SEROPREVALENCE OF HERPES SIMPLEX VIRUS INFECTIONS AMONG HIV POSITIVE INDIVIDUALS IN MBEYA REGION, TANZANIA

By

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CERTIFICATION

The undersigned certify that he has read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled ***Seroprevalence of Herpes Simplex Virus Infections among HIV Positive Individuals in Mbeya Region, Tanzania***, in partial fulfillment of the requirements for the degree of Master of Medicine (Internal Medicine) of Muhimbili University of Health and Allied Sciences.

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DEDICATION

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And to God, the Creator of all things, Who is the source of all knowledge and healing power.

ABSTRACT

Background

In Tanzania, HIV and AIDS remains a serious health problem, second only to malaria. Results from the Tanzania Household Indicator Survey for 2007/08 indicate that some 6% of Tanzanians in the age group of 15–49 years were infected with HIV. By 2008, regions that had a high HIV prevalence included Iringa (15.7%), Dar es Salaam (9.3%) followed by Mbeya (9.2%).

Synergisms between HSV and HIV have been documented in many studies and that Herpes Simplex Viruses increases the risk of HIV transmission 2 to 3 folds. Despite all that knowledge, the prevalence of HSV infection is largely unknown among HIV positive patients in the general Tanzanian population.

Objective

To describe the Seroprevalence of Herpes Simplex Virus type 1 and 2 infections among HIV infected individuals in Mbeya region.

Materials and Methods

This study was conducted in wards of Mbeya region among participants from the ongoing EU funded EMINI project. A specially designed questionnaire was used to identify social and demographic characteristics. Physical examination results were obtained from the database initially collected by NIMR MMRP research nurses and medical officers. Archived participants' sera were tested for Herpes Simplex Virus type 1 and 2 infections using Virotech HSV-1 (gG1) ELISA (Enzygnost, Behring, Germany) and Kalon HSV-2 (gG2) ELISA (Kalon Biological Ltd, Surrey, UK) respectively. Univariate and multivariate poisson regression models were used to identify factors associated with Herpes Simplex Virus infections.

Results

A total of 640 adults were randomly recruited after stratification by HIV status, age and sex. The overall Seroprevalence of HSV-1 was 92.1% while that of HSV-2 was 57.4%. Men and females were equally affected with HSV-1 (94.2% vs. 89.9%) (PR = 1.04, p-value > 0.05) while prevalence of HSV-2 was significantly lower in males as compared to females (64.5% vs. 50.2%) (PR = 0.72, p-value < 0.001). There was a strong and significant trend that HSV-2 increases with age (IRR = 1.18, p-value < 0.001), and HIV status (IRR = 2.05, p-value < 0.001) contrary to HSV-1 findings. HSV-1 was more frequent in hypertensives' (IRR = 1.08, p-value < 0.05) while HSV-2 was more common in over weights and obese participants (IRR = 1.67, p-value < 0.05)

Conclusion and Recommendations

There is a high prevalence of HSV-2 among females and this coincides with higher number of HIV infection in this group. Considering recent accumulating evidences of interaction of Herpes Simplex Virus infections and HIV; control and suppressive treatment of Herpes Simplex Virus should be considered as a possible strategy to control HIV infection.

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LIST OF ABBREVIATIONS

AIDS	-	Acquired Immunodeficiency Syndrome
CD4	-	Cluster of differentiation 4
CI	-	Confidence Interval
DCF(s)	-	Data Collection Form(s)
DNA/RNA	-	Deoxy/Ribo Nucleic acid
ELISA	-	Enzyme-linked immunosorbent assay
EMINI	-	Evaluate and Monitor the Impact of New Interventions
gG	-	Glycoprotein G
GPS	-	Global Positioning System
HAART	-	Highly active antiretroviral therapy
HIV	-	Human immunodeficiency virus
HSV	-	Herpes simplex virus
HSV-1/2	-	Herpes simplex virus type 1/2
N	-	Number
IDs	-	Identification(s)
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
MMRP	-	Mbeya Medical Research Programme
MS	-	Microsoft
NIMR	-	National Institute for Medical Research
PCR	-	Polymerase chain reaction
PDA	-	Personal digital assistant (=small handheld computer)
PR	-	Prevalence ratio
Prev.	-	Prevalence
RTI	-	Reproductive tract infection
STD(s)	-	Sexually transmitted diseases(s)
STI(s)	-	Sexually transmitted infection(s)

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Definition of Herpes Simplex Viruses

Herpes simplex viruses (HSVs) belong to the family *Herpesviridae* together with varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, human herpesvirus-6, human herpesvirus-7 and Kaposi's sarcoma-associated herpesvirus (Human Herpes Virus-8)[1].

HSVs are ubiquitous and cause diseases of the skin, mucous membranes, eyes and central nervous system. They exhibit a broad host range, being able to replicate in many types of cells and to infect many different animals. They grow rapidly and are highly cytolytic[2].

1.2. Epidemiology of Herpes Simplex Virus infections

There is a paucity of population-based literature on HSV-1 and HSV-2 studies. The literature cited hereunder includes studies that were done in America, Europe, Asia and Africa, and about seven studies were done in Tanzania.

The Seroprevalence of HSV-2 in adults has been studied extensively (compared to HSV-1) in several places in the world and shown to vary by country and population group[3].

