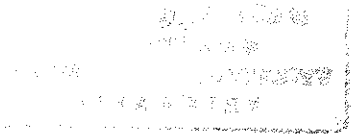
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Seroprevalence of *Herpes simplex* virus (HSV) type 2 in adult Ethiopians: its association with HIV, HSV-1, syphilis and some other risk factors



WUDE MIHRET

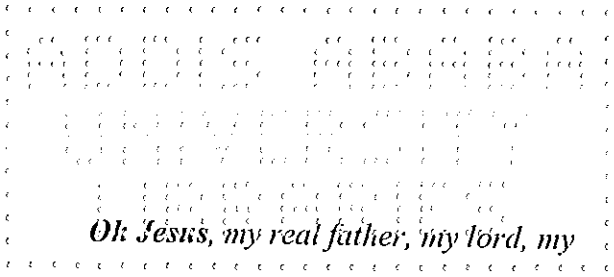
May, 2001

Seroprevalence of *Herpes simplex* virus (HSV) type 2 in adult Ethiopians: its association with HIV, HSV-1, syphilis and some other risk factors

A thesis submitted to the School of Graduate Studies, Addis Ababa University, in partial fulfilment of the requirements for the Degree of Master of Science in Biology (parasitology).

WUDE MIHRET

May, 2001



Oh Jesus, my real father, my lord, my saviour I have seen how strong your love is, how supportive your shoulders are, and how joyfull is the life you reveal at every inch of my step.

Dedication

This paper is dedicated to you, my husband

Dr Moges Alemu,

for your persistent love, support, encouragment, and admirable advices, as well as the basic back ground of knowledge that you lied starting from the very early years of my life, all of which have given the stamina to this work.

So much of my love also goes to my daughters **Kalkidan Moges, Wengelawit Moges** my son **Dagmawi Moges** and my dear mother **Bizunesh Kebede,**

whose patience and understanding love was kindling light of courage that helped me to uphold my work to the end of the tunnel.

Table of Contents

Content Page

Acknowledgements

I forward my immense gratitude to my advisors Dr Beyene Petros (associate professor), and Dr Tobias Rinke De Wit, who were giving me their timely advice and devoted assistance which has promoted this research project to the present level. I also forward my respect and deepest praise to Dr Arnaud Fontanet, who helped me in analysing the data and also was giving me his important advices, the sum of which has given the backbone to this work. A lot of my appreciation is also forwarded to Dr Dawit Wolday (the ENARP/EHNRI Laboratory Manager), who were following up my work closely, Dr Eduard Sanders and Dr Mathias Aklilu (ENARP managers), who were giving me articles closely related to my work and showed their collaboration that has helped me in accompalishing matters related with my work. My gratitude also goes to the ENARP (Ethio-Netherlands AIDS Research Project) EHNRI (Ethiopian Health and Nutrition Research Institute), supplying me with laboratory specimen and materials since this study is part of the Ethio-Netherlands AIDS Research Project (ENARP), a collaborative effort of EHNRI, the Amsterdam Municipal Health Service (GGGD), and the Academic Medical Center of the University of Amsterdam (AMC). ENARP is financially supported by the Netherlands Ministry of Foreign Affairs and the Ethiopian Ministry of Health (MOH) as a bilateral project. Lots of my thanks also goes to the staffs of the schools of graduate studies of Addis Ababa University, the Department of Biology, Region 14 schools office, and

the ENARP/EHNRI staff especially the PHD students Aster, Yared and Workenesh as well as the Laboratory technicians Asfaw, Mulu, Ermias and Tesfaye. I am also greatful to Dr Mekuria Lakew (my stream advisor) and Ato Belete Tebaru (EHNRI staff), who relentlessly were putting forward their critical comments. Lots of my appreciation also goes to Dr Abera Geyed and Ato Tilahun W/M, who helped me to join the ENARP/EHNRI Laboratory to carry out my research experiment. The wise pieces of advises that were being given to me by Dr Almaz Abebe, Dr Ayele Nigatu and Dr Tsehaynesh were those which have left their remarkable signs on this paper. My deep appreciation is forwarded to Dr Yohannes, who gave me his helpful comments right from the begning of this project. So much of my gratitude also goes to the AHRI (Armour Hanssen Research Institute) Library staffs especially Selome and Alemayehu who have been showing their cooperation in the search for articles. I also would like to appreciate my friends Misrak Kebede, Measho Hagos, Wondewossen Mekonen and Afework Kassu whose support has contributed a lot for my work. My greatest respect is also forwarded to my friend Wubalem Taye and family who trained me with computer programmes. Last but not least of my respect and appreciation also goes to my mother Bizunesh Kebede, My Father Betemariam Demissie, Mihret W/M, Telaye Yosef and family, my brothers Elias Bete and family, Fasil Bete, Girma Hailu, Esmelalem Mihret my sister Misrak Bete, my brother in law C/L Wondemu Alemu, and my uncle Asefa G/Yes , whose love and support has helped me in one way or the other to accompalish this work.

List of Figures **Page**

List of Tables **Page**

Abbreviations

ADCC – Antibody Dependent Cell Mediated Cytotxicity

AIDS – Acquired Immunodeficiency Syndrome

AMC – Academic Medical Center of the University of Amsterdam

CD - Cluster of differentiotion

CNS – Central Nervous System

EHNRI – Ethiopian Health and Nutrition Research Institute

ELISA - Enzyme linked Immunosorbent Assay

ENARP – Ethio Netherlands AIDS Research Project

GB -Glycoprotein B

GGGD- Amsterdam Municipal Health Service

GUD – Genital Ulcer Disease

HIV – Human Immunodeficiency Virus

HSV – *Herpes simplex* Virus

IFN – Interferon Gamma

IL – Interleukin

MOH – Ministry of Health

ND - Not Done

NK- Natural Killer cells

OR – Odd s Ratio

RPR – Rapid Plasma Reagin test

SIV - Simian immunodeficiency virus

STD – Sexually Transmitted Disease

STI - Sexually Transmitted Infection

TNF -Tumour Necrosis Factor

TPHA – *Treponema pallidum* Hemagglutination Assay

Abstract

This paper reports a preliminary data on HSV type 2 and type 1 from a retrospective cross sectional study on 1168 (530 female and 638 male) subjects aged 15-66, living at Akaki and Higher 23 (located at the vicinity of All African Leprosy Educational Research and Training (ALERT)), places around Addis Ababa, Ethiopia. The plasma samples were tested for antibodies against HSV-2 and HSV-1, by using glycoprotein g (gG2 and gG1) specific ELISA kit. Prevalence of antibodies against HSV type 2 was 44.5% (520/1168) in both sexes while the prevalence of antibodies against HSV-1 was found to be as high as 94.7% (231/244). HSV-2 serological data is presented in the context of HIV, syphilis, genital ulcer, genital discharge and various socioepidemiological information. An overall HSV-2 seroprevalence of 64% (124/193) in women 35-44 years old and 50% (28/56) in men 45-66 years old were determined. On the other hand seroprevalence of HSV-2 antibodies in women seropositive for HIV-1 antibodies was 80% (40/50) versus 47.5% (228/480) among HIV-negatives. Among the HIV positive men, the prevalence was 70% (46/66) and 36% (206/572) in the HIV-seronegatives. HSV-2 seropositivity was 71.3% (72/101) and 45.7% (196/429) in women seropositive and seronegative for syphilis antibodies respectively. In the men, it was 60% (94/157) and 32.8% (158/481) among the syphilis seropositives and syphilis-seronegatives respectively ($P < 0.001$). It was concluded that being older, HIV seropositive, and syphilis seropositive, were significantly associated ($P < 0.05$) with HSV-2 seropositivity in both sexes. Highly significant ($P < 0.05$) association was observed between HSV-2 seropositivity and the various sociodemographic factors examined in both women and men subjects.

1. Introduction

By the end of 2000 an estimated cumulative total of 36.1 million people were living with HIV/AIDS worldwide (UNAIDS/WHO, 2000). Out of these, 25.3 million belongs to sub-Saharan Africa, where heterosexual intercourse is the main mode of transmission (UNAIDS/WHO, 2000). The epidemiology of HIV infection in Africa, is strikingly different from the epidemiologic pattern in the United States or Europe (Mbopi-Kiou *et al.,* 2000). Other than sexually transmitted diseases (STDs), the factors considered to be important in contributing to the differences in HIV/AIDS disease progression between Africa and Europe or the US are, increased exposure to a multitude of highly pathogenic agents, late diagnosis of HIV infection, and lack of medical care (Anzala *et al,* 1995). Moreover the severity of HIV/AIDS in Africa is increased due to immune activation resulting from frequent or chronic parasitic infections, other infectious agents and nutritional factors all of which are resulting in faster disease progression in Africa than in Europe (Van de Perre *et al.,* 1984; Hunt, 1989; Anzala *et al,* 1995). Although sexual behaviour, may in part account for the rapid spread of HIV in many parts of Africa, the presence of a number of STDs serves as co-factor for the explosive heterosexual transmission in Afrcia (Martin, 1990; Mbopi-Kiou *et al.,* 2000). Large numbers of infections continue to occur in sub-Saharan Africa, the region most severely affected during the first decade of AIDS epidemic (Grosskurth, 1995). A severe HIV/AIDS epidemic is also experienced by Ethiopians as with most of Africans during the past 10 years (Fontanet *et al.,* 1998). It is estimated that there are about 2.6 million people infected with HIV in Ethiopia in the year 2000 [MOH, 2000]. Moreover this country has the third largest estimated HIV-infected population in the world (UNAIDS/WHO, 2000).

In many of the worst affected populations of the sub-Saharan Africa, STDs are highly prevalent (Grosskurth, 1995). This may be a good indication that STDs facilitate the transmission of human immunodeficiency virus (Martin, 1990; Grosskurth, 1995; Morse *et al.,* 1997). Although STDs in general facilitate the transmission of HIV, those causing genital ulcer diseases (GUD) have been recognized as the major risk factors in HIV transmission (Morse *et al.,* 1997). Genital ulceration may enhance acquisition of HIV by disrupting mucosal membranes, providing an easy portal of entry. *Herpes simplex* virus (HSV), which is also known as herpes virus hominis, *Treponema pallidum* and *Haemophilus ducreyi* are the three primary agents causing genital ulcer disease in STD clinic patients (Morse *et al.,* 1997). Among these, evolving evidence shows that genital HSV infection is a potent facilitator of sexual transmission of HIV (Corey and Handsfield, 2000). Genital herpes, which is one of the most common STDs throughout the world (Obasi *et al.,*1999; Corey and Handsfield, 2000) is mainly caused by HSV type 2 (HSV-2). Although the larger proportion of genital herpes is caused by HSV-2, genital infections with HSV type 1 (HSV-1) have also been increasingly reported (Myron, 1998; Whitley *et al.,* 1998; Corey and Handsfield, 2000). While, on the other hand, orolabial HSV-2 infection is rare, when it occurs, it is almost always seen with genital herpes and it's seroprevalence is a direct measure of genital herpes (Corey and Handsfield, 2000). This means that either genital or oropharyngeal infection could be caused by both HSV-1 and HSV-2 (Rosa-Santos *et al.,* 1996). Herpes simplex virus type 1 (HSV-1) is closely associated with orolabial infection, with one-fourth to one-half of all children under 18 testing seropositive for it (Straus *et al.,* 1985; Whitley and Gnann,1993).

The aetiological agents of recrudescent herpes labialis (RHL) are therefore HSV-1 and to a lesser extent HSV-2 (Scott *et al.*, 1997).

1.1 Herpes simplex viruses

The word "herpes" meaning to creep or crawl, is used by Greek scholars, particularly Hippocrates, to describe spreading lesions (Whitley *et al.*, 1998). The herpes group of viruses comprises some 70 or more members which infect a diverse range of hosts (Mahy,1991). It is in the late eighteenth century that the classification now in use came into being (Whitley *et al.*, 1998). The family (group) *Herpesviridae* is divided into three subfamilies on the bases of their biological properties. These are the α –, β –, and γ –*Herpesvirinae*, the prototypes of which are the human pathogens HSV type 1, HSV type 2 and *Varicella-zoster* virus, human cytomegalovirus (HCMV) and *Epstein-Barr* virus (EBV), respectively (Mahy, 1991; Mettenleiter, 1994). Antigenic analysis and definition of biologic properties, for example, host range and types of cytopathologic changes, are the most commonly used means of separating HSV from other herpesvirus*es* (Hirsch, 1994). The viruses in the subfamily *alpha-herpesvirinae* all grow rapidly, lyse infected cells, and establish latent infections in sensory nerve ganglia (White and Fenner, 1994). *Herpes simplex* virus (HSV) is a member of a family of viruses whose genomes consist of a single large double-stranded DNA molecule (about 100 x 10^6 in molecular weight) that encodes for more than 60 gene products (Harrisons, 1996). The approximate diameter of the DNA molecule of HSV is 180 nm (Mettenleiter, 1994; Ellis, 1998).

