1. INTRODUCTION

1.1 Background

Sexually transmitted diseases (STDs) are infections that are passed from one person to another through sexual contact, commonly spread by sex, especially vaginal intercourse, anal sex and oral sex. The causes of STDs are bacteria, parasites, yeast, and viruses (Newmann *et al*., 2000). There are more than 20 types of STDs, including Chlamydia, Genital Herpes, Gonorrhea, HIV/AIDS, HPV, Syphilis and Trichomoniasis. The majority of STIs worldwide are caused by eight infections: syphilis, gonorrhea, chlamydia, trichomoniasis, genital herpes, hepatitis B virus (HBV), and human papilloma virus (HPV) where more than 500 million are estimated to have infection with Herpes simplex virus (WHO, 2016c) and more than 290 million have HPV infection (De Sanjose *et al*., 2007). These infections, often silent and without symptoms, can result in serious or fatal health consequences increasing the risk of HIV transmission (Wasserheit, 1992). These infections can also lead to severe complications and long term sequale, including pelvic inflammatory disease, ectopic pregnancy, infertility, chronic pelvic pain and neurological and cardiovascular disease in adults, neonatal death, premature delivery, blindness or severe disability in infants, and increased risk of HIV acquisition and transmission (Nagarkar and Mhaskar, 2015).

Human Immuno-deficiency virus (HIV) is a lentivirus (slowly replicating virus) that causes Acquired Immuno-deficiency Syndrome (AIDS) (Douek *et al*., 2009) a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections and cancers to thrive. Trichomoniasis is a condition caused by the parasite *Trichomonas vaginalis* and this common STI is also known as “trich”. *T. vaginalis* is a flagellate which belongs to the order trichomonadida, having three to five flagellae and recurrent flagellum which may be attached to the body to form an undulating membrane (Ferrer, 2000). All five flagella arise from basal bodies grouped at the anterior end, just in front of the single nucleus and the organism can swim actively with the aid of these flagella (Kannel and Levine, 2003).

HIV is different in structure from other retroviruses. It is roughly spherical (McGovern *et al*., 2002) with a diameter of about 120 nm, around 60 times smaller than a red blood cell, yet large for a virus (Bruce *et al*., 2007). It is composed of two copies of positive single-stranded RNA that codes for the virus’s nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24 (Kuiken *et al*., 2008). The single stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle (Kuiken *et al*., 2008). This is, in turn, surrounded by the viral envelope that is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a

newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus particle (Kuiken *et al*., 2008). This protein, known as Env, consists of a cap made of three molecules called glycoprotein (gp) 120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle (Chan *et al*., 1997).

Two types of HIV have been characterized: HIV- 1 and HIV- 2. HIV- 1 is the virus that was initially discovered and termed both LAV and HTLV- III. It is more virulent, more infective (Gilbert *et al*., 2003) and is the cause of the majority of HIV infections globally. HIV- 1 (subtype C) and HIV- 2 were identified in Nepal (Nepal, 2007). The lower infectivity of HIV- 2 compared to HIV- 1 implies that fewer of those exposed to HIV- 2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV- 2 is largely confined to West Africa (Reeves and Doms, 2002).

The virus is primarily blood borne although it has been isolated in other body fluids including semen, vaginal and cervical secretions, tears, saliva, cerebrospinal fluid and breast milk (De Cock *et al.,* 2000). The main routes of transmission are: Sexual (often described as horizontal transmission), mother to child transmission (also known as vertical transmission), contaminated intravenous drug using equipment and blood borne transmission via infected blood, blood products, infected human organs (if used in transplantation) or infected donor semen (if used in artificial insemination procedures). Such transmission would also include cases of occupational infection and/or other transmission via contaminated medical equipment.

The clinical features of HIV infection can be classified into four stages.

1. Initial Infection: No symptoms during the first few years. The symptoms are fever, sore throat, headache, rash, malaise, etc. The symptoms last about two weeks and the person becomes asymptomatic for variable duration.

2. Asymptomatic carrier stage: This is the period during which a person can transmit the disease but show no symptoms except for persistent generalized lymphadenopathy.

3. AIDS related Complex (ARC): ARC is a stage during which a person exhibits one or more of the symptoms caused by viral damage to the immune system but does not have opportunistic infection.it shows the following symptoms: unexplained diarrhea for more than one month, loss of weight more than 10% of body weight, fever more

than one month, fatigue, malaise and night sweats and oral candidiasis, generalized lymphadenopathy or spleenomegaly.

4. AIDS: AIDS is the last stage of HIV infection during which many opportunistic infections and/ or cancers develop in addition to having symptoms of ARC. Death occurs due to the infection.

AIDS has become one of the world's most serious health and development challenges. The first case was reported in 1981 and today more than 30 years later, approximately 36.7 million people currently living with HIV and nearly 35 million people have died from AIDS related illnesses and 2.1 million people became newly infected with HIV since the start of the epidemic (UNAIDS, 2016a). The global prevalence of HIV was gradually increased from 29.8 million in 2001 to 36.9 million in 2014 (Sekar and Mythreyee, 2016). The most affected region with HIV is East and Southern Africa accounting 19 million people where women represent more than half the total number of people living with HIV (UNAIDS, 2016c). Similarly, Western and Central Africa (6.5 million), Asia and Pacific (5.1 million), Western and Central Europe and North America (2.4 million), Latin America and the Caribbean (2.0 million), Eastern Europe and Central Asia (1.5 million) and Middle East and North Africa (230,000) are the most affected region across the globe (UNAIDS, 2016a). According to the Global AIDS Update, key populations at increased risk of HIV infection include sex workers, people who inject drugs, transgender people, prisoners and gay men and other men who have sex with men (UNAIDS, 2016b). The rapid spread of HIV-1 infection in developing countries has been attributed in part to high STI morbidity as a cofactor (Kumar *et al*., 1995). The prevalence of STIs is estimated to be high, but there is very little data in Nepal (Chin *et al*., 1994). It is estimated that about 200,000 STIs occur every year in Nepal (NCASC, 1997). STI in Nepal is concentrated in the certain risk groups of people like sex workers (male and female) and their clients, MSM and TG. The scenario of HIV infection remains almost constant between 0.3-0.2% in Nepal, with comparatively higher transmission rate through sexual transmission than other key populations at risk (ECDC, 2015). Nepal's HIV epidemic is largely concentrated in most-at-risk populations, of which female sex workers, IDUs, MSM, transgender and some migrants to high risk districts in India accounted for total of 58% HIV infection. According to the annual report of DOHS (2010/11), terai region of Nepal had the highest frequency (21315) of STI while HIV/AIDS were the highest in hill region followed by terai and mountain region in the same year. Economic deprivation, low education, prostitution, social stigma, socioeconomic inequality and economically driven migration and mobility are the reasons found to be associated with the risk of STI/HIV infection (Bloom *et al*., 2002 and Bloom *et al*., 2004).

1.2 Objectives of the study

General Objective

To determine the prevalence of HIV and trichomoniasis among pregnant women and specific assessment of five years HIV trend in Dhanusha district.

Specific objectives

1. To determine the prevalence of HIV and trichomoniasis among pregnant women visiting Aastha Hospital in Dhanusha district.
2. To assess the risk factors of patients visiting Antiretroviral Therapy Centre of Janakpur Zonal Hospital.
3. To perform the retrospective assessment of five years trend of HIV in Dhanusha district.

1.3 Rationale of the study

As the numbers of patients with STIs and HIV are increasing day by day, the study will help to know about the number of people infected with STIs and HIV. These infections possess a high burden on pregnancy outcomes in developing country like ours. These infections also have potential adverse effects on neonates causing serious and life threatening sequel if untreated or undertreated. Thus, the study will highlight upon the prevalence of HIV and *T. vaginalis* infection among pregnant women of terai households which could ultimately lead to a big leap on knowledge about infections and therefore helping in the fight towards cutting down these infections. This study also aimed to identify the key populations at risk of HIV infection and socio-demographic characteristics of people living with HIV in Dhanusha district. The increased labour migration among males in terai have resulted in high HIV prevalence rates among them making their wives more vulnerable to infection. As a consequence, vertical transmission of HIV continues to play a prominent role in the increasing prevalence of the disease in Terai. This study will provide several insights into the challenging and critical task of preventing new HIV infection among the wives of labour migrants. The cultural, economic, household, ethnic, migration and employment factors increases the susceptibility to infection in Nepali context, the study will help raise awareness programmes targeting illiterate men and women in general and incorporating behavior change approaches particularly among migrant workers and their spouses which can play an important role in STI and HIV prevention. This will emphasize interventions at social and political level to improve the literacy and income of the people and ultimately reduce the risk factors.

2. LITERATURE REVIEW

Sexually transmitted infections, also known as Venereal Diseases (VD) or Sexually transmitted diseases (STDs) are diseases that are passed on from one person to another through sexual contact, genital contact, vaginal intercourse, oral sex and anal sex (Nordqvist, 2015). STDs constitute a tremendous epidemic with an estimated 357 million new cases annually for four curable STIs: *Chlamydia trachomatis, Neisseria gonorrhoeae*, syphilis or *Trichomonas vaginalis* (WHO, 2012a) with 151 million of them occurring in Asia (Balamurugan and Bendigiri, 2012) of which South East Asia Region only was estimated to have annual incidence of 78.5 million (7.2 million cases for *C. trachomatis*, 25.4 million cases of *N. gonorrhea*, 3.0 million cases of syphilis and 42.9 million cases of *T. vaginalis*) (IUSTI, 2008) which greatly increases the risk of acquiring HIV infection by two to three times in some populations (WHO, 2012a). New estimates reveal that there are more than 110 million (incidence and prevalence) STIs overall among men and women nationwide in the United States (CDC, 2013). Increased rates of several STIs (early syphilis, gonorrhea, chlamydia) and greater prevalence of unsafe sexual behaviors have been reported among MSM in the United States (Ciesielski, 2003). According to 2008 global STI estimates, over a quarter of new STIs like syphilis, gonorrhea, chlamydia and trichomoniasis occur in the Western Pacific Region (WHO, 2012a) while a quarter cases occurred in the Americas (PAHO, 2013) and European countries had shown stabilized chlamydia infections in the recent years whereas increased rates for gonorrhea (79%) since 2008 (ECDC, 2013).

STIs and reproductive tract infections (RTIs) can result in pelvic inflammatory diseases, infertility, adverse pregnancy outcomes, and increased susceptibility to HIV (Nagarkar and Mhaskar, 2015). STIs such as gonorrohea and chlamydia are important causes of infertility worldwide (WHO, 2016c). Pregnant women are at increased risk of STIs because of the physiological changes like congestion of the cervix, edema of the vaginal mucosa and alteration in the vaginal flora in pregnancy (Schwebke, 2005). Syphilis is believed to have infected 12 million people in the year 1999, with more than 90% cases in the developing world resulting in spontaneous abortions, still births and congenital syphilis during pregnancy (CDC, 2007b). Other individual studies of pregnant women in China, Mongolia, India, Nepal, Bangladesh, Thailand, Papua New Guinea, and Pacific Islands (Fiji, Kiribati, Samoa, Solomon Islands, Tonga, and Vanuatu) reported *Chlamydia* infection rates of 4.9–14%, 19.3%, 0.1–35.9%, 1%, 41–44%, 5.7–16.2%, 11–11.1%, and 11.9–26.1%, respectively (Adachi *et al*., 2016). A 12-study meta-analysis reported that chlamydial infection during pregnancy was associated with an increased risk of preterm labor, low birth weight, and perinatal mortality (Silva *et al*., 2011). In contrast, a few studies, including ones from India, Nepal, and South Africa, did not find significant associations, possibly due to issues with sample size and/or low prevalence of *C. trachomatis* (Alexander *et al*., 1993; Donders *et al*., 1993; Paul *et al*., 1999; Christian *et al*., 2005). Pregnant women may be less likely to have partners using condoms, with no option to leave their unsafe relationships (Mugo *et al*.,

2011). A study conducted among pregnant women in Botswana showed 15% positive case for any STI constituting 8% for *Chlamydia trachomatis*, 2% for *Neisseria gonorrhea* and 6% for *Trichomonas vaginalis* (Wynn *et al*., 2016). The prevalence of HIV and Syphilis was 0.17% and 0.22% respectively among pregnant women attending antenatal care in government hospital of Raigarh, India (Apparao and Siddharth, 2016).

According to a surveillance conducted at six HIV Surveillance Sites (HSS), i.e. AMDA hospital, Narayani Sub-regional Hospital (Birgunj), Maternity Hospital (Kathmandu), Western Regional Hospital (Pokhara), Bheri Zonal Hospital (Neplagunj) and Mahakali Zonal Hospital (Mahendranagar), from May to August, 2000 showed an overall HIV prevalence of 2.4% and 2% respectively, males constituting the major proportions of infections than females (AIDS, 2014). Various studies in Nepal (Kathmandu, Pokhara and 22 Terai districts) have concluded that STIs are more prevalent among high risk population and there is a low prevalence in general population (UNAIDS, 2011).

Trichomoniasis is an infection caused by the protozoan parasite *Trichomonas vaginalis*, is one of the most common sexually transmitted disease of non-viral origin (Van Der Pol B, 2007). The main symptom in women is vaginal discharge although most cases remain asymptomatic. *T. vaginalis* infection is associated with increased HIV susceptibility that has triggered the spread of HIV epidemic (Lemos and Garcia-Zapata, 2010; Mavedzenge *et al*., 2010). The prevalence of trichomoniasis remains globally and nationally high despite its easy diagnosis and prevention (Alves *et al*., 2011) as there is not a single program for trichomoniasis unlike for gonorrhea, syphilis and HIV (Soper, 2004). The estimated global prevalence for trichomoniasis was 5.0% among women aged 15-49 years in 2012 (WHO, 2012a). In a nationally representative sample from 2001-2004 in United States, 3.1% of women aged 14-49 years were found to be positive for *Trichomonas vaginalis* infection (Sutton *et al*., 2007). A study among female patients of 19 countries in Southwestern Rio Grande do Sul, Brazil revealed 9% prevalence of *Trichomonas vaginalis* infection (Ambrozio *et al*., 2016). Females of reproductive age group attending Gynaecological Outpatient Department of National Medical College and Teaching Hospital, Birgunj were tested for vaginitis resulting into 46.96% of vaginitis and 2.6% had trichomoniasis (Bhargava *et al*., 2016). The overall STI prevalence among women in Kailali district with migrant partners was 30% (Singh *et al*., 2005).

