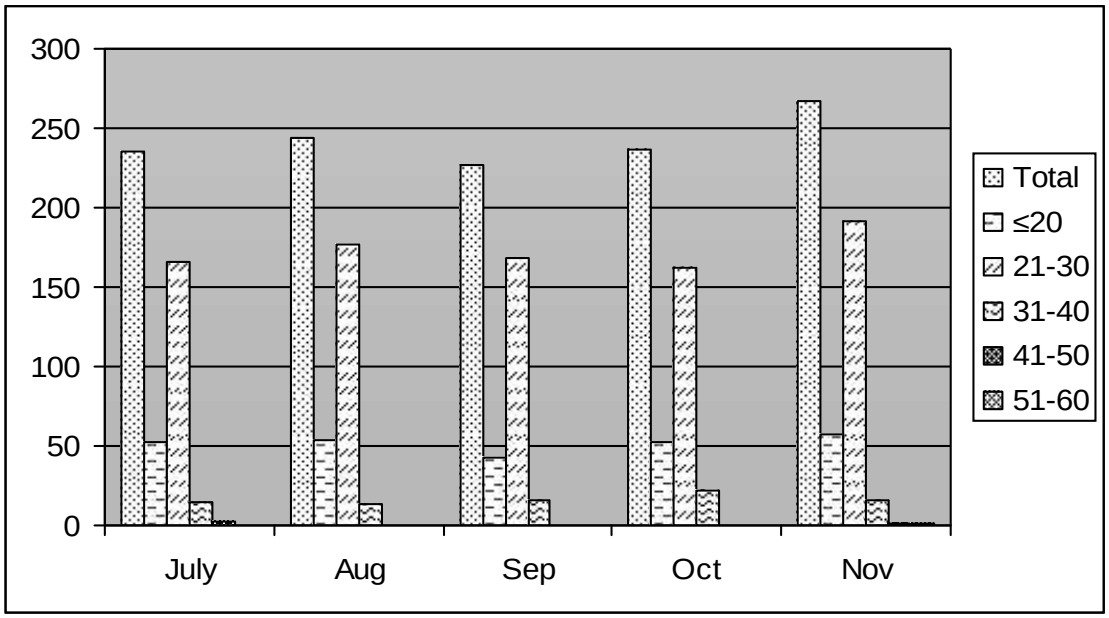


Figure No. 5.1.3: Month and Age Wise Distribution of Delivery Cases

5.2 Seroprevalence of HIV among the Blood Donors

Out of 795 blood donors 1 sample was reactive for anti HIV antibody (HIV-1). The overall seroprevalence of HIV was found to be 0.13%. Though 0.16% of male donors i.e. 1 sample was reactive for anti HIV antibody while no reactive case found in female. No significant different was found between male and female statistically.

Table No. 5.2: Seroprevalence of HIV among the Blood Donors

S.N.	HIV Test	Male		Female		Total	Overall Sero Prevalence %	P-value
		No.	%	No.	%			
1.	Reactive	1	0.16	0	0.00	1	0.13	P>0.05
2.	Non Reactive	637	99.84	157	100	794	99.87	
	Total	638	100	157	100	795	100	

5.3 Age and Sex Specific Seroprevalence of HIV among Blood Donors

There was only one reactive case i.e. in male donors which was in the age group of 21-30 years with the seroprevalence of 0.37% in male. And overall seroprevalence was 0.30%. There was no significant difference between the seroprevalence of age groups though no reactive cases found in other age group.

