PREVALENCE, INCIDENCE AND RISK FACTORS FOR HERPES SIMPLEX VIRUS TYPE 2 INFECTION IN COHORTS OF FACTORY WORKERS, AKAKI AND WONJI, ETHIOPIA, 1997-2002

By

YENEW KEBEDE TEBEJE

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Prevalence, incidence and risk factors for *Herpes simplex virus* type 2 infection in cohorts of factory workers, Akaki and Wonji, Ethiopia, 1997-2002

A Thesis submitted by:

YENEW KEBEDE JEBEJE

Department of Medical Microbiology, Immunology and Parasitology

Faculty of Medicine

Approved by the Examination Board:

Name	Signature
_____ External Examiner	_____
_____ Internal Examiner	_____
_____ Research Advisor	_____
_____ Research Advisor	_____
_____ Chairman	_____

SIGNED DECLARATION

I, the undersigned, declare that this thesis is my own work and it has not been presented in other Universities, Colleges or other institutions for similar degree or other purposes and that all sources of material used for the thesis have been duly acknowledged.

Yenew Kebede	_____	_____
Name	Date of submission	signature

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ABBREVIATIONS

AAU: Addis Ababa University

AIDS: Acquired Immunodeficiency Syndrome

CI: Confidence Interval

CNS: Central Nervous System

DNA: Deoxyribonucleic acid

EDTA: Ethylene Diamine Tetra acetic acid

EHNRI: Ethiopia Health and Nutrition Research Institute

ELISA: Enzyme Linked Immunosorbent Assay

ENARP: Ethio-Netherlands AIDS Research Project

ETB: Ethiopian Birr

GUD: Genital Ulcer Disease

HIV: Human Immunodeficiency Virus

HR: Hazard Ratio

HSV: Herpes simplex virus

HSV-1: Herpes simplex virus type 1

HSV-2: Herpes simplex virus type 2

MOH: Ministry of Health

OR: Odds Ratio

OD: Optical Density

PBS: Phosphate Buffer Saline

pys: person-years

RPR: Rapid Plasma Reagen

STDs: Sexually Transmitted Diseases

TPPA: Treponema pallidum Particle Agglutination Assay

UNAIDS: Joints United Nations Programme on HIV/AIDS

USA: United States of America

WB: Western Blot

ABSTRACT

Retrospective cohort study (1997-2002) was conducted to determine the prevalence, incidence and risk factors for Herpes simplex virus type 2 (HSV-2) infections among factory workers at two sites in Ethiopia. Among enrolled participants in a cohort study of HIV incidence and disease progression, 1222 (71.3%) were males and 491 (28.7%) were females. The median age was 35 years for males (range, 19-62) and 33 years (range, 19-46) for females. The serologic status of each stored plasma sample for HIV and syphilis has been routinely determined. Antibody to HSV-2 was detected by using the HerpeSelect ^{TM} 2 ELISA IgG, Focus Technologies, USA. The HSV-2 prevalence at enrollment was 41.1%, 57.2% among females and 34.6% among males ($p<0.001$). Seventeen-percent of females and 9.7% of males younger than 25 years had evidence of HSV-2 infection. Independent risk factors for HSV-2 seropositivity were HIV seropositivity, positive TPPA serology, older age, female sex, and ever being married. The incidence of HSV-2 during follow-up was 1.2/100 person-years (95%CI, 1.0-1.4), 2/100 person-years (95% CI, 1.2-3.2) among females and 0.9/100 person-years (95%CI, 0.7-1.3) among males ($p=0.02$, assuming Poisson distribution). Positive HIV serology at enrollment was an independent risk factor for HSV-2 seroconversion (adjusted hazard ratio [HR], 2.5; 95% CI, 1.1-5.6). Most of HSV-2 infections were asymptomatic or had minor symptoms, which were not recognized. The study showed high prevalence and incidence of HSV-2 infection. This large number of herpes infected individuals may continue to engage in unprotected sexual activity despite their infection facilitating the sexual transmission of HSV-2 and HIV making the vicious cycle. In the absence of protective vaccine or effective antiviral therapy, prevention of HSV-2 infection will rely on the widespread use of condoms and reduction in the number of sexual partners with emphasis to prevention of HSV-2 infection at early ages.

Key words: HSV-2, incidence, prevalence, seroconversion, Ethiopia

I. INTRODUCTION

1.1. Herpes viruses

Approximately 100 herpes viruses have been identified with at least 8 infecting humans. All human herpes viruses are adapted to their natural host, being endemic in all human populations studied and carried by a significant fraction of persons in each population. The human herpes viruses include: Herpes simplex virus, Varicella zoster virus, Epstein Barr virus, Cytomegalovirus, Human herpes virus 6, Human herpes virus 7 and recently discovered Human herpes virus 8 or Kaposi's sarcoma-associated virus (Roizman and Baines, 1991). All the viruses in this family have common characteristics like expression of a large number of viral enzymes, assembly of the nucleocapsid in the cell nucleus, destruction of the cell during productive infection, and ability to establish latent infections in the infected host. Based on biologic differences they are classified into three sub families. Among these the alpha herpes viruses are neurotropic viruses that replicate relatively rapidly and infect wide range of cells in cell culture. The herpes simplex virus is included in this family (Roizman and Baines, 1991; Ellis, 1998).

Herpes simplex viruses (HSVs) are double stranded DNA viruses with icosahedral capsid. They have a lipid envelope, which contains numerous glycoproteins inserted in it. The glycoproteins may be important for infectivity because some of them interact with cellular receptors on the different cell types. Their genome has an approximate diameter of 180 nm (Whitley and Gnann, 1993; Ellis, 1998). There are two antigenic types: Herpes simplex virus type 1 (HSV-1) and Herpes simplex virus type 2 (HSV-2). They share some antigenic cross-reactivity but they have different glycoproteins and hence have different neutralization

patterns and tend to produce different clinical symptoms (Roizman and Baines, 1991; Whitley and Gnann, 1993; Ellis, 1998).

1.2. The Herpes simplex virus types 1 and 2

The pathology and epidemiology of herpes virus infection depends not only on viral replication and associated cytotoxicity but also on the capacity of herpes viruses to establish latent infection. Latent infections or latency means that the genome of the infecting virus is stably maintained by the cell with only limited expression of viral genes, no production of progeny virus, and no evident virus induced cytotoxicity (Whitley *et al*., 1998). During primary infection, the virus enters peripheral sensory nerves and migrates along axons to sensory nerve ganglia in the central nervous system (CNS). This allows them to escape from the immune surveillance of the host and establish latent infections. Reactivation of latent virus leads to recurrent disease, the virus travels back down sensory nerves to the surface of the body and replicates, causing tissue damage. The site of latency and recurrences is mostly different for the two viruses: mostly sacral ganglia in the case of HSV-2, trigeminal ganglia and other nerve roots in the upper part of the body for HSV-1 (Benedetti *et al*., 1994; Sucato *et al*., 1998).

HSV-1 and HSV-2 cause infections, which are ranging from mild stomatitis to disseminated and fatal diseases. Infections by HSV-1 and HSV-2 can differ in their clinical manifestation and severity. HSV-1 infection is acquired during childhood and adolescence and is markedly more wide spread than HSV-2 infection (Smith and Robinson, 2002). It is closely associated with orolabial infections, with one-fourth to one-half of all individuals under 18 years of age

testing positive for antibodies of type 1 virus. Infection by this virus is therefore, universal and seropositivity increases with the age of individual but antibodies to HSV-2 are not routinely detected in sera before puberty (Sucato *et al*., 1998; Smith and Robinson, 2002).

1.3. Clinical significance

The clinical manifestations of the genital herpes infections are highly variable. The initial presentation can be severe with painful genital ulcers, dysuria, fever, tender local lymphadenopathy, and headache. In many patients, however, the infection is mild, sub clinical or entirely asymptomatic. Some studies have shown that the clinical manifestation of primary genital herpes tends to be more severe in women than men (Corey *et al*., 1983; Benedetti *et al*., 1994). Orolabial or genital HSV-1 infections can influence the clinical features of subsequent HSV-2 infection. Patients with serologic evidence of prior HSV-1 infection are less apt to have systemic symptoms and have a lower rate of complications and a shorter duration of disease than persons with true primary genital herpes. Neutralizing antibody to HSV has been found to inactivate extra cellular virus and interrupt the spread of HSV infections. In addition, a cellular immune response to HSV antigens appears earlier in persons with non-primary genital HSV infections than in persons with true primary infections. It is likely that both of these immune mechanisms account for the clinical differences between primary and non-primary first episodes of genital herpes (Benedetti *et al*., 1994; Stanberry *et al*., 1997).

Most infections with HSV-2 in individuals with existing antibodies for HSV-1 are sub clinical or asymptomatic (Stanberry *et al*., 1997). Recurrences are common following primary

genital herpes and the frequency of recurrence may correlate with the severity of primary infection. Administration of acyclovir as episodic therapy decreases duration of pain, induces faster healing of lesions and shortens the duration of HSV-2 shedding but did not influence recurrence rate. Suppressive therapy prevents or delays 80-85% of recurrences but this is expensive especially, in developing countries (Benedetti *et al*., 1994). When compared to primary infection, recurrent HSV infection is typically associated with fewer lesions in a unilateral, rather than bilateral distribution. The mean duration of lesion is generally shorter (10 days compared to 19) and systemic symptoms are infrequent. The duration of viral shedding is also short, usually 5 days (Nahmias *et al*., 1990; Benedetti *et al*., 1994; Stanberry *et al*., 1997).