Increased prevalence of STIs had public health consequences on the sexual and reproductive health of individuals (CDC, 2011) which pose economic burden for developing countries and account for economic losses in the community (Desai and Patel, 2011).

2.1 Global Scenario of HIV

The global prevalence of HIV was gradually increased from 29.8 million in 2001 to 36.9 million in 2014 (Sekar and Mythreyee, 2016), though the new HIV infections decreased from 3.4 million in 2001 to 2 million in 2014, and AIDS related death showed declining rate from the peak of 2.4 million in 2005 to 1.2 million in 2014 (UNAIDS, 2015). HIV is a leading cause of death and disease burden, especially in sub-Saharan Africa (Murray *et al*., 2014), of which 6 countries of sub-Saharan Africa i.e. Botswana, Lesotho, Namibia, Swaziland, South Africa, and Zimbabwe, had prevalence rates above 10% in 2015 (GBD, 2016). There were approximately 36.7 million people living with HIV, 2.1 million newly infected with HIV while 1.1 million died of AIDS related illnesses by the end of 2015, including 18.2 million PLWH accessing antiretroviral therapy as of June 2016 (UNAIDS, 2016a).

HIV not only affects the health of individuals, it impacts households, communities, the development and economic growth of nations. HIV endemic countries of the world also suffer from other infectious diseases, food insecurity, and other serious problems. Declines in new HIV infections among adults have slowed alarmingly in recent years, with the estimated annual number of new infections among adults remaining nearly static at about 1.9 million (1.7 million-2.2 million) in 2015 (UNAIDS, 2016c).

Latin America and the Caribbean

Latin America and the Caribbean (LAC) represent 8.3% of world's population (Garcia *et al*., 2014) having higher average per capita GDP than other developing regions, with 16 countries classified as middle-income and two as high-income (World Bank, 2014). The HIV epidemic in LAC began in the early eighties and has evolved since (Quinn *et al*., 1989), but the epidemic is not homogenous across the region (Bastos *et al*., 2008) as the general population prevalence in LAC is 0.4% while the prevalence varies from 0.5%-1% in eight Caribbean countries based on the report of twenty-six LAC countries in 2012 (UNAIDS, 2013a). HIV prevalence in Latin America is at stable levels while the Caribbean still has one of the highest high prevalence rates in the world after the sub-Saharan Africa (De Boni *et al*., 2014).

About 2 million people were estimated to be living with HIV, along with 100 000 new HIV infections and 21000 new HIV infections among children by the end of 2015 (UNAIDS, 2016a) in LAC. According to CDC (2015), HIV infection has been recorded the highest in reproductively potential age group particularly (25-29) years with high male population. Most of the LAC countries with generalized epidemics are located in the Caribbean Basin (WHO, 2000) and 12 countries of this region have an estimated prevalence of 1% or higher among pregnant women, with high prevalence among antenatal attendees in Brazil (Lewis, 2005). Two-thirds of PLWH lives in five countries- Argentina, Brazil, Colombia, Haiti and Mexico while countries of Caribbean and Central American sub Regions like Haiti, Bahamas and Belize have higher adult prevalence rates than the South America, Bahamas being one of

the region's high prevalence countries (USAID, 2012). Antiretroviral coverage among pregnant women increased from 2005 to 2011 in the Latin America and the Caribbean (PAHO, 2013).

Middle East and North Africa

The Middle East and North Africa (MENA) region comprises of geographically defined group of both high-income, well developed nations and low and middle income countries (El Beih *et al.,* 2013) with 10% of the world's population of reproductive age group (25-49) years (Abu-Raddad *et al.,* 2013). The annual number of new HIV infections declined by 38% globally, followed by a significant decline in AIDS-related deaths (WHO, 2015). In 2015, there were 230 000 people living with HIV in Middle East and North Africa, with 21,000 new HIV infections in the region which rose by 4% between 2010 and 2015 (UNAIDS, 2016a). There is substantial heterogeneity in HIV epidemic dynamics across MENA (Gokengin *et al.,* 2016). 88% of PLWH belong to five countries of MENA- Algeria, Iran, Morocco, Somalia and Sudan, with Iran ranking first among other countries regarding new HIV infections and AIDS-related deaths (WHO, 2015). Though there had been a significant increase in ART coverage within the last decade, it accounted for only 11% by the end of 2013 which was still the lowest throughout the world (UNAIDS, 2013). Middle East and North Africa also made important gains in the treatment coverage but achieved lower level of coverage (17%) (UNAIDS, 2016c).

Western and Central Africa

The Western and Central Africa region (WCA) is made up of 25 countries with relatively small populations where average HIV prevalence rates are relatively low (2.3%) as compared to Southern Africa in 2014 (Garmaise, 2016).

There were 6.5 million people living with HIV in Western and Central Africa (WCA) accounting Women for nearly 60% of the total number of people living with HIV in 2015, with a decline in new HIV infections by 8% between 2010 and 2015 (UNAIDS, 2016a). Nigeria, a West African country, is an enormous country with a very high number of people living with HIV despite a very low HIV prevalence where the epidemic is concentrated mainly among heterosexuals followed by a growing rate of HIV infection among MSM, FSWs and IDUs (Awofala and Ogundele, 2016). Of all people living with HIV globally, 9% of them live in Nigeria, a West African Country (UNAIDS, 2014). According to the Doctors without Borders report, 3 out of 4 HIV positive people do not have access to antiretroviral treatment and 45% of the global total of children are born HIV positive in WCA.

East and Southern Africa

The estimated number of people living with HIV in East and Southern Africa was 19 million in 2015 where women accounted for more than half the total number of people living with

HIV (UNAIDS, 2016a). Sub-Saharan Africa is home to only 12% of the global population which accounts for 71% of the global burden of HIV infection (Kharsany and Karim, 2016). Ten countries, mostly in southern and eastern Africa, *viz*. South Africa (25%), Nigeria (13%), Mozambique (6%), Uganda (6%), Tanzania (6%), Zambia (4%), Zimbabwe (6%), Kenya (6%), Malawi (4%) and Ethiopia (3%), account for almost 80% of all people living with HIV while the epidemics in Botswana, Namibia and Zambia appear to be declining and the epidemics in Lesotho, Mozambique and Swaziland seem to be plateauing (UNAIDS, 2014). HIV incidence rates among young women of reproductive age group are the highest of any age-sex subgroup (Harrison *et al*., 2015). Though the trends in new HIV infections across countries in sub-Saharan Africa have shown a decline by more than 33% from an estimated 2.2 million in 2005 to 1.5 million in 2013 due to increased coverage of ART (Tanser *et al*., 2013), it accounts for 46% of the global total of new HIV infections between 2010 and 2015 (UNAIDS, 2016b). In Kenya, an analysis in 2014 found that 65% of new HIV infections occurred in just nine of the country's forty-seven countries (NACC, 2014). In South Africa, surveillance data published in 2015 estimated HIV prevalence among sex workers was 71.8% in Johannesburg, 39.7% in Cape Town and 53.5% in Durban (UCSF, Anova Health Institute and WRHI, 2015).

There has been a parallel increase in the number of pregnant women receiving ART for the prevention of mother to child transmission of HIV and significantly more women and children are receiving ART and AIDS related deaths overall have declined by 39% in the period 2005 to 2013 in sub-Saharan Africa with dramatic declines in Rwanda (76%), Eritrea (67%), Ethiopia (63%), Kenya (60%), Botswana (58%), Burkina Faso (58%), Zimbabwe (57%), Malawi (51%), South Africa (48%) and Tanzania (44%) attributable to the rapid increase in the number of people on ART (WHO, 2010a).

Eastern Europe and Central Asia

The region of Eastern Europe and Central Asia consist of 23 countries and Eastern Europe and Central Asia (EECA) represent one of the few regions globally where there is a continued increase in the incidence of HIV infection (Dehovitz *et al*., 2014). In 2015, there were 1.5 million people living with HIV in EECA where new HIV infections rose by 51% and the number of AIDS-related deaths increased by 22% between 2010 and 2015 (UNAIDS, 2016). A world report published in "The Lancet" (Clark, 2016) draws attention to the growing HIV epidemic in Russia, but Russia is not alone as eight out of 12 countries in EECA have reported increases in new HIV infections (UNAIDS, 2016c) and the epidemic continues to grow by about 10% every year (Saldanha and Buse, 2016).

In recent years, rates and overall numbers of people diagnosed with HIV were highest in the East of region and lowest in the Centre (WHO, 2015). Although the number of AIDS cases has continued to decline steadily in the West and EU/EEA, it has increased by 80% in the East during the last decade reporting the highest rates of new HIV diagnoses from Estonia

(20.6; 270 cases), Latvia (19.8; 393 cases), Malta (14.2; 61 cases) while the lowest rates were reported by Slovakia (1.6; 86 cases), Slovenia (2.3; 48 cases) and the Czech Republic (2.5; 266 cases) in 2015 (ECDC, 2016).

Asia and the Pacific

The Asia and Pacific Region has about 55% of the world's population with some most populous countries as a part of this region (Kaldor *et al*., 1994). Asia and the Pacific is the region with the second highest number of people living with HIV in the world – an estimated 5.1 million in 2015 (UNAIDS, 2016d). Three countries - China, India and Indonesia – account for around three-quarters of the total number of people living with HIV in the region indicating India as the highest estimated number of HIV-infected people in the Asia- Pacific region (Dore *et al*., 1996). There were an estimated 300 000 new HIV infections among people living with HIV in the region which declined by 5% between 2010 and 2015 (UNAIDS, 2016a). The epidemic is largely characterized by concentrated and growing epidemics in a variety of countries, particularly among key affected populations including men who have sex with men (sometimes referred to as MSM), sex workers, people who inject drugs (also referred to as PWID) and transgender people (UNAIDS, 2013a). The epidemics in South and South-East Asia are decreasing, while in East Asia, HIV infections are rising (UNAIDS, 2013c). The four states of south India (Andhra Pradesh, Karnataka, Maharastra and Tamil Nadu) account for 53% of all the HIV-infected population (Phanuphak *et al*., 2015). Globally, every minute one young woman is infected by HIV and AIDS- related illness is the leading cause of death among women of reproductive age group (UNICEF, 2010). Women diagnosed with HIV infection face widespread stigma and discrimination along with negative attitudes of health staffs which prevent them from disclosing their status or seek treatment (Solomon *et al*., 2016). A study comprising 757 women living with HIV who had been pregnant within the past 18 months in six Asian countries (Bangladesh, Cambodia, India, Indonesia, Nepal and Vietnam) reported that significant number of pregnant women living with HIV in these regions are denied their right to health care and are subjected to discrimination from health professionals, including refusal of care provision and antenatal care, coerced abortion and sterilization (Head *et al*., 2014).

Prevention of Mother-To-Child Transmission has been significantly scaled-up across Asia and the Pacific (UNAIDS, 2013c) as period between 2009 and 2015 saw a decline of 30% in new HIV infections among children (UNAIDS, 2016a). However, PMTCT treatment coverage throughout the region remains shockingly low at 19% (UNAIDS, 2013c) much lower than other regions of the world including sub-Saharan Africa (59%) (UNAIDS, 2012b) and the global average of 62% (UNAIDS, 2013a). Malaysia is one example of a country that has made substantial progress with its PMTCT programme, increasing treatment coverage from 68.5% in 2012 to nearly 85.5% in 2013 and MTCT rates have also declined, from 18% in 2012 to 8% in 2013 (MOH, 2014).

Western and Central Europe and North America

In 2015, there were 2.4 million people living with HIV in Western and Central Europe and North America. There were an estimated 91000 new HIV infections in the region (UNAIDS, 2016a).

The United States of America (USA) accounts for the majority of people living with HIV in the region (56%) (UNAIDS, 2014), with men accounting for most of the HIV or AIDS diagnoses among adults and adolescents in the United States (CDC, 2007a). The HIV epidemics continue to disproportionately affect African Americans in the United States (Anderson *et al.,* 2002) and Aboriginal persons in Canada (Boulos *et al*., 2006). Four countries in Western Europe account for a further quarter of this number - France (8%), Spain (6%), United Kingdom (5%) and Italy (5%) (UNAIDS, 2014). In Western and Central Europe, the United Kingdom continues to have a large HIV epidemic, together with France, Italy and Spain (Bruckova *et al*., 2007). The HIV epidemic continues to be concentrated in London accounting for 41% of new HIV diagnoses in 2006 with significant increase in new diagnoses in East Midlands, Northern Ireland and Wales (Health Protection Agency, 2007).

In Western and Central Europe, People who inject drugs (PWID) and their sexual partners, transgender people, prisoners and sex workers are also at a heightened risk of HIV (UNAIDS, 2014). Between 2010 and 2015, the number of AIDS-related deaths in the region decreased by 24% (UNAIDS, 2016a).

2.2 Key population at the risk in global context

Key populations at increased risk of HIV infection include sex workers, people who inject drugs, transgender people, prisoners and gay men and other men who have sex with men (UNAIDS, 2016b). The HIV epidemic in WHO South East Asia Region is concentrated among key populations. Of people living with HIV, 99% are found in five member states- India, Indonesia, Myanmar, Nepal and Thailand (Singh, 2016).

Latin America and the Caribbean:

Most incident cases in Latin America and the Caribbean occur among MSM (De Boni *et al*., 2014). In 2015, among all adults and adolescents, the diagnosed infections attributed to male-to-male sexual contact (70%, including male-to-male sexual contact and injection drug use) and those attributed to heterosexual contact (24%) accounted for approximately 94% of diagnosed HIV infections in the United States (CDC, 2015).