Herpes simplex viron has four concentric layers: an electron-dense core packaging the viral genonme (DNA); surrounded by an icosahedral capsid, a regular icosahedral protein shell composed of 162 capsomeres; a tightly adhering, amorphous at times eccentric layer of proteins designated tegument (a structure composed of a number of viral proteins whose properties and functions are largely vague), surrounding the capsid; and finally an envelope (Fig 1) (White and Fenner, 1994; Harrisons, 1996; Whitley *et al.*, 1998). The envelope is an external covering of the virus, which is a lipid-containing membrane derived from modified cell membrane (Harrisons, 1996).

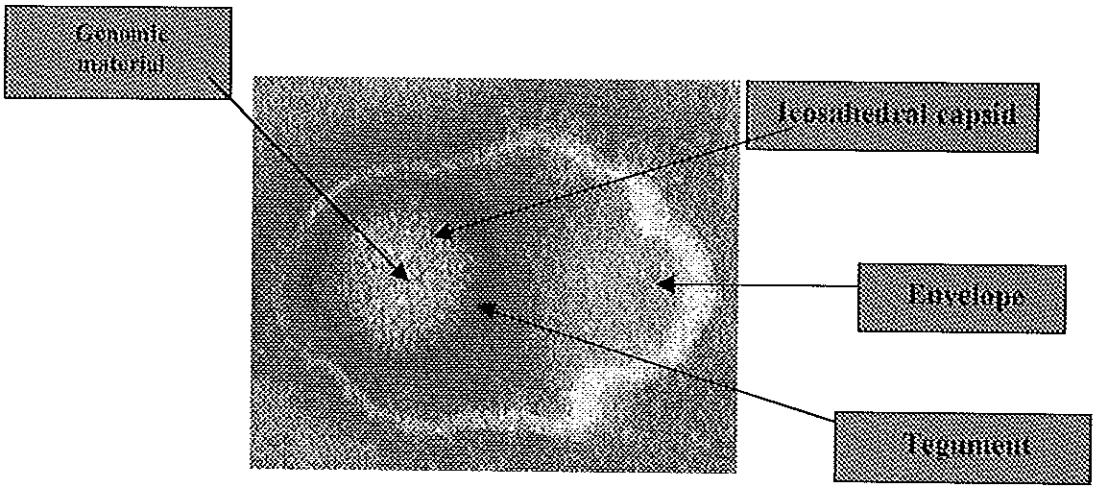


Figure 1. "*Herpes simplex* viruses have an envelope which is surrounding an icosahedral capsid of approximately 100 nm. University of Cape Town, Department of Medical Microbiology.

HSV-1 and HSV-2 encode a serine protease, which is needed for viral replication, and represent a viable target, which can be used for therapeutic intervention. *Herpes simplex* virus type 1 and type 2 have almost identical three dimensional structures and their proteases share high sequence homology (Hoog *et al.,* 1997). Fifty percent sequence homology is detected between HSV-1 and HSV-2 (Pelczar *et al.,* 1988; Gull Laboratories, 1997). The homologous sequences are distributed over the entire genome map and most of the polypeptides specified by one viral type are antigenically related to polypeptides of the other viral type (Hirsch, 1994).

At least 10 glycosylated and several nonglycosylated viral proteins, lipids, and polyamines are contained in the envelope (Whitley *et al.,* 1998). Certain glycoproteins like gB, are antigens known to be shared by HSV-1 and HSV-2 (Hirsch, 1994) while, many-type specific regions unique to HSV-1 and HSV-2 proteins do exist, and many of these regions appear to be useful in host immunity (Harrisons, 1996). Glycoprotein G (gG-1, gG-2) are found to be type-specific for HSV-1 and HSV-2 respectively, and help to identify one from the other type through serological assays (Pelczar *et al.,* 1988; Gull Laboratories, 1997). Distinguishing between the two subtypes and among strains of the two subtypes is also possible through restriction endonuclease analysis of viral DNA (Harrisons, 1996). Moreover, identifying one from the other is also possible through some of their main clinical manifestations since HSV type 2 is usually associated with genital (and hence neonatal) infections, while HSV type 1 is responsible for oral-facial and most of the other forms of HSV infections (Harrisons, 1996).

Infection with HSV has an incubation period that ranges between 2-12 days, with an average of 6 days (Harrisons, 1996). Infections occur throughout the year and get established for life in humans (Harisons, 1996; Posavad *et al.*, 2000). Although experimental animals can easily be infected, there are no known animal reservoirs for HSV. The only natural reservoir of HSV appear to be humans (Hirsch, 1994). Initial infection with HSV is generally self limited in the normal host (Stanberry, 1995). HSV is transported by retrograde movement to the nuclei of sensory ganglia following entry and infection of nerve endings.

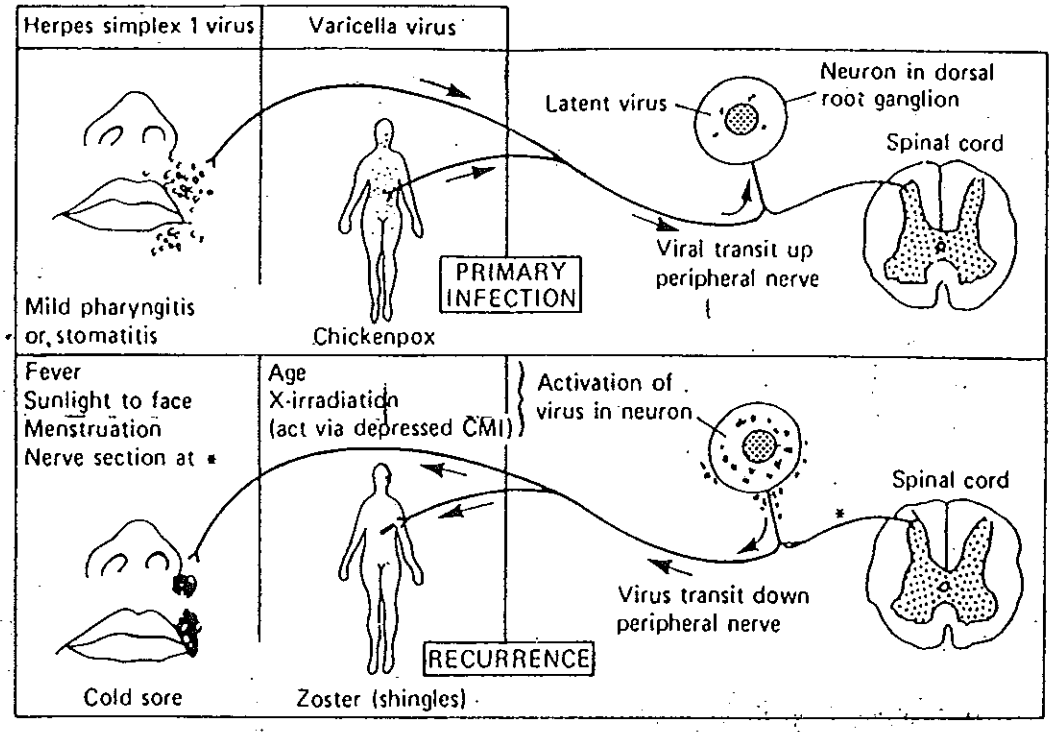


Fig. 2. Mechanisms of latency: in *Herpes simplex* and zoster. Reactivation of the *Herpes simplex* virus causes recurrent *Herpes simplex*; reactivation of varicella virus causes zoster. Primary infection occurs in childhood or adolescence. (Mims and White, 1984).

The viral genome remains in an episomal state in the majority of the infected neurons for the entire life of the individual (Whitley *et al.*, 1998). HSV then appears to avoid detection by the cells of the immune system once latent in the sensory nerve ganglia (Richards *et al.*, 1998). This is a unique strategy evolved by some viruses to evade the immune system and establish persistent infection (Fig 2) (Stanberry, 1995). Periodical reactivation of the latent virus into a replication competent form (Stanberry, 1995; Fleming, 1997) results in viral replication in the neurons without neuronal lysis, followed by travel along the sensory nerve pathway and viral assembly in the anatomically appropriate and predominantly squamous epithelial cells (Ellis, 1998). This then may cause recurrent mucocutaneous HSV infectons (recurrent Herpes labialis or recurrent genital Herpes) after the viruses transport to the periphery at mucosal surfaces of the skin under situations like stress or exposure to UV light (Stanbery, 1995; Fleming, 1997; Richards *et al.*, 1998). The latent virus may also sometimes reactivate in the presence of stimuli like hormonal or emotional disturbances, environmental factors such as heat and cold (Pelczar *et al.*, 1988). Immunosuppression and trauma are also other factors for reactivation of HSV though, we do not understand what is the common link between these apparently disparate events known to trigger reactivaion (White and Fenner, 1994).

1.2 Epidemiology of HSV-1 and HSV-2 infection

It is in ancient Greece that HSV infections of humans were first documented and it was not until 1893 that Vidal specifically came to recognize person-to-person transmission of HSV infections (Whitley *et al.*, 1998). Close person-to-person

contact e.g. sexual contact, kissing, contact sports like wrestling ('herpes gladiatourm') or contact with active ulcerative lesions or asymptomatically excreting patients (Harrisons, 1996), corneal skin abrasion, mucosal contact with infected secretions are the ways through which transmission of HSV occurs (Ellis, 1998). Non-genital personal contact is the primary route of transmission for HSV-1, which commonly causes oropharyngeal infection (Fleming, 1997). Insertive oro-genital contact is also an important risk factor for acquisition of HSV-1 (Edwards and Carne, 1998) since asymptomatic salivary excretion of HSV-1 occurs in infected individuals. It has been reported that asymptomatic salivary excretion of HSV-1 takes place in 2 to 9 percent of adults and 5 to 8 percent of children (Harrisons, 1996).

A very high prevalence of *Herpes simplex* virus type 1 and type 2 are detected in human populations world wide representing a 30% increase over the past two decades (Fleming *et al.*, 1997). The age-related patterns of infection are different for HSV-1 and HSV-2. HSV-1 antibodies rapidly rise during childhood. Common primary infections with HSV-1 may be contracted by children over 6 months old (Pelczar *et al.*, 1988). The prevalence of antibodies to HSV-1 is approximately two fold higher among Caucasians through adolescence than it was in their childhood (Whitley *et al.*, 1998). For instance HSV-1 seroprevalence among 20-49 years of age in Switzerland natives is 77% (Laubereau *et al.*, 2000).

Seroconversion to HSV-1 occurs early in life, in developing countries (Whitley *et al.*, 1998) where by puberty nearly all members of lower socio-economic groups will

be infected with HSV-1 and sub-clinical form of primary herpes could be developed by more than 90% of adult persons, who have initial contact with HSV-1 (Pelczar *et al.*, 1988). While the prevalence of antibodies among adult asymptomatic individuals is slightly higher in females than in males (Whitley *et al.*, 1998). The incidence of HSV-1 is somewhat lower in higher socio-economic groups (Pelczar *et al.*, 1988).

On the other hand HSV-2 typically affects the genital area and sexual contact is the usual transmission path (Hoog *et al.*, 1997; Posavd *et al.*, 2000). Important factors in the sexual transmission of HSV infection are found to be undiagnosed symptomatic genital lesions and sub-clinical episodes of viral shedding in the genitourinary tract (Koutsky *et al.*, 1992; Brugha *et al.*, 1997; Arvaja *et al.*, 1999). Transmission of HSV-2 may also occur from active lesions, which are recognized or are atypical (Lauberau *et al.*, 2000; Smith *et al.*, 2000). Because most persons are less likely to have intercourse in the presence of genital symptoms, most STDs are transmitted primarily by the subset of infected persons with mild or absent clinical manifestations (Corey and Handsfield, 2000). Harrisons in (1996) forwarded that the efficiency of transmission is greater during symptomatic than asymptomatic periods of viral excretion. This is due to the reason that the titer of HSV in cultures from lesions is 100 to 1000 times higher than from salivary or genital tract secretions in asymptomatically excreting persons (Whitley *et al.*, 1998). HSV-2 infection leads to the production of lifelong antibodies and seropositivity to HSV-2 infection is associated with high risk sexual behaviour (Farland, *et al.*, 1999). Prevalence of HSV-2 genital infections increase with numbers of life time sexual partners (Brugha *et al.*, 1997; Hughes *et al.*, 2000). Almost all HSV-2 infections are acquired in the

range of 15 to 40 years of age, as expected for an STD (Whitley *et al.*, 1998; Corey and Handsfield, 2000). Life time number of partners, years of sexual activity, sexual orientation, lower income, are demographic characterstics associated with increased HSV-2 prevalence. Gender (i.e., more women than men), lower education level, history of other sexually transmitted diseases are markers of sexual exposure which are correlated also with HSV-2 seropositivity (Brugha *et al.*, 1997; Farland *et al.*, 1999; Hughes *et al.*, 2000).