The complications of genital herpes are related to both local extension and spread of the virus to extra genital sites. Central nervous system involvement as aseptic meningitis, transverse myelitis or sacral radiculopathy may be encountered. Autonomic nervous system involvement leads to hyperesthesia or anesthesia of the perineal, lower back or sacral regions and urinary retention and constipation. Fungal super infection of the lesions may occur and in general, complications of the primary genital herpes are more frequent in women than men (Corey *et al*., 1983; Wasserheit, 1992; Benedetti *et al*., 1994).

1.4. The public health impact of genital herpes

The public health impact of genital herpes is increasingly recognized. The disease is characterized by long latency and episodic symptoms. Unlike patients with other genital ulcerative diseases, those with genital herpes are often unaware of prior infections making

serologic evidence a more accurate predictor of prior genital herpes (Benedetti *et al*., 1994; Wald *et al*., 1995). Genital herpes is usually caused by HSV-2, HSV-1 accounting for small proportion of the infection. Symptomatic recurrences and asymptomatic viral shedding is also less frequent as compared to those with HSV-2 infection (Wald *et al*., 1995; Stanberry *et al*., 1997). Therefore, emphasis is given to HSV-2 genital infection.

HSV-2, a major cause of genital ulcer disease (GUD), is highly prevalent in human populations worldwide (Nahmias *et al*., 1990). It is a major global health problem. The high prevalence of the infection, its associated morbidity, the frequent recurrences of the clinical episodes and its complications such as aseptic meningitis and neonatal transmission render this disease of great concern to patients and health care providers (Wasserheit, 1992; Brown *et al*., 1997). Multiple interactions between HSV and HIV both on the epidemiological and clinical levels, the fact that there is no effective vaccination against the disease and possibilities of recurrences even after treatment have further emphasized the importance of this infection. Increasing proportions of genital ulcers may be attributed to HSV-2 in many parts of Africa (O'Farell, 1999). Especially, recurrent genital ulcer is most commonly caused by HSV-2. In addition to genital lesions, infection with HSV-2 can result in encephalitis and neonatal herpes. In African countries there is a great concern about the effects of HSV-2 (genital ulceration, neonatal herpes) and its potential to boost the HIV epidemic (Auvert *et al*., 2001; Morand, 2002).

The frequency and recurrences of HSV-2 infection appear to increase during pregnancy and transmission to neonates is common from asymptomatic mothers who acquire primary genital herpes infection during the third trimester. Transmission rate is much higher when the mother

is experiencing a primary or initial genital infection (> 50%) versus a recurrent infection (<5%). Most mothers (70%) who transmit HSV to their children are asymptomatic at delivery (Brown *et al*., 1997; Morand, 2002). The neonate acquires the infection at the time of delivery through contact with HSV-2 in the birth canal. Because the survival out of the oral-genital secretions is weak, indirect and /or nosocomial transmission of HSV are very rare and should be controlled by common-sense precautions (Morand, 2002). Seropositivity for HSV-2 is therefore, associated with viral shedding in the genital tract, even in subjects with no history of genital ulcer and most HSV-2 infections are acquired from a person with no history of symptomatic genital herpes infection (Benedetti *et al*., 1994; Wald *et al*., 1995; Bossi, 2002).

The prevention of the acquisition of genital or neonatal HSV infections is a challenge because it is based on the understanding and the control of asymptomatic shedding. Currently, there is no proven completely effective means of prophylaxis of HSV. Condoms decrease the transmission of the disease. The high prevalence of asymptomatic and atypical HSV infection implies that development of an effective HSV vaccine is the best approach for the prevention of HSV infections. There are two main categories of HSV-2 vaccines on trial: prophylactic vaccines aimed at protecting against HSV-2 infection in the uninfected individuals have been shown to work in animal experiments, therapeutic vaccines are expected to reduce frequency and/ or severity of recurrences in the infected individuals. The availability of an effective therapeutic vaccine would be useful in addition to prophylactic vaccines because of the high prevalence of HSV-2 in many countries. Their main disadvantage is the likely need for frequent vaccination. The effort has concentrated on glycoproteins D and B as the immune response to these glycoproteins appears to induce high levels of neutralizing antibodies.

Recently a DNA vaccine encoding gD2 has shown some protection in both human and animal models (Stanberry *et al*., 2002). These products are currently either in preclinical or early clinical stages of development.

1.5. The Human immunodeficiency virus pandemic and role of sexually transmitted diseases

From its discovery in 1981, the Human immunodeficiency virus (HIV) has been a global problem. According to UNAIDS estimates (2002), 42 million people all over the world are living with HIV. About 29.4 million are in sub-Saharan Africa and 2.7 million are children under 15 years of age. Five million people are newly infected per year, which approximately is the same as fourteen thousand new HIV infections per day and three million people died of the Human Acquired Immunodeficiency Syndrome (AIDS) in the same year.

According to Ethiopian Disease Prevention and Control Department of Ministry of Health (2002), an estimated 2.2 million people are living with HIV/AIDS, including 2 million adults and 200,000 children. Most people do not know that they are infected because they have no visible symptoms they can easily recognize. However, almost all will develop AIDS and die within the next 10 years or so.

The HIV pandemic continues to worsen, particularly in many developing countries where more than 80% of HIV transmission occurs heterosexually (Hayes *et al*., 1995; Nelson *et al*., 1998). Several investigators have proposed that one important reason for the more extensive heterosexual transmission of HIV in Africa is that other sexually transmitted diseases (STDs)

are quite common and act as additional risk factors facilitating the sexual transmission of HIV. In addition to systemic factors such as immunosupression, hormonal contraceptive use, or micronutrient deficiency, local factors such as the presence of other STDs, specially those causing genital ulceration are believed to increase the risk of HIV transmission by increasing the amount of HIV shedding through genital lesions and by providing an easier portal of entry of the virus in to the host (Hook et al., 1992; Sittitrai and Brown, 1994; Hayes *et al*., 1995; Bonell *et al*., 2000). The local host defense for both syphilis and herpes for example, predominantly involves mononuclear cells in ulcer bases, patients with these infections may potentially transfer more HIV infected lymphocytes or free HIV virions to sexual partners. The hypothesis that the relatively high prevalence of untreated STDs in sub-Saharan Africa is a contributing factor for the higher prevalence of heterosexually transmitted HIV compared with the industrialized world is supported by the success of an HIV prevention intervention based on community-wide enhanced STD treatment in Mwanza, Tanzania (Gross-Kurth *et al*., 1995).

However, the roles of specific STDs in HIV infection have not been easy to evaluate because, the wide spread availability and use of antibiotics in some countries by populations commonly infected with STDs has rendered their diagnosis difficult. Availability of diagnostic facilities is also limited. In addition, it is difficult to separate the behavior that led to STDs from the potential causal effect of the STDs in HIV transmission (Fleming and Wasserheit, 1999). Most therapeutic decisions made by infected patients or medical practitioners are based on syndromic diagnosis rather than microbiologic identification of specific pathogens. Even when microbiologic laboratory support is available in developing countries, *Haemophilus ducreyi* is often difficult to isolate and culturing of HSV-2 may not be

available or may be too expensive to perform routinely. Recently, multiplex polymerase chain reaction, a more sensitive and specific method, has been reported for detecting the organisms that commonly cause genital ulcers (Morse *et al*., 1997). But this is even more expensive.

1.6. The Human immunodeficiency virus and Herpes simplex virus type 2

HIV infection results in destruction of the immune system leading to profound immunosupression and development of opportunistic infections. It is unclear whether opportunistic infections contribute directly to HIV clinical progression through T-cell activation with subsequent HIV replication or through T-cell destruction or both. HSV-2 infections and recurrences would be expected to increase in frequency and severity in areas with high HIV prevalence, because of immunosupression (Nahmias *et al*., 1990). Particularly, reactive HSV-2 infections are found with increased frequency in HIV infected patients. Many studies have shown that the genital shedding of HSV occurs with higher frequency among HIV infected individuals. The other conditions associated with increased frequency of asymptomatic HSV shedding include use of oral contraceptive pills, pregnancy and vitamin A deficiency (Mostald *et al*., 2000). This asymptomatic shedding of HSV, which frequently occurs in individuals with genital herpes, accounts for the majority of HSV infections transmitted to sexual partners and neonates.

An acute HSV-2 episode can result in increased HIV replication and plasma viral load (Mole *et al*., 1997). International studies show that replication-competent HIV-1 persists in active herpetic genital ulcers and is often in higher titer, whether the plasma HIV titers are high or low (Mole *et al*., 1997; Schaker *et al*., 1998). These findings suggest that HSV-2 may act as a

local cofactor in sexual transmission of HIV. It has the potential to contribute to the continual spread of HIV infection because of its recurrent nature, due to the high prevalence of genital herpes in populations at risk to HIV infection and to the large number of herpes infected individuals who continue to engage in sexual activity despite their infection (Gwanzura *et al*., 1998; Schaker *et al*., 1998).