MENA:

The key populations at risk in MENA region are: People who inject drugs (PWID), Men who have Sex with Men (MSM), Female Sex Workers (FSWs), mobile populations and intimate partners of key populations. The major route of infection in the MENA region seems to be sexual transmission as heterosexual sex was the most common reported mode of HIV transmission among men in Tunisia, UAE, Syria, Jordan, Morocco, Kuwait and Palestine in 2011 (Bozicevic *et al.*, 2014). Injected Drug Use is a growing and persistent problem in MENA as the number of IDU in MENA is estimated to have a mean prevalence of 0.24% (Dutta *et al.*, 2013). It was the most common mode of HIV transmission among men in Iran and Afganistan in 2011 followed by Pakistan and Egypt with the lowest number in Somalia (Mumtaz *et al*., 2014). Surveillance data of eight countries (out of sixteen) the most commonly reported mode of transmission was heterosexual sex followed by unknown mode in four countries, suggesting the possibility of male-to-male transmission as the unknown mode (Bozicevic *et al*., 2014). High HIV prevalence rates among MSM were reported from Sudan (Elrashied, 2008). The HIV prevalence among FSWs in the IBBS including 14 cities in Iran revealed an HIV prevalence of 4.5% in 2011 (Sajadi *et al*., 2013) with quite high prevalence rates among FSWs in Tripoli and Libya (Valadez *et al*., 2013). Also, intimate partners of key populations at risk are at high risk of infection which is shown by a study in Iran where HIV prevalence among non-injecting female sexual partners of male PWID was 2.8% which is twenty times the estimated adult prevalence in Iran (UNAIDS, 2013a).

Western and Central Africa

The epidemic in West Africa is deemed to be heterosexually driven but recent data revealed that MSM may play a significant role in the spread of HIV infection (De Cock *et al*., 2012 and Samuelsen *et al*., 2012) which is being justified by data from Senegal, The Gambia, Ghana and Nigeria indicating a substantial number of infections among MSM (Merrigan *et al*., 2011; Vuylsteke *et al*., 2012; Mason *et al.*, 2013; Vu *et al.*, 2013), many of whom also report having sex with women (Sheehy *et al*., 2013) In Western and Central Africa region, sex workers accounted for 15% of new HIV infections followed by clients of sex workers and other sexual partners of key populations (10%) in 2014 (UNAIDS, 2016b) as FSWs are identified as a high prevalence and incidence core group in West Africa (Forbi *et al*., 2011; Labbe *et al*., 2012**)**. Heterosexual intercourse is the major route for HIV transmission in Nigeria accounting for over 80% of infections (NNACA, 2014). According to 2010 IBBS survey, men who have sex with men constitute the second most-at-risk populations affected by the epidemic in Nigeria with average prevalence rate of 17.2% (FDH, 2010). Also, the barriers like cultural and social norms, legal and human right issues and economic commitment are the major factors contributing to a heavy HIV burden among key populations in West Africa (Djomand *et al.*, 2014).

East and Southern Africa

In sub-Saharan Africa, key populations accounted for more than 20% of new infections and HIV prevalence among these populations is often extremely high (UNAIDS, 2016d). For example, surveillance data published in 2015 in South Africa estimated HIV prevalence of 71.8% in Johannesburg, 39.7% in Cape Town and 53.5% in Durban among sex workers (UNAIDS, 2014).

Studies from South Africa and Kenya show that HIV prevalence was almost three fold higher in men who had sex with men than in men who had sex with women only (Dunkle *et al*., 2013) and Similarly, HIV incidence rates have also been three to four fold higher among men who have sex with men only, than among men who have sex with men and women (Sanders *et al*., 2013)

Harmful gender norms and inequalities, insufficient access to education and sexual and reproductive health services, poverty, food insecurity and violence, are at the root of the increased HIV risk of young women and adolescent girls accounting for 56% of new HIV infections among adults in sub-Saharan Africa (UNAIDS, 2016b).

Eastern Europe and Central Asia

The number of new HIV cases especially in key and most at risk populations continues to grow (WHO, 2014). HIV infections in central Asia and Europe in 2014 were among people from key populations and their sexual partners whereas people who inject drugs accounted for 51% of HIV infections in Eastern Europe and Central Asia (UNAIDS, 2016b). Sexual transmission between men was the most common mode in the EU/EEA and transmission through heterosexual contact and injecting drug use were the main reported transmission modes in the east of the Region (ECDC, 2015).

HIV epidemics in Eastern Europe and Central Asia continue to be driven by injecting drug use (IDU) and by flawed policy responses to people who use drugs (Beyrer *et al.*, 2010). There are an estimated 3.1 million people who inject drugs (PWID) in Central and Eastern Europe and Central Asia, of whom one million are estimated to be HIV infected (Mathers *et al*., 2008). HIV infections are increasing in other population groups, including female sex workers and their clients, prisoners, and migrants in Central Asia (Thorne *et al*., 2010).

Asia and the Pacific

In Asia and the Pacific, key populations at risk are sex workers (SWs), injecting drug users, men who have sex with men (MSM) and transgender people (WHO, 2012b). According to UNAIDS, at the end of 2013, there were 4.8 million people living with HIV in Asia and the

Pacific, with China, India, Indonesia, Myanmar, Thailand and Vietnam accounting for more than 90% of the people living with HIV in the region. In a systematic review in low- and middle-income countries, the burden of HIV infection was disproportionately high among FSWs in Asia and the Pacific, with a 29-fold increase in odds of living with HIV compared with women of reproductive age group (Baral *et al.,* 2012). PWID accounted for 13% of new HIV infections in Asia and the Pacific in 2014 as low national prevalence masks much higher prevalence among these groups and in specific locations, particularly urban areas (UNAIDS, 2016b).

In 2007, street based FSW in Thailand had an especially high HIV prevalence (22.7%) than among venue-based FSW (2.5%) (Manopaiboon *et al.,* 2013). TG women are 50 times more likely to acquire HIV than adult males and females of reproductive age 18% of surveyed male sex workers in Indonesia and Thailand tested HIV positive, 31% of TG sex workers in Jakarta, and 19% in Maharastra (UNAIDS, 2013b). An estimated 3-4 million people living in Asia are PWID (Phanuphak *et al*., 2015). In 2012, HIV prevalence among PWID was 36.4% in Indonesia (UNAIDS, 2013c), 13.6% in the Philippines (NAC, 2012) with an explosive epidemic in Cebu (53.8%) and 11.6% in Vietnam (VAAC, 2013). MSM is the risk group with rapidly increasing HIV infection in China (Wang *et al.*, 2008; Guo *et al.,* 2009; Zhao *et al.*, 2014). In 2013, 64% of new HIV infections occurred among key populations, 42% among MSM, while only 12% for FSW and clients and 10% for PWID in Thailand (UNAIDS, 2012a). India continues to portray a concentrated epidemic where HIV prevalence is high among risk groups like FSW, PWID, MSM and TG, is about 20 times higher than in general population accounting the highest HIV prevalence in PWID (7.1%) followed by MSM (4.4%), FSW (2.7%) and STI clinic attendees (2.5%) (Mishra *et al.,* 2012; NACO, 2012; Godbole *et al.,* 2014). In 2015, HIV prevalence among men who have sex with men was higher than 5% in nine of the nineteen countries that reported data and cities such as Bangkok in Thailand, Yangon in Myanmar and Yogyakarta in Indonesia have estimated HIV prevalence rates of between 20% and 29% (UNAIDS, 2016c). A further example of the HIV epidemic among men who have sex with men in a high-income country within the region is in Australia as for example: a 2012 study found that while HIV prevalence in Australia had declined overall, HIV prevalence rose among men who have sex with men (Beyrer, 2012) with 11.2% of men who have sex with men living with HIV (UNAIDS, 2012a). In 2015, 20 – 65% of all new adult HIV infections were among people who inject drugs in Afghanistan, Myanmar, Pakistan and Vietnam (UNAIDS, 2016d). In Katmandu, Nepal, increases in the use of safe needles led to a dramatic reduction in HIV prevalence - from 68% in 2002 to 6.3% in 2011 (UNAIDS, 2013a). HIV remains a critical concern for many transgender populations across Asia where high HIV prevalence rates have been found particularly among transgender populations in cities such as Delhi (49%) and Mumbai (42%) in India, and Phnom Penh (37%) in Cambodia in comparison to MSM (APCOM, 2013). Stigma, discrimination and legal barriers remain a major obstacle for providing and accessing services for transgender populations in Asia (UNDP, 2012).

Western and Central Europe and North America

The five key populations at risk in Western and Central Europe and North America are MSM, Sex workers, PWID, Transgender people with a focus on transgender women and people in prisons and closed settings (UNAIDS, 2013a). In Western and Central Europe, in 2014, 37% of all new HIV infections occurred among migrants from outside of this region (ECDC, 2015). African Americans are one of the groups most affected by HIV in the USA and accounted for 46% of all people living with HIV (506,000) in 2013 (CDC, 2015). Gay men and other men who have sex with men accounted for 49% of new HIV infections in Western and Central Europe and North America (UNAIDS, 2016b). In Western and Central Europe, People who inject drugs (PWID) and their sexual partners, transgender people, prisoners and sex workers are also at a heightened risk of HIV (UNAIDS, 2014).

2.3 National Distribution

The first case of AIDS in Nepal was reported in 1988. The estimated prevalence of HIV in total population was 5% in 2014 (DOHS, 2014/15) where young population aged 15-24 years accounted for 0.03% of HIV infections in 2015 and women aged 15-49 years constituted 40.6% of all people living with HIV (PLWH) with HIV prevalence of 21.4% among population aged 50 years and above (NCASC, 2015a). Nepal is experiencing the transition of HIV epidemic (Suvedi, 2006) where the spread of HIV is fuelled by poverty, ignorance, poor accessibility to services, illiteracy, stigma and discrimination (NCASC, 2014c). By sex, males accounted for two-thirds (64.8%) of the infections while remaining more than one-third (35.2%) of infections were in females of which 82% belonged to reproductive age group, 15-49 years (DOHS, 2014/15). The annual number of new HIV positive pregnant women was 414 in 2015 and there were 107 children aged 0-14 years with new HIV infections in 2015 (NCASC, 2015b).

The key populations infected for HIV were people who inject drugs (PWID), sex workers and their clients, men who have sex with men and transgender people, male labour migrants and their wives and prison inmates (DOHS, 2010/11). HIV infection rate among street-based sex workers in the Kathmandu Valley was 4.2% whose clients (4.4%) were mainly transport workers, members of the police or military, and migrant workers, not using condoms (World Bank, 2012) while prevalence was 15.7%, 2.1% and 1.4% in 2001, 2004 and 2006 respectively (UNDP, 2012). According to IBBS (2008), 42 % of FSWs were having at least one STI symptom in Kathmandu valley and prevalence of syphilis among them was 1 % while prevalence of syphilis was 2.3 % & Chlamydia was 3.6 % among men who have sex with men (IBBS, 2007) (NCASC, 2014a). A study conducted in the department of dermatology and venerology of Dhulikhel hospital, 74.5% had STIs and 17.9% had HIV/AIDs related symptoms with males accounting for the major proportions (53.3%) (Karna *et al.*, 2011). A survey conducted in 1993 reported higher number of STI cases in the

plain (terai) region (along the border of India in eastern Nepal) and in urban areas than in other parts of the country (Burathoki, 1993). In addition, the survey reported high risk-behaviours and high prevalence of STIs in the plain region of eastern Nepal, closely linked to India. Furthermore, 25 to 27% of young men from border towns reported multiple partners (Brown *et al.,* 2000).

According to World Bank 2012, the male labor migrants comprise of 27% of total estimated HIV infections in Nepal as of 2011 and estimates of internal and external migration for seasonal and long-term labour ranges from 1.5 to 2 million people. According to NCASC 2007 estimations, 41% of HIV cases in labour migrants with terai region alone accounting for 49.7% of HIV, and 21% among housewives or their partners.

2.4 Key populations at risk in Nepal

In context of Nepal, scenario of HIV infection remains almost constant between 0.3- 0.2% with comparatively higher transmission rate through sexual transmission than other key populations at risk (ECDC, 2015). Nepal's HIV epidemic is largely concentrated in (most-at-risk populations) MARPs, 58% of which are accounted for by key populations like female sex workers (FSW), IDUs, MSM, transgender and some migrants to high risk districts in India (World Bank, 2012).

Female Sex Workers (FSW) and their clients

The IBBS studies conducted since 2004 among FSWs in Kathmandu, Pokhara and other districts 22 Terai Districts suggests that the country has successfully kept the HIV prevalence below 2% (New Era, 2004). It is suggested that the sex workers largely contribute to increase the HIV prevalence in Nepal (Karki, 2008). Despite the consistent trend of low prevalence of HIV among FSWs for the last decade, STI prevalence among them have varied considerably over the last decade as the prevalence of active syphilis among FSWs of terai districts has decreased from 9.0% in 2003 to 0.3% in 2012 while prevalence of gonorrhea among the same population soared from 1.5% in 2009 to 4.5% in 2012 (MOHP, 2015). A study of sex workers in Nepal's terai region revealed that 4% of sex workers were positive for HIV among 17% sex workers who worked in Indian brothels.

The migrant workers, wage labors, transport workers, highway drivers, army and police usually become the clients of sex workers (Karki, 2008). One study showed that the proportion of having sex with FSWs increased by 20% for each group (42% to 62% for transport workers and from 10% to 30% for migrant workers within one year period of time (2000-2001) (FHI, New Era and SACTS, 2002).The country has limited data to assess the HIV situation among the clients of FSW.

Labour migrant and mobile population

There is risk of HIV infection among labour migrants in origin and destination countries due to social and economic factors (UNAIDS, 2008). An estimated 42% of all HIV infections in Nepal are among labour migrants (New Era and FHI, 2010). There is increasing trend in migration for both male and female between 2008/09-2013/14 (Shah, 2016). Male Labour migrants (particularly going to India) account for about 16% of HIV infections among reproductive age group in Nepal (NCASC, 2013). HIV prevalence among male labour migrants shows considerable variation by district and work-related migration is higher from the Western, Mid-Western, and Far Western Hill regions than anywhere else in Nepal (NCASC, 2014a). Among labour migrants in the Mid and Far West Regions surveyed in the 2012, overall HIV prevalence was 1.4% (IBBS, 2012), compared to 0.8% in 2008 and 2.8% in 2006 (NCASC, 2013). The estimated number of population that migrated to India was 722,255 (DOFE, 2014).