In a Caribbean island population, a blinded serosurvey from blood donors, pregnant women, and HIV-positive patients reported the Seroprevalence of HSV-1 and HSV-2 being 81% and 34%, respectively, in blood donors, 84% and 40% in the antenatal population and 89% and 77% in the HIV-positive group. The HSV-2 Seroprevalence was significantly higher in HIV-infected adults than in the other groups[4].

Other studies have reported HSV-2 prevalence in the general population to be 22% in the United States[5], 3 to 5% in the United Kingdom[6], and between 10% and 16% in Sweden, depending upon age[7]. Seven percent of pregnant women in Japan have HSV-2 antibodies[8].

In African continent, the epidemiology of HSV infections is not well characterized but available data suggest that HSV-2 Seroprevalence is higher than in the United States[3, 9].

HSV-2 is more common in Sub-Saharan Africa than in Europe or the North America. Up to 82% of women and 53% of men in Sub-Saharan Africa are seropositive for HSV-2. These are the highest levels of HSV-2 infections in the world, although exact levels vary from country to country in this continent[9].

Genital herpes appears less common in Northern Africa compared to Sub-Saharan Africa. For example, only 26% of middle-aged women have antibodies for HSV-2 in Morocco. Women are more likely to be infected with HSV-2 once they are over the age of 40[10]. Algerian children are also likely to acquire HSV-1 infections at a young age (under 6) and 81.25% of the population has antibodies to HSV-1 by the age of 15 years[11].

In a rural Ugandan population it was found that Seroprevalence rates in adults were 91.2% for HSV-1 and 67.9% for HSV-2. Males were significantly less likely to be HSV-2 seropositive than females. Reactivity to HSV-2 rose with increasing age[12].

Studies done worldwide have reported the risk factors for genital herpes caused by HSV-2 being strongly related to the number of lifetime sexual partners, number of years of sexual activity, female gender and a history of previous STI infection[13].

Sera collected from Tanzanian children and young persons from 1 to 20 years old in Muhimbili National Hospital reported the prevalence of HSV-1 antibodies to be 92% for the age group of 17 to 20 years and 40% for HSV-2. There was no statistically significant association between seropositivities for HSV-2 and HIV[14].

In a cohort of female facility workers in north-western Tanzania, the Seroprevalence of HSV-2 and HIV infections were reported to be 80% and 30%, respectively and both infections were significantly and independently associated with older age, being a bar worker, working at a truck stop, and having more lifetime sexual partners. HSV-2 infection was also associated with lower socioeconomic status, increased alcohol intake, younger age at first sex, inconsistent condom use, and vaginal douching[15].

A cross-section study on Tanzanian STD patients and non-STD patients found that the prevalence of HSV-2 antibodies was 70% in STD patients and 35% in Tanzanian blood donors and pregnant women. A higher HSV-2 Seroprevalence was associated with female sex, increasing age, previous STDs, history of genital HSV infection, coitarchal age (age at first intercourse) and HIV seropositivity[16].

A random serosurvey in 12 rural communities of Mwanza region, found 75% of women and 60% of men having HSV-2 antibodies. HSV-2 Seroprevalence was higher in women who were married in a polygamous marriage. Seroprevalence was higher in men who were married, had lived elsewhere, had more lifetime partners, or had used condoms. The association between HSV-2 infection and lifetime sex partners was strongest in both sexes[17].

In a study that was done in a cohort of female bar workers in Mbeya region, found HSV-2 Seroprevalence to be 87%, and HIV was 68%. HIV infection was associated with syphilis and HSV-2 seropositivity[18].

1.3. Properties of the Herpes Simplex Viruses

There are two distinct herpes simplex viruses; type 1 and type 2 (HSV-1, HSV-2). Their genomes are similar in organization and exhibit substantial sequence homology. They can be distinguished by restriction enzyme analysis of viral DNA. They differ in their mode of transmission: HSV-1 is mainly spread by contact, usually involving infected saliva, whereas

HSV-2 is principally transmitted sexually or from maternal genital infections to a newborn[1, 2].

1.3. Herpes Simplex Virus infections

Infections with herpes simplex virus are among the commonest human viral infections throughout the world[19]. HSVs are natural pathogens for humans, with particular affinity for the nervous tissue. The virus spreads from person to person by infected secretions, classically oral secretions for HSV-1 and genital secretions for HSV-2[20, 21].

HSV-1 is the most constantly present virus in humans than any other virus. Primary infections occur early in life and are usually asymptomatic. Highest incidence is noted in children aged 6 months to 3 years. By adulthood, 70-90% of persons have antibodies. Antibodies do not eradicate the virus from the body[1, 20, 21].

Low class societies have higher infection rates than high class ones. The source of infection for children is usually an adult with symptomatic lesions or with asymptomatic viral shedding in saliva. The recurrence of infections with HSV-1 is frequently triggered by stimuli such as exposure to sunlight, trauma, menstruation and pyretic infections. The frequency of reactivations and recurrences varies with individual[22].

HSV-2 is a highly prevalent infection acquired through sexual contact and therefore antibodies to this virus are seldom found before puberty[3, 9] HSV-2 is the main cause of genital ulcers worldwide[23]. Recurrent genital infections may be symptomatic or asymptomatic and either situation provides a reservoir for transmission[24].