Table No. 5.3: Distribution of HIV Seropositive among Blood Donors according to Age Groups

Age Group (years)	Male	No. of Sero Positive	Female	No. of Sero Positive	Overall Sero Prevalence%
≤ΛI|	86	0(0.00%)	36	0(0.00%)	0.00
21-30	272	1(0.37%)	66	0(0.00%)	0.30
31-40	183	0(0.00%)	38	0(0.00%)	0.00
41-50	74	0(0.00%)	16	0(0.00%)	0.00
51-60	23	0(0.00%)	21	0(0.00%)	0.00
Total	638	1(0.16%)	157	0(0.00%)	0.13

5.4 HIV Seroprevalence among Volunteer and Replacement Blood Donors

The overall seroprevalence of HIV was observed only in male volunteer donors i.e. 0.24% whereas no reactive case was observed in other type of donors. Although the difference between male and female volunteer and replacement donors was not statistically significantly (P>0.05) overall seroprevalence of volunteer donor was 0.19%.

Table No 5.4: Distribution of HIV Seropositive Males and Females according to Type of Donation

S.N	Subjects	male		female		Overall seroprevalence %
		Total	No.of sero positive	Total	No. of sero positive	
1	Volunteer donor	419	1(0.24%)	114	0(0.00%)	0.19
2	Replacement donor	219	0(0.00%)	43	0(0.00%)	0.00
		P>0.05		P>0.05		

5.5 Seroprevalence of HIV among Delivery Cases

Out of 1210 delivery case 1 case was reactive for anti HIV antibody (HIV-1). The overall seroprevalence was found to be 0.08%.

Table 5.5: Seroprevalence of HIV among Delivery Cases

S.N.	HIV test	Delivery cases	Sero Prevalence %
1)	Reactive	1	0.08
2)	Non – Reactive	1209	99.92
	Total	1210	100

5.6 Age Specific Seroprevalence of HIV among Delivery Cases

The reactive case was in the age group of 21-30 years with the seroprevalence of 0.12%. And though no reactive case was found in other age groups difference observed between the age group was statistically not significant.

Table 5.6: Distribution of HIV Seropositive Delivery Case according to Age Group.

Age Groups(Years)	Delivery Cases	No. of Sero Positive	Sero Prevalence %	P-value
≤20	259	0	0.00	P>0.05
21-30	865	1	0.12	
31-40	82	0	0.00	
41-50	3	0	0.00	
51-60	1	0	0.00	
Total	1210	1	0.08	

5.7 Comparative HIV Seroprevalence among Blood Donors and Delivery Cases

The overall seroprevalences was 0.10%. Though seroprevalence among the blood donors was slightly higher than that of delivery cases there was no significant difference statistically (P>0.05).

Table No. 5.7: Seroprevalence of HIV among the Blood Donors and Delivery Cases

S.N.	Sample Tested	Blood Donors	Delivery Case	Total	Overall Sero Prevalence %	P-value
1.	Reactive	1(0.13%)	1(0.08%)	2(0.10%)	0.10	P>0.05
2.	Non-reactive	794 (99.87%)	1209 (99.92%)	2003 (99.90%)		
	Total	795	1210	2005		

5.8 Age Specific Comparison of HIV Seroprevalence among Blood Donors and Delivery Cases

The highest percentage of samples was in the age group 21-30 years in both blood donors and delivery cases. The overall seroprevalence i.e. 0.17% was observed in the age group 21-30 years and any non-reactive cases found in other age groups in both blood donors and delivery case.

Though the seroprevalence was higher in blood donors (0.30%) than in delivery case (0.12%) the difference was not statistically significant.

Table No. 5.8: Distribution of HIV Seropositive Blood Donors and Delivery Cases according to Age Group

Age Group (Years)	Blood Donors	No. of Sero Positive	Delivery Cases	No. of Sero Positive	Overall Sero Prevalence %
≤20	122	0(0.00%)	259	0(0.00%)	0.00
21-30	338	1(0.30%)	865	1(0.12%)	0.17
31-40	221	0(0.00%)	82	0(0.00%)	0.00
41-50	90	0(0.00%)	3	0(0.00%)	0.00
51-60	24	0(0.00%)	1	0(0.00%)	0.00
Total	795	1(0.13%)	1210	1(0.08%)	0.10