Spouse of Migrant Workers

Nepal is one of the major sources for migrant labourers, helping to fulfill the demands of rapidly industrializing countries in Asia and abroad. Foreign employment provides an alternative livelihood for many young Nepalese (CARAM/BCHR, 2007; ADB, 2009). A large number of Male Labour Migrants from Western, Mid and Far Western Regions migrates to HIV burden areas of India and their mobility and frequent return back home to their families, spouses are also at a higher risk of HIV transmission (Shah, 2016). A study done in HIV testing and Counseling unit in Bangladesh showed prevalence of 6.2%, notably 75% of adults testing positive had a history of migrant work or was the spouse of a migrant worker (Urmi *et al.*, 2015). A qualitative and quantitative study conducted in Achham, a hilly district of FWDR with the highest number of people living with HIV in Nepal (DOHS, 2014/15), found that illiteracy, low socio-economic status, gender inequality, migration of men to India, and HIV stigma were the social factors that interacted with individual's knowledge and behavior to determine women's vulnerability to HIV infection in FWDR (Thapa *et al.*, 2015). Trends of HIV testing and counseling in VCT centre of Dang plus showed maximum among married females (75.3%) whose spouse were labour migrant within or outside the country (Ghimire, 2004). Similarly, a study done in Bardiya district among spouses of male migrant workers and non-migrants in 2009, revealed that spouses of migrants were 15 times more likely to perceive risk of HIV infection than spouses of non-migrants people (Sharma *et al*., 2012).

Vertical Transmission

Vertical transmission also known as Mother-To-Child Transmission (MTCT) is transmitted from an HIV positive woman to her child during pregnancy, childbirth and breastfeeding. MTCT accounts for over more than 90% of new HIV infections among children (De Cock *et*

al., 2000). Mother-To-Child Transmission is the largest source of HIV infections in Nepal where one in three people living with HIV in Nepal are women, representing the single largest group of people living with HIV in Nepal (UNICEF, 2014). For HIV positive women who become pregnant, early detection of HIV and initiation of treatment is the key to preventing the transmission of HIV to their children (UNICEF, 2014). The annual estimated pregnancies in 2014/15 was 751,490 with 414 women requiring Prevention of Mother-To-Child Transmission (PMTCT) while only 145 pregnant women were receiving antiretroviral therapy to reduce the risk of MTCT (NCASC, 2016). There are still challenges in engaging pregnant women in the health system in Nepal, and stigma and discrimination is a barrier to HIV infected mothers accessing the treatment and care they need to ensure their children are born HIV-free (UNICEF, 2014).

Clients of sex workers

Heterosexual sex involving female sex workers (FSWs) is widely documented for its role in facilitating the spread of sexually transmitted infections (STIs/HIV) (Jin *et al.*, 2010; Braunstein *et al.*, 2011; Pruss-Ustun *et al.*, 2013). A growing body of research shows high rates of inconsistent condom use, multiple sex partners, substance use and HIV/STI among male clients of FSWs (Goldenburg *et al*., 2010). These findings have prompted increasing recognition of male clients of FSWs as a critical bridge population by which STI/HIV infection may spread from high risk populations (eg. FSWs) to the general population (eg. wives and steady female partners) (Shrestha, 2013; Shrestha and Copenhaver, 2014). Research to date has revealed that the use of FSWs in Nepal may be common among truck drivers and migrant workers, and often involves young men (Ghimire *et al.*, 2011; Sagtani, *et al.*, 2013). A study conducted using sample of 4,121 men aged 15-49 years from the data of Nepal demographic Health Survey, 2011 revealed that approximately 5% of Nepali men reported a sexual relationship with FSWs in their lifetime (Shrestha *et al*., 2016). Despite a growing body of research on FSWs and their role in HIV epidemic (Rana *et al.*, 2013), considerably little research has been devoted to their male clients in the Nepali Context.

Housewives

Housewives have higher proportion of HIV incidence among the HIV infected female population. The new reported HIV cases in housewives among all the HIV females are constantly increasing, that was 65% in 2005, and then reached to 87% in 2006, and 78% in 2007 indicating ¾ of the total women infected to be housewives (Karki, 2008). A study showed the transition of HIV from high risk behviour groups (such as FSWs) to low-risk behavior groups such as housewives (Suvedi, 2006). The prevalence of HIV among housewives was 10.6% in a retrospective study carried during the period 1988 to 2004 (Joshi *et al*., 2004). A vast majority of housewives are illiterate and do not have access to services that would protect them from HIV/AIDS (Pokhrel *et al*., 2001).

People who inject drug (PWID)

The survey conducted by Central Bureau of Statistics in 2012 estimated that there were around 52,174 PWID in the country. Nepal has successfully reduced the HIV prevalence since 2002 (IBBS, 2002) when HIV prevalence was recorded 68% in Kathmandu data (NCASC, 2014a). In the same period of time the behavioural indicators (condom use, needle syringe, prevention reach) showed consistent improvement. In the Eastern Highway districts, the HIV prevalence declined from 35.1% in 2002 (IBBS, 2002) to 8.1% in 2009 (IBBS, 2009) and has remained 8.1% since then (IBBS, 2012) while in the Western Highway districts, the HIV prevalence also declined from 8% in 2009 (IBBS, 2009) to 5% in 2012 (IBBS, 2012) (NCASC, 2015a). The HIV prevalence in Pokhara has dropped from 22% in 2003 to 4.6% in 2011 (USAID, 2011). Likewise, in Kathmandu, the HIV prevalence declined from 68% in 2002 (IBBS, 2002) to 6.3% in 2011 (IBBS, 2011). Likewise the HIV prevalence in Pokhara has dropped from 22% in 2003 to 4.6% in 2011 (USAID/NCASC, 2011). Females account for 7% of the total PWID of Nepal (CBS, 2012). A study conducted by UNODC in 2011 reported 4% HIV prevalence among female who inject drugs.

MSM

The term "men who have sex with men" denotes all men who have sex with men, regardless of their sexual identity, sexual orientation and whether or not they also have sex with females (WHO, 2010b). Men who have sex with men are among the small number of remaining groups for which the HIV epidemic remains uncontrolled worldwide. In low- and middle-income countries, men who have sex with men are 19 times more likely to be living with HIV compared with people in the general population and represent an estimated 10 % of all new infections each year (Baral *et al*., 2007). MSM are comprised of following sub-populations a) Male sex workers (MSWs) (estimated population size of 12,639) b) Transgender sex workers (TSW) (estimated population size of 9,474) and c) Men who have sex with men (MSM) with the population size estimated at 196,270 (NCASC, 2014a). Data from Kathmandu valley shows that overall HIV prevalence among MSM has not changed much and remains around 3.8% since 2009 while the prevalence among MSW has increased from 4.8% in 2004 to 6.8% in 2012 (NCASC, 2015a).

3. MATERIALS AND METHODS

3.1 Study Area

The study area for the research is Dhanusha district situated in Janakpur Zone of Central region of Nepal. Janakpur is one of the fourteen zones of Nepal, reaching from the Indian border in the south to the Tibetan border in the north and Sagarmatha zone in the east and Bagmati and Narayani zones in the west. Janakpur is divided into six districts: Dhanusha, Dolakha, Mahottari, Ramechhap, Sarlahi and Sindhuli district. The Dhanusha district with Janakpur as its district headquarters, covers an area of 1,180 square km and has a population of 754,777 (CBS, 2011), 50% female. Dhanusha is spread over latitude 26° 35- 27° 5 N to longitude of 85° 52- 86° 20 E, the average North- South length of the district is about 45 km and East-West width about 27 km. The district is divided into three topographical zones: the Sivalic area in the north which ranges in elevation from 300 to 600 metres above sea level, the Bhanwar area (150- 300m), and the Terai area in the south (60- 150m). More than 50 % of the district is in the Terai, which is densely populated with Madhesi people. The district is surrounded by Mahottari District in the west, Siraha District in the east, Sindhuli District in the north and Indian State of Bihar in the south. The district comprises 101 Village Development Committees (VDCs) and one municipality, 17 Ilakas and seven electoral constituencies.

The age profile of Dhanusha reveals a rather young population. The population of males aged 20- 34 is noticeably smaller than that of females due to the dynamics of migration. In 2011, 90% of Dhanusha's population is Hindu, 9% Muslim, 2% Buddhist and smaller shares are of other religions. Dhanusha is predominantly an agriculture dependent district, as are most districts in Nepal. In 2003/ 2004 around half of economically active population was engaged in agriculture followed by trade and commerce (14%) and production and recycling (13%) and to lesser degrees construction, general administration and education. Between 2003-2009, Dhanusha led the country in number of migrants heading overseas to work – an estimated 7% of the district's population migrated overseas.

The study was conducted in two different hospitals- private hospital (Aastha Hospital) and government hospital (Janakpur Zonal Hospital (JZH)). Aastha hospital is a private hospital famous for its Gynaecology and Obstetrics Department located nearby hospital road in Janakpur. The hospital is making plannings to set up other departments as well within 2-5 years. The primary research on HIV and trichomoniasis was done in Aastha Hospital with the help of doctor, staff nurse and lab technician. Janakpur Zonal Hospital is located in the hospital road with many departments running successfully. Janakpur Zonal Hospital serves as the main referral point for Dhanusha, Mahottari, Siraha and Sarlahi Districts. The data for HIV/AIDS cases were collected from Antiretroviral Therapy Centre (ART) of JZH.

3.2 Materials Used

Different materials were used for two different studies (HIV and Trichomoniasis). The materials used during laboratory examination are listed below:

For HIV

Centrifuge machine

Blood lancet

RDT Kits

Stop watch

Test tube

Cotton spirit

Capillary tube

Buffer solution

Syringe

Gloves and mask

For Trichomoniasis

Microscope

Saline water

Swab stick

Glass slides

Sterile test tube

Cover slip

Test tube stand

Disposable container

Marker

Dropper

Slide stand

Chemicals required

Saline water

Giemsa's stain

3.3 Study Design

The study was conducted in two parts.

a) First part:

Study design: A prospective analytical study was carried out using purposive sampling method to analyse the prevalence of *T. vaginalis* in pregnant women visiting Aastha Hospital, Janakpur, Nepal.

Place of study: The study was done in outpatient department of Gynaecology and Obstetrics of Aastha hospital.

Duration of the study: The total study period was during the months of March-May between 9 am to 4pm six days in a week.

Study population: A total of 120 pregnant women were included in the study regardless of the complaint of vaginal discharge or not.

Inclusion criteria: Women of reproductive age group in their pregnancy were included in the study.

Exclusion Criteria: Non pregnant women, older women and patients in complicated stage of pregnancy were excluded in the study.

3.3.1 Sampling techniques and procedural steps

Among the women attending Hospital, only pregnant women were selected for the study. They were first examined by the doctor and recommended patients were tested for trichomoniasis. Every pregnant woman was tested for HIV infection by using a rapid kit by lab technician in the hospital. They were then interviewed with other socio-demographic characteristics.

3.3.2 Blood Examination

3.3.2.1 Vein puncture method

After extracting about 2.5 ml blood from vein, the blood was transferred to test tube and was centrifuged for 30 minutes. After centrifuge plasma and serum was separated and the serum was transferred by micropipette to RDT kits and buffer solution was added in the kit. After five minutes it gives result. If two bands appear in the kit, it shows positive result and if only one band appears it shows negative result. HIV positive patient was then referred to ART Centre of Janakpur Zonal Hospital for their Antiretroviral treatment. They were provided proper course of medicines and condoms at the centre. They were counseled by the counselor to take complete dose of medicines and take precaution during sexual intercourse.

3.3.3 Swab Test

3.3.3.1 Sample collection

Before the clinical examination, patients were asked to micturate and then in each case general and local examination was done by the doctor. For local examination patients were kept in Lithotomy position. Any abnormality in the vulva or perineum was inspected. Then per speculum examination was done with good source of light. Then, the abnormality in the vagina, cervix was noted. With all aseptic precautions swab was then taken from the posterior formix of vagina with two cotton tipped sterile swab sticks and kept in a sterile test tube. The test tube was then marked.

3.3.3.2 Laboratory examination

As soon as the sample was collected the wet mount preparation of the sample was done. Smear was prepared in clean, dry sterile glass slide. The edge of the glass slide was marked like as the test tube. On the smear a drop of normal saline was dropped with the help of dropper and an even solution was made. Then, it was covered by a cover slip and seen under the microscope.

3.3.3.3 Microscopic examination

The smear prepared was observed under the microscope in 10×10 and 10×40 magnification. The presence of *Trichomonas vaginalis* could be identified by the pear shaped anterior flagella and typical jerky rotatory movement. The interval between the collection of sample and microscopic examination was maximum of 10 minutes. The confirmation of microscopic examination was done by the attending pathologist on the duty of lab of Aastha Hospital. None of the sample was processed for Giemsa's staining because of the absence of positive case among 120 pregnant women.

Recording: After the history taking, clinical examination and microscopic examination all the findings were recorded in the printed questionnaire.

3.3.4 Questionnaire survey

Questions were asked verbally to each patient during questionnaire survey. The questions asked to them were about their general information i.e. name, age, address, sex, smoking habits, occupation, marital status, socio-economic status, education level, contraception used, number of children, symptoms like pruritus, itching, odour, consistency, color of vaginal discharge, physical appearance and microscopical findings.

3.3.5 Data Analysis

Different age groups of pregnant women participating in the study were categorized into three groups: <20 years, 21-45 years and >46 years. Also, caste groups of these patients

were divided into three major categories: Upper caste, Middle caste and Lower caste. Upper caste groups included castes like Brahmin, Bhumihar, Rajput and Kayastha while middle caste groups constituted Yadavs, Sah, Mandal and Mahato and Lower caste included Dom, Chamar, Tatma, Mukhiya and Paswaan. Muslims included Ali, Ansari, Khatun and Shekh.

b) Second Part

Study design: Retrospective assessment of patients visiting ART centre of JZH was done to analyse the prevalence of HIV in Dhanusha district, Janakpur, Nepal. A total of 3770 individuals attending ART centre from 2010/11-2014/15, the complete five years records were obtained from ART centre for further analysis. The name of HIV positive patients were kept confidential according to the rules of organization.

3.3.6 Data Analysis

Data obtained from government authorities of JZH were analysed using Statistical Package for Social Sciences (version 20) and MS Excel. Descriptive statistics were used to describe the socio-demographic characteristics of the HIV positive patients. The p-value was calculated to determine the significant difference between the variables and odds ratio (OR) was calculated to find out the risk assessment of individuals visiting ART centre of JZH. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.