Classically, infections with HSV-1 cause nongenital disease, usually oro-labial manifestations, while HSV-2 is usually sexually transmitted and therefore related to urogenital organs. However, both viruses can cause either clinical syndrome[8, 24]. While most genital herpes infections are caused by HSV-2, there has been a trend toward an increasing prevalence of

HSV-1 causing genital herpes[24-26], suggesting changes in the age-specific epidemiology of HSV infections.

Genital HSV-1 infections are usually both less severe clinically and less likely to recur. HSV-1 infection might render a certain protection against an HSV-2 infection and seems to mitigate the HSV-2 illness. It is not yet clear whether the advent of HSV-1 genitally will reduce the general occurrence of HSV-2[7].

The virus must encounter mucosal surfaces or broken skin in order for an infection to be initiated. HSV-1 infections are usually limited to the oropharynx and virus is spread by respiratory droplets or by direct contact with the infected saliva[24].

Following primary infection, both types establish lifelong latent infections that periodically reactivate and may be associated with recurrent episodes of disease, with or without clinical symptoms, and as such the infection may often be transmitted without knowledge[24, 27, 28]. The lifelong latent infections by HSV-1 and HSV-2 are established in trigeminal and sacral ganglia, respectively.

1.4. Types of Herpes Simplex Virus infections

There are three types of herpetic infections: lytic infection, latent infection and transforming infection.

1.4.1. Lytic infections of Herpes Simplex Virus

The virus multiplies inside the nucleus of infected cell. This is followed by production of infectious virions before lysis of infected cells, partly due to suppression of host protein svnthesis by a structural protein named virus host shutoff protein[29, 30].

1.4.2. Latent infections of Herpes Simplex Virus

The viral DNA is maintained in a non-replicative state and persists in the nucleus as an episome for the entire life of the individual[31].

1.3.3. Transforming infections of Herpes Simplex Virus

Numerous studies have shown that, both HSV-1 and HSV-2 are able to transform the morphological phenotype of rodent cells. It has been proposed that HSV-2 participates as a cofactor in the development of invasive cervical carcinoma[3]. No human tumour has so far been shown to be directly caused by HSVs[32].

1.5. Herpes Simplex Virus diseases

HSV-1 and HSV-2 are common human pathogens that can cause primary and recurrent infections of mucous membranes. Primary HSV infections are usually symptomatic but may be sub-clinical. Recurrent infections are generally less severe than primary ones because of existing cellular and humoral immunity from prior exposures[33].

1.5.1. Common Herpes Simplex Virus disease entities

The most common clinical manifestations include oro-facial and genital lesions. Ocular infections may include any part of the eye including the retina, conjuctiva, cornea and eyelids[21, 34, 35]. Meningitis is usually benign, but the HSV encephalitis has been associated with high mortality[36]. Neonates are particularly at risk for serious HSV infections; early treatment appears to be an important determinant of the outcome[37].

Herpetic whitlow is an infection of fingers among dentists and other health care workers[38], whereas infection on bodies of wrestlers is called herpes gladiatorum. Erythema multiforme is frequently associated with HSV infection[39, 40].

Immunocompromised patients are at risk of developing frequent and more severe HSV infections. Herpetic lesions may spread and involve the respiratory tract, oesophagus and intestinal mucosa. Although cutaneous HSV infections are uncommon in healthy persons, they may be seen in a number of skin disorders such as eczema herpeticum and atopic dermatitis[41].

1.5.2. Clinical Presentation of Herpes Simplex Virus infections

Lesions may appear as grouped blisters that rupture, crust, and heal in 7 to 10 days accompanied with fever. More commonly, ulceration is the finding with no prior history of blisters. Once severely immunosuppressed, HIV-infected persons often experience chronic lesions that continue to expand and form large, crusted erosions 2 to 10 cm or larger in diameter. Lesions may be quite painful, especially if located perianally or periorally. Periungual infection is another characteristic manifestation of HSV-2 infection in the HIV-infected patients. All paronychial lesions should be cultured for HSV[42]. Like in HIV uninfected patients, HSV affected HIV positive individuals may be asymptomatic or symptomatic.

1.6. Laboratory diagnosis of Herpes Simplex Virus infections

Current laboratory methods used to diagnose HSV infections include: virus isolation, antigen detection, DNA detection and serology.

1.6.1. Isolation of Herpes Simplex Virus

Virus isolation is the definitive diagnostic approach. Specimens include vaginal or urethral swabs, throat washings, CSF and stool. The specimens are put into appropriate virus transport medium. The virus grows in many cell lines. They grow very fast: a cytopathic effect usually develops within 24-48 hours after inoculation of any specimens containing infectious virus. Immunofluorescent staining of cultured cells helps in quickly identifying HSV as being type 1

or 2. However, since the virus causes persistent infections, virus isolation is not in itself sufficient evidence to indicate that the virus is the causative agent of a disease in question.

1.6.2. Detection of Herpes Simplex Virus in specimens

Virus detection methods include DNA detection, particularly using polymerase chain reaction (PCR), and antigen detection. Antigen detection method can be nearly as sensitive as culture method, which is usually done by immunofluorescence staining[42].

1.6.3. Serology of Herpes Simplex Virus infections

Serological tests allow identification of silent carriers of HSV infections and provide useful information in symptomatic patients when virological tests such as culture, antigen detection and PCR are not helpful[43].