1.7. Herpes simplex virus antibody as biological marker of sexual activity

An estimated 90% of all HIV infections occur in low-income settings where heterosexual intercourse is the predominant mode of transmission. Prevention is still the main tool for controlling HIV epidemics. Programmes aiming to change sexual behaviors have formed the backbone of prevention efforts and have been heavily utilized since the start of epidemic. Ideally, evaluation of behavioral change interventions directed at HIV transmission would be based on the HIV incidence (Van de Laar *et al*., 1998), but its use may be problematic because of ethical considerations surrounding testing and the large sample sizes that are needed. The problem of large samples is particularly acute where HIV incidence is still low but where there is also the greatest potential to prevent large-scale epidemics. An alternative outcome for evaluation is the incidence of other sexually transmitted infections, which, sharing a common mode of transmission with HIV may provide an indication of pattern of HIV risk behavior. The usefulness of any such biological marker depends on the availability of accurate diagnostic tests, incidence and prevalence of infection, the pattern of sexual networking in the target population (Stephenson *et al*., 2000).

In view of the HIV epidemic and the great burden of ill health caused by other STDs in the region, the study of sexual behavior, the design, evaluation and implementation of interventions to decrease sexual risk behavior have assumed increasing importance. However, the study of sexual behavior is problematic. By its nature it cannot be directly observed, and consequently only indirect information may be obtained from questionnaires, interviews and focus group discussions and other qualitative methods. Due to the sensitive nature of the issues raised, these methods are subject to considerable bias and can be difficult to reproduce. An inexpensive and reliable biologic marker of sexual activity would be an invaluable tool in HIV and STD research (Van de Laar *et al*., 1998; Whittington *et al*., 2001).

HSV-2 has been suggested as a suitable biological marker to capture patterns of HIV risk behavior. HSV-2 infection is responsible for considerable morbidity in populations of reproductive age and share a common route of transmission with HIV (Stanberry *et al*., 1997). Culture and antigen detection techniques for HSV-2 are insensitive in the absence of ulcers. These methods cannot be used for large scale epidemiological studies because the disease has long period of latency and most infections are asymptomatic. The fact that HSV-2 is almost exclusively sexually transmitted and infection leads to the production of life long antibodies which can be detected in patients' serum, makes antibody detection an important biological marker of sexual activity. Seropositivity is associated with high-risk sexual behavior in all populations (Wasserheit, 1992; Langeland *et al*., 1998).

1.8. Recent development of type-specific assays for herpes simplex viruses

Seroepidemiologic studies of HSV infections have been hampered because of difficulty in accurately identifying antibodies to HSV-1 and HSV-2, especially in persons who have been infected with both viruses. Knowledge of HSV diagnosis is useful in explaining the potential infectivity during episodes of lesions, identifying persons at risk for transmitting infection sub clinically, selecting women at future risk for transmitting infection for neonates, and confirming the diagnosis in those in whom antiviral chemotherapy is prescribed. New immunologic assays that use type-specific glycoproteins such as glycoprotein G1 (gG1) and glycoprotein G2 (gG2) distinguish HSV-2 from HSV-1 infection and permit better understanding of the epidemiology of genital herpes. These assays allow the detection of HSV-2 in the presence of HSV-1 antibodies and vice versa. The identification based on the gG showed that there is no known cross-reactivity between the gG in HSV-1 (gG1) and that in HSV-2 (gG2) (Whittington *et al*., 2001). The type-specific serologic tests can be used to assess the prevalence of HSV-2 in populations, to monitor changes over time and to identify asymptomatic and sub clinically infected individuals. Serologic testing for genital herpes identified more individuals infected with HSV-2 than were recognized clinically (Langeland *et al*., 1998; Obasi *et al*., 1999; Whittington *et al*., 2001). Therefore, monitoring antibodies for HSV-2 may be used to evaluate interventions programs in age cohort over time and new infection with HSV-2 is considered a valid proxy for unprotected sexual behavior (Cowan *et al*., 1994).

1.9. Studies of Herpes simplex virus type 2

There is an urgent need to consider potential control measures for HSV-2 that might be applied in an effort to reduce HIV transmission. For this purpose data on the HSV-2 prevalence and incidence must be available. Most previous studies of prevalence and risk factors for HSV-2 infection have been done in selected groups, such as antenatal and STD clinic attendants and blood donors in industrialized countries (Langeland *et al*., 1998; Bonell *et al*., 2000). Few population based surveys reporting high seroprevalence rates in adults (60-80%) have been recorded (Cowan *et al*., 1994; Obasi *et al*., 1999; Mbopi-Keou *et al*., 2000). In sub-Saharan Africa, studies of HSV-2 have been scarce, owing to lack of available laboratory tests and facilities. This is the area where increasing proportion of genital ulcers is attributed to HSV-2 and with high prevalence of HIV-1 infection. In addition, HSV-2 shedding from the genital tract of asymptomatic and seropositive people is similar to those with history of symptomatic herpes infection (Gilson and Mindel, 2001). So, both have significant role in disease transmission. Only few longitudinal studies have addressed the impact of HSV-2 on HIV transmission and vice versa in prospective manner. In Ethiopia, preliminary work on the Akaki cohorts showed that HSV-2 prevalence at enrollment was high; 61.5% for females and 44.7% for males (Mihret *et al*., 2002) but incidence of HSV-2 infection and risk factors for disease transmission could not be investigated in this cross-sectional study. Data on HSV-2 prevalence and incidence are scarce in this setting. This work is, therefore, aimed at determination of incidence and seroprevalence of HSV-2 and to know risk factors for prevalent and incident infections.

1.10. Objectives

General objective

- To study the seroepidemiological patterns of HSV-2 infection in cohorts.

Specific objectives

- To determine incidence of HSV-2 infection in Ethio-Netherlands AIDS Research Project cohorts (Akaki and Wonji).
- To determine prevalence of HSV-2 infection in the cohorts.
- To assess risk factors for incident and prevalent infections.
- To know possible correlation of HSV-2 with HIV seropositivity and vice versa.

II. MATERIALS AND METHODS

2.1. Study population

The study was conducted on the cohort participants of factory workers (Wonji and Akaki) enrolled to study HIV incidence and disease progression. At admission and 6-months intervals, the participants followed procedures such as signing an informed consent, pretest counseling for HIV testing, gender-matched interview on socio-demographic characteristics and sexual behaviors using a structured questionnaire. Factors suggestive of risky sexual behavior like history of genital discharge, genital ulceration and lifetime number of sexual partners were included in the questionnaire. Blood was drawn in ethylenediamine tetra acetic acid (EDTA) and plain vacutainer tubes (Becton and Dickinson) for serologic analyses. Plasma was isolated by centrifugation at room temperature for 10 minutes at 1180 revolution per minute (rpm) and stored at $-80^{O}C$.

Plasma samples from cohort participants collected at date of enrollment between 1997-2002 (n = 1714) were tested for HSV-2 IgG. From the participants negative for HSV-2 antibody at enrollment, 57 (5.6%) do not have follow-up data. For participants HSV-2 negative at enrollment and with follow-up data (n=953), the last sample from follow-up samples taken routinely every six months was selected and tested for HSV-2 IgG. For individuals whose last sample is positive for HSV-2 IgG, at least four follow-up samples between the first and the last samples were tested to determine the moment of seroconversion.

2.2. Laboratory methods

Plasma samples of the cohort participants were routinely tested for HIV-1 antibodies by HIVSPOT (Genelabs Diagnostics, Singapore) and ELISA (Vironostika HIV Uni-Form II Plus O; Organon Teknika, Boxtel, The Netherlands). Plasma samples reacting positive with any one or both tests were confirmed by Western Blot test (HIV Blot 2.2; Genelabs Diagnostics, Singapore). Plasma samples are also tested for syphilis antibodies first by the rapid plasma reagen (RPR) (RPR Nostican II, Organon Teknika, Boxtel, The Netherlands) and then reactive samples were routinely confirmed by *Treponema pallidum* particle agglutination assay (TPPA) serodia-TPPA (Fujirebio, Tokyo, Japan). Participants with reactive test results in both tests were considered to have current syphilis.

Testing for HSV-2 antibody was performed using gG2 coated antigens in a commercially available ELISA test kit (HerpeSelect ^{TM} 2 ELISA IgG, Focus Technologies, USA). Recently, this test kit has been evaluated using sera from individuals in different countries of Africa showing sensitivity of 100% and specificity of 88%, compared to Western blot (WB) and it was 100% sensitive and 96% specific compared to the gG2 inhibition assay (Hogrefe *et al*., 2002). In studies quoted by the manufacturer in their notice to the users, the test kit has sensitivity of 96.1% and specificity of 97.0% in sexually active adults, and sensitivity of 100% and specificity of 96.1% in expectant mothers as compared to WB in diagnosing HSV-2 infections.

In the procedure, all the samples, controls and calibrators were diluted to 1:101 by adding 10µl of each to 1 ml of diluent (protein, surfactant, and non-azide preservatives in PBS).

The 100µl of diluted specimen, control and calibrator was used for analysis. After one hour of incubation, the ELISA wells were washed three times by using automatic washing machine (Washer 430, Organon Teknika, Austria). Washing step was repeated after adding conjugate (peroxidase-conjugated goat anti-human IgG) and incubating for 30 minutes. Hundred-microlitre of substrate reagent (tetramethylbenzidine and organic peroxide in buffer) was added to each well and incubated for 10 minutes. Blue colour development at this step indicates positive test result. An equal amount of stopping reagent (1 M sulfuric acid) was added and the intensity of colour was quantified using ELISA reader (Reader 530, Organon Teknika, Austria) at 450 nm within 10 minutes of stopping the reaction. The ELISA reader was programmed to calculate index values automatically from the optical density (OD) reading value by dividing the OD values by mean OD value of calibrators.