The seroprevalence of HSV-2 in the United States is higher among persons attending STD clinics than in the broad population varying from 30 to 70% in most clinics (Whitley *et al.*, 1998). HSV-2 seroprevalence ranges from 60% to 90% among gold miners and commercial sex workers in South Africa (Corey and Handsfield, 2000). Though the number of initial visits to physicians for genital HSV infection in the United States increased from about 75,000 per year in 1978, to more than 150,000 per year in the early 1990s, it is not known whether this elevation was because of a true increase in incidence or due to increased public awareness and improved diagnosis (Fleming *et al.*, 1997).

1.3 Pathogenicity of HSV-1 and HSV-2

HSV-1 and HSV-2 are pathogens, which infect the mucosal surfaces of the eye, mouth, as well as genitalia, causing ulcerative lesions at these sites (Richards *et al.*, 1998) and producing infections which are ranging from mild stomatitis to disseminated and fatal disease (Straus *et al.*, 1985; Whitley and Gnann,1993).

Infection of the local epithelial cells with cell lysis, followed by lymphatic drainage to regional lymph nodes and hence viraemia with distant dissemination is the proposed mechanism of infection and disease (Ellis, 1998). Besides this HSV possess two unique biological properties, which can influence human diseases. These properties are the capacity to invade and replicate in the CNS and the capacity to establish a latent infection. More severe and even fatal infections like encephalitis, could thus be caused occasionally (Pelczar *et al.*, 1988). HSV-1 is found to be responsible to cause encephalitis while HSV-2 is more responsible for myelitis (Chretien *et al.*, 1996). The cytopathic changes caused by the two viruses (HSV-1 and HSV-2) looked different during the early stages. Small size and globular shape is gradually attained by all cultured cells whether cultured with HSV-1 or HSV-2. The cytopathic effects induced by HSV-1 infection took 24 more incubation hours than those induced by HSV-2 infection to manifest *in vitro* (Su *et al.*, 1995).

A typically less severe disease is manifested during recurrences of disease. There is also a decrease in the frequency of the disease with time (White and Fenner, 1994). The recurrence is usually characterized by superficial vesicles known as cold sores and fever blisters (Pelczar *et al.*, 1988). Frequency of future reactivations of infection is influenced by the anatomic site and virus type (Harrisons, 1996). Although smaller proportion of genital herpes can be caused by HSV-1, HSV-2 is the cause of most cases of recurrent genital herpes. Conversely HSV-2 is replicating to a higher titer than does HSV-1 in genital mucosa (Rosa Santos *et al.*, 1996; Sucato *et al.*, 1998). While oral-labial HSV-1 infections are recurring more frequently than

oral-labial HSV-2 infecions (Harisons, 1996), this situations show that the two HSV types display a degree of selectivity in their tissue tropism (White and Fenner, 1994).

Prior genital HSV-1 infection is more protective than prior oral HSV-1 infection. Re-infection with HSV-2 would reactivate a latent HSV-1 infection and therefore, infection with HSV-1 provides partial protection from HSV-2 acquisation (Sucato *et al.*, 1998). Primary and recurrent disease caused by HSV-2 infection, involve the genital tract in adults (Pelczar *et al.*, 1988), resulting in severe systemic disease in neonates born to infected women and in immunosupressed hosts that may end up in painful recurrent genital lesions and permanent neurological handicap and death, respectively (Brugha *et al.*, 1997; Fleming, 1997). Mucocutaneous disorders are found to be more common and frequent in individuals infected by HIV or AIDS than in non-infected ones (Hachem *et al.*, 1998).

1.4 Clinical Spectrum of *Herpes simplex* Viruses

The disease, caused by HSV type 1 or 2 viruses is characterized by the formation of thin-walled vesicles that ulcerate, crust and heal. The occurrence of the vesicles is often in clusters, on skin and/or mucous membranes (Harrisons, 1996, Whitley *et al.*, 1998). The anatomic site of the infection, the age, immune status of the host, and the antigenic type of the virus determine the clinical manifestations as well as the course of HSV (Harrisons, 1996). *Herpes simplex* viruses can infect almost any area of the skin (Hirsch, 1994), for instance, the local epithelial cells (Ellis, 1998) of ears, face and hands among wrestlers as in the case of Herpes Gladiatorum (Corey, 1994).

It can also infect the finger (herpetic whitlow), which may occur as a complication of primary oral or genital herpes by inoculation of virus into the hand through occupational or some other type of exposure. Other than mucosal surfaces of the eye, mouth, genitalia *Herpes simplex* viruses can also infect (Richards *et al.*, 1998) visceral organs due to viremia, involving multiple-organs (Harrisons, 1996). A vesiculoulcerative lesion therefore may occur at any mucocutaneous site. Although lesions of HSV-1 are most commonly detected in the mouth or on the lips, and that of HSV-2 in genital and peri-anal areas, either virus may cause lesions at any location, based on the site of primary mucocutaneous inoculation (Broder *et al.*, 1994).

1.5 Immune Response Against *Herpes simplex* Viruses

Like all herpesviruses, HSV remains in a latent state for the life of the host, after a primary infection (Gull Laboratories, 1997). Moreover, acquisition of disease, resistance to the development of latency, maintenance of latency, and frequency of HSV recurrences are also situations influenced by host responses to infection (Harrisons, 1996). A major role is played by the immune response of the host to determine the severity of both primary and reactivated infections with HSV (Gull Laboratories, 1997). Therefore the likelihood of viraemia is dependent upon the host immune integrity (Ellis, 1998). A variety of host immune responses which suppress or restrict virus replication are mounted for most viral infections (Stanberry, 1995).

For instance mucosal immunization protects the female genital tract from sexually transmitted viral infections (Gallichan and Rosenthal, 1998) and protective immune response is potentially gained not only at the site of administration but also at other mucosal surfaces during mucosal administration of antigen (Richards *et al.*, 1998).

Following primary infection with clinical or subclinical infection by HSV, people develop neutralizing antibodies, to be maintained life long (Pelczar et al., 1988). The neutralizing antibodies have been shown to recognize surface glycoproteins, which are antigens and mediate neutralization as well as immune mediated cytolysis antibody-dependent cell-mediated cytotoxicity, (ADCC). Primary infection by HSV may be successfully prevented by neutralizing antibody directed against envelope glycoproteins, notably gB and gD so as to limit spread of *Herpes simplex* virus from epithelial cells to nerve endings (White and Fenner, 1994).

On the other hand cell-mediated immunity plays a more important role in the immune defences against intracellular pathogens like that of viruses. More severe and extensive HSV infections are experienced by immunocompromised patients with defects in cell-mediated immunity when compared with those patients with defects in humoral immunity like in the case of agammaglobulinemia (Harrisons, 1996; Posavad *et al.*, 2000). A recent study has also suggested that the frequency of HSV-2 recurrences as a cause of coetaneous ulcerations rises sharply as CD4 cell counts drop below $50/mm^3$ (Broder *et al.*, 1994). Other than variety of T lymphocyte populations, host defence against HSV infections is also supported by multiple cell

populations involving natural killer (NK) cells, macrophages and lymphokines generated by these cells (White and Fenner, 1994; Ellis, 1998). For instance following immunization with heat-attenuated HSV-1 significant increase of interleukin (IL)-12, IL-6, IL-10, and IFN-gamma were detected in peritoneal cells of mice (Halford *et al.*, 1998). Viral antigens are presented on dendritic cells and macrophages to CD4+ Th1 lymphocytes at the site of epidermal infection. This initiates viral clearance by secreting cytokines such as interferon γ (IFN-γ), which recruit and activate macrophages and natural killer (NK) cells. Infected cells will be lysed by CD4+, CD8+ T cells, NK cells and through antibody-depenent cell-mediated cytotoxicity (ADCC) (White and Fenner, 1994). Both CD4+ and CD8+ TNF-beta-producing T cells could also be stimulated by HSV (Schmid *et al.*, 1997). Activation of multiple T cell populations, including cytotoxic T cells, confer maximum protection (Harrisons, 1996). There is also frequent occurrence of viral escape from T cell surveillance which results in prolonged survival of the virus in its host (Burgert, 1996).

1.6 Human Immunodeficiency Virus (HIV) and immunopathogenesis

The human immunodeficiency virus (HIV) is a retrovirus, a virus whose genome consists of RNA. Most retroviruses result in the uncontrolled growth of their host cells, but HIV which is a lentiviruse causes pathological damage by killing the host cell like several other lentiviruses. HIV-1 and HIV-2 are the two known types of HIV that share about 40% of their genome (Janeway and Travers, 1994). HIV-1 is being distributed through out the world while HIV-2 is found primarily in West

Africa. A close relationship is found between HIV-2 and simian immunodeficiency virus (SIV) (Hirsch *et al.*, 1989). HIV-1 is more virulent than that of HIV-2 (Marlink *et al.*, 1994). Though the degree of virulence of HIV-2 is a reduced one, it is equally associated with HIV-1 in causing AIDS (Popovic *et al.*, 1984).

HIV causes a persistent infection in which the infected individual will eventually develop acquired immunodeficiency syndrome (AIDS), which is the ultimate clinical stage of infection and is characterized by opportunistic infections and specific malignant diseases in the patients (Chiu *et al.*, 1985). The relentless destruction of CD4+ T lymphocytes is the hallmark of HIV-1 (Godard *et al.*, 1997). AIDS has evolved from a mysterious syndrome which was initially reported from gay men, to a viral illness affecting every segment of society (Reiter, 1998).

HIV is transmitted through exposure to the virus via bodily fluids like blood, semen or vaginal fluid (Janeway and Travers, 1994). In Africa sexual contact is the primary mode of transmission of HIV the genital, mucosal cells serving as viral target cells (Poss and Overbaugh 1999). Moreover the presence of a number of STDs in Africa, contributes a lot for the explosive transmission of HIV (Mbopi-Kiou *et al.*, 2000).

1.6.1 Frequency and interaction of HSV-2 infection with HIV infection

Though syphilis and chancroid are STDs which are emphasized as risk factors for HIV infection, studies now are directly implicating that HSV infection, also contributes to HIV transmission (Corey and Handsfield, 2000). This is because of the reason that these diseases (HSV-2, syphilis, chancroid) cause genital ulcers (Stamm

et al., 1988; Fleming, 1997; Gwanzura *et al.*, 1998) and hence increase the amount of HIV shedding through genital lesions by providing an easy portal of entry of the virus into the host (Gwanzura *et al.*, 1998; Martin, 1990). Immunosuppressed patients, particularly those with impaired cell-mediated immunity, are more likely to show greater frequency and severity of HSV infection. Mucocutaneous lesions due to HSV are found to be less prone to be resolving spontaneously in HIV-infected individuals than in immunocompetent patients (Broder *et al.*, 1994). The fact that genital ulcer diseases are risk factors for sexual acquisation and transmission of HIV infection has began to be appreciated in the late 1980s, soon after the viral etiology of AIDS was defined (Corey and Handsfield, 2000). The risk of HIV transmission or acquisition rises substantially, typically by 2-fold to 8-fold or even as much as 32-fold if either person in an HIV-discordant sexual partnership has an inflammatory STD (Corey and

Handsfield, 2000). No difference appears to occur between the frequency of HSV-1 infection in HIV-infected persons from that found in the general population, while rates of HSV-2 antibody are generally higher in individuals with HIV (Broder *et al.*, 1994). A study suggests that high HSV-2 prevalence rate among adolesescents implicates HSV-2 to be an important risk factor for HIV-1 infection (Wagner *et al.*, 1994). Many different studies forward epidemiological evidences suggesting an association between HIV infection and other sexually transmitted infections STIs, especially ulcerative STIs like that of HSV-2 and syphilis (Bonell *et al.*, 2000). Increasingly, HSV-2 is also being recognized as an important cause of genital ulcer disease in the developing world (Laubereau, *et al.*, 2000; Smith *et al.*, 2000).