HSV-1 and HSV-2 are highly homologous genetically and antigenically[44]. This results in an antigen-sharing profile for the two types. The recent development of reliable HSV type-specific antibody assays using either the complete glycoprotein G (gG)[45] or specific epitopes in gG[46] has provided the means to identify past infection with a given HSV type, regardless of whether it was clinically apparent or not.

HSV-1 and HSV-2 have glycoprotein G-1 and G-2, respectively. These two antigens are used in preparing ELISA systems that distinguish the two viruses. Spiezia et al reported a sensitivity of 92.9% and a specificity of 98.7% for a gG-1 and gG-2 based ELISA assays[47].

1.6.4. Herpes Simplex Virus polymerase chain reaction

PCR is the preferred diagnostic method for HSV infection but not affordable in resource poor countries like Tanzania. Evaluation of Cerebrospinal fluid specimens from patients with biopsy-proven HSV encephalitis and in those with other proven diseases has a sensitivity of greater than 95% at the time of clinical presentation and a specificity that approaches 100%[48].

1.7. Herpes Simplex Virus and Human Immunodefiency Virus co-infection

Herpes simplex virus (HSV) disease is a major cause of morbidity in immunocompromised individuals, with a greater frequency, severity, and chronicity that is proportionate to the degree of immunosuppression[49].

Anogenital herpes was one of the first opportunistic infections described in persons with AIDS, and persistent herpetic ulceration is an AIDS-defining illness[50].

1.7.1. Epidemiology of Herpes Simplex Virus among HIV patients

Among HIV-1 infected individuals, HSV-1 and HSV-2 infections are common, with prevalence that approximate or exceed those in the general population. In recent years, a number of studies have focused on the prevalence of HSV-2 among HIV-1 infected individuals, finding seroprevalences of 50-90% in some populations, significantly higher than among those without HIV-1[51].

In sub-Saharan Africa, where the HIV pandemic has hit hardest, more than 60% of the general population may be infected by HSV-2[52]. At this prevalence, it has been estimated that as many as half of the incident cases of HIV infection can be directly attributed to infection with HSV-2[53].

The highest prevalences of co infection with HSV-2 among HIV-1-infected individuals have been seen in heterosexual women and men in sub-Saharan Africa and in men who have sex with men in America.

1.7.2. Clinical presentation of Herpes Simplex Virus among HIV patients

1.7.2.1. Asymptomatic Herpes Simplex Virus reactivation

Prospective studies show that individuals coinfected with HIV-1 and HSV-2 experience more frequent episodes of mucosal shedding of HSV-2 than do HIV uninfected individuals, most of whom are subclinical[54, 55]. HSV-2 is frequently shed even in persons with CD4+ cell counts more than 400 cells/mm3, with substantial variability observed between persons at all CD4+ cell counts[52, 55].

In addition, HSV-2 reactivations in HIV-infected men are more often at multiple anatomic sites, compared with reactivations in HIV-uninfected men[54].

1.7.2.2. Symptomatic Herpes Simplex Virus infections

Among HIV-1 infected persons, the clinical presentation of symptomatic HSV-2 infection can vary considerably. HIV-infected persons can have more frequent or persistent anogenital herpetic lesions, which may become extensive, deeply ulcerated, and necrotic, primarily in patients with low CD4+ cell counts[17, 56, 57].

Preexisting antibody to HSV-1 is associated with milder or asymptomatic primary HSV-2 infection. Primary infection may be accompanied by systemic symptoms, including fever, headache and myalgia.

Mucocutaneous manifestations of HSV-2 infection may have an atypical location or appearance, leading to delays in diagnosis and in initiation of appropriate therapy[55, 58, 59].

More serious and systemic manifestations of HSV infection include esophagitis, meningoencephalitis, hepatitis, pneumonitis, retinal necrosis, and disseminated infection, all of which are relatively rare, even among those with advanced HIV-1 infection[59, 60].

1.7.3. Herpes Simplex Virus and HIV-1 Transmission

There is increasing evidence that genital herpes is facilitating the perseverance of the global HIV-1 epidemic[52]. Among those who become infected with HIV-1, HSV-2 seropositivity and genital ulcer disease resulting from HSV have been associated with significantly higher HIV-1 plasma viral loads[61].

A number of observational studies have found that HSV-2 reactivation, including asymptomatic shedding, also increases the concentration of HIV-1 in plasma and genital secretions[62].

A prospective study among heterosexual HIV-1 discordant couples in Rakai, Uganda, found that genital ulcer disease in the HIV-1-infected partner, primarily resulting from HSV-2, was associated with a 4-fold increase in the likelihood of HIV-1 transmission[63].

It is not known how coinfection with HSV affects hosts immune control of HIV. HIV susceptibility may be related to macroulceration or microulceration during reactivation[64] and/or to the increased number of HIV target cells and activated CD4+ T cells, even in the absence of HSV reactivation[65].

HSV can alter HIV-1 cell tropism, as the result of a phenotypic mixing between HIV-1 and HSV[66-68]. This allows infection of cells that are not normally susceptible to HIV-1 infection, such as CD4-negative cells.

Enhanced expression of HIV-1 receptors and coreceptors[69, 70] and expression of Fc receptors[71] have been demonstrated as potential modes for expanding HIV tropism to CD4-negative cells.