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

A total of 2005 sera samples, 795 of the blood donors and 1210 delivery case were screened for the Anti HIV-1 and HIV-2 antibodies. According to WHO guideline screening of anti HIV antibody was performed using rapid test kits HIV TRI-DOT, Determine and Capillus. In this study blood donors and the delivery cases were taken as study population because these particular groups represent healthy status and as a low risk group in the society. Any HIV positive result indicates that the individuals are unknown to their infection and they act as the potential carrier of HIV which can be transmitted to other healthy individuals through different life behavior. The trend of new or incident infections, especially in young people who have recently become sexually active, is the most sensitive marker to track the course of the HIV epidemic**.** Unfortunately, incidence is hard to measure directly, but prevalence in young women is an indirect but useful proxy.

Among the total, seroprevalence of anti HIV antibodies was observed to be 0.10%. Although higher seroprevalence was observed among the blood donors 0.13% than among the delivery cases 0.08% but the difference observed was statistically in significant which indicates the uniform distribution of HIV seroprevalence among the general healthy people of Damak in Jhapa district. In both cases prevalence was found only in the age group 21-30 years with overall seroprevalence of 0.17% though the difference was statistically insignificant, it is fact that this age group is highly sexually active and also mobile behavior. Our study revealed a quite low seroprevalence (0.10%) than described for general Nepalese population (0.5%) (UNDP, 2005). This difference might be due to the low population of the town, health consciousness of the people and

high literate proportion of the people of the town and few samples were taken for this study.

Downward trends in HIV prevalence are occurring in a number of countries where prevention efforts aimed at reducing new HIV infection since 2000 and 2001 are showing results. In most of Sub-Saharan Africa, national HIV prevalence has either stabilized or is showing signs of a decline. In South East Asia, the epidemics in Cambodia, Myanmar and Thailand all show declines in HIV prevalence (UNAIDS-WHO, 2006).

Among the 795 blood donors screened, the overall seroprevalence of anti HIV was observed to be 0.13 % (1/795) with 100% seropositvity in male donors. This study revealed a low seroprevalence of HIV among blood donors of Damak BTS compared with the seropositivity rate described for Kathmandu valley (0.13% vs 0.16%)(NRCS, BTS annual report 2006/07). Relatively lower seroprevalence was observed in the present study than reported by Karki et al., 2008 (0.19%), Thapa (2004) 0.41% among the blood donors of Kathmandu valley.

This difference might be due to the highly varied life style of Kathmandu than of Damak, due to the use of different test kits for screening, centralized urban setting of Kathmandu valley and due to the wide difference in the geographical condition. Similarly Chander et al., (2004) has reported 3.2% seroprevalence of HIV in patient attending teaching hospital from Bhairahawa, which is much higher than the result of present study.

Higher seroprevalence of HIV among blood donors than observed in present study was also reported from researches carried in foreign countries like: Dey et al., (2002) in India (0.32%), Sonwane et al., (2003) in India (1.9%), Mathai et al., (2002) in India (0.2%), Luksamijarkul et al., (2002) in Thailand (0.69%), Sarkodie et al., (2001) in Ghana (2.4%), Rukundo et al., (1997) in Uganda (3.9%), Ogunkolo et al., (2006) in

Nigeria (0.87%) by Matte et al., (2006) in Tanzania (3.8%), Mwangi (1999) in Nairobi Kenya.

Though present seroprevalence observed was quite higher than in other regional blood transfusion services of Nepal in Morang (0.019%), Banke (0.095%), and Kaski (0.05%) as reported by Tiwari et al., (2008) it might be due to stringent donor selection criteria, self exclusion of high risk group from blood donation and increased public awareness and intervention programs.