Previous analysis of longitudinal reliability of Focus glycoprotein G-based type-specific enzyme immunoassay showed the possibility of seroreversion (the initial positive test result becoming negative during follow-up) (Cherpes (a) *et al*., 2003). This occurred when the mean positive index value of women who seroconverted was relatively low (less than 2.5). More than 87% of positive test results for HSV-2 in their longitudinal analysis have index values greater than 3.0. This situation warrants care, especially in the longitudinal studies of HSV-2 acquisition. To minimize the possibility of false positive results in persons with low index values in this longitudinal study, a cutoff value of 3.0 was used. All the laboratory tests were conducted in Ethio-Netherlands AIDS Research Project (ENARP) laboratory.

2.3. Statistical methods

HSV-2 prevalence was calculated as the number of subjects testing HSV-2 positive at enrollment divided by the number of the enrollment specimens screened. HSV-2 incidence was calculated among subjects testing HSV-2 negative at enrollment by dividing the number of seroconversions by the person-years of observations. For subjects remaining HSV-2 seronegative, person-years (pys) of observation were calculated as the interval of time from enrollment to the most recent date of follow-up. For subjects seroconverting for HSV-2, person-years were calculated as the interval of time from enrollment to the mid point between the last HSV-2 seronegative test and the first HSV-2 seropositive test. Ninety-five percent confidence intervals (CIs) for incidence estimation were based on a Poisson distribution.

HSV-2 incidence and prevalence were calculated for sub-populations defined by demographic characteristics and reported risk factors relating to sexual behavior. The prevalence and incidence of HSV-2 was compared between males and females by using the X^2 test. Risk factors for HSV-2 prevalence were identified by use of logistic regression. To determine independent association for HSV-2 acquisition, we first considered the variables separately in a series of bivariate analyses. All variables significant in bivariate analysis at $p<0.05$ were entered in to a full model; those remaining significant were retained in the final model. Risk factors for HSV-2 and HIV incidence were assessed by Cox proportional hazard analysis. Independent associations for HSV-2 incident infection were determined by multiple Cox proportional hazard analysis. The continuous variables of age, income, and lifetime number of sexual partners were assessed as ordered categories. Odds ratio (OR) and hazard ratio (HR) were calculated for each stratum separately and for the linear trends across increasing strata.

The final multivariate models assess the linear trends across increasing strata. In all cases p-value of <0.05 was taken to indicate level of significance and for all the analysis Stata Intercooled Version 7 (Stata Corporation, College station, Texas, USA) was used.

2.4. Ethical clearance

This study was within the frame work of the Ethio-Netherlands AIDS Research Project of the HIV incidence and disease progression study, which has got ethical clearance from National Ethical Clearance Committee, Ethiopian Science and Technology Commission to collect and use socio-demographic, behavioral and medical information and to test for antibodies to HIV and other STDs. In addition, ethical clearance specifically for this study was obtained from Faculty of Medicine, Addis Ababa University and Ethiopia Health and Nutrition Research Institute (EHNRI).

III. RESULTS

3.1. HSV-2 prevalence at enrollment

The kit is standardized for serum samples, but we compared 40 paired samples of serum and plasma taken approximately on the same date (just to exclude discrepant results due to new infections) and found that the plasma test result is 88% sensitive (only 3 out of 40 positive serum test results tested negative in plasma), and 100% specific as compared with serum test results (Table 1) by using an index value of 3.0 as cut off point. The interassay agreement value (kappa) was 86%. Another study conducted on the evaluation of serum and plasma samples for ELISA-based detection of HSV-2 antibody showed the agreement between the two samples of 98.9% (Cherpes (b) *et al*., 2003). Detection of HSV-2 IgG from plasma samples may be slightly less sensitive probably because of the interference of clotting factors in binding process of antibody and its conjugate.

From 1997 to December 2002, a total of 1714 subjects were enrolled in the HIV incidence and disease progression study in both cohorts. One thousand two hundred twenty two (71.3%) were males and 491 (28.7%) were females. The median age was 35 years for males (range, 19-62 years) and 33 years (range, 19-46 years) for females. Only 8.8% had education above grade 12 and 53.8% had more than primary education. The monthly income was low, 61.7% of the study subjects had a monthly income of less than 300 ETB (≈ 22 US$ in 1997). At the time of the survey, 78.5% of males and 83.5% of females were married and 18 males (1.5%) and 8 females (1.6%) reported no lifetime sexual partners.

The HSV-2 seroprevalence at enrollment in this longitudinal study was 41.1% (704 participants positive for HSV-2) and the prevalence was higher in females than males, 57.2% compared with 34.6% ($p<0.001$). Genital ulcers and discharge were viewed as clinical manifestations rather than risk factors for HSV-2 infections. History of genital ulcer in the past five years preceding enrollment was reported by 4% of HSV-2 seropositive participants and by 1.7% of HSV-2 seronegative participants ($p=0.01$). History of genital ulcer before enrollment had a predictive value of 62.2% for HSV-2 infections and recurrent genital ulcer in the past five years had a predictive value of 84.6% for HSV-2 infections. History suggestive of sexually transmitted diseases was reported by 13.9% of HSV-2 seropositive participants and 8.5% of HSV-2 seronegative participants and 11.8% of males and 17.1% of females with serologic evidence of HSV-2 infections had previous historical evidence of STDs. Out of 18 males and 8 females who reported no lifetime sexual partners 1 male (5.6%) and 4 females (50%) had evidence of HSV-2 infections. The HIV prevalence in females (11.7%) was higher than that in males (7.6%) ($p=0.006$). The past or current syphilis prevalence defined as positive TPPA serology, and positive RPR and TPPA serology respectively; in this study population was 25.9% with no difference by gender.

One hundred forty-eight subjects (8.6%) were HIV-1 positive at enrollment. Out of these, 98 (66.2%) were positive for HSV-2 IgG. In participants enrolled as HIV-negative, the prevalence of HSV-2 was only 38.8% (600 out of 1546; $p<0.001$). In the same manner, the HIV prevalence among participants who were HSV-2 positive at enrollment was 98/698 (14%) and this was found to be significantly higher when compared to 50/996 (5%) HIV prevalence among HSV-2 negative participants at enrollment ($p<0.001$).

3.2. Risk factors for prevalent infection

Table 2 presents HSV-2 prevalence by demographic characteristics and reported risk factors. HSV-2 prevalence increased with increasing age, beginning at 11.4% among participants aged less than 25 years and reaching 55.3% among participants 45 years and older. The prevalence by age group was higher in females than males of the same age group ($p < 0.001$). At the age of 45 years and older the HSV-2 prevalence in females reached maximum of 87.5% but in males of the same age group the maximum prevalence was 50.0%.

Of all groups examined, HSV-2 prevalence was highest (66.2%) among workers who were also HIV-positive at enrollment. This difference of HSV-2 seropositivity in HIV-positive and negative participants persisted after adjusting for sexual behaviors and history of sexually transmitted diseases (OR, 2.4; 95% CI, 1.6-3.6). HSV-2 prevalence was high among participants who divorced (62.9%) and the association of marital status with HSV-2 seropositivity persisted after adjustment for age and number of lifetime sexual partners (OR, 2.3; 95% CI, 1.6-2.3). There was no uniform trend observed between income and HSV-2 seropositivity but lower education is associated with a relative increase in HSV-2 seropositivity even after adjustment for age and gender.

In multiple logistic regression analysis (Table 3), HSV-2 seropositivity was significantly associated with HIV seropositivity at enrollment (adjusted OR, 2.4; 95% CI, 1.6-3.6), positive TPPA serology (OR, 1.8; 95% CI, 1.4-2.4), older age (OR, 1.1 per age group; 95% CI, 1.03-1.1), married (OR, 1.6; 95% CI, 1.1-2.4), divorced (OR, 2.3; 95% CI, 1.2-4.6), widowed (1.2; 95%CI, 0.4-3.5), and female sex (OR, 2.8; 95%CI, 2.1-3.7)

Table 1. Comparison of the serum and plasma test results by HerpeSelect ™ 2 ELISA IgG test kit, Focus technology, USA of 40 paired samples taken approximately on the same date.

Plasma test results	Serum test results			
		Positive	Negative	Total
	Positive	21	0	21
	Negative	3	16	19
	Total	24	16	40

Note: sensitivity of the test using the plasma is the ratio of positives in the plasma confirmed using the serum to the total positive test results in the serum and specificity of the test in the plasma is the ratio of positives in the plasma confirmed as positives in the serum to the total positives in the plasma.

Table 2. Prevalence of Herpes simplex virus type 2 (HSV-2) specific antibodies among factory workers at enrollment in a cohort, by demographic characteristics and risk factors, Akaki and Wonji, Ethiopia, 1997-2002.