HSV-1 and HSV-2 can play a role as cofactors in HIV-1 infection. Genital herpes, more than other sexually transmitted diseases, appears to be linked to HIV-1 transmission both by providing a portal of entry or exit for the virus and by resulting HIV-1 load boosting[54].

Chronic infection with or reactivation of HSV has been associated with a higher HIV plasma viral load in several[61, 62, 72] but not all[73] studies. HSV can activate HIV transcription directly and also activates host Toll-like receptors, which have been linked to systemic inflammation and HIV progression[74].

1.7.4. Herpes Simplex Virus Diagnosis in HIV co-infections

Among HIV-1-infected persons, the potential for atypical presentations of genital HSV may further increase the chance of inaccurate diagnosis.

Clinical diagnosis of genital HSV infection in HIV-infected persons is unreliable, and both false-negative and false-positive diagnoses occur[75].

PCR of genital lesions has 2- to 4-fold higher sensitivity than viral culture in determining the etiologic diagnosis of herpes simplex virus infections[51, 76].

For asymptomatic individuals, type-specific serologic testing based on glycoprotein G can accurately diagnose and distinguish HSV-1 from HSV-2 with high sensitivity and specificity (>95%) should be used[51, 75, 77]. These assays appear to perform well among persons with HIV-1 infection.

Some experts have adopted routine HSV serologic testing as part of the initial evaluation of persons presenting for HIV-1 care, in order to diagnose asymptomatic or unrecognized HSV-2 infection[51]. Concerns about the psychological burden of a diagnosis of HSV-2 should not prohibit testing; studies have found that the effect is limited[78].

1.8. Treatment of Herpes Simplex Virus infections

There is currently no curative therapy of HSV infections. Available drugs cannot act on the virus during latency in the dorsal root ganglia. The current most successful drugs such as acyclovir, valacyclovir, famciclovir are all nucleoside analogues inhibiting replication. They cause monophosphorylation by virally encoded thymidine kinase and concentrates in infected cells. Host enzymes, cellular kinases further phosphorylate the drug to the active triphosphate form that inhibits viral DNA polymerase and consequently viral replication[79-81].

Foscarnet is a pyrophosphate analogue which directly inhibits viral DNA polymerase without prior activation by thymidine kinase. In contrast to nucleoside analogues, foscarnet is a non-competitive inhibitor of the DNA polymerase[81].

Cimetidine, a common component of heartburn medication, has been shown to lessen the severity of herpes zoster outbreaks in several different instances[82-84].This is an off-label use of the drug. It and probenecid have been shown to reduce the renal clearance of acyclovir[85]. These compounds also reduce the rate, but not the extent, at which valaciclovir is converted into aciclovir.

Limited evidence suggests that low dose aspirin (125 mg daily) might be beneficial in patients with recurrent HSV infections[86, 87].

1.9. Treatment of Herpes Simplex Virus infections coinfected with HIV

Antiviral treatment of HSV infection with nucleoside analogues acyclovir, valacyclovir, and famciclovir has been the mainstay of therapy. These antivirals inhibit the replication of HSV-1, HSV-2, and varicella-zoster virus selectively, because phosphorylation requires a virally encoded thymidine kinase. They have a high therapeutic index and are safe, well tolerated, and effective in HIV-infected patients[87].

Studies among HIV-1-infected individuals have shown that these medications are well tolerated in this population and, importantly, demonstrate no interaction with antiretroviral medications used in the treatment of HIV-1.

Antiviral chemotherapy provides clinical benefits both as episodic treatment of symptomatic patients and as suppressive therapy for prevention of recurrent disease.

1.9.1. Herpes Simplex Virus episodic therapy

Episodic therapy for symptomatic reactivation of genital HSV can shorten the duration of lesions. Symptomatic outbreaks of genital herpes tend to respond more slowly to anti-HSV therapy in HIV-infected patients, especially patients with lower CD4+ cell counts, and higher doses of anti-herpes medications may be required for resolution of symptoms.

Typically, symptomatic herpes lesions in an HIV-infected person respond to episodic therapy in 5–14 days, whereas HIV-uninfected adults will usually have complete resolution of lesions in 3–5 days[88, 89]. Episodic treatment can be initiated with acyclovir, valacyclovir, or famciclovir[88, 90]. Severe outbreaks can require use of intravenous acyclovir.

1.9.2. Herpes Simplex Virus suppressive therapy

Symptomatic HSV infection can be more severe and asymptomatic shedding can be more frequent in HIV-1–infected persons and therefore long-term anti-HSV suppressive therapy should be considered. Several studies have shown that acyclovir, valacyclovir, and famciclovir effectively suppress HSV-2 reactivation in persons coinfected with HIV-1 and HSV-2[54, 91].

Suppressive oral therapy, taken daily, effectively reduces the rate of recurrence of symptomatic genital herpes as well as the frequency of asymptomatic genital HSV shedding. When breakthrough reactivations do occur, they tend to be short and asymptomatic.

Long-term suppressive antiviral therapy against HSV should be considered for persons coinfected with HSV-2 and HIV-1. Higher-dose treatments (eg, acyclovir 800 mg 3 times per day, and similar bioequivalent doses for famciclovir and valacyclovir) can be used for those who have frequent HSV outbreaks despite receiving standard suppressive therapy[51].