An association between HIV infection and genital ulcer disease is demonstrated in recent studies in Africa whose study participants are highly sexually active population of heterosexual men and women (Stamm *et al.*, 1988). Compared with the industrialized world, the relatively high prevalence of untreated STDs in sub-Saharan Africa has been proposed as a contributing factor in the higher prevalence of heterosexually transmitted HIV in that region (Gwanzura *et al.*, 1998). For instance more frequent previous history of genital ulcerative diseases is recorded in HIV-infected-Nairobi prostitutes than the non-infected ones (Martin, 1990). HSV-2 seroprevalence was 35.7% among HIV-seronegative subjects and 82.7% among HIV-seropositive subjects of male factory workers in Zimbabwe who were screened for HSV-2 specific antibodies (Gwanzura *et al.*,1998).

STDs are highly prevalent in Ethiopia (Duncan *et al.*, 1995). The first HIV positive sera in Ethiopia was found in 1984 (Tsega *et al.*, 1988). The HIV/AIDS epidemic has spread to the entire country to reach a prevalence ranging 7-20% in the 15-50 year age groups in the urban areas by 1998 (Fontanet *et al.*, 1998). Different prevalence of HIV is recorded among different social sectors, commercial sex workers having the highest prevalence (Mehret *et al.*, 1990). The HIV prevalence was 6% among commercial sex workers in Addis Ababa in 1985-1986 (Ayehunie *et al.*, 1987), a figure which has grown to be 25% in 1989 and 74% in 1998, an incredible increase over a relatively short period of time (Mehert *et al.*, 1990). HIV-I prevalence was 3.6% in antenatal clinic attendants in Addis Ababa in the late 1980s, 6% in prisoners, 2.6% in military recruits, and 3.2% in healthy individuals with no

identified risk behaviour (Mehret *et al.*, 1990; Zewdie *et al.*, 1990; Kebede *et al.*, 1991; Kefenie *et al.*, 1992). STDs are found to be highly prevalent among females practicing multiple partner sexual contact in Addis Ababa in a study conducted in sex workers in 1990 (Solomon, *et al.*, 1990). 17.7% of 2663 female sex workers in the city of Addis Ababa in 1989 were reported to experience one or more episodes of STD, gonorrhea being the major cause (Mengistu *et al.*,1990). Genital discharge and genital ulcer was found to be approximately in 3:1 ratio in both sexes caught with STDs, involving multiple episodes in STD patients who were attending four health centres in Addis Ababa (Workneh *et al.*, 1990). Solomon (1992) has also reported that seropositivity for HIV antibodies among students in Addis Ababa, attending clinics for STDs from 1991-1992, increase with age and in female sex. One cross sectional study was carried out to assess the prevalence of STD among Ethiopian women attending antenatal clinic in Addis Ababa from 1975-1976 in two teaching hospitals and a mother and child health care (MCH) center. The prevalence of anti *Herpes simplex* virus type 2 (HSV-2) antibodies in these Ethiopian pregnant women attending antenatal care (ANC) was 35% (108/306) (Duncan *et al.*, 1995).

HIV-1 seroprevalence has also shown significant difference ($P < 0.001$) by age, with the highest prevalence in the age group 20-24 in another study among pregnant women of Addis Ababa from 1995 to 2000 (Yared *et al.*, 2000). No study was carried out to determine risk factors that associate with HSV-2 infection in this country. The present study focuses on the prevalence of HSV-2 and HSV-1 and the association of HSV-2 with various epidemiological risk factors as well as HIV, HSV-1 and syphilis infection among adult Ethiopians.

Objectives of the study

General Objectives

To study the epidemiology of HSV-2 and HSV-1 infections and the relations of HSV-2 with various risk factors among Ethiopians.

Specific Objectives

1. To determine the seroprevalence of HSV-2 and HSV-1 infections in the adult population of Addis Ababa.

2. To identify and examine risk factors for HSV-2 infection, including HSV-1, HIV, syphilis infections and, some clinical, behavioural, sociodemographic factors.

2. Material and methods

2.1 . Study population

From a total of 1375 plasma specimens, 56.7% (779/1375) originated from factory workers cohort participants and 43.3% (596/1375) originated from community based study participants: Test for the presence of anti-HIV-1 antibodies was done for all the 1375 subjects and test for the presence of syphilis antibodies had already been carried out for 98.8% (1358/1375) of the study subjects through the ENARP cohort and community based study program.

During the present cross-sectional retrospective study, 91.1% (1252/1375) of the plasma specimen were used to test the presence of anti-HSV-2 antibodies. While only 37.2% (511/1375), i.e., 85.7% (511/596) of the community based study subjects plasma samples were used to test the presence of anti-HSV-1 antibodies.

56.7% (779/1375) of the study subjects are factory workers in Akaki enrolled in the Ethiopian-Netherlands AIDS Research Project (ENARP) cohort study, that aims at studying HIV infection progression. 43.3% (596/1375) of the subjects are community-based study participants who were enrolled for the purpose of pilot study to choose a cohort site. The community-based study subjects live in the kebeles of higher 23 at the vicinity of the African Leprosy Educational Research and Training (ALERT) center.

The Akaki cohort subjects were enrolled since 1997 while, the community based study was done in 1996 only. 46% (358/779) females and 54% (421/779) males, were enrolled in the factory workers cohort while 46% (275/596) females and 54% (321/596) males were enrolled in the community-based study. Results of HIV testing were made available to participants willing to know their HIV status through post-test counselling.

Participants got counselling for HIV testing, answered a questionnaire on socio-demographic characterstics, medical history, and sexual behaviour, had clinical examination by a physician, provide blood, stool and urine samples for various laboratory analysis after an informed consent has been obtained. The same procedures were repeated during regular follow up visits every six months for the Akaki cohort particiapants. The cohort participants are treated free of charge, according to the national standards of care, for any condition diagnosed during and between the follow-up visits every six months. The same was also done for the community based study subjects for any condition diagnosed during the period of the study.

In the present retrospective cross-sectional study, plasma specimens of only 1252 adults were analysed because of insufficiency of samples for the rest of the 123 subjects. Out of the 1252 plasma specimens 661 came from Akaki factory workers while 591 came from subjects for whom random community based survey was done. Therefore a total of 1252 plasma samples of the study participants were used to test for the presence of anti-HSV-2 antibodies.

HSV-2 seroprevalence was estimated by associations between immunological, some clinical, various epidemiological and behavioral risk factors, while HSV-1 seroprevalence was estimated by sex only.

2.2. Laboratory methods

2.2.1. Test for HIV antibodies

A total of 1375 plasma samples were tested for HIV-1 antibodies using two enzyme-linked immunosorbent assays (ELISA) at the EHNRI-ENARP laboratory. The first test was done by using (Vironistika HIV Uni-Form II plus 0, Organon Teknika, Boxtel, The Netherlands). Further analysis was done for all positive samples by using the Wellcozyme recombinant ELISA (Wellcome Diagnostics, Dartfor, Kent, UK). Samples were considered to be positive when they were positive in both assays. Further tests were carried out using Western blot (Diagnostic Biotechnology, Singapore) for samples with discordant results by the two ELISAs. Those samples with positive results in the last test were considered as confirmed positives.

2.2.2. Test for syphilis antibodies

Syphilis serology at baseline was measured for a total of 98.8% (1358/1375) plasma samples because of insufficiency of sample for the rest 1.2%. Test for syphilis seropositivity was done by Rapid Plasma Reagin (RPR slide-Test, Biomerieux, France)

assay and if found to be positive a sample was further tested using the *Treponema pallidum* hemagglutination assay (TPHA; Serodia-TP, Fujirebio, Tokyo, Japan). A positive RPR result with a positive TPHA result were interpreted as indicating active or recently treated syphilis infection, whereas a negative RPR result and a positive TPHA result were interpreted as indicative of a cured syphilis infection.

2.2.3. Test for *Herpes simplex* virus (type 1 and type 2) antibodies

A total of 1252 plasma samples stored at -81 °C, were tested for the presence of anti-HSV 2 antibodies while only 511 plasma samples were tested for the presence of anti-HSV-1 antibodies at EHNRI-ENARP laboratory using Gg2 and Gg1 coated antigens in a commercially available ELISA test kit (Gull laboratory GmbH, Germany).

The relative sensitivity and relative specificity of the HSV-2 specific IgG ELISA test is 98% and 96.7% respectively when compared to Gg2 Western Blot test results (Gull Laboratories, 1997). Moreover the relative sensitivity and the relative specificity of the HSV-1 specific IgG ELISA test was 94.6% and 96.2% respectively when compared to the Gg1 Western Blot test results (Gull Laboratories, 1997). Gg2 and Gg1 Western blot assays are the gold standard for the determination of type specific HSV type 2 and type 1 antibodies respectively (Arvaja *et al.*, 1999).

The tests were carried out according to the instructions of the manufacturer. Briefly eight by twelve Glycoprotein g2 (gG2) antigen coated ELISA plate wells and

glycoprotein g1 (gG1) antigen coated ELISA plate wells were used to determine the presence or absence of HSV type 2 and type 1 specific antibodies, respectively. 10 µl of each study subject's plasma sample was diluted in 200 µl of the specimen diluent (7.2 pH phosphate buffered saline (Na_2HPO_4), with 0.01% sodium azide (NaN_3) in a 1 to 21 proportion. Dilutions of negative control, positive control, and reference serum were also carried out within the same proportion and vortexed during which time the lavender colour turns blue. 100 µl of the diluted plasma samples, controls, reference sera and specimen diluent (blank well) were pipeted into corresponding ELISA plate wells. The wells were then incubated for 30 minutes at 37°C.

After incubation ELISA plate wells were washed manually three times, using 250µl of wash solution (tris-buffered saline, pH 7.5, with detergent and 0.01% NaN_3). Using a multi-channel pipet, 100µl conjugate (alkaline phosphatase-labeled antihuman IgG (caprine) containing 0.01% NaN_3) was added to all wells, including the blank well and incubated for 30 minutes at 37°C. Para- nitrophenyl phosphate (p-NPP) substrate solution was prepared by dissolving p-NPP tablets in substrate buffer (tris buffer, pH 9.5 solution containing 0.01% NaN_3) and 100µl p-NPP substrate solution was added to each well, including the blank well. The wells were then incubated for 30 minutes at 37°C. The blue color of the p-NPP substrate solution was changed to green for the positive control and the samples containing detectable levels of anti-HSV-1 IgG antibodies or anti-HSV-2 IgG antibodies, while the rest remained blue. Then after 100µl stopping reagent (1.5 N sodium hydroxide) was added to each well to stop the reaction, during which time the green color changed to yellow, showing the presence of the respective antibodies in those plasma samples, and the

blue color changed to pink showing the absence of the respective antibodies in those plasma samples. Plates were then read at 405 nm within 1 hour using ELISA reader (Reader 230, Organon Teknika, The Netherlands). Optical density (OD) readings were considered to be positive if they were found to be above the cut off value which was calculated by finding the mean absorbance value for the three reference sera (expected to be ≥ 0.100 and ≤ 1.000).

2.3. Statistical analysis:

Prevalence of HSV-2, HSV-1, HIV and syphilis antibodies as well as associations between prevalences of HSV-2 antibody and various risk factors were statistically analysed using univariate analysis by: chi-square test, with STATA 6.0 software (Stata Corporation, College Station, Texas, USA). Briefly, HSV-2 seropositive and seronegative subjects were compared with respect to their HIV, Syphilis, HSV-1 serostatus, some clinical findings (genital ulcer and genital discharge), different sociodemographic risk factors (age, sex, marital status, educational status, monthly income) as well as behavioural factors (history of alcohol consumption and number of life time sexual partners). P values, Odds ratios (ORs) and 95% confidence interval were estimated for each risk factor using logistic regression analysis. Multivariate analysis was performed after controlling all potentially relevant variables, in order to get adjusted Odds ratios.

2.4. Ethical consideration

This study is part of a pilot and long-term cohort study on the progression of HIV infection in Ethiopia which is performed by ENARP and is ethically cleared by both EHNRI and National Ethical Clearance Committee to collect and use socio-demographic, behavioural, and medical information and to test for antibodies of HIV and other infectious diseases. Informed consent was obtained from each subject. In this study no additional material was collected. Confidentiality of the data was preserved by using coded information.