Variable	n	Number HSV-2 seropositive (prevalence,%)	Unadjusted OR (95% CI)
Total	1714[a]	704 (41.1)	_
Age categories (in years)			
<25	149	17 (11.4)	referent
25-29	330	111 (33.6)	3.9 (2.3-6.9)[b]
30-34	408	169 (41.4)	5.5 (3.2-9.4)
35-39	371	178 (48.0)	7.2 (4.2-12.3)
40-44	408	203 (49.6)	7.7 (4.6-13.1)
>=45	47	26 (55.3)	9.6 (4.5-20.7)
Marital status			
Single	240	47 (19.6)	referent
Married	1371	592 (43.2)	3.9 (2.2-4.4)
Divorced	70	44 (62.9)	6.9 (3.9-12.4)
Widowed	28	18 (64.3)	7.4 (3.2-17.1)
Education			
Secondary or more	922	306 (33.2)	referent
Less than secondary	791	398 (50.3)	2.0 (1.7-2.5)
Number of lifetime Sex partners			
0	23	3 (13)	referent[c]
1	410	169 (41.2)	2.7 (0.8-9.7)
2-4	448	184 (41.1)	4.9 (1.4-17.6)
5-9	279	105 (37.6)	6.1 (1.7-22.2)
>=10	421	184 (43.7)	8.3 (2.3-29.7)
Positive HIV serology			
No	1546	600 (38.8)	referent
Yes	148	98 (66.2)	3.1 (2.2-4.4)
Positive TPPA serology			
No	1139	396 (34.8)	referent
Yes	398	225 (56.5)	2.4 (1.9-3.1)

Note: [a], totals do not always add to 1714 because of missing data
[b], p<0.001 for all age categories
[c], adjusted for gender

Table 3. Independent association with herpes simplex virus type 2 seropositivity among factory workers at enrollment in a cohort, Akaki and Wonji, Ethiopia, 1997-2002.

Variable	Adjusted OR[a]	95% CI
Age	1.1[b]	1.04-1.1[c]
Enrolled HIV positive	2.4	1.6-3.5
Positive TPPA serology	1.8	1.4-2.4
Female sex	2.8	2.1-3.8
Married	1.6	1.1-2.4
Divorced	2.3	1.2-4.6
Widowed	1.2	0.4-3.5

Note: [a], multiple logistic regression analysis OR adjusted for the other variables in the table
[b], OR per age category
[c], all p values <0.001, except for widowed (p=0.7)

Figure 1 shows the association between sexually transmitted infections (HIV, Syphilis and HSV-2) and age among participants in the cohort study, 1997-2002. The prevalence of HSV-2 and past/current syphilis is increasing with age but the HIV prevalence declined approximately after 35 years in males and 30 years in females, probably as a result of excessive mortality in HIV infected individuals.

Figure 2 shows the association between sexually transmitted infections and lifetime number of sexual partners. As expected the prevalence of the three infections increased with increasing number of sexual partners. The p value for the trend was less than 0.01 for each association tested. The prevalence levels of HSV-2 were highest of all the three, followed by syphilis and HIV infection. This may be because of the differences in the infectiousness of the three pathogens even though all are transmitted sexually. Four females and one male had at least one sexually transmitted infection while reporting no sexual partners in their lifetime. Although the possibility of non-sexual transmission of HSV-2 needs to be studied, some underreporting of sexual behaviors is suspected, especially among females. The seemingly high prevalence of HSV-2 among women who reported no lifetime sexual partners was just based on the 4 observations from 8.

Figure 3 indicates in all age groups the prevalence of HSV-2 is higher in females than males of the corresponding age. The difference is significantly high especially in younger age groups. The p value for the trend is less than 0.001. Four hundred twenty three men and 281 women were tested positive for HSV-2 antibodies. Total number of men tested in each group was 113 (age <25), 211 (age 25-29), 211 (age 30-34), 255 (age 35-39), 319 (age 40-44), and 38 (age >=45) and corresponding figures for women were 36, 119, 122, 116, 90, and 8.

The exaggerated difference seen in the younger age groups is probably because many girls at younger age due to economic dependence, tend to have sexual activity with older men who may have been infected with HSV-2 as a result of their lifetime exposure to risk factors but this is not usually the case in males.

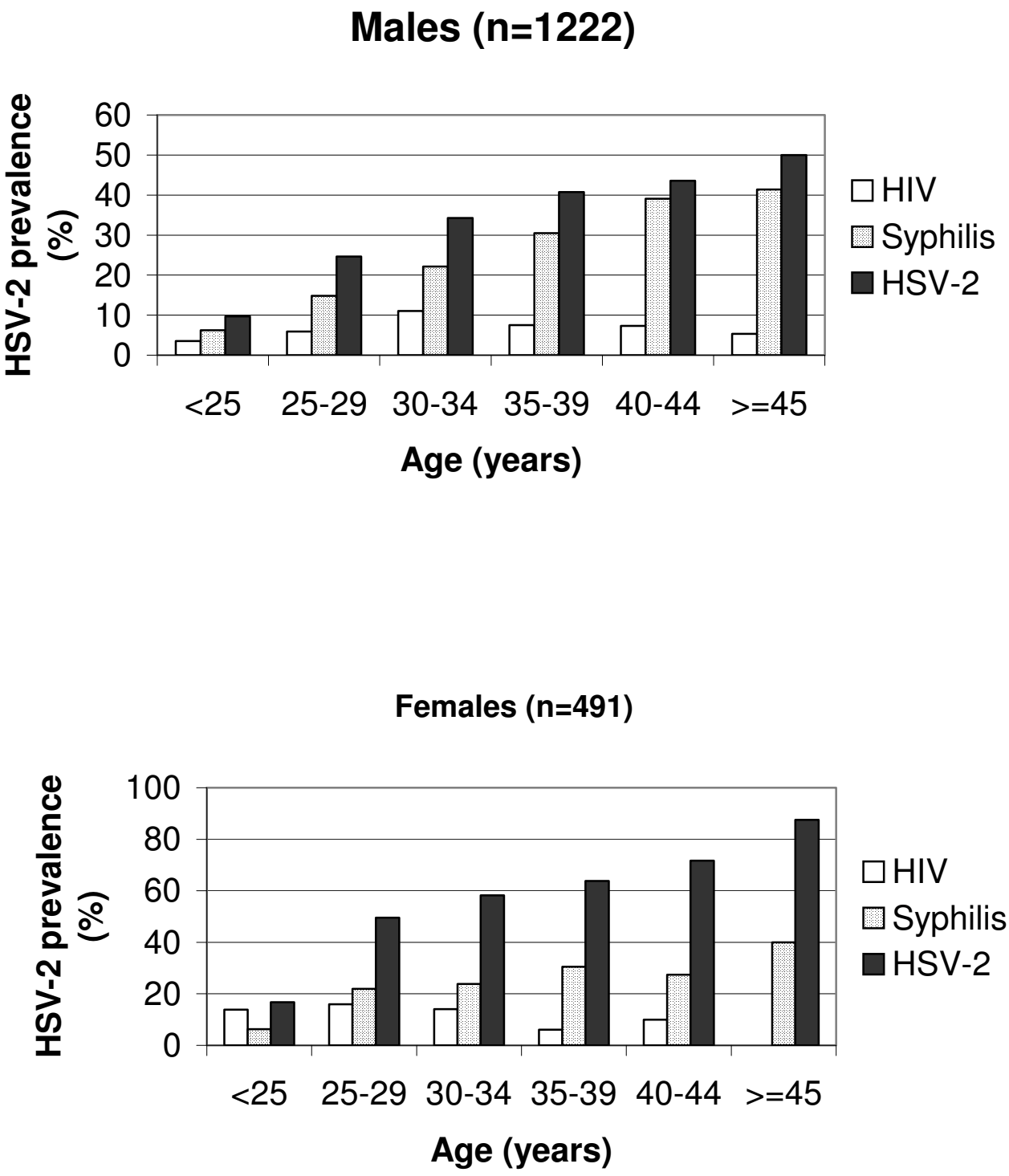

Figure 1. Prevalence of antibodies against HIV, Syphilis and Herpes simplex virus type 2 infections by age group and gender among factory workers at enrollment in a cohort, Akaki and Wonji, Ethiopia, 1997-2002.