Similarly present HIV seroprevalence was much higher than researches in foreign countries reported by Gupta et al., (2004) in Ludhiana, India (0.084%), Rhahaman et al., (2002) in Pakistan (0.001%), Kakepoto et al., 2002 in Pakistan (0.007%), Elhazmi et al., 2004 in Saudi Arabia (0.00%), Yumiko et al., (2007) in Philliphines (0.006%), Ayala Gayalan et al., (1997) in Mexico (0.02%), Tserenpuntsag et al., (2008) in Mongolia (0.00%).

However the similar seroprevalence has been reported by Shrestha (2008) in blood donors of Kathmandu valley (0.12%) (27/21716), Chattoraj et al., (2008) in Calcutta, India (0.13%), Andrade-Neto et al., (2002) in Brazil (0.14%).

The prevalence rate was higher in volunteer donors (0.19%) as compared to replacement donors (0.00%) but the difference was not statistically significant. This might be due to the study period and low sample number of blood donors.

Estimating the seroprevalence of HIV in a low risk population such as pregnant women provides essential information for an effective implementation of AIDS control programmes, and also for the monitoring of HIV spread within a country. As I know, very few studies are available from Nepal showing the current trend in HIV prevalence in the pregnant women and antenatal population, which led us to carry out this study.

HIV infection in the pregnant woman poses a dilemma for the mother as well as for her child.

According to NCASC, 2008 estimation nearly 1% of the pregnant women are HIV infected in Nepal. In our study we screened total of 1210 delivery cases and overall seroprevalence was found to be 0.08% i.e. (1/1210). This difference may be due to the variation in the study population i.e. here in our study we screened for delivery case which is later stage of pregnancy and also could be due to the difference in surveillance methodologies. However similar prevalence has been reported from the TUTH, Maharajgunj 0.073% whereas few sites has lower prevalence among pregnant and delivery cases such as 0.04% in Bharatpur Hospital, Chitwan, 0.00% in Baglung District Hospital, 0.00% in Koshi Zonal Hospital, Biratnagar, 0.00% in Narayani Sub Regional Hospital, Birgunj and most of the PMTCT Sites from Nepal has higher prevalence of HIV such as 1.10% in Achham District Hospital, 0.484 in Mahakali Zonal Hospital, Mahendranagar, 0.438% in Bheri Zonal Hospital, Nepalgunj, 0.254% in Mechi Zonal Hospital, Jhapa, 0.217% in Maternity Hospital, Thapathali, 0.19% in Western Regional Hospital, Pokhara, 0.165% in BPKIHS, Dharan, (NCASC, 2008). These results indicate that HIV is also spreading to “low risk” women. It is more than likely that they contracted the virus from their husband who was earlier infected through casual sex or drug injecting patterns and monogamy may be strong reason for low rates of HIV in delivery cases.

Similar studies have been carried out through out the world in pregnant women. The present seroprevalence of HIV among the delivery cases of Damak admitted in the maternity ward of AMDA hospital was more or less similar to the result of John et al., (1995) who reported 0.054% of HIV seroprevalence in pregnant women in Vellore region of India.

The present study finding is much lower than the most of the similar studies carried out in rest of the world: Gupta et al., (2008) in north India New Delhi (0.88%), Ukey et al.,

(2005) in Nagpur India (1.38%), Phuapradit et al., (1995) in Bangkok Thailand (0.36%), de lima et al., (2000) in Brazil (0.6%.), Mahomed et al., (2008) in greater Harare Zimbabwe (18%), Stringer et al., (2008) in Lusaka, Zambia (22.5%), in antenatal clinic attendees, Northwest Ethopia (11.9%), Yahva-Malima et al., (2006) in Northern Tanzania (2.0%), Harry et al., (1995) in Maiduguri Nigeria (2.3%).

Similarly present HIV seroprevalence in delivery case was slightly higher than the research done by Aidaoui et al., (2005) in Annaba region in Algeria showing the HIV prevalence of 0.01% in pregnant women.