3. Results

3.1. Seroprevalence of HSV-2, HSV-1, HIV and syphilis antibodies.

The seroprevalences of HSV-2 was found to be 36.2% and 52% in the community and factory workers study, respectively. The observed differences in HSV-2 prevalence between the two study populations is mainly attributable to differences in age and gender distribution of the two population. It was then found to be necessary to combine the data of the two study population and the rest of the analysis was done in terms of the combined data set. During combining the data, the anti-HSV-2 antibody test result of 84 subjects (80 from the community based and 4 from the factory subjects) were excluded from data analysis, and the sample size was reduced to 1168 (530 female and 638 male). The exclusion of the samples was necessitated by the absence of certain sociodemographic data for the subjects excluded. The analysis was done iu terms of gender, because gender was found being a highly significant risk factor for HSV-2 acquisation. Certain clinical, and sociodemographic factors examined in association with HSV-2 acquisation in this study, were also significantly associated with female gender. The total HSV-2 seroprevalence after combining the data of the two study populations was therefore found to be 44.5% (520/1168) which was 50.6% (268/530) in the female and 39.5% (252/638) in the male subjects ($P < 0.05$). A total prevalence of 93.7% (479/511) was found when samples were tested for anti-HSV-1 antibodies which was 94.7% (231/244) in the female subjects and 92.8% (248/267) in the male subjects ($P > 0.05$). Screening for anti-HSV-1 antibodies was not done for the factory workers plasma samples because HSV-1 seroprevalence in the community based study population was already found

to be extremely high as it is worldwide and hence the seroprevalence of anti-HSV-1 antibodies was assumed to be similar as that of the community based study subjects.

HIV as well as syphilis prevalence were also analysed in terms of the combined data set. The seroprevalence of HIV-1 was 9.4% (50/530) in the female subjects and 10.3% (66/638) in the male subjects ($P > 0.05$). While seroprevalence of anti-syphilis antibodies was found to be 19.1% (101/530) in the female and 24.6% (157/638) in the male subjects ($P < 0.05$) (Table 1).

Table 1. Prevalence of antibodies against HSV-2, HSV-1, HIV, and syphilis in adult community based study subjects (1996) and factory workers (1997) in Ethiopia.

Antibodies against	Number (%)
HSV-2	
Female	268/530 (50.6) **
Male	252/638 (39.5)
Total	520/1168 (44.5)
HSV-1	
Female	231/244 (94.7)
Male	248/267 (92.8)
Total	479/511 (93.7)
HIV	
Female	50/530 (9.4)
Male	66/638 (10.3)
Total	116/1168 (9.9)
Syphilis	
Female	101/530 (19.1)
Male	157/638 (24.6) **
Total	258/1168(22.1)

**** $P < 0.001$, P refers to likelihood ratio test for association.**

3.2. Association of HSV-2 seroprevalence with seroprevalence of HSV-1, HIV, syphilis antibodies, and presence of genital ulcer as well as genital discharge.

There was no significant association between HSV-2 and HSV-1 seropositivity among females ($P > 0.05$, OR = 0.69, CI = 0.23-2.13) whereas HSV-1 infection has shown a marginal protective effect in males ($P = 0.06$, OR = 0.39, CI = 0.16-1.02) (Table 2). Whereas significant association was observed between HSV-2 and HIV-1 seropositivity as well as HSV-2 and syphilis seropositivity in both females and males $P < 0.001$ (Table 2). Furthermore HSV-2 seropositivity was significantly associated with genital ulceration in females ($P < 0.001$, OR = 8.1, CI = 2.4-27.3). Although the association was not statistically significant ($P>0.05$), HSV-2 seroprevalence was higher in males with genital ulceration (50%) compared to those having no genital ulceration (39%) (Table 2). Similarly, HSV-2 seropositivity was significantly associated with genital discharge in females ($P < 0.001$, OR = 2.56, CI = 1.5-4.3), although it did not reach significant level among males (Table 2).

Table 2. Percentage HSV-2 Seroprevalence and measure of possible risk of infection, in community based study subjects (1996) and factory workers (1997) in Ethiopia, in association with various risk factors

Predictable variable	Female HSV-2		Male HSV-2	
	HSV-2 sero+ve (%)	OR (95% CI)	HSV-2 sero+ve (%)	OR (95% CI)
HSV-1				
-ve	ND	ND	10/19 (52.6)	1 -
+ve	86/231 (37.2)	0.69 (0.23-2.13)	76/248 (30.6)	0.39 (0.16-1.02) [m]
HIV				
-ve	228/480 (47.5)	1 -	206/572 (36)	1 -
+ve	40/50 (80)	4.4 (2.2-9.0) [**]	46/66 (69.7)	4.1 (2.4-7.1) [**]
Syphilis				
-ve	196/429	1 -	158/481 (32.9)	1 -
+ve	72/101 (71.3)	2.95 (1.8-4.7) [**]	94/157 (59.9)	3.05 (2.1-4.4) [**]
Genital ulcer				
No	245/504 (48.6)	1 -	241/616 (39)	1 -
Yes	23/26 (88.5)	8.1 (2.4-27.3) [**]	11/22 (50)	1.6 (0.7-3.6)
Genital Discharge				
No	215/454 (47.4)	1 -	220/575 (38.2)	1 -
Yes	53/76 (69.7)	2.56 (1.5-4.3) [**]	32/63 (50.8)	1.7 (0.9-2.8)

[**] P < 0.001, [m] P = 0.06. Abbreviations: P refers to likelihood ratio test for association. CI = Confidence Interval. OR = Odds ratio. ND = Not Done.

3.3 Association of HSV-2 seroprevalence with behavioural risk factors among female and male subjects:

The seroprevalence of HSV-2 was significantly higher in the female subjects who reported to have 2-4 life time sexual partners when compared with the female subjects reported to have 0-1 life time sexual partners.

Table 3. Percentage seroprevalence of HSV-2 infection in community based study subjects (1996) and factory workers (1997) in Ethiopia when associated with behavioural factors.

Predictable variables	Female HSV-2		Male HSV-2	
	Number (%)	OR (95 % CI)	Number (%)	OR (95 % CI)
Life time sex partner				
0-1	141/322 (43.8)	1 -	22/128 (17.2)	1 -
2-4	102/170 (60)	1.87 (1.28 – 2.72) **	57/147 (38.8)	2.28 (1.35 – 3.84) *
5-9	ND	ND	68/150 (45.3)	2.98 (1.78 – 5.00) **
10+	ND	ND	97/203 (47.8)	3.29 (2.02 – 5.37) **
Alcohol consump				
Never	243/488 (49.8)	1 -	32/137 (23.4)	1 -
Some times	22/39 (56.4)	1.29 (0.67 – 2.51)	172/385 (44.7)	2.4 (1.59 – 3.63) **
Frequently	ND	ND	41/98 (41.8)	2.14 (1.25 – 3.68) *

*** P < 0.05 ** P < 0.001 Abbreviations: P refers to likelihood ratio test for association. CI confidence interval. OR = Odds ratio. ND = Not done (because sample size is < 10).**

The number of female subjects with 5-9 and > 10 life time sexual partners were excluded because their low number (< 10) has less statistical power (Table 3). While HSV-2 seroprevalence in the male subjects, was significant in all categories of life time sexual partners, when compared with male subjects who reported to have 0-1 life time sexual partners.

No significant association was observed (P = 0.435, OR = 1.29, 95% CI = 0.67-2.51) between HSV-2 infection and alcohol drinking in the female subjects who reported to drink alcohol sometimes compared with those females who reported not to drink alcohol totally. The female subjects who reported to drink alcohol frequently, were excluded from the data analysis because of their low number (i.e., < 10). Whereas infection with HSV-2 was observed to be significantly associated in the male, who reported to drink alcohol both sometimes (P < 0.001) and frequently (P < 0.05) than those male subjects who reported not to drink alcohol at all (Table 3).

3.4. Association of HSV-2 seroprositivity with different socio demographic risk factors

HSV-2 seroprevalence iucreased significantly (P ≤ 0.001) in both female and male subjects as age increased when compared with the younger subjects of the same sex. HSV-2 seroprevalence in female of younger age, 25-34 was found to be more or less similar (51.9%) with HSV-2 seroprevalence of males aged 45+ (50%) (Table 4).

Table 4. Percentage seroprevalence of HSV-2 infection in community based study subjects (1996) and factory workers (1997) in Ethiopia when associated with age in years.

Predict-able variable	Female HSV-2		Male HSV-2	
	Number (%)	OR (95% CI)	Number (%)	OR (95% CI)
Age in years				
15-24	24/109 (22)	1 -	17/123 (13.8)	1 -
25-34	107/206 (51.9)	3.8 (2.26-6.49) **	76/183 (41.5)	4.42 (2.45-7.99) **
35-44	124/193 (64.3)	6.4 (3.71-10.93) **	131/276 (47.5)	5.63 (3.21-9.90) **
45+	13/22 (59.1)	5.1 (1.95-13.4) **	28/56 (50)	6.23 (2.99-12.97) **

**** P < 0.001 Abbreviations: P refers to likelihood ratio test for association. OR= Odds ratio. CI = Confidence Interval.**

HSV-2 seroprevalence reached it's peak, 64.3% (OR = 6.4) in the age range of 35-44 in the females while being 50% (OR= 6.23) at the age of 45 and above in the male subjects. The odds of being seropositive for HSV-2 antibody ranges from 3.8 to 6.4 for age groups 25 to 45+ in both sexes, when compared with the reference age (15-24) (Table 4).

Table 5. Percentage seroprevalence of HSV-2 infection in community based study subjects (1996) and factory workers (1997) in Ethiopia when associated with other socio-demographic factors.

Predictable variable	Female HSV-2		Male HSV-2	
	Numbers +ve (%) (HSV-2)	OR (95% CI)	Numbers +ve (%) (HSV-2)	OR (95% CI)
Level of education				
Non-formal	104/182 (57.1)	1 -	42/101 (42)	1 -
Elmentary (1-6)	109/204 (53.4)	0.9 (0.58-1.28)	89/212 (42)	1 (0.63-1.64)
Juni-sec (7-12)	45/124 (36.3)	0.4 (0.26-0.68) **	85/233 (36.5)	0.8 (0.50-1.30)
Monthly income				
≤199 (low)	133/316 (42.1)	1 -	74/239 (31)	1 -
200-399 (med)	122/189 (64.6)	2.5 (1.74-3.66) **	139/306 (45.4)	1.9 (1.35-2.74) **
400+ (high)	13/24 (54.2)	1.6 (0.71-3.76)	38/83 (45.8)	2 (1.18-3.26) *
Marital status				
Unmarried	87/195 (44.6)	1 -	70/241 (29.1)	1 -
Married	181/335 (54)	1.46 (1.02-2.08) *	182/396 (45.9)	2.1 (1.47-2.92) **

**** P < 0.001, * P < 0.05, Abbreviations P refers to likelihood ratio test for association. CI Confidence Interval. OR = Odds Ratio.**

HSV-2 seroprevalence was significantly lower in the female subjects who reported to attend Junior secondary school (36.3%) in comparison to the female subjects who reported to have non-formal education (57.1%). Although not statistically significant a relatively lower HSV-2 seroprevalence was observed in female subjects who

reported to attend elementary school (53.4%) than the female subjects who reported to have non-formal education (Table 5). Female subjects who reported to attend grade 7-12 were therefore significantly ($P < 0.001$) protected from being infected by HSV-2.

Because the number of female subjects who reported to complete grade 12 and attend higher education were low (< 10), the analysis was not done for both sexes.

Although no statistical significance ($P > 0.05$) was observed HSV-2 seropositivity was seen to be decreased from 42% to 36.5% as the reported grade level of education for the male study subjects increased from elementary to Junior secondary (Table 5).

Moreover Female subjects reported to have medium monthly income were found to have the highest prevalence (64.6%) (122/189) of anti-HSV-2 antibodies with highly significant association ($P < 0.001$) when compared to that of the female subjects who reported to have low income (42.1%). While a relatively less HSV-2 seroprevalence (54.2%) was detected in the female subjects who reported to have high monthly income than the female subjects who reported to have medium monthly income (Table 5).

Male subjects who reported to earn medium and high incomes were observed to be significantly at higher risk ($P < 0.001$ and $P< 0.05$ respectively) to be infected by HSV-2 when compared with male subjects with low income.

HSV-2 seropositivity was significantly higher in both female ($P < 0.05$) and male ($P < 0.001$) married subjects when compared with the unmarried female and male subjects (Table 5).

3.5. Adjusted association of HSV-2 seropositivity with some behavioural, sociodemographic, clinical factors, HIV as well as Syphilis seropositivity

As was shown in the univariate analysis, the significant association of HSV-2 infection in the increasing age trend is not lost for both female and male subjects even after the adjustment of different variables like education, marital status, genital discharge, and alcohol consumption (Table 6). The highest OR is recorded in the women of age 35 to 44 (OR = 5.24) and in the males of age 45 + (OR = 3.94).

The significant association in the univariate analysis, between having more number of life time sexual partners, consuming alcohol, having genital discharge, being married and being infected by HSV-2 is not maintained in the multivariate analysis. However, the significant association observed in the univariate assocation between HSV-2 infection and having medium income, was not lost ($P = 0.002$) in the multivariate analysis after adjusting for education, marital status, genital discharge, alcohol consumption and life time sexual partners for the female subjects while it is totally lost for the male subjects of all income categories.