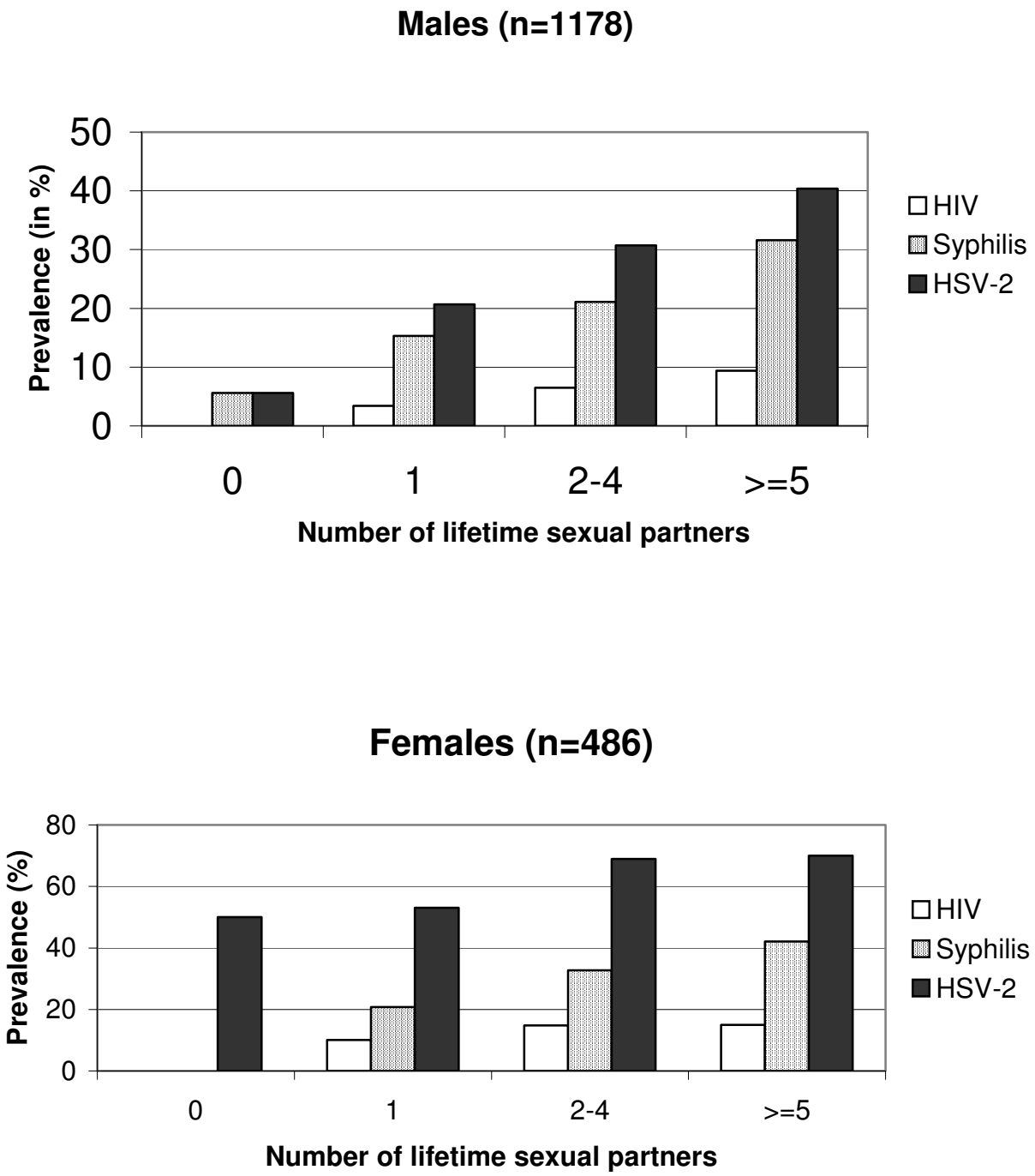

Figure 2. Prevalence of antibodies against HIV, Syphilis and Herpes simplex virus type 2 infections by lifetime number of sexual partners and gender among factory workers at enrollment in a cohort, Akaki and Wonji, Ethiopia, 1997-2002.

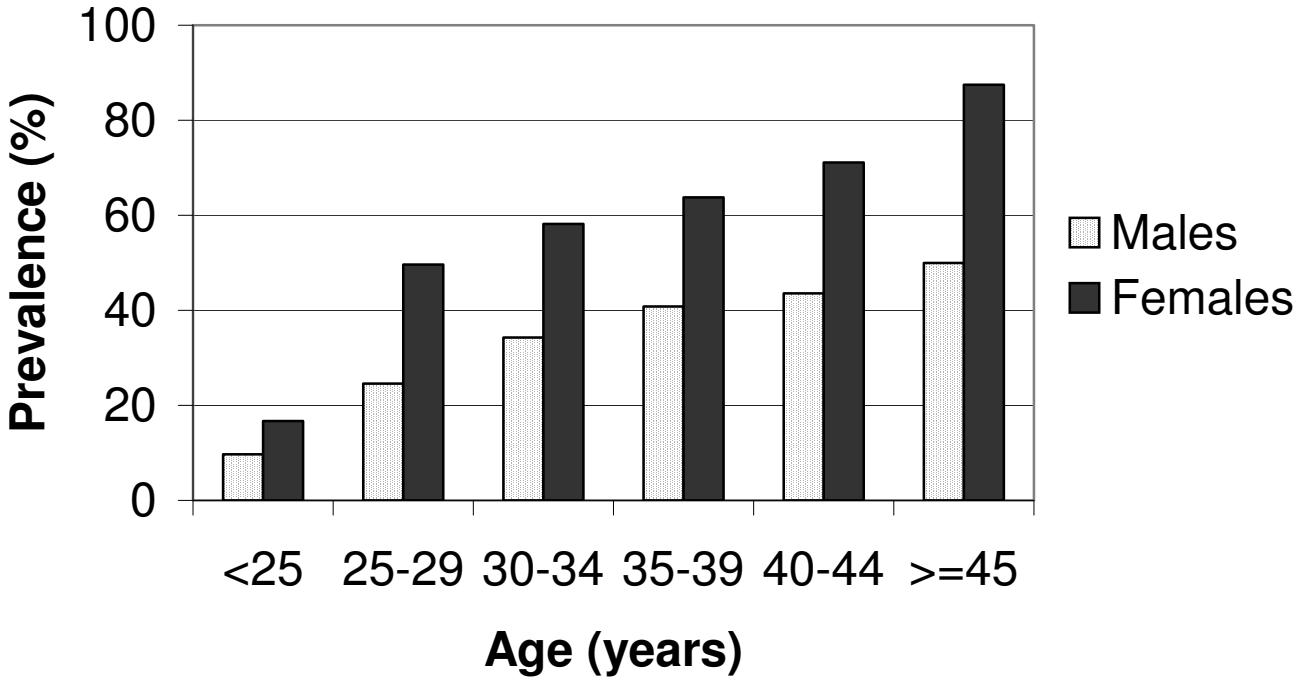

Figure 3. Percentage of Herpes simplex virus type 2 (HSV-2) seropositive men and women in different age groups at enrollment in a cohort, Akaki and Wonji, Ethiopia, 1997-2002.

3.3. HSV-2 incidence over the study period

The incidence of HSV-2 was 1.2/100 pys (95% CI, 1.0-1.5), based on 55 seroconversions among the 953 participants who enrolled seronegative and were observed for 4842 pys of follow-up. History of genital ulcer during the follow-up period was reported by 7.2% of participants who seroconverted for HSV-2 and by 1.4% of those who did not seroconvert.

3.4. Risk factors for the incident infection

Table 4 displays HSV-2 incidence by demographic characteristics and reported risk factors. Unlike HSV-2 prevalence, HSV-2 incidence did not differ significantly by age, education and number of lifetime sexual partners. In contrast to HSV-2 prevalence, single participants did not have a lower incidence of HSV-2 compared with married ones. The incidence was higher in females than males; 2/100 pys (95% CI, 1.2-3.2) as compared with 0.9/100 pys (95% CI, 0.7-1.3) (p=0.02, assuming Poisson distribution). HSV-2 incidence was 2.8/100 pys (95% CI, 1.4-5.6) among participants who were HIV-positive at enrollment and 1.0/100 pys (95% CI, 0.7-1.3) in those who were HIV-negative at enrollment (p=0.007, assuming Poisson distribution). The incidence among participants with evidence of past or current syphilis was1.8/100 pys (95% CI, 1.1-2.9) as compared with 1.0/100 pys (95% CI, 0.6-1.3) among those with no evidence of past or current syphilis (p=0.03, assuming Poisson distribution).

Table 5 indicates multiple Cox proportional hazard analysis model for determination of independent associations of HSV-2 incident infections with the sociodemographic and reported risk factors. HIV-positive serology at enrollment was the only independent risk

factor for HSV-2 acquisition (adjusted HR, 2.5; 95% CI, 1.1-5.6). The association of HSV-2 incident infection with past or current syphilis disappears after adjustment for age, history suggestive of past STDs, HIV status, and lifetime number of sex partners (adjusted HR, 1.6; 95% CI, 0.2-10.2 and p=0.1, assuming Poisson distribution). HSV-2 incidence was not independently associated with age, sex, education, income and marital status.

3.5. Acquisition of HSV-2 and HIV

Among participants negative for HIV and HSV-2 at enrollment, 44 seroconverted for HSV-2 alone, 6 seroconverted for HIV alone and 3 seroconverted for both HIV and HSV-2 during follow-up. Among participants positive for HSV-2 but negative for HIV at enrollment, 11 seroconverted for HIV and among participants negative for HSV-2 but positive for HIV at enrollment, 8 seroconverted for HSV-2. The incidence of HIV among participants who were HSV-2 positive at enrollment was 0.8/100 pys (95% CI, 0.2-1.6) compared with 0.4/100 pys (95% CI, 0.2-0.9) among those who were HSV-2 negative at enrollment indicating a 2.2 risk of HIV acquisition even though it was not statistically significant probably because of very small number of HIV seroconversters (only 20 HIV seroconverters). The incidence of HSV-2 among participants HIV-positive at enrollment was 2.8/100 pys compared with 1.0/100 pys among those HIV-negative at enrollment (p=0.007, assuming Poisson distribution). The temporal relation of HSV-2 and HIV in this longitudinal study however, could not be established because, majority of the participants with evidence of both HIV and HSV-2 infections have base line positive test result at enrollment (98 out of 109) and there were only few seroconverters for HIV.

Table 4. Incidence of Herpes simplex virus type 2 among cohort participants during follow-up by demographic characteristics and risk factors, Akaki and Wonji, Ethiopia, 1997-2002.