The secondary data was analysed in two different tables.

i) Risk assessment: The major risk groups were divided into nine categories like IDUs, Sex workers, MSM, clients of sex workers, migrant workers, spouse of migrant workers, vertical transmission, housewives and others. Tested and prevalence percentages for each of the variables from 2010/11-2014/15 were calculated. P value was calculated to determine the significance difference between the different risk groups. Similarly, OR was calculated to figure out the probability that by what folds each risk group acquired HIV infection.

ii) Trend analysis: The complete five years records was analysed to determine the distribution of people living with HIV with the help of six important variables. These were: age group, sex, marital status, caste, district and current pregnancy status. P value was calculated for each of these variables. Age groups were classified into five categories as done in NCASC, HIV +ve case reporting form. These groups were: 0-14 years, 15-19 years, 20-24 years, 25-49 years and 50 years and above. Sex was categorized as male, female and transgender. The married, single, divorced and widow were divided individually to determine the prevalence according to marital status. Caste included two divisions: (uppercaste, middle caste and lower caste) and Muslim. Caste with titles like Sharma, Jha, Thakur, Singh, Chaudhary, Tiwari, Pandey, Karki and Mishra were categorized in upper caste. Middle caste groups constituted Yadav, Mandal, Sah, Mahato, Gupta, Mahaseth and Ray. Caste like Ram,

Kapar, Raut, Chamar, Paswaan, Mahara, Das, Sada, Bika, Tatma, Mijhar, Mukhiya, Bin, Khataune, Sahani, Pandit, Goet, Khatwe, Khadka, Gosai, Kwyat, Pariyar, Nayak, Malaha, Lahari, Tamang, Gurung, Musahar and Sattar were classified as lower caste groups. Muslims constituted Khatun, Sekh, Raien, Ali, Ansari, Husen and Mansuri. HIV positive patients belonging to different districts were divided into five categories: Dhanusha, Mahottari, Sarlahi, Siraha and Others. Others included places like Okhaldhunga, Udaypur, Morang, Saptari, Sindhuli, Gaighat, Gaur and Sitamadi, Jaynagar and others from India. Pie chart was used to represent HIV infection among key populations at risk.

3.3.7 Some photographs during the study period

Photo 1: Aastha Hospital, Janakpur

Photo 2: Pregnant woman

Photo 3: Vaginal swab collection

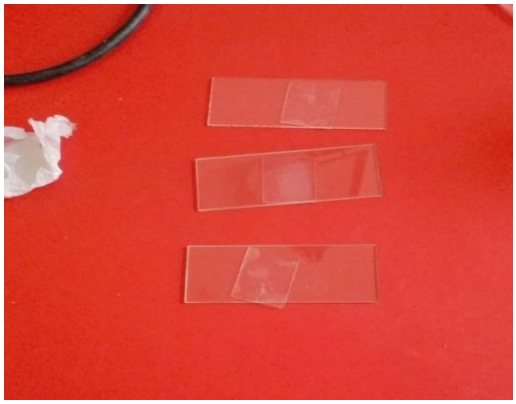

Photo 4: Slides preparation

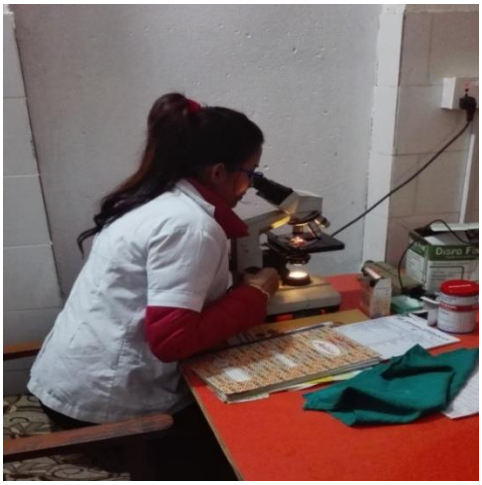

Photo 5: Microscopic examination

Photo 6: Interview with pregnant woman

Photo 7: Blood sample collection

Photo 8: RDT test for HIV

Photo 9: Entrance of ART Centre, JZH

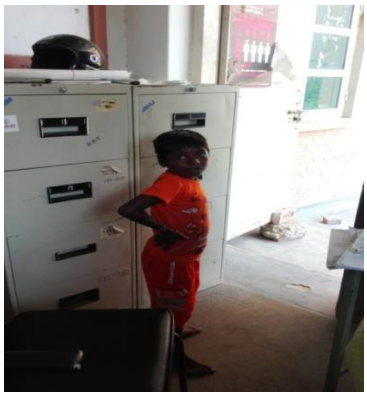

Photo 10: HIV positive child in ART Centre

4. RESULTS

A total of 120 pregnant women were randomly selected in order to determine the prevalence of HIV and Trichomoniasis during their visit to Aastha Hospital for Antenatal check up. They were interviewed to assess other demographic characteristics as well. Along with this, secondary data of 3770 patients visiting ART centre of Janakpur Zonal Hospital from 2010/11 to 2014/15 were analysed in order to determine the trend of HIV in Dhanusha District of Janakpur Zone within these five years.

4.1 Prevalence of HIV and trichomoniasis in Aastha Hospital, Janakpur

Primary data on HIV and trichomoniasis were collected from 120 pregnant women visiting Aastha Hospital, Janakpur, for ANC check up during the month of March-May, 2016. During the study period, pregnant women belonging to 21-45yrs age groups participated in the study constituted the major proportions (67.5%).

Table 1: Age wise prevalence of trichomoniasis and HIV among pregnant women visiting Aastha hospital, Janakpur.

Age group (Yrs)	Frequency of pregnant women N= 120	Trichomoniasis	HIV
<20 yrs	37 (30.83%)	0	0
21 – 45 yrs	81 (67.5 %)	0	1 (0.83 %)
>46yrs	2 (1.66 %)	0	0

Among the 120 pregnant women tested both for HIV and trichomoniasis, revealed 0.83% of HIV infection belonging to age group 21-45 years which is the most potential reproductive age group while none of the tested pregnant women were found positive for trichomoniasis.

Religion wise distribution of the pregnant women participating in the study showed maximum percentage belonging to Middle and Lower caste community people compared to upper caste Hindu and Muslim community people. Pregnant women from Middle Caste group constituted the highest number (39.16%) followed by Lower caste (29.16%) and Muslim (18.33%) whereas the least belonged to Upper caste group (13.3%). Also, HIV was found to be prevalent in Middle Caste Hindu pregnant women (0.83%).

Table 2: Religion wise prevalence of HIV and trichomoniasis among pregnant women visiting Aastha Hospital, Janakpur.

Religion	Caste	Frequency of pregnant women	Trichomoniasis	HIV
Hindu	Upper caste	16 (13.3 %)	0	0
	Middle caste	47 (39.16 %)	0	1(0.83 %)
	Lower caste	35 (29.16 %)	0	0
Muslim	Muslim	22 (18.33%)	0	0

Among the total pregnant women who came for ANC check up without any symptom of HIV, one pregnant woman (0.83%) belonging to middle caste Hindu religion was found to be positive for HIV while none of the pregnant women were found to be positive for trichomoniasis infection.

4.2 Risk assessment of patients visiting ART Centre of Janakpur Zonal Hospital

Risk assessment of patient visiting ART centre was done to characterize the nature and likelihood of HIV prevalence. Risk groups reported in the data represent the chance of acquiring HIV. The study was focused to identify the individuals with HIV in order to make the community people aware. It is well accepted that HIV in Nepal remains concentrated among the key behavioural populations like male labor migrants and their wives, VT, MSM, clients of sex workers, Intravenous drug users and others.

ART centre in JZH, Dhanusha was established in 2008 with the aim of providing treatment to people living with HIV infection. Patients are tested for HIV and provided with two most common medicines Zidovudine and Lamivudine, first line ART, for positive cases. Patients are counseled regarding the dose of medicines, follow up procedures, improvement of quality of life, Prevention of Mother to Child Transmission (PMCT) and Post Exposure Prophylaxis (PEP). The lifetime risk of someone with latent TB developing TB disease in HIV negative individual is 5- 10% where as in HIV positive individual is 50%. Managing TB among HIV infected individuals is one of the major responsibilities of the ART clinician. Patients are mostly provided medicines for opportunistic infections like candidiasis and TB at this ART centre.

Table 3: HIV Testing and counseling of patients visiting ART centre of Janakpur Zonal Hospital, 2010/11 – 2014/15

	2010/2011 (N)	2011/2012 (N)	2012/2013 (N)	2013/2014 (N)	2014/2015 (N)	Total	P value Comparing 5 years
Total Tested	656	723	691	854	846	3770	0.968
No. of HIV Positive	85	96	97	120	115	513	
Prevalence (%)	12.9	13.3	14	14	13.5	13.6%	

During the period of five years, ART centre of Janakpur Zonal Hospital recorded 3770 individuals tested for HIV. The result revealed 13.6% prevalence in Dhanusha District of Janakpur Zone showing no significant difference ($p>0.005$) It showed that the trend of HIV is almost similar within five years from 2010/11- 2014/15.

Table 4: Risk assessment of patients visiting ART centre in Janakpur Zonal Hospital, 2010/11 – 2014/15

Variable	2010/11		2011/12		2012/13		2013/14		2014/15		P Value	OR
	Tested (%) N=656	+ve (%) N=85	Tested (%) N=723	+ve (%) N=96	Tested (%) N=691	+ve (%) N=97	Tested (%) N=854	+ve (%) N=120	Tested (%) N=846	+ve (%) N=115		
IDU's	0	0	0.13	0	1.15	0	0.35	0	0.11	0	0	0
Sex workers	0	0	0.55	0	0.14	0	0	0	0	0	0	0
MSM	0.6	2.3	0.55	1.04	0.14	0	0.11	0	2.3	7.8	0.000	4.310
Clients of sex workers	1.5	1.2	0.82	0	2.7	2.06	0	0	0	0	0.383	0.593
Migrant workers	25.7	45.8	34.16	46.8	32.4	45.3	32.7	40	29.4	33.0	0.000	1.725
Spouse of MW	26.37	0	26.6	18.7	20.2	30.9	22.8	33.3	24.8	25.2	0.440	0.917
Vertical Transmission	7.6	32.9	6.6	21.8	11.2	7.2	13.11	13.3	4.3	9.5	0.000	2.405
House wives	0	0	2.7	3.1	7.9	4.12	5.7	4.1	15.2	10.4	0.048	0.649
Others	38	14.1	27.6	5.2	23.8	10.3	25.05	12.5	23.6	15.6	0.000	0.313

Men having sex with men (MSM) are more likely to perceive HIV infection indicating 4.3 times high odds of infection. They are the most vulnerable risk groups as they are more likely to be living with HIV than the general population. The probability of acquiring HIV infection is 1.725 times higher among Migrant Workers.

Vertical transmission (VT) or Mother-to-child transmission (MTCT) is by far the largest source of HIV infection in children below the age of 15 years. The risk of a baby acquiring the virus from an infected mother increases by 2.4 times in VT.

The risk of exposure to HIV infection had shown to increase by less than 1 time among spouse of migrants (OR, 0.917), housewives (OR, 0.649), clients of sex workers (OR, 0.593) and others (OR, 0.313).

4.3 Retrospective assessment of people living with HIV, 2010/11-2014/15

This was a retrospective assessment of patients enrolled in ART centre from 2010/11-2014/15 at Janakpur Zonal Hospital of Dhanusha District. Between 2010/11- 2014/15, a total of 3770 patients were enrolled in ART centre. Of these, 513 were assessed retrospectively based on the positive case report. In this study, the baseline explanatory variables were age, sex, marital status, caste, district, current pregnancy status, number of children, client clinical staging, risk groups, major routes of transmission, etc.

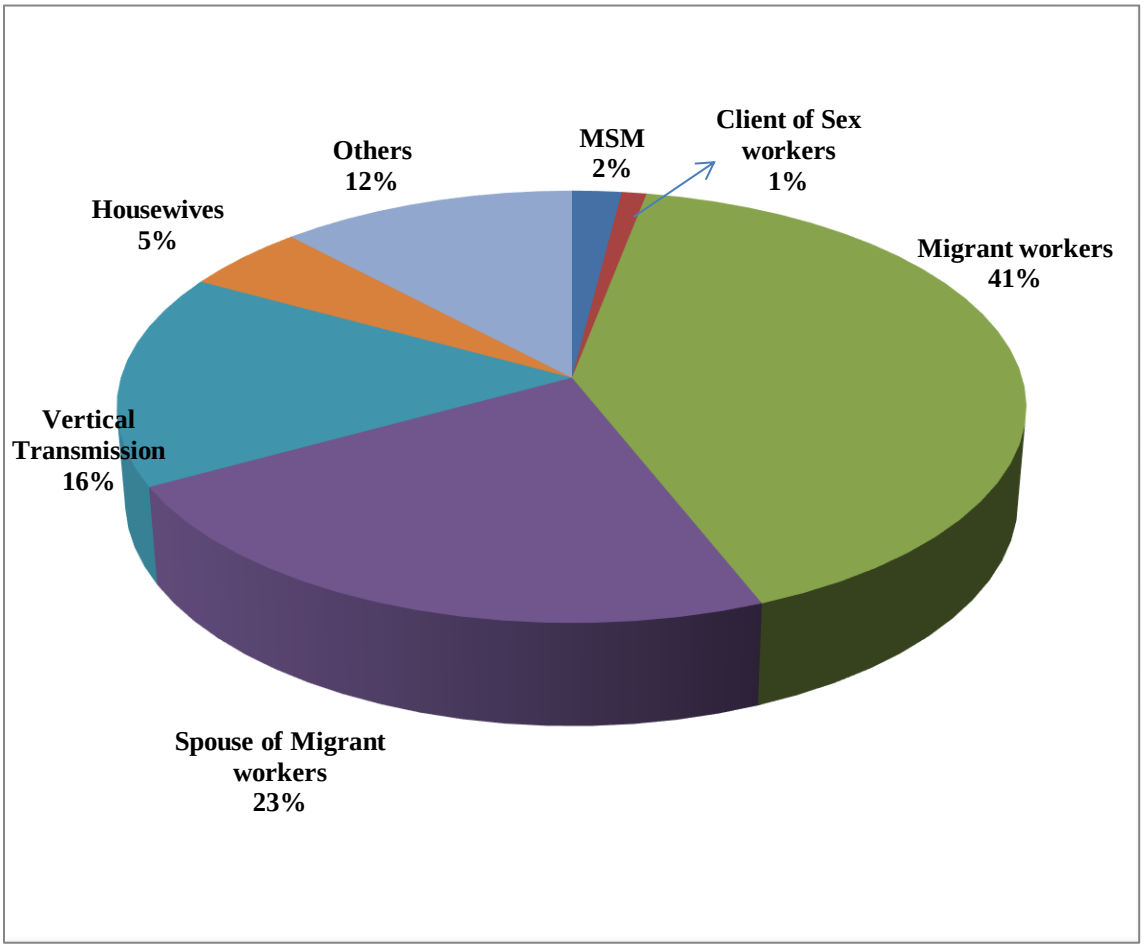

Figure 1: Distribution of people living with HIV, 2010/11-2014/15, in JZH

The retrospective study noted that out of 513 positive cases recorded from 2010/11-2014/15, the vast majority of HIV infections were found to be dominant among MW (41%) followed by spouse of migrants (23%), Vertical Transmission (16%), Others (12%), Housewives (5%), MSM (2%) and client of sex workers (1%).