Table 6. Adjusted association of HSV-2 infection with selected risk factors for community based study subjects (1996) and factory workers (1997) in Ethiopia. [a]

Predictable variables	Female HSV-2 OR (95% CI)	Male HSV-2 OR (95% CI)
Age in years		
15-24	1 -	1 -
25-34	3.2 (1.63-6.27) **	2.75 (1.35-5.60) *
35-44	5.24 (2.49-11.03) **	3.63 (1.67-7.89) *
45+	4.27 (1.35-13.49) *	3.94 (1.55-10.06) *
Monthly income		
≤199 (low)	1 -	1 -
200-399 (medium)	2.06 (1.30-3.26) *	1.29 (0.83-2.00)
400+ (high)	2.64 (0.88-7.94)	1.37 (0.68-2.89)
Genital ulcer	5.25 (1.42-19.35) *	1.55 (0.60-3.99)
TPHA	1.8 (1.06-3.04) *	2.09 (1.39-3.15) **
HIV	5.32 (2.39-11.8) **	3.37 (1.85-6.14) **

After adjusting for education, marital status, genital discharge, alcohol consumption and life time sexual partners. ** P < 0.001, * P < 0.05, abbreviations P refers to likelihood ratio test for association. CI = Confidence Interval. OR. [a] = Only significant predictors are presented.

The significant association of genital ulcer with HSV-2 seropositivity in the female subjects ($P < 0.05$) is not lost in the multivariate analysis, while it remained insignificant for the male subjects. Syphilis and HIV infection have remained significantly associated with HSV-2 infection for both female and male subjects, after adjusting for education, marital status, genital discharge, alcohol consumption and life time sexual partners (Table 6).

4. Discussion

This is a cross sectional study which examined the prevalence of HSV-2 and HSV-1, in Ethiopia. The association between HSV-2 and the risk factors like HIV, syphilis, number of life time sexual partners, genital ulceration, genital discharge and different sociodemographic characterstics were examined. The total HSV-2 seroprevalence was 44.5%, being 50.6% in the female subjects and 39.5% in the male subjects. As acquisition of HSV-2 is influenced by sex (Whitley *et al.*, 1998), females are more likely than males to have higher seropositivity for HSV-2 antibody (Morse *et al.*, 1997). A similar finding of a higher seroprevalence of HSV-2 antibody among females (25.6%) than males (17.8%) was reported from the United States of America in a longitudinal study done from 1976-1980 (Fleming *et al.*, 1997). Likewise, a seroprevalence of HSV-2 antibody in females was much higher than in males both in Uganda and Tanzania (Morse *et al.*, 1997; Obasi *et al.*, 1999).

Long *et al.* (2000) had suggested that the higher susceptibility of females may be due to the greater mucosal surface area of exposure when compared with that of the males' genitalia. As reported by Whitley *et al.* (1998) the prevalence of antibodies against HSV-I was also determined to be slightly higher among females than in the males. However, the difference was not significant as that of HSV-2's. The total HSV-1 sero-prevalence was found to be 93.7% which is in agreement with the high worldwide seroprevalence of HSV-1 (Whitley *et al.*, 1998). Moreover, Pelczar (1988) has also reported that, primary herpes could be developed by more than 90% of adult persons in developing countries.

Furthermore, previous HSV-1 infection reduces the risk of subsequent HSV-2 infection (Hirsch, 1994). The cross protection of HSV-1 against HSV-2 was marginally significant (P = 0.06) for the men subjects of this study, whereby HSV-2 prevalence was only 30.7% in the HSV-1 seropositives as compared to 52.6% in the HSV-1 seronegatives. This may be because that seroconversion for HSV-1 antibodies occurs early in life in developing countries (Whitley *et al.*,1998), while people in the developed countries (Hughes *et al.*, 2000) may be less exposed to HSV-1 at a young age and thus are more susceptible to symptomatic genital HSV infections as adults.

The reason for the insignificant HSV-1 cross-protection against HSV-2, in the female subjects, may be because the anti-HSV-1 IgA antibodies are not sufficiently protective against HSV-2 infection through their susceptible mucosa with relatively larger surface area (Long *et al.*, 2000).

Acquiring HSV-2 is also influenced by age (Brugha *et al.*, 1997; Farland *et al.*, 1999) and hence older age is more associated with HSV-2 seropositivity than younger age (Morse *et al.*, 1997). The seroprevalence of HSV-2 antibodies is virtually nonexistent in persons younger than 12 years while it reaches peak by the age of 40 years, and remains stable thereafter (Corey and Handsfield, 2000; Whitley *et al.*, 1998).

This fact is reflected in our study that revealed an increasing prevalence of anti-HSV-2 antibodies with increase in age in both sexes. On the other hand only very small

number of subjects (< 10) were detected with anti-HSV-2 antibodies at age 18 in both the female and male subjects.

According to the study held from 1976-1980 anti-HSV-2 antibody was detectable in roughly one of five persons age 12 years or older, nationwide in the United States (Fleming *et al.*, 1997). Increment in the prevalence of HSV-2 with increasing age, is also detected in male Zimbabwean factory workers, where it was 6.5% at age 18-20 years and reached 62.2% at age 46 years and older (Farland *et al.*, 1999).

Moreover, seroprevalence of HSV-2 antibody rose steeply with increasing age to a plateau of around 75% at the age of 25 and above in females ($P < .001$) and around 60% at the age of 30 and above years in males (P value < 0.001) in the rural Tanzania (Obasi *et al.*, 1999).

Other than with sex and age, HSV-2 seropositivity is also correlated with other STDs (Farland *et al.*, 1999). The seroprevalence of HSV-2 in the United States is higher among persons attending STD clinics than in the broad population varying from 30 to 70% in most clinics (Corey and Handsfield, 2000).

It ranged from 14% to 90% in STD clinic attendees in Sweeden; and 5% to 40% in various other populations in Europe (Corey and Handsfield, 2000); while its prevalence is as high as 83% in homosexual men in United States (Whitley *et al.*, 1998).

Studies by other workers have shown that HSV-2 is common in adult populations in Africa and that its prevalence across diverse populations shows a wide variation (Gwanzura *et al.*, 1998). For example HSV-2 seroprevalence ranged from 20% in surgical patients to as high as 96% among prostitutes in a study that screened stored sera from Dakar (Gwanzura *et al.*, 1998). The prevalence was 28% among Rwandan military recruits, 71% among adults in Congo (Brazzaville) (Nahmias *et al.*, 1990; Langeland *et al.*, 1998) 36% among Genital Ulcer Disease (GUD) patients in Kampala, Uganda (Mostad *et al.*, 2000).

Much higher seroprevalence (50-80%) of HSV-2 has been recorded in some highly sexually active populations in many developing countries (Mostad *et al.*, 2000).

Seropositivity for syphilis antibodies has been shown to be associated with a significantly higher prevalence of HSV-2 infection (OR = 5.18; $P < 0.05$) in females of rural communities in Tanzania. This association of HSV-2 and syphilis infection in the rural communities of Tanzania, remained strong in multivariate analysis (Obasi *et al.*, 1999).

Similarly in this study, HSV-2 seroprevalence was determined to be significantly associatied with seropositivity of syphilis antibody (OR = 1.8; $P < 0.05$) in the female and (OR = 2.0; $P < 0.001$) in the male Ethiopian subjects, after adjusting for education, marital status, genital discharge, alcohol consumption and life time sexual partners.

HSV-2 has been determined to be the main etiologic agent of genital ulcer (White and Fenner, 1994; Myron, 1998; Nascimento *et al.,* 1998). Likewise, although not statistically significant, increased HSV-2 seroprevalnece was detected in both female and male Ethiopian subjects with genital ulceration The ulceration was much more severe in the female subjects, which may be due to susceptibility of the mucosa induced by its larger surface area in this sex. Similar conditions were reported from patients in Kampla, Uganda (Gwanzura *et al.,*1998) and from rural communities of Tanzania (Obasi *et al.* 1999).

Furthermore, genital ulcer disease (GUD) is believed to increase the risk of HIV acquisition per sexual exposure (Gwanzura *et al.,* 1998; Fleming, 1997) and seropositivity for HSV-2 antibody is also significantly associated with HIV seropositivity (Morse *et al.,* 1997). Similarly HSV-2 seroprevalence in HIV seropositive subjects was found to have significant association in both the female and the male subjects in this study. Comparable determination on HSV-2 infections among HIV/seropositive persons in Brazili was reported to be 73% (Rosa-Santos *et al.,* 1996).

Similar association was reported for male Zimbabwean factory workers (Farland *et al.,* 1999). However, HSV-2 was detected in a significantly higher proportion of HIV seropositive than in HIV seronegative GUD patients of Lesotho at a level of 47% versus 16%, respectively (Morse *et al.,* 1997). Seroconversion for HIV was significantly associated with the antecedent occurrence of genital ulcer during a follow-up in seronegative prostitutes of Nairobi.

This association persisted after controlling for other variables like sexual activity and condom use (Moss *et al.*, 1990). Similarly in a cohort of 75 HIV seropositive men attending an STD clinic in Zimbabwe, a history of genital ulcer was associated with HIV seroconversion in the wives who were serodiscordant with their husbands (Moss *et al.*, 1990).

Increased prevalence of HSV-2 is also influenced by factors like history of genital discharge (Farland *et al.*, 1999). In this study HSV-2 seropositivity and history of genital discharge was significantly associated ($P < 0.001$) in the female subjects while only marginally significant in the male subjects ($P = 0.06$). The genital discharge may possibly be an access for genital shedding of HSV-2 in those subjects who were seropositive for HSV-2 antibodies and hence increase transmission of HSV-2 to the sexual partner. In both the female and male Ethiopian subjects seroprevalence of HSV-2 was relatively lower in the absence of genital discharge.

Furthermore, increased prevalence of HSV-2 has been shown to be influenced by factors like number of life time sexual partners (Farland *et al.*, 1999). This influence of increasing number of life time sexual partners on the increasing prevalence of HSV-2 antibodies is also seen in the female and male Ethiopian study subjects.

The female subjects in this study did not report to have more than 4 life time sexual partners while there were male subjects who have reported to have up to 10 or more

life time sexual partners. Similar high-risk sexual behaviour was more commonly reported by males when compared with females in Ethiopia (Tefera, *et al.*, 1999).

The statistically significant associations detected in the females with 2-4 life time sexual partners and in the males with 2 or more (up to 10+) life time sexual partners were not maintained after adjusting for education, marital status, genital discharge, and alcohol consumption. There was a significant association between increasing prevalence of infection and increasing number of lifetime sexual partners in female and male farmers of rural communities of Tanzania. Unlike the finding of the present study, the association remained significant after adjustment for age and residence stratum (Obasi *et al.*, 1999).

On the other hand, HSV-2 seropositivity in the currently unmarried female and male Ethiopian subjects was found to be much less in comparison with the currently married female and male subjects although this significance is lost after adjusting for education, marital status, genital discharge, alcohol consumption and life time sexual partners.

The increased prevalence of HSV-2 in married subjects may be because that married subjects are relatively older than unmarried subjects and hence older age showed a confounding effect over being married in the statistical analysis.

In this study the married females' HSV-2 seroprevalence was relatively higher than that of the married males' HSV-2 seroprevalence. The same situation was reported

for wives and husbands, in studies of married couples in Atlanta, USA and Seville, Spain. These findings suggest an increased biologic susceptibility among females, which possibly is accounted for the anatomical or hormonal differences between the sexes (Johnson *et al.*, 1989; Long *et al.*, 2000).

A more or less similar prevalence (around 50%) of HSV-2 was found among the females, who were between the age of 25 and 34 and males, who were at the age of 45 and above. Obasi *et al.* (1999) explained the comparable prevalence of HSV-2 infection in younger females with that of the older males, to be a reflection of the age gap in marriage between males and females in Tanzania. This explanation may hold true in the Ethiopian subjects since there is similar trend of males marrying females much younger than themselves.

Income is also a factor that is associated with the increased prevalence of HSV-2 (Obasi *et al.*, 1999). HSV-2 seroprevalence was detected to be higher in those female subjects who earn medium income than those females with a higher income, a situation which remained significant even after adjusting for education, marital status, genital discharge, alcohol consumption and life time sexual partners.

Earning less money was shown to be a risk factor for increased prevalence of HSV-2 in female subjects since it may have forced them to have commercial sex to fulfil their economic needs and hence are more exposed to the infection. On the other hand, the significant association of HSV-2 seroprevalence with medium and high income in the male subjects was lost after adjusting for variables like education,

marital status, genital discharge and alcohol driniking. HSV-2 seropositivity was similarly associated with higher income in the Zimbabwean male factory workers as in the case in the male subjects in this study.