1.10. Herpes Simplex Virus resistance to Antiviral drugs

The emergence of acyclovir-resistant HSV strains was first documented soon after acyclovir was introduced[92] Acyclovir resistance has been described in persons with and without prior antiviral exposure[93]. Usually, the mechanism is a deficiency of HSV thymidine kinase or, less commonly, enzymatic alterations in thymidine kinase or DNA polymerase[94-96].

Isolation of drug-resistant HSV from immunocompetent persons has remained infrequent (less than 1% of isolates)[97, 98]. Surveillance studies indicate that acyclovir resistance is somewhat more common, but rates of acyclovir resistance are still fewer than 5% among HIV-infected persons[98, 99].

At times, acyclovir-resistant HSV presents a clinically important problem for HIV-1–infected persons. The detection of drug-resistant virus in vitro is not always clinically relevant, because many infections still respond well to standard therapy.

HSV resistance testing should only be ordered for patients who have experienced clinical failure, rather than as part of routine surveillance of HIV-1 infected persons[100].

If there is a poor response to therapy, and the isolate is found to be resistant to acyclovir, intravenous foscarnet is indicated, because all acyclovir-resistant strains are resistant to valacyclovir and most are also resistant to famciclovir[101].

Although foscarnet is the drug of choice for the treatment of drug-resistant HSV infection, several topical alternatives offer limited efficacy when treatment is constrained by renal toxicity.

Cidofovir gel (1%) and foscarnet cream (1%), when applied to herpetic lesions, have been shown to promote healing, cessation of viral shedding, and pain relief for patients with acyclovir-resistant HSV infection[102, 103].

1.11. Prevention and control of Herpes Simplex Virus infections

For genital herpes, condoms are highly effective in limiting transmission of herpes simplex infection[104, 105]. The virus cannot pass through latex. Condom use also reduces the transmission risk by 50%[78, 104, 105]. Condom use is much more effective at preventing male to female transmission than vice-versa[104].

Topical microbicides which contain chemicals that directly inactivate the virus and block viral entry are currently being investigated[24].

To prevent neonatal infections, seronegative women are recommended to avoid unprotected oral-genital contact with an HSV-1 seropositive partner and conventional sex with a partner having a genital infection during the last trimester of pregnancy. A seronegative mother that contracts HSV at this time has up to a 57% chance of conveying the infection to her baby during childbirth, since insufficient time will have occurred for the generation and transfer of protective maternal antibodies before the birth of the child, whereas a woman seropositive for both HSV-1 and HSV-2 has around a 1-3% chance of transmitting infection to her infant[106, 107].

Mothers infected with HSV-2 are advised to avoid traumatic delivery (e.g., fetal scalp electrodes, forceps, and vacuum extractors) but elective caesarean section can reduce exposure of the child to infected secretions in the birth canal[24].

The use of antiviral treatments, such as acyclovir, given from the 36th week of pregnancy limits HSV recurrence and shedding during childbirth, thereby reducing the need for caesarean section[24].

1.12. Herpes Simplex Virus infections in the Era of HAART

Few studies have explored the effect of HAART on the clinical course of HSV-2 infection. HAART decreases the frequency of symptomatic herpetic lesions[108].

Specifically, persons receiving HAART had significantly fewer days with HSV lesions, compared with persons not receiving HAART, whereas, mucosal HSV-2 shedding was similar[108].

Severe genital HSV-2 lesions have been reported in patients after HAART initiation as an unusual manifestation of immune reconstitution with a delayed response to antiviral therapy, despite documented acyclovir sensitivity[109].

Even when it is not clinically evident, HAART may be associated with an initial period of increased asymptomatic genital HSV shedding[110].

A study to determine the prevalence of infection with HSV-1 and HSV-2 among Americans women with and at high risk for HIV infection, and to evaluate the effect of HAART on the recurrence of genital lesions found that the use of HAART and subsequent immune recovery did not completely eliminate the effect of HIV infection on genital lesions among women with concurrent HSV-2 infection[111].

Likewise, a study by Posavad et al[108] suggested that a longer duration of HAART (median 21 months) could only decrease clinical but not subclinical HSV-2 reactivations, but also Mayaud et al[112] found that clinical and subclinical HSV-2 reactivations occur more frequently among HIV-infected women, particularly those with lower CD4 cell counts. The use of HAART only

partly reduces the frequency of HSV-2 genital shedding. HSV therapy may benefit HIV-infected individuals, including patients responding well to HAART[113].

But beside all that in Cuba it was discovered to be more likely that HIV individuals receiving HAART to have a better recovery of CNS infections than those who are not receiving it[114].

In Burkina Faso, a study to demonstrate a causal relationship between HSV-2 and increased genital HIV-1-RNA shedding in women on HAART found that HSV-2 facilitates residual genital HIV-1 replication among dually infected women taking HAART despite HIV-1 suppression at the systemic level[115].

Studies have shown HSV suppressive therapy significantly reduces genital and plasma HIV-1 RNA levels in dually infected women and these findings may have important implications for HIV control[116].

Antiherpetic therapy could reduce HIV transmission by decreasing HIV plasma load and/or mucosal HIV shedding, but a proof-of-concept trial is needed to demonstrate this. It also appears that individuals co-infected with HIV and HSV-2 has more frequent HSV recurrences than individuals infected with HSV-2 alone. There is a strong correlation between decreasing CD4 count and increasing rates of HSV reactivation, suggesting that reactivation is linked to immunosuppression[116].