Table 5: Distribution of people living with HIV in JZH, 2010/11 – 2014/15

Variable		2010/11 HIV (%) N = 85	2011/12 HIV (%) N = 96	2012/13 HIV (%) N = 97	2013/14 HIV (%) N = 121	2014/15 HIV (%) N = 115	Total HIV (%) N= 513	P value Comparing 5yrs
Age group	0-14 yrs	7	1	5	8	12	7.01	0.023
	15-19yrs	4	7	2	4	3	4.09	
	20-24yrs	14	15	8	8	9	10.5	
	25-49yrs	74	74	84	73	68	74.07	
	50yrs & above	1	3	1	7	8	4.2	
Sex	Male	59	54	61	57	62	58.6	0.389
	Female	39	45	39	43	38	40.7	
	Transgender	2	1	0	0	0	0.58	
Marital Status	Married	79	68	82	78	79	77.1	0.008
	Single	11	14	11	16	18	14.6	
	Divorced	1	0	1	0	0	0.38	
	Widow	9	18	6	6	35	15.2	
Caste	Upper Caste	12	14	3	10	11	10.1	0.004
	Middle Caste	64	47	43	43	44	47.3	
	Lower Caste	14	22	38	33	35	29.2	
	Muslim	10	17	15	14	10	13.2	
District	Dhanusha	46	48	35	44	32	40.7	0.066
	Mahottari	36	26	39	29	37	33.3	
	Sarlahi	13	7	18	11	16	12.8	
	Siraha	4	7	4	8	6	6.04	
	Others	1	12	4	8	9	7.01	
Current pregnancy Status	Yes	2.35	3.12	1.0	1.6	0	1.5	0.661

Among 513 people living with HIV (PLWH), 74% of them belonged to age group 25-49yrs followed by 20-24yrs (10.5%) while 0-14yrs, 15-19yrs and 50yrs & above were comparatively less affected with HIV. The distribution of HIV in different age groups showed insignificant difference ($p>0.005$).

The distribution of PLWH on the basis of sex revealed that males are affected more (58.6%) compared to female (40.7%) whereas transgender constitutes the least proportion (0.58%). The prevalence of HIV infection was found to be independent of the sex group (p>0.005).

Out of total positive cases (513), maximum individuals were married (77.1%) compared to Single, Divorced and Widow. The distribution of HIV infection among marital status was statistically insignificant (p>0.005).

HIV infection was found to be dominant in Middle caste (Hindu) (47.3%) among total number of PLWH while Lower caste (29.2%), Muslim (13.2%) and Upper caste (10.1%) showed comparatively lesser prevalence. HIV infection was significantly related with caste (p<0.005).

District wise distribution of HIV showed HIV infection was more widely spread in Dhanusha (40.7%) compared to two other nearby districts Mahottari (33.3%) and Sarlahi (12.8%). There was insignificant relation between HIV infection and District (p>0.005).

Among 513 HIV positive cases, 1.5% of women in their current pregnancy status were receiving antiretroviral treatment from ART Centre of Janakpur Zonal Hospital.

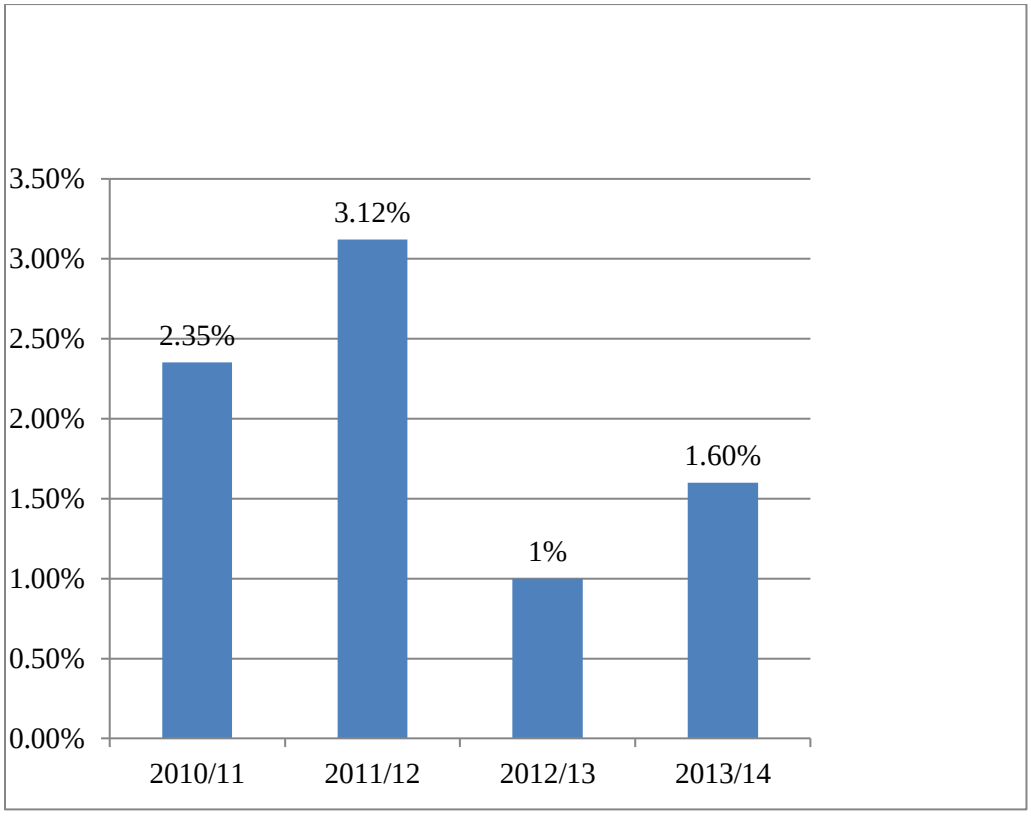

Fig 2: Pregnant women receiving ART from JZH 2010/11- 2014/15

Table 6: Client Clinical Staging (CC Staging)

	2010/2011 CC Staging (%) N= 85	**2011/2012** CC Staging (%) N= 96	**2012/2013** CC Staging (%) N= 97	**2013/2014** CC Staging (%) N= 120	**2014/2015** CC Staging (%) N= 115	**Total** CC Staging (%) N=513	**P value Comparing 5 years**
Asymptomatic	2.35	6.2	53.6	15.7	21.0	20.0	0.000
Mid Symptoms	0	6.2	21.6	18.1	2.6	10.1	
Advance Symptoms	4.7	8.3	4.1	1.6	15.7	7.0	
Severe Symptoms	2.3	4.1	4.1	1.6	15.7	5.8	
Not assessed	90.5	75	16.4	62.8	58.7	60.3	

Among 513 PLWH, 5.8% people had tested after having severe HIV symptoms while 10% and 7% people had tested for HIV after having mid as well as advanced symptoms respectively. The maximum 60% of people living with HIV had been tested for HIV infection without any symptoms. Majority of PLWH were found be in asymptomatic stage indicating the highest percentage (53.6%) in 2012/13 while mid symptoms (21.6%) were also mostly seen in 2012/13 in these five years. Advance symptoms and severe symptoms were mostly prevalent in 2014/15.

5. DISCUSSION

Sexually transmitted infections (STIs) remain a global public health challenge. World Health Organization estimated that 498.8 million new STIs occur annually among people aged between 15-49 years whereas the four infections (chlamydia, gonorrhea, trichomoniasis and syphilis) only accounted for 357 million new infections in adults in 2012 and sub-Saharan Africa has the highest STI incidence (240/1000) in the world where new cases of *Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis* infections take place annually (WHO, 2012a).

In developing countries STIs are among the leading causes of disability-adjusted life years (DALYs) lost for women of reproductive age (CDC, 2011). Studies conducted in various parts of South East Asia have shown almost double prevalence of STIs among women (Hawkes *et al*., 2002; Thomas *et al*., 2002) as women have limited access to information or health services than men and they are too busy in their household chores and child caring activities (Dixon-Mueller, 1999; Voeten *et al*., 2004). They have symptoms for longer periods and less often they seek treatment than men. Also, women are economically dependent on men and less often they visit a health centre without a male relative. The analysis based on the nationally representative cross-sectional sample survey, NDHS 2011, revealed that women who reported intimate partner violence (IPV) in the last 12 months had about two times higher risk of getting STI compared to those who did not have IPV. Women from the terai region, from poor households, with no education, who worked manual jobs, had been married more than once, and those who lived with co-wives had a higher prevalence of IPV compared to their counterparts (Dhakal *et al*., 2014). A study done among 791 married women of reproductive age from rural parts of six districts (Dadeldhura, Nawalparasi, Makwanpur, Siraha, Sindhupalchowk, and Sankhuwashava) of Nepal revealed that 16.6% experienced sexual violence from intimate partners at any point of life and 7.1% had sexual violence from an intimate partner in the last 12 months (OPCM, 2012). A cross-sectional study conducted among women attending four weeks long health camps during December 2005 and 2006, in Rauthat, Mahottari and Saptari districts located in the terai region of Nepal, showed STI prevalence of 30.1 % followed by pelvic organ prolapsed, menstrual disorders and sub-fertility (Dangal, 2008).

STI are among the most important cause of maternal morbidity and perinatal morbidity and mortality. They can have severely debilitating effects on pregnant women, their partners and fetuses. STI in pregnant women can lead to early onset of labor, abortion, premature rupture of the membranes and uterine infection after delivery. It has harmful effects on babies like stillbirth, low birth weight, conjunctivitis, pneumonia, neurological damage, congenital syphilis etc. STIs and pregnancy-related issues are among these historically neglected health problems and continue to be important sources of healthy life years lost for women (Glasier *et al*., 2006). In Africa, it is estimated that 92.6 million new cases of the curable STIs

(*Chlamydia trachomatis, Neisseria gonorrhoeae, Treponema pallidum, and Trichomonas vaginalis*) occur, whereas 78.5 million and 128.2 million new cases are estimated to be in Southeast Asia and the Western Pacific (WHO, 2012b). A study conducted among pregnant women from January 1999 through March 2001 in a south rural eastern district of Sarlahi, Nepal concluded very low rates of gonococcal (2.3%) and chlamydial infections (1%) among them (Christian *et al*., 2005). Few studies have reported the prevalence and risk factors for STIs in rural populations in South Asia. Newman *et al*., in 2012 reported that globally 900 000 pregnant women were infected with syphilis resulting in approximately 350 000 adverse birth outcomes including stillbirth in 2012.

Trichomonas vaginalis, a flagellated protozoon, is the causative agent of trichomoniasis, which is the most prevalent non-viral sexually transmitted infection worldwide with an estimated 180 million infections acquired annually (Molero *et al*., 1998; Nelson and Macones, 2002) with South East Asia Region alone accounting for 42.9 million annual incidence (NCASC, 2014c). The pregnant women infected with this parasite may be at risk of unfavorable birth outcomes such as premature rupture of membranes, premature labor, and low birth weight (Adada *et al*., 2001). Trichomoniasis is associated with a 30% increase in low birth weight infants and a 30% increase in risk of preterm births (Coleman *et al.*, 2013). Vaginal trichomoniasis has been associated with increased HIV virus seroconversion in women (Laga *et al*., 1993). In spite of the fact that trichomoniasis is the most common of STIs, data on prevalence and incidence are limited. Trichomoniasis prevalence rates amongst pregnant women in Latin America and Caribbean in the 1990s ranges from 2.1% in Brazil (Simoes *et al*., 1996), 3.6% in Barbados (Levett *et al*., 1995), 8% in Nicaragua (Espinozoa *et al*., 1993) and 5% in Chile (Franjola *et al*., 1989). Prevalence studies amongst pregnant women in Africa show rates from 9.9% in Central African Republic to 41.4% in South Africa (WHO, 2001). Few prevalence studies have been conducted amongst men. A study conducted among pregnant women visiting Paropakar Maternity and Women's Hospital in Kathmandu showed that 29.5% had candidiasis, 52.6% bacterial vaginosis and 1.3% trichomoniasis and vaginitis was common in women with third pregnancy (52.6%) (Shrestha *et al.*, 2011). The present study carried out in Aastha hospital, Janakpur among 120 pregnant women, showed contrasting results as compared to other studies mentioned above as the prevalence of trichomoniasis was 0% while 0.83% of pregnant woman was found to be positive for HIV. The zero prevalence for trichomoniasis could be because of the less number of pregnant women (PW) participating in the study which is supported by one of the studies in Ebonyi state where 12.3% PW were found to be positive among 1025 PW (Okonkwo *et al*., 2010). Also, prevalence of trichomoniasis was 2.6% among 330 females attending gynaecology department of National Medical College and Teaching Hospital in Birgunj, Nepal (Bhargava *et al*., 2016). In contrasting to this, a study conducted among 100 PW in a teaching hospital of South-western Nigeria revealed 2% prevalence of trichomoniasis among them (Olowe *et al*., 2014) which was opposite to the findings from this study where none of the PW was found to be infected with *T. vaginalis* infection. Similar contrasting results to the

present study were seen from a cross-sectional study conducted among women attending four weeks long health camps during December 2005 and 2006, in Rauthat, Mahottari and Saptari districts located in the terai region of Nepal, which showed STI prevalence of 30.1 % followed by pelvic organ prolapsed, menstrual disorders and sub-fertility (Dangal, 2008). Most of the PW visited the hospital with their in-laws and some with their husbands, feared of extra payment for the test and thus refused to take part in the study resulting into low participation of PW in the study. Being a Madhesi daughter-in-law from terai, traditionally they would be under the guardianship of mothers-in-law and husbands and have little say in household and other major decisions (Gram *et al*., 2016). It was found that decision-making was higher among women living separately with their in-laws and whose husbands have migrated to foreign for employment. Further, PW stayed silent in front of their in-laws unable to negotiate for the test as speaking against their in-laws is considered as disrespectful to them. The prevalence of trichomoniasis appears to be relatively lower than the other vaginal infections and seems to have declined when compared with earlier reports (Taylor *et al*., 2013) which justifies the reason for no prevalence of trichomoniasis in this study. It is estimated that more than 14 million females between 15 and 19 years of age give birth each year, and more than 90% of these births occur in developing countries (WHO, 2004). Rates of pregnancy are reported to be as high as 89 births per 1000 females aged 15 to 19, but births among younger females are also frequent (WHO, 2004). Pregnant women from 21-45 years age constituted the major proportion (67.5%). This could be because of the marriages taking place at an early age in terai which is supported by the latest study done by Gram *et al*., where 88% of sampled women were married between ages 12 and 18 resulting in the first pregnancy at an early age. Bajracharya and Amin (2012), hypothesized that harmful traditional practices such as dowry might be driving poor families to marry their daughters off early to avoid having to pay large sums in dowry.