Contrary to this, in the USA, it is rather the lower income group which is associated with increased HSV-2 prevalence as in the medium income female Ethiopian subjects. A possible explanation for the higher HSV-2 prevalence of male Ethiopian as well as Zimbabawean male shows that men with high income can afford increased access to sexual partners; while the lower HSV-2 seroprevalence in the women with higher income could be explained by the cultural value of sexual restriction as a matter of high class dignity.

Lower education is also one of the risk factors for the increased prevalence of HSV-2 (Obasi *et al.*, 1999). HSV-2 seropositivity was detected to progressively decrease from Ethiopian female subjects who reported to have only informal education to females who reported to attend elementary school and finally to females who reported to have attended junior and secondary (grade 7-12) schools.

The possible explanation for this is that the relatively better educational status in females gives better awareness about the risks of unrestricted sex and hence would lead to reserved sexual behaviour. A decreasing trend in HSV-2 prevalence in the male subjects was also seen, as the level of education increased from non-formal to higher education. In agreement with the finding of the present study, Solomon,

(1992) reported that high school students are at a greater risk of being infected with STDs when compared with that of college students in Addis Ababa.

Gayle *et al.* (1990) reported that the use of alcohol and other drugs impair judgment and may lead one to unsafe sexual behaviour. The highly significant association of anti-HSV-2 antibody seropositivity in the male Ethiopian subjects who reported to drink alcohol some times and frequently could possibly be explained by such behavioural change induced through alcohol intoxication leading to sexual contact with commercial sex workers who possibly are carriers of HSV-2.

On the other hand, HSV-2 seroprevalence is not significantly associated with alcohol drinking iu the females who reported to drink alcohol sometimes. This may suggest that behavioural change leading to sexual contact may not be readily brought about in the females, after drinking alcohol, as a matter of dignity as it is demanded by the cultural values of most females.

5. Conclusions and recommendations

Strong association was found between HSV-2 seropositivity and different epidemiological as well as clinical risk factors examined in this study. Female sex, being older, seropositivity for syphilis and seropositivity for HIV were factors found to have more strong and significant association with HSV-2 seropositivity.

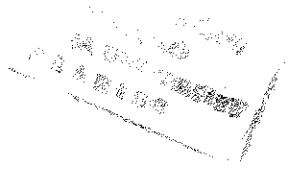
The analysis of findings showed that female sex has profoundly vivid association with HSV-2 seropositivity in the face of the other risk factors. The seroprevalence of HSV-2 in this study, 50.6% in the female and, 39.5% in the male subjects, is similar to what was reported from other African countries. The different HSV-2 prevalence rates in the female and male subjects can be explained by the anatomical as well as immunological differences between females and males with regard to HSV-2 infectivity (Long *et al.*, 2000).

The high prevalence of HSV-2 in older subjects is a phenomenon reported from several other studies. This indicates that it is most likely that people will get infected with HSV-2 as they begin to have multiple sexual partners, and hence the older they are the higher is the risk of being infected with HSV-2. Furthermore, the high prevalence to HSV-2 among younger females is comparable to the HSV-2 seroprevalence of older males. This suggests that younger females frequently have sexual contact with older males.

In general the ever changing epidemiology of STDs may in part account for the rapid spread of HIV in many parts of Africa (Moss *et al.*, 1990). STDs causing genital ulcer disease (GUD) may be considered major risk factors in facilitating HIV transmission by disrupting mucosal membranes, and providing an easy portal of entry (Morse *et al.*, 1997).

Although a very high prevalence of HSV-1 was determined in this study, no significant difference was found between HSV-1 seropositivity and HIV-1 infection. This insignificant association of HSV-1 seropositivity also holds true with the other sociodemographic factors, which were found to be significantly associated with that of HSV-2 infection in this study.

This study has confirmed the high prevalence of HSV-2 infection among adults of Addis Ababa. Such high prevalence is worrisome in view of its potential role as a co-factor for HIV transmission.



Finally, from the results obtained in this study, the following recommendations can be put forward:

1. Availability of HSV vaccines with proven safety and long term efficacy may change this situation. In the absence of a protective vaccine, prevention of HSV-2 infection will rely on the reduction of the number of sexual partners, faster treatment for other STDs, especially those causing genital ulceration and the use of condoms. As *Herpes simplex* virus is known to cause mucocutaneous ulcers in AIDS patients (Flaitz *et al.*, 1996) comprehensive intervention programmes for the prevention of STD and HIV must include HSV-2 control as well.

2. Since identification of modifiable co-factors such as HSV-2 that are associated with the sexual transmission of HIV have consistently been shown to facilitate transmission of HIV, special attention should be given to their accurate diagnosis and specific treatment (Martin, 1990). If the relationship between HSV-2 infection and HIV acquisition is indeed causal, then suppressive treatment of HSV-2 has biologic plausibility as an HIV prevention intervention. Suppression of HSV-2 among HIV-positive persons may be particularly important in preventing secondary transmission.

3. HSV-2 seropositivity can be considered as a serologic end point in the assessment of HIV prevention interventions that seek to reduce high-risk sexual behaviours. The presence or acquisition of antibodies to HSV-2 is an objective measure of high-risk

sexual behaviour. HSV-2 seropositivity among HIV negative subjects may be useful in identifying a subpopulation of persons at high risk of acquiring HIV. Thus, prevention resources and recruitment for prevention intervention studies may be targeted to such persons. In general, as Fleming *et al.* (1997) and James *et al.* (1998) observed earlier, attenders of Genito-Urinary Medicine (GUM) clinics have to be identified as a target population for educational and prevention interventions that aim to reduce the spread of STDs including HIV.

4. Increasing public awareness of genital herpes and other STDs should also be included in the national program to prevent and control HIV infection.

The draw backs of this study is that precise measure of sexual behaviour was not possible because of the information source, the subjects themselves, who may not forward the correct information.

Being unable to know the timing of seroconversion, i.e., whether HSV-2 seroconversion preceded HIV seroconversion or vice versa, whether or not clinical pictures like genital discharge as well as genital ulcer are generated because of HSV-2 were litmitations on this study. Moreover Physical examination rather than answering questioniare was a better way to reach at a correct diagnosis for the presence or absence of genital discharge and genital ulcer. Further studies should thus be encouraged by taking these points into account.

6. References

Anzala, O.A., Nagelkerke, N.J., Bwayo, J.J. (1995).Rapid progression to disease in African sex workers with human immunodeficiency virus type 1 infection. *Journal of Infectious Disease.* 171: 686-689.

Arvaja,M., Lehtinen, M., Koskela, P., Lappalainen, M., Paavonen, J., Vesikari, T. (1999). Serological evaluation of *Herpes simplex* virus type 1 and type 2 infections in pregnancy. *Sexually Transmitted Infections.* 75:168-171.

Ayehunie, S., Britton, S., Yemane-Berhane, T., Fehniger, T. (1987). Prevalence of human immunodeficiency virus (HIV) antibodies in prostitutes and their clients in Addis Ababa, Ethiopia. *IIIrd International conference on AIDS, Washington DC.* Abstract MP214.

Bonell, C., Weatherburn, P., Hickson, F. (2000). Sexually transmitted infection as a risk factor for homosexual HIV transmission: a systematic review of epidemiological studies.*AIDS.* 11: 697-700.

Broder, S., Meritan, T.C., Bolognesi, D. (1994). Immunopathogenesis. *Text Book of AIDS Medicine.* Williams and Wilkins. 55-75.

Brugha, R., Keersmaekers, K., Rentorn, A., Mehesus, A. (1997). Genital Herpes infection (review). *International Journal of Epidemiology.* **26:** 698-709.

Chiu, J.M., Yaniv, A., Dahlberg, J.E. (1985). Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. *Nature.* **317:** 366-368.

Chretien, F., Belec, L., Hilton, D.A., Flament-Saillour, M., Guillon, F., Wingertsmann, L., Baudrimont, M., De-Truchis, P., Keohane, C., Vital, C., Love, S., Gray, F. (1996). *Herpes simplex* virus type 1 encephalitis in acquired immunodeficiency syndrome. *Neuropathology and Applied Neruobiology.* **22:** 394-404.

Corey, L (1994). *Herpes simplex* virus. *Infectious Diseases and Their Etiologic Agents.* 1st ed. W.B.saunders company. U.S.A.1080-1089.

Corey, L and Handsfield, H.H. (2000). Genital herpes and public health: addressing a global problem. *Journal of Amenrican Medical Association.* **283:** 791- 794.

Duncan, M.E., Tibaus, G., Pelzer, A., Mehari, L., Peutheer, J., Young, H., Jamil, Perine, P.L. (1995). Prevalence and significance of sexually transmitted diseases among Ethiopian women attending antenatal clinics in Addis Abeba. *Ethiopian Journal of Health* Development. **1:** 31-40.

Edwards, S., Carne, C. (1998). Oral sex and the transmission of viral STIs. *Sexually Transmitted Infections.* **74:** 6-10.

Ellis, M. (1998). Specific viral lower respiratory tract infection of adults. *Infectious Diseases of the Respiratory Tract.* 1st ed. Cambridge University Press. U.S.A. 150-170.

Farland, W.M., Gwanzura, L., Bassett, N.T., Machekano, R., Latif, A.S., Ley,C., Pasonnet, J., Burke, R.L.,Katzenstein, D.(1999). Prevalence and incidence of *Herpes simplex* virus type 2 infection among male Zimbabawean factory workers. *The Journal of Infectious Diseases.***180:** 1459-65.

Flaitz, C.M.,, Nichols, C.M., Hicks, M.J. (1996). Herpesviridae-associated persistent mucocutaneous ulcers in acquired immunodeficiency syndrome. *Oral Surgery,Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics.* **81:** 433-41.

Fleming, D.T., Mcquillan, G.M., Johnson, R.E., Nahmias, A.J., Aral, S.O., Lee, F.K., Louis, M.E. (1997). *Herpes simplex* virus type 2 in the United States, 1976 to 1994. *The New England Journal of Medicine.* **337:** 1105-1111.

Fontanet, A.L., Tsehaynesh, M., Amare, D., Fikre, E., Almaz, A., Cutts,F,T., Rinke de Wit, T. Tefera, S., Bindels, P., Hailu Yeneneh., Coutinho,R.A., Nokes,D.J. (1998). Age-and sex-specific HIV-1 prevalence in the urban community setting of Addis Ababa, Ethiopia. *AIDS.* **12:**315-322.

Gallichan, W.S., Rosenthal, K.L. (1998). Long-term immunity and protection against *Herpes simplex* virus type 2 in the murine female genital tract after mucosal but not systemic immunization. *Journal of Infectious Diseases.* **177:** 1155-61.

Gayle, H.D., Keeling, R.P., Gracia-Tunon, M. (1990). Prevalence of HIV among University students. *New England Journal of Medicine.* **323:** 1538-1541.

Godard, C.M., Serries, H., Fulachier, C., Chermann, J.C. (1997). Apoptosis of CD4+ T cells induced after contact with HIV1-infected or non-infected macrophages. *Research in Virology.* **148:** 383-96.

Grosskurth, H., Mosha, F., Todd, J., Mwijarubgi, E., Klikke, A., Senkore, K., Mayaud, Philippe., Changalucha,J., Nicol, A., Ka-Gina, G., Newell,J., Mugeye, K., Mabey, D., Hayes, R. (1995). Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet.* 346: 530-36.

Gull Laboratories, Inc., (1997). *Herpes simplex* virus type –2 specific IgG ELISA test instructions. *GULL Lab.* USA.1-9.

Gwanzura, L., McFarland, W., Alexander, D.A., Burke, R,L., Katzenstien, D. (1998). Association between Human Immunodeficiency virus and *Herpes simplex* virus type 2 seropositivity among male factory workers in Zimbabwe. *The Journal of Infectious Diseases.* **177:** 481- 4.

Hachem, M.E., Bernardi, S., Pianosi, G., Krzysztofiak, A., Livadiotti, S., Gattinara, G.C. (1998). Mucocutatenous manifestations in children with HIV infection and AIDS. *Pediatric dermatology.* **15:** 429-434.

Halford, W.P., Veress, L.A., Gebhardt, B.M., Carr, D.J. (1998). Immunization with HSV-1 antigen rapidly protects against HSV-1 antigen rapidly protects against HSV-1 induced encephalitis and is IFN-gamma independent. *Journal of Interferon and Cytokine Research.* **18:** 151-8.

Harrison's (1996). *Principles of internal medicine.* (13th ed) Mc Graw-hill. U.S.A. **1:** 782-787.

Hirsch, M.S. (1994). *Herpes simplex* virus. *Infectious Diseases and Their Etiologic Agents.* (1st ed). W.B.Saunders company. England. 1336-1345.