Growing data describing the benefits of herpes suppression in the context of individuals co-infected with HIV/HSV, with additional clinical trials poised to further elucidate these issues in the near future.

2. PROBLEM STATEMENT

There is considerable biological and epidemiological evidence that HSV-2 infection increases the risk of acquiring and transmitting HIV infection. HSV-1 has also been identified in genital ulcers and thus likely to increase the risk of acquiring and transmitting HIV infection, though its course is less severe than that of HSV-2 infection.

Results from the Tanzania Household Indicator Survey for 2007/08 indicate that some 6% of Tanzanians in the age group of 15–49 years were infected with HIV. By 2008, Mbeya region was among three regions in Tanzania that had a highest HIV prevalence in the order of Iringa (15.7%), Dar es Salaam (9.3%), and Mbeya (9.2%)[117].

HSV co-infection is not only an important risk factor for HIV acquisition, but it also affects the subsequent course of HIV disease and secondary transmission of HIV infection[118]. Infection with HSV has been associated with higher HIV viral load during acute infection[52, 72, 119] and also enhances retroviral transcription at the peripheral blood mononuclear cell level has HSV reactivation during chronic HIV infection[62, 72].

Studies have shown that chronic HSV-2 infection increases HIV acquisition rates approximately 3-fold for both men and women[120] and up to 6-fold for high-risk female sex workers[121].

Despite all these advances on understanding of HSV infections and their association with HIV infection; the epidemiology of HSV infections in Tanzanian HIV positive patients is largely unknown.

3. RATIONALE

Apart from the morbidity due to symptomatic episodes, infection with HSVs may have severe consequences in immunosuppressed hosts or neonates[122].

Geographic and socioeconomic differences affect the frequency of HSV infections, so the acquisition patterns of these infections vary greatly in different areas and populations[8, 123, 124]. Continuous changes in societies might therefore affect these acquisition patterns, and frequent studies would therefore be useful for monitoring purposes.

Although it has now been generally recognized that HSV facilitate HIV transmission and HIV infection may simultaneously prolong or augment the infectiousness of individual with HSV or other STIs[125-127], yet little information exist on the prevalence and pattern of HSV infections and disease among HIV patients.

It is therefore justifiable to have this study, which describe the epidemiology of HSV infections in HIV infected and uninfected individuals in Mbeya region, which is among regions most affected with HIV[117], to identify the magnitude of the problem and help in designing the appropriate control and treatment measures of HSV infections, so as to reduce morbidity and transmission of HIV.

4. OBJECTIVES

4. 1. Broad objective:

To determine the sero-prevalence of Herpes Simplex Virus infections among HIV infected individuals in Mbeya region, Tanzania.

4. 2. Specific Objectives:

1. To determine the Seroprevalence of HSV-1 and HSV-2 infections among HIV infected individuals in Mbeya region, Tanzania

2. To compare the Seroprevalence of HSV infection in the HIV infected and uninfected populations in Mbeya region, Tanzania

3. To determine socio-demographic factors associated with HSV-1 and HSV-2 infections among HIV infected and uninfected individuals in Mbeya region, Tanzania

5. METHODOLOGY

5.1. Study design

This was a population based cross sectional study that was conducted between September 2009 and March 2010.

5.2. Study site

This study was done in Mbeya region situated in the southern highland areas of Tanzania. The region is divided into eight administrative districts namely Chunya, Ileje, Mbeya rural, Mbeya City, Mbozi, Mbarali, Kyela and Rungwe. The districts are further divided into 25 divisions which are subdivided into 135 wards and 577 villages. Only nine chosen wards are shown in figure 1 below.