The NDHS 2011 showed that still at least 17 percent of adolescent women are already involved in childbearing activities; fertility rates are highest among 20-24 years women and only 24 percent in this age group use modern family planning methods (Pathak, 2012). A study conducted in three clinical trials in South Africa showed that the median age of women screened for STIs was 28 years (Naidoo *et al*., 2014) which is consistent with this study results where reproductively potential age group (21-45 yrs) constituted the highest frequency (81) followed by women below 20 years age. The reasons for high incidence of STIs in this age group includes low levels of protective cervical antibodies, increased sexual activity, new influence of reproductive hormones causing vast changes in tissues that may lead to increased susceptibility to STIs (Barousse *et al*., 2004).

Most of the PW visiting hospital was Hindu (81.62%) while 18.33% were Muslim. This is very consistent with data obtained from among 800 women of reproductive age from Anganwadi Centre register of Tirupati Town in India which showed that 84.5% women were Hindu with higher prevalence of STI among Hindus (28.7%) than Muslims (Sri Devi and

Swarnalatha, 2007). This might be because Hindu women families do not pose much restriction in going outside of their homes as compared to the Muslims. This could be best justified with one of the study's findings on Hindu and Muslim's behavior in India which stated that Muslim women were more likely to participate in veiling, less likely to go on family outings to places like fairs and movie theaters, and less likely to be employed (Desai and Temsah, 2014). Among the Tarai/Madhesi groups, Muslim women had the highest percentage (47 percent) reporting no participation in any of the four areas (education, urban/rural residence, wealth status, occupation) of decision making (Benett *et al*., 2008).

Majority of PW belonged to middle caste group (39.16%) followed by lower caste (29.16%), Muslim (18.33%) and upper caste (13.3%). This is because the population of Dhanusha district in particular, is dominated by these middle caste (Yadav) and ethnic groups such as Muslims and Tharus (Amin *et al.,* 2014). The high frequency of middle caste PW attending private hospital could be also because of their sufferings from health problems to a greater extent as these caste groups are mostly illiterate or less educated (up to primary levels only) while upper caste PW were found to be educated, some employed as well and had scored the highest points regarding knowledge on HIV/ AIDS and its prevention steps in an interview with them. They seemed to be in a good health visiting hospital for their normal ANC check up while middle caste women and lower caste women were meant to attend the hospital either for the safe abortion for unwanted pregnancies or to get confirmed if they were pregnant. They were not educated as well with no knowledge about HIV/AIDS. Majority of these caste groups had not heard about HIV/AIDS while some identified this disease as being transmitted from other men (who are not their husbands). This is supported by the study done by Benett *et al*., which reported that Terai/Madhesh-origin groups– with the notable exception of the Madhesi "high caste" groups – are doing worse on almost all health and education indicators. Also, Gyawali *et al*., in their study found that participants from the hill and mountain districts were 1.54 times and 1.82 times more likely having knowledge on HIV and its transmission respectively when compared with those living in plane district (Gyawali *et al*., 2014). Another reason could be the foreign remittances these middle caste, lower caste and muslim women would have been receiving from their husbands living abroad for employment. They have more economic freedom compared to those whose husbands are not migrating. In an interview with a middle caste woman from a nearby VDC Ghodghas of Dhanusha district, mentioned a separate secret account for wife which she did not have told to any of her in-laws and could spend that money wherever she would like. Also, husbands creating a pressure on their parents for the proper care and treatment of their wives in their absence had driven the parents' responsibilities of seeking proper treatment to their daughters-in-law in a private hospital.

While no PW were found to be positive for *T. vaginalis* parasite, one PW showed positive infection for HIV. The study result was almost similar to the findings from a tertiary care centre of Dhulikhel hospital where two patients out of 106 women, suffering from STI, were

positive for HIV (Karn *et al*. 2011). Worldwide, 1% of pregnant women are HIV-positive (Hampanda, 2013). The study findings revealed that the 0.83% of HIV prevalence belonged to middle caste group and her husband worked as a police, considered as high risk group. This shows the possibility that her husband might have acquired the infection through FSWs which in turn transmitted to his pregnant wife. According to World Bank 2012, almost 60 percent of the clients of FSWs, mainly transport workers, members of the police or military, and migrant workers, did not use condoms.

The Human Immuno Deficiency Virus infection (HIV) continued to be the pandemic since the first case of Acquired Immune Deficiency Syndrome (AIDS) was identified in 1981 (Haider *et al.,* 2009). According to UNAIDS Fact Sheet of 2016, globally 36.7 million people were living with HIV by the end of 2015 and sub-Saharan Africa is the most affected region with about 25.6 million people living with HIV in 2015, 2.1 million (India), 65400 (China) and 28,865 (Nepal). The prevalence of HIV among adult population has been declining steadily in Nepal as the number of new HIV infections declined from 7512 in 2000 to 1331 in 2015 (NCASC, 2015a). This was almost similar to the findings of present study where HIV positive cases in five years (2010/11-2014/15) were in steady phase (12.9% in (2010/11), 13.3% in (2011/12), 14% in (2012/13), 14% in (2013/14) and 13.5% in (2014/15). These five years data were analyzed in order to determine the trend of HIV and key populations at risk in Dhanusha District of Janakpur Zone. The highest prevalence was found in the age group 25-49 years: a potential reproductive age group which was similar to the findings of Paranjape (2016) that reported the majority of HIV infections to occur in 15-49 years. This dominant age group could be because of the marriages taking place at an early age in Dhanusha and nearby districts of Janakpur Zone. This age group includes sexually active individuals with least of them using protective measures. Also, this age group constituted the majority of males migrating to foreign countries for employment. It might increase their chances of getting infected with HIV in the conditions in which they lived with no access to health services. This study's results showed that adults were found to be positive for HIV infection which is consistent with the CDC report for the United States where 37% of HIV positive cases were in age group 20-29 years followed by 24% in 30-39 years and 17% in 40-49 years age group (CDC, 2013).

A study in Dang (DACC, 2012) recorded that male were infected more than female, that was similar to the present study in which males are affected more (58.6%). Both genders can acquire HIV/AIDS if they do not practice safe sex, and gender roles are in every area, which increases or decreases the chances of HIV infection (Haghdoost *et al.,* 2011). The findings in this study show that HIV is more prevalent in men (58.6%) than women (40.7%) which are similar to the report from ART centre of Burdwan district, West Bengal where 63.1% were males (Chatterjee *et al*., 2014). Also, Van Griensven *et al*., (2010) supported that men were sexually active with a combination of strong sexual desire, sexual opportunities, and HIV risk factors and behaviours likely fuel their chance of getting infected. On the other hand,

globally, women are more vulnerable to the infection because of their reproduction role and their low socio-economic position in the society. Males, usually being the decision maker in the family in terai, have more freedom compared to women as they do not need any permission to go out of their home which might be one of the reasons for higher chances of acquiring HIV infection either through sex workers or extramarital affairs. Men generally acquire HIV through multiple sexual partners, including high risk groups, such as female sex workers, while the majority of women acquire HIV from their husbands (Gangakhedkar *et al*., 1997; Newmann *et al*., 2000; CDC, 2009; CDC, 2011). A report prepared by UNFCO in 2013 showed that new HIV-positive cases were found in 59% of male in which was almost similar to the findings (58.6%) from this study.

Marital status of the HIV positive patients showed maximum 77.1% which was similar to the previous studies (Gangakhedkar *et al*., 1997; Bhattacharya, 2004; Sharma, 2005; Newmann *et al*., 2000; Mehendale *et al*., 2007). The high prevalence of HIV among married individuals in Dhanusha district could be because of their exposure to sexual relationships with their spouses or other intimate partners as single people are presumed not to have sexual relationships until they are married and this pose high HIV infection among married people. HIV transmission risk to the married women is even exacerbated through husbands who might have unprotected sex outside of the marriage with multiple partners or sex workers (Solomon *et al*., 1998; Bhattacharya, 2004). Out-migration from terai to urban centres in the country and abroad is rapidly increasing (Gartaula and Niehof, 2013) causing higher prevalence of HIV among them which in turn makes their spouses more vulnerable to HIV infection.

Health outcomes are affected by many interrelated factors. These include culture, religious and social beliefs and norms especially those that reflect the entrenched gender, caste and ethnic hierarchies as well as economic, institutional and location related specificities. There are significant variation in access to health care between women from different caste and ethnic groups. Access to health and family planning services for rural women are lowest among Dalit and terai middle caste women (Tiwari, 2008). They are not allowed to go outside their homes as it affects their family's reputation. Knowledge level is also very low in middle caste, lower caste and Muslim women in terai. Most of them do not have heard about AIDS and its prevention methods. They are not even able to negotiate with their husbands for safer sex. Also, middle caste men are not well educated. Most of them are engaged in labour migration and thus get infected with HIV from FSWs or through injecting drug use. These could be the reasons that Middle Caste individuals constituted the major proportions (47.3%) of HIV infections in Dhanusha District.

According to the IBBS Survey, 1999-2016, the key populations at risk in Nepal are FSWs, MSM and Transgender, males and females who inject drugs, clients of sex workers, male labour migrants, wives of migrants and street involved children and youth. In recent years overseas migration has become more popular among skilled labourers in Dhanusha. Being a

poor economic status, majority of migrants are from the hill and terai region and destination of one third male migrants is India and other popular countries are middle-eat and Malaysia (MOHP, 2012). Between 2003 and 2009, Dhanusha led the country in number of migrants heading overseas to work- an estimated 7% of the district's population (upward of 58,000 people) migrated overseas (DOFE, 2012). This study results showed highest prevalence of HIV among migrant workers (41%) which was similar to the findings of (Gurubacharya and Gurubacharya, 2004) where HIV prevalence was found to be 2.3% among migrants within Nepal and 8.5% among migrants within India. Migrant workers might not receive proper living conditions and lack of access to HIV prevention services put them at a higher level of vulnerability to HIV infection. Unfortunately, 66.7%-80% migrants from Nepal did not use condom during the last sex with FSW and 82.8% during last sex with their spouses (New Era, 2008). Similar might be the conditions with MW from terai ; lack of awareness about the measures for HIV infection, unsafe sex with FSWs and multiple sex partners might be the reasons for HIV prevalence among MW of terai people. Also, the results revealed that the probability of receiving HIV infection is 1.725 times higher among MW. Spouse of migrants were the second major risk groups among HIV positive patients visiting ART centre of JZH, constituting 23% of the total HIV positive cases. MWs are the most high risk group transmitting HIV infection to their spouses. Further, though married women from terai culture are not supposed to engage in other extramarital sexual affairs, there is a gap in perceptions and realities on this issue. The lack of sexual intimacy between husband and wife because of them being separated causes instabilities in their sexual relation as well as financial status, would have enabled them to seek sexual pleasure from someone else or to have sex with multiple partners as an economic supplement to their needs. Sex with multiple partners and often, lack of access to information and condom affect their abilities to protect themselves from HIV infection. This study result is consistent with a study conducted in Bangladesh where HIV prevalence in migrant workers or their spouses was 75% among individuals attending HIV Testing and Counseling Unit (Urmi *et al.,* 2015). As per IBBS survey in 2008, the prevalence of HIV among wives of migrants was 3.3%. Furthermore; some of the norms promoting men's multiple partnership and gender power role in the society have increased the women's susceptibility to HIV infection (Shedlin *et al*., 2000).

The proportion of housewives is increasing among the new cases of HIV in Nepal. According to the national level report, housewives constituted 25.8% of HIV cases (NCASC, 2010). This study revealed HIV prevalence among housewives to be 5% within these five years while a hospital based study of HIV patients in Seti Zonal Hospital, in Far Western Nepal, stated that more than 50% cases were housewives (Poudel *et al*., 2008). Several studies have shown wives residing in sending countries have higher rates of HIV infection, largely, due to their husbands' increased sexual risk behaviours (Grawert, 1992; Quinn *et al*., 2009). Housewives stay at home and are involved in household chores with very less movement outside their homes in terai. Housewives, in general have only one sexual partner. Their level of risk to HIV exposure, therefore, is very much defined by the sexual practices

of their husband. The 5% HIV prevalence seen among housewives in this study could be because of the extra marital affairs of their husbands or they being the clients of FSWs, leading to transmitting infections to their wives. Furthermore, it has been estimated that almost 60% of clients of FSWs do not use condoms (World Bank, 2012). This situation is alarming since the likelihood of transmitting HIV to unborn children will be high.