Variable	Person -years	HSV-2 seroconversions	HSV-2 incidence /100 pys (95% CI)[a]	Unadjusted HR (95% CI)
Total	4842	55	1.2 (1.0-1.5)	_
Age categories (in years)[b]				
<25	474	1	0.2 (0.03-1.5)	referent
25-29	947	9	0.9 (0.5-1.8)	1.1 (0.8-1.9)
30-34	1163	15	1.3 (0.8-2.1)	0.9 (0.5-1.5)
35-39	981	13	1.3 (0.8-2.3)	1.2 (0.7-2.4)
40-44	1133	14	1.2 (0.7-2.1)	0.8 (0.3-2.1)
>=45	87	0	0	-
Sex				
Male	3974	36	0.9 (0.7-1.3)	referent
Female	814	16	2.0 (1.2-3.2)	2.1 (1.1-3.6)[c]
Marital status[b]				
Married	3648	38	0.9 (0.8-1.4)	referent
Single	1136	14	1.2 (0.7-2.1)	1.8 (0.4-1.9)
Education[b]				
Secondary or more	2794	24	0.9 (0.3-1.7)	referent
Less than secondary	1994	28	1.4 (0.5-1.5)	1.2 (0.6-1.4)
Number of lifetime Sexual partners				
0	206	3	1.4 (0.5-4.5)	referent
1	1048	10	1.0 (0.5-1.8)	1.8 (0.4-2.5)
2-4	1238	15	1.2 (0.7-2.0)	1.9 (0.2-2.4)
5-9	922	7	0.8 (0.4-1.6)	1.5 (0.3-1.6)
>=10	1306	17	1.3 (0.8-2.1)	1.4 (0.4-1.6)
Positive HIV serology				
No	4455	44	1.0(0.7-1.3)	referent
Yes	285	8	2.8 (1.4-5.6)	2.8 (1.3-6.0)[d]
Positive TPPA serology				
No	3806	34	1.0 (0.6-1.3)	referent
Yes	953	17	1.8 (1.1-2.9)	1.9 (1.1-3.5)[e]

Note: [a], 95% CI on the basis of Poisson distribution [b], at enrollment
[c], Cox proportional hazard analysis, p=0.02 [d], Cox proportional hazard analysis, p=0.007
[e], Cox proportional hazard analysis, p=0.03

Table 5. Multiple Cox proportional hazard analysis model for determination of independent associations with Herpes simplex virus type 2 (HSV-2) seroconversion among participants during follow-up in a cohort, Akaki and Wonji, Ethiopia, 1997-2002.

Variable	Adjusted Hazard ratio[a]	95% CI	p-value
HIV positive at enrollment	2.5	1.1-5.6	0.02
Positive TPPA serology	1.7	0.9-3.1	0.1
Married	0.5	0.3-1.1	0.07
Number of lifetime sexual partners[b]	1.1	0.9-1.5	0.4
Female sex	1.7	0.8-1.8	0.2
Education	0.8	0.6-1.0	0.1
Age[b]	1.0	1-1.1	0.5

Note: [a], Multiple Cox proportional hazard analysis, hazard ratio adjusted for the other variables in the table.
[b], hazard ratio across category

IV. DISCUSSION

This study addresses the prevalence, incidence and risk factors for HSV-2 infection among participants enrolled in a cohort study of HIV infection and disease progression at two factories in Ethiopia from 1997 to December 2002. Prevalence of HSV-2 antibodies varies greatly in studies performed in various geographic areas and study populations. The prevalence may also vary with the use of different serological tests. Reports show particularly high prevalence in USA (Nahmias *et al*., 1990) and on the African continent. Prevalences reaching 60% in males and 75% for females in rural Tanzania (Obasi *et al*., 1999) and 60% for males in urban Zimbabwe (McFarland *et al*., 1999) were reported. STD clinic patients usually have markedly higher prevalence of HSV-2 antibodies than the general population (Hook *et al*., 1992). The finding in the present study of an overall HSV-2 seroprevalence of 41.1% was therefore, not surprisingly high.

The HSV-2 prevalence among females was 57.2% and that among males was 34.6% ($p<0.001$). This higher prevalence among women than men has been noted by others (Langeland *et al*., 1998; Malkin *et al*., 2002; Gottlieb *et al*., 2002; Turner *et al*., 2003). The significant difference of HSV-2 prevalence by sex was maintained in all age categories (Figure 3). The probable explanation could be anatomic differences that lead to greater and more vulnerable surface area exposed in the genital tract of women compared to men. The higher frequency of recurrences among men (20% higher than in women (Benedetti *et al*., 1994; Stanberry *et al*., 1997)), and possibly a lower perception of discomfort in men with active lesions than in women are also other reasons for the higher prevalence of HSV-2 in women. Males with active lesions may increasingly be engaged in sexual activity despite the

infection. This facilitates efficient transmission of HSV-2 from males to females than in the opposite direction. The finding in this study showing higher rate of HSV-2 acquisition in females (2.0/100 pys) than in males (0.9/100 pys; p=0.02) is therefore, in line with this explanation.

HSV-2 seropositivity was also associated with older age, lower education and being married. An increase in HSV-2 prevalence with increasing age in both sexes may not directly imply older age as a risk factor for HSV-2 infection. High HSV-2 prevalence at older age could be because of the cumulative effect since HSV-2 remains latent in the body after infection, then after the individual will be tested positive for HSV-2 IgG. This is evidenced in this study by the finding in risk factor analysis for incident infection, where older age is not a risk factor for HSV-2 acquisition. Information on age and sex-specific prevalence of HSV-2 infection is useful to optimize genital herpes control strategies, which increase in importance because accumulating data indicate that HSV-2 infection may increase acquisition and transmission of HIV. HSV-2 prevalence by age varies markedly by country, region within country and population sub group. Age-specific HSV-2 prevalence is usually higher among women than men and in populations with high-risk sexual behaviors (Smith and Robinson, 2002). The 16.7% HSV-2 prevalence among females younger than 25 years and the 9.7% prevalence among males of the same age group in this study is an important finding indicating efforts to prevent genital herpes to begin at early ages.

The higher prevalence of HSV-2 among married participants in this study even after adjustment for sex, age and number of lifetime sexual partners (adjusted OR, 1.6; 95% CI, 1.1-2.4) has been reported by another study in rural West Africa (Halton *et al*., 2003).

This has an important implication in HSV-2 prevention intervention programmes. Much transmission of HSV-2 in this setting may occur within marriage where opportunity for personal protection is limited owing to high fertility desire in married people, limited communication between partners and greater sexual freedom for men. High levels of transmission within marriage may undermine the impact of sexual behavior change programmes aiming to reduce HSV-2 and HIV incidence and may complicate their evaluation.

As HSV-2 is more readily transmitted sexually than HIV and infection leads to the production of life long antibody which can be detected in individual's serum, HSV-2 serology may be useful marker for changes in sexual behavior in HIV intervention studies (Langeland *et al*., 1998). However, the persistent nature of the infection implies the seroprevalence may not be a sensitive marker of behavioral changes, although it will be more discriminating at the lower prevalence seen in the younger age groups (Obasi *et al*., 1999). HSV-2 seroincidence would be a preferable marker of behavioral changes, especially in countries in sub-Saharan Africa where there is high incidence among young people. This important role of HSV-2 antibody as biological marker of sexual activity (Van de Laar *et al*., 1998; Kim *et al*., 2002), has been confirmed in this urban African setting as well, where HSV-2 infection shows dose-response relationship with life time number of sexual partners (Figure 2).

Chancroid, syphilis and genital herpes are all common causes of GUD in developing countries. However, there is some evidence that there have been changes in the etiology of GUD in recent years (O'Farell, 1999). There is a rapid increase in the proportion of herpetic ulcers, in both HIV-positive and HIV-negative subjects. As a result, the frequency of

treatment failures when using syndromic management is increasing. The etiology of genital ulcer remains largely unknown in Ethiopia; no study aiming at direct identification of pathogens in genital ulcer material has yet been conducted. In this study repeated genital ulcer has a high positive predictive value (84.6%) for HSV-2 infection, indicating HSV-2 may be common cause of genital ulceration. This high positive predictive value of reported genital ulcer should not, however, obscure the fact that large proportion of women (94%) and men (97.6%) were HSV-2 infected but did not report any genital ulcer in the past five years; this suggests that most of these infections were asymptomatic or had minor symptoms which were underreported. Reports even from high-risk populations such as STD clinic patients showed that the majority of HSV-2 seropositive persons (84.7%) had never received a diagnosis of genital herpes (Gottlieb *et al*., 2002). This could be a great concern in the area because infected participants despite their infection with HSV-2, may continue to engage in unprotected sexual activity and may be responsible for the widespread dissemination of HSV-2 infection. As has been shown in the other studies, most transmission events were not associated with a clinically recognized HSV-2 infection (Bossi, 2002).

HSV-2 prevalence was highest (66.2%) among participants who were also HIV-positive at enrollment. Base line HIV seropositivity was an independent risk factor for prevalent HSV-2 infection (adjusted OR, 2.4; 95% CI, 1.6-3.6). This strong association of HSV-2 and HIV was consistent with cross-sectional and longitudinal seroepidemiological studies on the African continent (Langeland *et al*., 1998; McFarland *et al*., 1999; Eis-Hubinger *et al*., 2002). Extremely higher HSV-2 prevalence (88.1%) among HIV-positive sugar estate male factory workers in Malawi has been reported recently (Sutcliffe *et al*., 2002).