Almost all the above researches done worldwide among the pregnant women found highest HIV seroprevalence in the age group 21-30 years which is in consistent with the present research. Heterosexual contact remains the major mode of transmission; thereby resulting in a growing population of HIV infected house wives (23.53%) (NCASC, 2008). HIV infection in women occur primarily during their reproductive years, hence pregnancy provides a unique opportunity for implementing prevention strategies against HIV infection. If we estimate seroprevalence in pregnancy, the effective & timely intervention will reduce the transmission of infection to newborns.

With over 30.8 million adults infected worldwide, and 2.1 million new cases each year, the ultimate solution to the epidemic is to prevent new cases of HIV, especially among young people. Even though, our study population is not representative of whole Nepal because of ours being a hospital and BTS based study with limited sample size, the data indicate the silent presence of HIV within the general members of society which can affect the decreasing trend of HIV within the nation. Here it does not bother that HIV seroprevalence is lower in particular subgroups, but the presence of HIV reminds us to take specific precaution to be away from HIV infection.

Therefore, it may be recommended that even though the curative treatment for HIV is not available at present we can minimize, if not prevent, the pediatric HIV infection by

early screening of pregnant mothers for HIV followed by perinatal short term chemotherapy, safe delivery practices, modified infant feeding and by counseling the HIV infected blood donors.

Conclusion

Though lower HIV seroprevalence was observed in delivery cases than in blood donors (0.08% vs 0.13%) there was no significant difference statistically. The overall HIV seroprevalence i.e. (0.10%) observed in this study is very low than the national adult HIV prevalence i.e. (0.5%).

Interestingly in both case HIV infection was observed in the age group 21-30 years which is considered as sexually active age.

Though current study does not show any sign of alarming condition of HIV infection it indicates the silent presence of HIV among the general low risk group people so it is concluded that prevention, treatment and intervention programme should not only focused on High Risk Groups population like sex workers, their clients, IDUs but also it should be focused on the general low risk groups.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

This study was conducted at NRCS, BTS and Amda hospital Damak during the period of five months July 2008 to November 2008 with the aim of estimating seroprevalence of HIV among blood donors and delivery case and compare the seroprevalence between them.

1. Total number of 2005 serum samples were included in this study.

2. A total of 795 blood donors were included in the study. Out of total donors, 638 (80.25%) were males and 157 (19.74%) were females.

3. Blood donor's age ranged from 18-60 years, with the mean age of 29.92 years with the highest percentage (42.52%) of donors in the age group 21-30 years.

4. 533 (67.04%) donors were volunteer donors whereas 262 (32.95%) donors were replacement donors.

5. A total of 1210 delivery cases were included in the study .

6. The age of the delivery case ranged from 17-60 years, and mean age of delivery cases was 24.11 years with the highest percentage (71.49%) of delivery cases in the age group 21-30 years.

7. The overall seroprevalence of HIV among the total serum samples was 0.10%.
8. Though the seroprevalence was higher in blood donors than in delivery case (0.13% vs. 0.08%) but the difference was not statistically significant (P value >0.05).

9. The seropositivity among blood donors was 100% in male donors.

10. The seroprevalence of HIV was higher in Volunteer donors than in Replacement donors (0.17% vs 0.00%) but the difference was not statistically significant (P value >0.05).

11. In both case blood donors and delivery case HIV infection was found in the age group 21-30 years with overall seroprevalence 0.17% and with individual seroprevalence of 0.30% and 0.12%. The difference observed between the age groups was also statistically not significant (P value >0.05).

7.2 Recommendations

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

1. This kind of research should be carried out through out the nation for effective implementation of HIV intervention, treatment, care and support programme.
2. Effective post donation counselling should be done among the blood donors and also in pregnant womens.
3. The positive sample should be repeatedly tested and further confirmed by using WHO guidelines.
4. For Prevention of Mother To Child Transmission (PMTCT) HIV intervention programe should be focused among the house wives and they should be inspired for the HIV test in the early stage of pregnancy.

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