Vertical transmission or Mother-To-Child Transmission is the most common way that children become infected with HIV. Vertical transmission is the dominant mode of acquisition of infection for HIV infection in children, and about 1600 infants are newly infected each day worldwide (Newell, 2000). In the absence of treatment, the risk of vertical transmission of HIV is as high as 15-45% (WHO, 2016a). Vertical transmission in this study was 16% which was the third highest dominant risk group among HIV positive patients visiting ART centre of Janakpur Zonal Hospital. Illiteracy and lack of knowledge towards prevention of mother-to-child transmission (PMTCT) might be the reason for higher prevalence of HIV among the children in Dhanusha and nearby districts. Furthermore, HIV infected mothers might not be going for their treatment with the fear that their status would be disclosed among others and they would be misbehaved in their society. This is supported by a body of research that highlighted how HIV-related stigma and differentiation affect a pregnant woman's decision to enroll on PMTCT programmes and interrupt adherence to treatment and retention in care (Turan and Nyblade, 2013). It has been estimated that over 50% of vertical HIV transmissions from mother-to-child globally can be attributed to the cumulative effect of stigma (ICRW, 2014). Unfortunately, disclosure rates remain extremely low; a multi-site mixed methods study in Burkino Faso, Kenya, Malawi, and Uganda found that only 37% of HIV-positive pregnant women disclosed their HIV status to their husband (Hardon *et al*., 2012). Furthermore, socio-economic barriers like persistent unequal power between men and women; legal discrimination against women; women's low economic status; women's low educational status; and domestic violence could be the reason for disengagement of PW for the treatment. Despite the increase in ART uptake by women attending ANC and tested HIV positive, the coverage for elimination of vertical transmission was relatively low though improving over the last three years as during the entire period of 2014, only 162 pregnant women (out of 498) received ARV prophylaxis compared to 142 PW in 2013 and 130 in 2012 (MOHP, 2015). The study result is contrasting with the NCASC report (2015a) as the findings showed 16% HIV prevalence through vertical transmission among total HIV positive patients attending ART centre, in these five years (2010/11-2014/15). Also, the chance to acquire HIV infection through vertical transmission was 2.405 times higher among children.

Men having sex with men constituted 2% of HIV positive infection in these five years which was not similar to the findings from a study in china where HIV prevalence among Chinese MSM were 8% in six years from January 2009 to October 2015 (Tang *et al*., 2016). The prevalence of HIV among MSM in Nepal was 3.8% in 2009 (WHO, 2010b). The study

results revealed that MSM were four times more likely to acquire HIV and thus representing one of the most vulnerable groups. Though showing lesser prevalence of only 2%, the chances of getting infected with HIV is four times higher among them. This could be because of not using condoms during the last anal sex with a male partner. Also, some MSM might have sex with FSWs or injecting drug use. They have multiple sexual partners of all types- regular, casual, commercial (paid) and paying (WHO, 2010b). And, clients of sex workers constituted the least prevalence of 1% only within these five years. Though clients of sex workers are high risk groups in Nepal, the low percentage in this study might be due to not disclosing their status that they ever had sex with FSWs fearing the societal consequences. Similarly, sex workers and IDU's were the risk groups within the study period but they accounted for lower prevalence rates comparatively. The NCASC country progress report had also shown significant decrease in the prevalence of HIV among PWID in Eastern and Western Highway Districts. The client clinical staging of HIV positive individuals showed the highest rates for being not assessed (60.3%) followed by asymptomatic stage (20.0%), mid symptoms (10.1%), advance symptoms (7.0%) and severe symptoms (5.8%). Individuals might have been tested for HIV but were not found to be positive resulting into higher percentages for not being assessed.

6. CONCLUSION AND RECOMMENDATIONS

A total of 120 pregnant women attending Gynaecology Department of Aastha Hospital, were tested both for HIV and trichomoniasis and showed 0.83% of HIV positive prevalence while none were found to be positive for *T. vaginalis* infection. Pregnant women of reproductive age group constituted the major proportions (67.5%). Among the patients attending hospital, the highest frequency was found to be from middle caste groups (39.16%).

HIV trend in five years (2010/11-2014/15) was found to be in a steady state accounting for the total prevalence of 13.6%. The major risk groups tested for HIV infection were IDUs, sex workers, MSM, clients of sex workers, migrant workers, spouse of migrant workers, vertical transmission, housewives and others. While these were the tested individuals, HIV positive infection was concentrated among migrant workers, spouse of migrant workers, vertical transmission, housewives, others, MSM and clients of sex workers among patients attending ART centre of JZH. Though, IDUs and sex workers are risk groups in context to Nepal, the risk assessment of records showed zero prevalence of HIV among them in Dhanusha district. Very few individuals injecting drug use (1.74%) and sex workers (0.69%) were tested for HIV as compared to other risk populations in these five years. Similarly, retrospective assessment of people living with HIV showed maximum prevalence among migrant workers (41%), followed by spouse of migrant workers (23%), vertical transmission (16%), housewives (5%), MSM (2%), clients of sex workers (1%) and others. Age group 25-49 years accounted for the highest prevalence rates (74.07%) with males constituting the major proportions (58.6%). Also, married individuals were the most affected ones (77.1%). Middle caste groups were 47.3%. Most (40.7%) of them were from Dhanusha district. And, only 1.5% of women were in their current pregnancy status. The client clinical staging showed maximum were not assessed (60.3%) and 20% of them were in asymptomatic stage.

Based on the findings of the study following are the recommendations

1. Knowledge regarding HIV/AIDS, transmission, prevention, family planning methods and the importance of HIV testing should be raised by different programmes in the village level.

2. The rapid scale up of primary prevention, testing and treatment services for high risk individuals like migrant workers, spouse of migrants, pregnant mothers, housewives and MSM are needed to prevent further transmission

3. The stigma and discrimination in association with HIV should be minimized by organizing programmes on behavior change strategies targeting specifically to migrant workers and their wives in rural settings.

4. NGOs and INGOs working in rural areas of Dhanusha and Mahottari districts should focus on comprehensive HIV testing programs among men and women which would help them identify their HIV status and thus proper treatment could be provided to them.

5. Programmes of preventing the vertical transmission of HIV and to increase access to treatment should be launched.

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APPENDIX 1

Informed Consent Form

Central Department of Zoology,
Institute of Science and Technology,
Tribhuvan University

Name of the researcher: Neha Sharma
Supervisor: Mahendra Maharjan, PhD
Co- supervisor: Neelam Chaudhary, MBBS
Title of thesis: "HIV and *Trichomonas vaginalis* infection among pregnant women and specific assessment of five years HIV trend in Dhanusha district".
Informed consent form for: Doctor and health staffs

I am a student of this esteemed university enrolled in CDZ with special paper "Parasitology", Zoology. This is the research work for the fulfillment of requirements for Master's degree in Science. We are doing research on the prevalence of infections, specifically, HIV and trichomoniasis among pregnant women attending private hospital (Aastha Hospital) in Janakpur. I am going to give you information and invite you to be part of this research and that, you can take your time to reflect on whether you want to participate or not.

Parasitic infections are more common in rural or developing areas like ours. There are more than 30 parasitic infections that cause several pathogenic diseases in human beings. HIV is a very dangerous disease that has engulfed many people in our country. Although *T. vaginalis* infection does not seem to be prevalent very often, it poses a high risk of health, fertility, infant mortality ectopic pregnancy, fatal and prenatal deaths and there is increased risk of HIV among STI patients. The reason we are doing this research is to find out the prevalence rates of above mentioned infections in Janakpur and if there is need for screening to pregnant women for such infections at regular intervals during the antenatal check ups.

The main reason for doing this study is to help answer the following research question:
"Whether there is the prevalence of sexually transmitted protozoan parasite *Trichomonas vaginalis* and HIV among pregnant women or not?
The research will include collecting blood samples and testing it for HIV infection by using RDT kits and by collecting vaginal swabs for *T. vaginalis* infection to be seen under microscope for the parasite's motility to determine the occurrence of *T. vaginalis*. For this, we will seek support from your health professionals for the purpose of lab work. This will help figure out the prevalence of these infections among pregnant women attending Gynaecology and Obstetrics department in Aastha hospital of Janakpur. The second part of study includes obtaining HIV positive cases data from ART centre and their retrospective and risk assessment.

Your participation in this research is entirely voluntary. Either you choose or not to participate, all the services you receive will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

Because we do not know about the prevalence rates of these infections here, in Janakpur, we will need pregnant women to take part in this research. We will conduct a structured interview with suspected pregnant women. Their participation in the study will be just a 30 minutes interview which will be kept confidential. They will be asked for consent prior to participation.The study will be carried out 6 days a week or changeable according to the availability of the health staffs/ health facility. At the end of three months, this research will be finished. Your participation in this research will be a boon for beginners like us and there will not be any potential risk to you. There may not be any specific benefit for you but your participation is likely to help us find the answer to the research questions. There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.

The information collected from this research will be kept confidential and will not be shared to anyone except the researchers. The knowledge that we get from doing this research will be shared with you before it is made widely available to public. Later on, the results will be published in order that other interested persons may learn from our research.

You can share your any queries or questions regarding this research activity.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a technical person in this research.

Name of the Doctor/ Health staffs: …….

Signature of the Doctor/ Health staffs ……..

Date: …….

APPENDIX 2

Consent form for participants

Central Department of Zoology,
Tribhuvan University

I am a student from Central Department of Zoology, with Parasitology as a specialized paper. Currently, I am studying the prevalence of HIV and *Trichomonas vaginalis* infection among pregnant women attending Aastha hospital. I ask you to participate in this study and interview as I am very much interested to know about the risk factors for these infections among pregnant women of our community.

This is important for beginners like us, aiming to conduct research in our field of study. Particularly, you can really help us by agreeing to participate in the study and responding to the questions in the interview. By, doing this, the public health community can positively expand research into the areas of our country and into the diseases where that information is lacking. The information generated in this study can be used to guide public health policy and the allocation of resources within local governments and by international agencies towards improving the women's health. Certainly, this will benefit your community.

Your participation: Your participation in this study will be an half an hour long interview. You may pass on any question that you find uncomfortable and at any time you may tell me you would like to stop the interview.

Please note that your responses will not affect your accessibility to health services at this institution. Neither will it affect your relationship with the concerned health authority.

Confidentiality: Your name and identifying information will not be associated with any part of the written report of the research. All of your information and responses will be kept confidential. [Is that ok?] If you have any questions or concerns please let us know.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason.

Date: ……

Signature …….

Thumbprint (if illiterate) ……

APPENDIX 3

QUESTIONNAIRE

I. Demographic information

Form no: …….. S. No…….
OPD No: …… Date of examination: …….

1. Identification:

Name …….. Husband's name: ……..
Age ……. Present address : ……
Permanent address: …….. Phone no: …….

2. Ethnic Group:

*Brahmin *Bhumihaar *Yadav *Kayastha * Rajput

*Sudi/teli/Haluwai *Dom/ Chamar/ Musahar *Bin/Mukhiya

3. Religion:

***Hindu *Muslim *Buddhist *Others (specify)…….

4. Socio economic status:

a) Occupation :

WIFE:

*Housewife *Business *Service *Daily wages/Labour

*Farmer *Unemployed

HUSBAND :

*Business *Service *Farmer *Daily wages/ labour

*Remittances * Unemployed

b) Education :

WIFE :

*Doctorate *Graduate *Intermediate *Secondary school

* Primary School *Iliterate

HUSBAND :

*Doctorate *Graduate *Intermediate *Secondary school

*Primary school *Illiterate

c) Source of water:

***Piped *Handpump *Tube well/closed well *Open well *Ponds

d) Type of latrine in use

*Flush *Pour flush * Pit *Open field

e) Type of fuel used

***Electricity *Gas *Kerosene *Firewood * Cow dung

f) Type of vehicle used

***Car *Motorbike *Bicycle *Bus *None

5. Marital Status

*Unmarried *Married *Divorced/ separated *Widow

II. Natural and behavioural practices:

Menstrual History:

……..Cycle…….Flow…….LMP……

Obstetrical History:

a) No. of pregnancy: Place of delivery: ……

b) No. of live birth:

c) No. of still birth:

d) No. of living children:

e) No. of miscarriages:

f) No. of abnormal pregnancy:

g) How many months pregnant (for current pregnant situation) ? …….

Instrumental:

LSCS:

Subfertility:

Primary	Yes	No
Secondary	Yes	No

Contraceptions:

i. Type:

None	*	*
Natural barrier	*	*
OCP	*	*
DMPA	*	*
Cu T	*	*
Minilap/ Vasectomy	*	*

ii. Duration of use:

Months:

Year:

Personal, medical, surgical and family history:

Personal History:

Smoker: Yes No Duration: Months/Year

Alcoholic: Yes No Duration: Months/Year

Frequency of coitus: Once/wk Thrice/wk >Thrice/wk None

Medical History:

	Yes	No
a. Diabetes	*	*
b. HTN	*	*
c. TB	*	*
d. Bleeding Disorders	*	*

Systemic examination:

i. Breast:

ii. Chest:

iii. CVS:

iv. Abdomen:

v. Per speculum examination:

A. Cervix:

Normal* *Abnormal

Congestion*

Erosion*

Hypertrophied*

Atrophied*

B. Discharge:

Normal* *Abnormal

a. Amount: Scanty Copious

b. Colour: White Yellow Greenish Blood stained

c. Odour: Odourless Foul smelling

d. Consistency: Watery Thick

vi. PV- examination:

III Knowledge and attitude

Knowledge about STD:

*HIV AIDS *Trichomoniasis *Gonorrhoea

*Syphilis *Others

Transmission:

*Blood transfusion *Sexual intercourse

*Mother to child (Congenital) *Use of common latrine

Symptoms:

*Itching *Burning *Vaginal discharge a) Normal

b) Yellowish

c) Others

*Inflammation

*Odour: a) Foul b) Normal

Suggestion to prevent STD transmission

……

……

……

……

Finding of microscopic examination:

IV Surgical history:

	Yes	No
Gynaecological	*	*
If yes type/ indication	……..	
Other than Gynaecological	*	*
If yes type/indication	……..	

Family History:

a. Diabetes	*	*
b. TB	*	*
c. Any other illness	……	

Specific History (Vaginal Discharge):

a) Amount : Scanty Copious

b) Colour: White Yellow Green Blood stained

c) Odour: Odourless Foul smelling

d) Consistency: Watery Thick

e) Pruritus: Yes No

Examination record:

a. General appearance:

Physical: a) Obese

b) Normal

c) Malnourished

b. Pulse:

c. BP:

d. Pallor:

e. Icterus:

f. Oedema:

V HIV infection: Yes No

VI If yes, mode of transmission: ……

VII Occupation of Husband: ……..

The End