The observed rate of HSV-2 seroconversion was 1.2/100 pys. The estimate is relatively low when compared with 6.2/100 pys seroconversion rate among male factory workers in Zimbabwe (McFarland *et al*., 1999), the estimates to populations in USA at higher risk for STDs, such as multipartnered heterosexual STD clinic patients in Atlanta (5% over 6 months), gay men in San Francisco (5/100 pys) (Stanberry *et al*., 1997). The incidence was 2.6/100 pys in persons seeking a repeat HIV counseling and testing in San Francisco (Turner *et al*., 2003). However, all these studies, except the Zimbabwean case, were conducted at high-risk groups which may not represent the general population and hence incidence rates are expected to be high. One longitudinal study from the African continent (rural Uganda) estimated HSV-2 incidence among men 11.8% over 12 months (Wagner *et al*., 1994); however, the sample size was relatively small (2 seroconversons among 17 men, 95% CI, 1.4%-42.5%). The lower seroconversion rate in this study may be as a result of changes in sexual behavior of cohort participants during follow-up. The incidence of HIV in this particular population is also declining, now at the level of 0.4/100 pys (95% CI, 0.3-0.6). To minimize the problem of seroreversion, which has been reported at relatively lower index values in HSV-2 longitudinal studies, we used a higher cutoff value (3.0). In addition, several serologic tests for HSV-2 are in use and there is at present no agreed gold standard, therefore, comparisons between studies should be with caution.

In contrast to HSV-2 prevalence, the incidence of HSV-2 was not significantly associated with age, marital status, number of lifetime sexual partners (Table 4). The difference is likely explained by HSV-2 seropositivity reflecting lifetime risk, where as HSV-2 seroconversion reflects exposure during the study period. Unlike HSV-2 prevalence, single participants did not have lower incidence rate of HSV-2 when compared with married participants.

The tendency was even vice versa although it was not significantly associated. The most likely explanation is that single individuals may be more likely to have higher rates of new partner acquisition than married ones. Educational status of participants showed borderline significance with HSV-2 seroconversion (HR, 0.8, p=0.05) but the association disappeared after adjustment for age, sex, lifetime number of sexual partners and HIV status (Table 5).

Positive TPPA serology was significantly associated with the seroconversion rate of HSV-2 (HR, 1.9; p=0.02), but after addition of HIV status in to Cox proportional hazard analysis model, the association disappeared (adjusted HR, 1.6, p=0.1). This may be because syphilis seemingly acted as a risk factor for HSV-2 incident infection either by predisposing individuals for HIV infection (HIV is an independent risk factor for HSV-2 acquisition) or behavioral risk factors that lead to syphilis are also risk factors for HSV-2 acquisition. However, in the risk factor analysis for the prevalent HSV-2 infection (Table 2), in both sexes, there was a strong association between TPPA positive serology and HSV-2 infection even after adjusting for the other variables (adjusted OR, 1.8; 95% CI, 1.4-2.4) (Table 3); therefore, it is possible that syphilitic ulcer increases susceptibility of an individual for HSV-2 infection or vice versa.

Enrollment while HIV-positive in this cohort study remained significantly associated with HSV-2 seroconversion after the analysis was controlled for other markers of sexual exposure (adjusted HR, 2.5; 95% CI, 1.1-5.6, p=0.02). The prevalence of HIV is higher (14%) among participants positive for HSV-2 (p<0.001) when compared with 5% prevalence of HIV in HSV-2 negative participants at enrollment. Another study has shown that the acquisition of HIV during follow-up was also associated with 3.9 higher risk of HSV-2 incident infection

($p<0.001$) and on the other hand, preexisting infection with HSV-2 at enrollment (HR, 3.5; 95%CI. 2.2-5.8) and HSV-2 seroconversion during follow-up (HR, 6.7; 95% CI, 4.2-10.7) were strong predictors of HIV acquisition even after being controlled for age, marital status, education, income, history of genital ulcer, history of genital discharge, number of sex partners, and prostitute contact (McFarland *et al*., 1999). This finding was based on the 68 HIV seroconverters. In this study, there were only 20 seroconverters for HIV and hence it is difficult to draw associations. In addition, among 109 participants showing evidence of infection for HSV-2 and HIV, 98 have positive test results at enrollment for both infections. This made determinations of the temporal sequence of seroconversions difficult.

HIV and HSV-2 manifest bi-directional interactions. HSV-2 increases the efficiency of HIV acquisition and transmission where as HIV may increase susceptibility to HSV-2 and increase HSV-2 shedding, HSV-2 recurrence rate and severity of clinical manifestations. Studies have shown that HSV-2 reactivation and duration of recurrences are significantly increased in HIV infected individuals and the frequency and severity of recurrences increase as CD4 cell count decreases. HIV infection is also likely to increase transmission of HSV-2, as there is evidence that the prevalence and quantity of genital HSV-2 shedding is significantly increased among HIV seropositive individuals (Schaker *et al*., 1998; Mbopi-Keou *et al*., 2000). Co-infection may enhance transmission of HIV and HSV-2 to others through increased reciprocal viral replication, increased viral shedding, and increased frequency of genital ulceration as a portal for viral excretion.

The interaction between HSV-2 and HIV infections are not fully understood and establishment of cause effect association is not simple (Suligoi (a) *et al*., 2002). Among HIV-positive individuals, HSV-2 associated GUD may enhance HIV shedding and infectiousness by disrupting the genital mucosal integrity. Among HIV-negative individuals, HSV-2 associated GUD may increase susceptibility by disrupting mucosal integrity, and also by the recruitment and activation of HIV target cells, and possibly by HIV taking the advantage of chemokine receptors (Fleming and Wasserheit, 1999). A number of cohort studies have demonstrated that HSV-2 seroconversion and prevalent HSV-2 infections, with or without obvious clinical disease, were risk factors for HIV seroconversion (Fleming and Wasserheit, 1999; McFarland *et al*., 1999). Studies to explain the mechanism by which the largely asymptomatic genital shedding of HSV-2 may act as a cofactor for HIV infection are lacking. This study cannot demonstrate a causal role of HSV-2 in the HIV transmission and vice versa, and further longitudinal and intervention studies will be necessary to elucidate this point. For example, data from HIV discordant couples should be analyzed stratifying by HSV-2 status, to estimate risk of HIV transmission between HSV-2 positive and negative couples.

In the face of the severe and worsening HIV epidemic in Africa, the high prevalence and incidence of HSV-2 (as shown in this study) and HIV, and the high frequency of asymptomatic HSV-2 shedding, and a possible synergistic effect between the infections may be cause of concern. It has been learned in the last decade that no single approach will contain the HIV/AIDS epidemic, and there is a need to consider all available means of control. The Mwanza study in Tanzania (Gross-Kurth *et al*., 1995) demonstrated that improved STD case management was an important additional HIV prevention strategy resulting in a 40% reduction in HIV incidence in the general population. No such reduction

was observed in the Rakai study in Uganda (Waver et al., 1999) and one reason postulated for this was a higher prevalence of STDs in Rakai, such as genital herpes, that were not targeted by the antimicrobial agents of the syndromic management (Hitchcock and Fransen, 1999). Therefore, the situation may warrant consideration of modification of the current syndromic management approach of STDs, especially in HIV-high prevalent areas.

Some limitations of this study deserve mention. It was not possible to determine the timing of seroconversions for the vast majority of participants with both HSV-2 and HIV infection (98 out of 109). One may question the validity of self-reported sexual behavioral data collected through a structured questionnaire (Ankirah, 1989). Despite the assurance of privacy and confidentiality during the interview, some cohort participants may misreport sexual behavior, which might bias the results. Precise measures of sexual behavior, sexual exposures, period of active genital lesions, viral replication, viral shedding, and physical examinations were lacking.

Conclusion and recommendations

The study showed high prevalence and incidence of HSV-2 infection among participants of the cohort study of HIV infection and disease progression at two factories in Ethiopia. The prevalence and incidence of HSV-2 was higher in females and the relatively higher prevalence among married participants indicates transmission of HSV-2 within marriage where opportunity for personal protection is limited. Most infections of HSV-2 in both sexes were without symptoms or with minor symptoms that were not noticed. An increase in HSV-2 prevalence with increasing number of lifetime sexual partners suggests HSV-2 antibody as a good biological marker of sexual activity. HSV-2 seroincidence would be a preferable marker of behavioral change and can possibly be used for evaluation of intervention programmes, especially in sub-Sahara African countries where there is high incidence among young people. HIV is an independent risk factor of HSV-2 prevalent infection and HSV-2 seroconversion in both sexes. The high prevalence and incidence of HSV-2 is worrisome in view of the significant morbidity attached to HSV-2 infection and its potential role as a cofactor for HIV transmission, especially in developing countries where HIV prevalence is high. The situation is being further complicated because of emergence of antiviral drug resistant HSV-2 mainly in HIV infected individuals (Suligoi (b) *et al*., 2002; Reyes *et al*., 2003). In the absence of a protective vaccine or effective therapy, prevention of HSV-2 infection will rely on the use of condoms and reduction in number of sexual partners. Emphasis should be given to prevention of HSV-2 infection at early ages.

Based on the important findings in this study, the following recommendations were made:

- In the absence of effective vaccine or antiviral therapy, efforts for the prevention of HSV-2 infection should continue targeting widespread condom use and reduction of the number of sexual partners.
- Such intervention programmes focused on prevention of HSV-2 infection should be done intensively at early age.
- The high rates of undiagnosed HSV-2 infection likely contribute to ongoing transmission. HSV-2 serologic counseling and testing at HIV counseling and testing sites might help prevent the spread of both infectious diseases.
- The etiology of GUD is largely unknown in Ethiopia; pathogen identification-based study should be conducted for GUD.
- HSV-2 prevalence is high in females of all ages but the impact of HSV-2 on pregnant women and pregnancy outcome is not known in this setting. Studies estimating the burden of neonatal herpes should be conducted.
- The main constraint to the widespread use of acyclovir in developing countries is likely to be the cost of the drug. The drug should be made more readily available at least for the treatment of severe cases of genital herpes, especially in HIV infected individuals.

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