Hirsch, V.M., Olmsted, R.A., Murphey-Corb, M., Purcell, R.H and Johnson, P.R. (1989). An African primate lentivirus Simian immunodeficiency virus (SIM_{sm}) closely related to HIV-2. *Nature* . **339:** 389-392.

Hoog, S.S., Smith,W.W., Qiu,X., Janson, C.A., Hellmig, B., McQueney, M.S., O'Donell, K.,Shannessy, D.,Dilella, A.G., Debouck, C., Abdel-Meguid, S.S. (1977). Active site cavity of Herpes virus proteases revealed by the crystal structure of *Herpes simplex* virus protease/inhibitor complex. *Biochemistry.* **36:** 1423-9.

Hughes, G., Catchpole, M., Rogers, P.A., Brady, A.R., Kinghorn, G., Mercey, D., Thin, N. (2000). Comparison of risk factors for four sexually transmitted infections: results from a study of attenders at three genitourinary medicine clinic in England. *Sexually transmitted infection.* **76:** 262-267.

Hunt, C.W. (1989). Migrant labour and sexually transmitted disease: AIDS in Africa. *Journal of Health and Social Behaviour.* **30:** 353-373.

James, N.J., Gillies, P.A., Bignell, CLJ. (1998). Evaluation of a randomised controlled trial of HIV and sexually transmitted disease prevention in a genitourinary medicine clinic setting. *AIDS.* **12:**1235-1242.

Janeway, C.A., Travers, P. (1994). Acquired immune deficiency syndrome (AIDS). *Immuno Biology: The immune system in health and disease.* Current Bilogy Ltd., Middlesex House. **10:**27-10:42.

Johnson, R.E., Nahmias, A.J., Magder, L.S., Lee, F.K., Brooks, C.A., Snowden, C.B. (1989). A seroepidemiologic survey of the prevalence of *Herpes simplex* virus tye 2 infection in the United States. *The New England Journal of Medicine* **321:** 7-12.

Kebede, Y., Pickering, J., McDonald, C., Wotton, K., Zewdie, D.W. (1991). HIV infection in an Ethiopian prison. *American Journal of Public Health.* **81:** 625-627.

Kefenie, H., Desta, B., Zewdie,D.W. (1992). HIV-1 seroprevalence in Ethiopia over 5 years interval. *VIIIth International conference on AIDS/STD World Congress, Amsterdam, The Netherlands.* Abstract 4017

Koutsyk, L.A., Stevens, C.E., Holmes, K.K., Ashley, R.L., Kiviat, N.B., Critchlow, C.W., Corey, L. (1992). *The New England Journal of Medicine.* **326:** 1533-1539.

Langeland, N., Haarr, L., Mhalu, F. (1998). Prevalence of HSV-2 antibodies among STD clinic patients in Tanazania. *International Journal of Sexually Transmitted Diseases and AIDS.* **9:** 104-7

Laubereau, B., Zwahlen, M., Neuenschwander, B., Heininger, U., Schaad, U.B., Desgrandchamps, D. (2000). *Herpes simplex* virus type 1 and 2 in Switzerland. Schweiz. Med. *Wochenschr.* **130:**143-50.

Long, E.M., Martin,H.L., Kreiss, J.K., Rainwater, S.M., Lavreys, L., Jackson, D.J., Rakwar, J., Mandaliya, K., Overbaugh, J.(2000). Gender differences in HIV-1 diversity at time of infection. *Nature Medicine.* **6:** 001-008.

Mahy, B.W.J.(1991). Growth, assay and purification of *Herpes* viruses. *Virology a practical approach.* (1st ed). Oxford University Press. England. 207-236.

Marlink, R., Kanki, P., Thior, I. (1994). Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science.* **265:** 1587-1590.

Martin, D.H. (1990).The interrelationship between human immunodeficiency virus infection and other sexually transmitted diseases. The medical clinics of north America: *Sexually Transmitted Diseases.* (1st ed). W.B.Saunders Company. U.S.A.74: 1647-1660.

Mbopi-Keou, F.X., Gresenguet,g., Mayaud, P., Weiss, H.A., Gopal, R., Matta, M., Paul,, J.L., Brown, D.W., Hayes, R.J., Mabey, D.C., Belec, L. (2000). Interactions between *Herpes simplex* virus type 2 and human immunodeficiency virus type 1 infection in African women: opportunities for inervention. *Journal of Infectious Disease.* **182:** 1090-6.

Mehret, M., Kodakevich, L., Zewdie, D.W., Shanko, B. (1990). Progression of human immunodeficiency epidemic in Ethiopia. *Ethiopian Journal of Health Develpoment.* **4:** 183-90.

Mehret, M., Khodakevich, L., Zewdie, D,W. (1990). HIV-1 infection and some related risk factors among female sex workers in Addis Ababa. *Ethiopian Journal of Health Development.* **4:** 171-176.

Mengistu, M., Khodakevich, L., Bekele, S., Fikirte, b. (1990). Sexual behaviours and some social features of female sex workers in the city of Addis Ababa. *Ethiopian Journal of Health Development.* **4:** 133-137.

Mettenleiter, T.C. (1994). Initiation and spread of *α-Herpes* virus infections. *Trends in Microbiology.* **2:** 2-4.

Mindle, A., Taylor, J., Tideman, R..L., Seifert, C., Berry, G., Wagner, K., Page,J., Marks, C., Trudinger, B., Cunningham, A. (2000). Neonatal Herpes prevention: a minor public health problem in some communities. *SexuallyTransmitted Infections.* **76:** 287-291.

MOH, (2000). The HIV/AIDS Pyramid. AIDS in Ethiopia. 6

Morse, S.A., Trees, D.L., Htun, Y., Radebe, F., Orle, K.A., Dangor, Y., Beck-Sague, C.M., Schmid, S., Fehler, G., Weiss, J.B., Ballard, R.C. (1997). Comparison of clinical diagnosis and standard laboratory and molecular methods for the diagnosis of gental ulcer disease in Lesotho: Association with Human immunodeficiency virus infection. *The Journal of Infections Diseases.* **75:** 583-9.

Moss, G.B. and Kreiss, J.K.(1990). The interrelationship between Human immunodeficiency virus infection and other sexually transmitted diseases. *Sexually Transmitted Diseases* **74:** 1147- 1661.

Mostad, S.B., Kreiss, J.K., Ryncarz, A.J., Mandaliay, K., Chohan, B., Ndinya, J., Bwayo, J.J., Corey, L. 2000. Cervical shedding of *Herpes simplex* virus in Human Immunodeficiency virus-infected women: Effects of hormonal contraception, pregnancy, and vitamin A deficiency. *The Journal of Infectious Diseases.* **181:** 58- 63.

Myron,S.C.1998. Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis. *Lancet* **351**:5-7.

Nahmias, A.J., Lee, F.K., Beckman-Nahamias, S. (1990). Sero-epidemiological and sociological patterns of *Herpes simplex* virus infection in the world. *Scandinivian Journal of Infectious Diseases.* **69:** 19-36.

Nascimento, M.C., Sumita, L.M., Souza, Vanda, A.U.F., Souza, V.A., Pannuti, C.S. (1998). Detection and direct typing of *Herpes simplex* virus in prerianal ulcers of patients with AIDS by PCR. *Journal of Clinical Microbiology.* **36:** 848-849.

Obasi, A., Mosha, F., Quigley, M., 1999. Antibody to *Herpes simplex* Virus Type 2 as a Marker of Sexual Risk Behaviour in Rural Tanzania. *Journalof Infectious Disease.* **179:**16- 24.

Pelczar, M. J., Chan, E.C., Krieg, N.R. (1998). *Herpesviridae. Microbiology.* 2nd ed. McGraw-hill book company. U.S.A. 840-841

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

Poss, M and Overbaugh, J. (1999). Variants from the diverse virus population identified at seroconversion of a Clade A human immunodeficiency virus type infected woman have distinct biological properties. *Journal of virology.* **73:** 5255-5264.

Posavad, C.M., Huang, M.L., Barcy, S., Koelle, D.M., Corey, L. (2000). Long term persistence of *Herpes simplex* virus-specific CD8+ CTL in persons with frequently recurring genital herpes. *The Journal of Immunology.* **165:** 1146-1152.

Reiter, G.S. (1998). Human immunodeficiency virus (HIV) in America. *Seminars in Ultrasound, CT and MR.* **19:** 122-7. Richards, C.M., Shimeld, C., Williams, N.A., Hill, T.J. (1998). Induction of mucosal immunity against *Herpes simplex* virus type 1 in the mouse protects against ocular infection and establishment of latency. *The Journal of Infectious Diseases.* **177:** 1451-7.

Rosa-Santos, O.L., Silva, A.G., Pereira, A.c. (1996). *Herpes simplex* virus type 2 in Brazil seroepidmiologic survey. *International Journal of Dermatology.* **35:**794-796.

Scott, D.A., Coulter, W.A., Lamey, P.J. (1997). Oral shedding of *Herpes simplex* virus type 1: a review. *Journal of Oral and Pathological Medicine.* **26:** 441-7.

Schmid, D.S., Thieme, M.L., Gary, H.E., Reeves. W.C. (1997). Characterization of T cell responses to *Herpes simplex* virus type 1 and type 2 (HSV-1 and 2) using a TNF-beta ELISpot cytokine assay. *Virology.* **142:** 1659-71.

Smith, A., Denham, I., Keogh, L., Jacobs, D., Harg, V.N., Marceglia, A. and Wood, V. (2000). Psychosocial impact of type-specific *Herpes simplex* serological testing on asymptomatic sexual health clinic attendees. *International Journal of STD and AIDS.* **11:** 15-20.

Solomon Belayneh. (1992). *Assessment of HIV among student of high schools and colleges attending clinics for sexually transmitted diseases in Addis Ababa.* MSc Thesis. School of Graduate Studies, Addis Ababa University. 39.

Solomon, D., Workneh, F., Mohammed, Y., Mengistu, M., Aberra, G., Massimo, G., Khodakevich, L., Debrework, Z., Carosi, G. (1990). Prevalence of STD and STD related risk factors in sex workers of Addis Ababa. *Ethiopian Journal of Health Development.* **4:** 149-153.

Stamm, E., Handsfield, H., Rompalo, A.M., Ashley, L., Roberts, P.L., Correy, L. (1988). The association between genital ulcer disease and acquistion of HIV infection in homosexual men. *Journal of American Medical Association.* **260:** 142

Stanberry, L.R. (1995). *Herpes simplex* virus vaccines as immunotherapeutic agents. *Trends in microbiology.* **3:** 244-247.

Straus, S.E.1985. *Herpes simplex* virus infection: Biology, treatment, and prevention. *Ann..Intern..Med.* **103**:404-19.

Su, S.J., Wu, H.H., Lin, Y.H., Lin, H.Y. (1995). Comparative studies of type 1 and 2 *Herpes smplex* virus infection of cultured normal Keratinocytes. *Developments in Biological Standardaization.* 48: 75-9.

Sucato, G., Wald, A., Wakabayashi, E., Vieira, J., Corey, L. (1998). Evidence of latency and reativation of both *Herpes simplex* virus (HSV)-1 and HSV-2 in the genital region. *The Journal of Infectious Diseases.* **177**: 1069-72.

Tefera Sahlu., Elias Kassa., Tsehay Agonafer., Aster Tsegay., Rinke de Wit, T., Hiruy, Gebremariam., Doorly, R., Spijkerman., Hailu Yeneneh., Coutinho, R.A., Fontanet, A. (1999). Sexual behaviours, perception of risk of HIV infection, and factors associated with attending HIV post-test counselling in Ethiopia. *AIDS.* **13:** 1263-1272.

Tsega, E, Mengesha, B., Nordenfelt, E. (1988). Serological survey of human immunodeficiency virus infection in Ethiopia. *Ethiopian Medical Journal.* **26:** 179-184.

UNAIDS/WHO, (2000). Adults and children estimated to be living with HIV/AIDS at the end 2000. 3

Van de Perre, P., Rouvroy,D., Lepage, P. (1984). Acquired immunodeficiency syndrome in Rwanda. *Lancet.* **2:** 62-65.

Yared, M., Aster, T., Rinke, W.T, Asfaw, B., Sanders, E., Fontanet, A. (2000). Stabilisation of HIV-1 prevalence in Addis Ababa. Evidence from sentinel surveillance among pregnant women. *Ethiopian Medical Journal.* **38:** 212

Zewdie, D.W., Teferi, N., Kebede, T., Gebrehiwot, B. (1990). Seroprevalence of HIV and syphilis among child bearing age women in Central Ethiopia. *VI International conference on AIDS, San Francisco.* Abstract FC600.