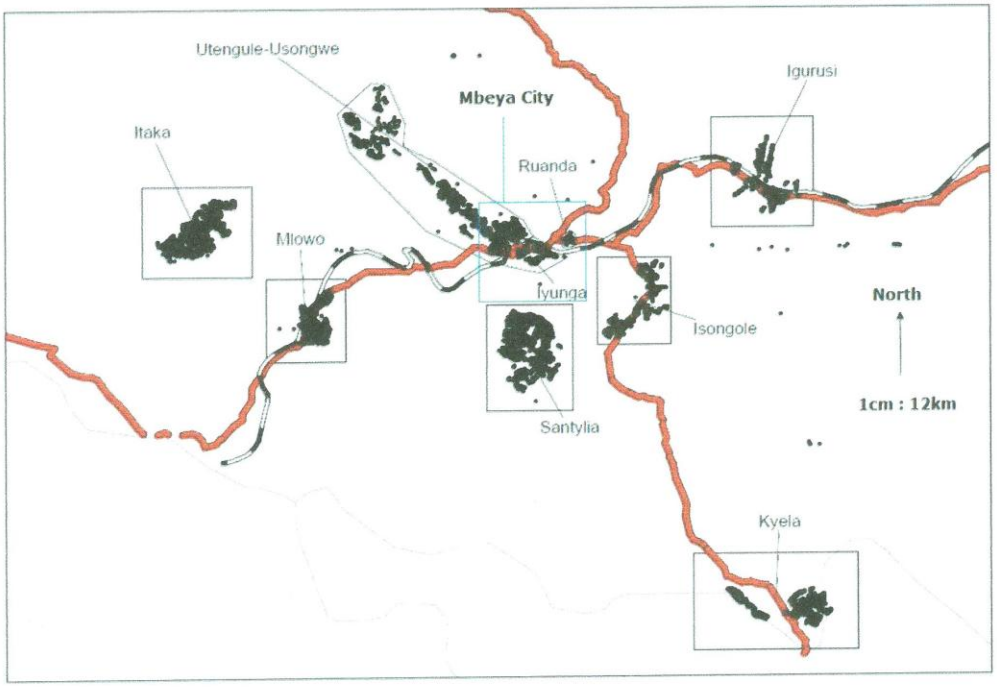


Figure 1. Herpes Simplex Virus Infections study sites in Mbeya region

According to Tanzania National Census, the population of Mbeya region grew from 1,476,199 in the 1988 census to 2,070, 046 in 2002. This was about 6 percent of the total Tanzania Mainlands population, making Mbeya region one of the most populous regions after Shinyanga and Mwanza[128].

5.3. Study Population

The current study includes a subset of participants who were initially recruited into the ongoing EMINI cohort study between July 2008 and August 2009.

EMINI (Establishment of the Infrastructure to Evaluate and Monitor the Impact of New Interventions) is a longitudinal study conducted in Mbeya region and has established a cohort of about 20,000 people in nine selected communities. Study households have been visited once each year since 2006, when the study was initiated.

This project is under the Mbeya Regional Medical Office and conducted by NIMR - Mbeya Medical Research Programme (NIMR-MMRP), a collaborative joint research institution between the Government of Tanzania, namely the National Institute for Medical Research, Mbeya Regional Medical Office, the Mbeya Referral Hospital, and the University of Munich, Germany.

The overall objective of the EMINI project is to contribute to the general improvement of health by controlling communicable diseases in Mbeya Region. This project also assists in the set-up and maintenance of the health information system and disease surveillance.

Data from the official 2002 Population and Housing Census were used to determine suitable sites to conduct the EMINI cohort study. For logistic reasons the sites were supposed to be within a radius of 120 km from the Mbeya Referral Hospital. All wards that belonged to selected sites had to have a health centre or a dispensary. Sites were selected in such a way

that the population of each site was around 20,000. For some sites this meant that two or three wards were combined in order to reach this number of inhabitants

At least two urban sites were to be included (Ruanda and Iyunga/Iwambi) as well as two sites along the main road within the Rift Valley area so that some areas with a high HIV transmission would be captured (Mlowo and Igurusi). In comparison to that, at least one site in a remote rural area (Itaka) was to be part of the Census. One border site (Njisi) was included, as well as two sites at high altitude or with low temperatures (Isangati/Santilya and Isongole/Simambwe) and one site at the Lake Nyasa (Kyela). Mbarali and Mbozi were taken into the Census because these sites had shown exceptionally good cooperation during previous surveys conducted through NIMR MMRP.

This process resulted in the selection of nine sites with a total of two Health Centers and 16 dispensaries. Initially a total population census was done in these sites from August up to November 2005 to identify the EMINI study population and the location of all households in these sites (Figure 1). The staff for the population survey underwent courses in computer training and the use of PDAs, to ease the work load on the data entry staff.

Out of the census population a subsample of households were chosen as the population for the first and subsequent population surveys. This sample was a geographically stratified random sample covering 10% of about 42,000 households that were interviewed during the census: All census households were mapped and then each site was divided into 7-9 subareas. Next, 10% of households from each of these geographically defined areas were randomly selected to participate in the surveys. During the first survey, households that did not participate (e.g. because all members refused to participate, the household had moved etc.) were replaced with other households. However, if households withdrew from the study or could not be found during one of the later surveys this was recorded in EMINI dataset but no replacement

Within the 4283 selected households all consenting members were included into the surveys and new household members (i.e. newborn children or people joining the household after the first survey) were also included. Core data (location of the household and the name, sex and age of its members) for the first survey were retrieved from the census data and updated during the survey.

Ten percent of the census population (approximately 20,000 participants) were randomly chosen for the survey and interviewed on the size of the household, income, and other demographic characteristics. Parallel to the interviews the geographical position of each household was determined using handheld GPS receivers for future reference and tracking. This activity was finished in December 2005.

During each of the annual study visits, every household was visited by a research team to collect blood, conduct interviews and do physical examination to volunteers. Initially the collected data were entered into paper questionnaires and then into a PDA. From the PDA, the data were directly downloaded onto a laptop (MS Access) in the field. Once the use of the PDAs was fully established and checked that there are no technical or human errors, paperless data collection using PDA was done.

5.4. Study procedure

5.4.1. Clinical intervention

The index study used blood, demographics characteristics, sexual risk behaviors and physical examination data that were initially collected by research nurses and assistant medical officers between July 2008 and August 2009. HIV testing was done by NIMR MMRP laboratory scientist and technicians during the same timeline.

5.4.2. HIV testing

The blood specimens collected from the participants were tested serially for HIV-1 and 2 in the NIMR-MMRP main laboratory at the Mbeya Referral Hospital using Abbott Determine HIV-1 or 2 (Abbott Laboratories, Abbott Park, IL) tests followed by Enzygnost anti HIV-1 and 2 plus (DADE-Behring, Marbug, Germany) ELISA if positive, followed by a second ELISA rLAV (Biorad Laboratories, Redmond WA, USA) if discordant and confirmed by Western Blot, MPD HIV Blot 2.2 (MP Biomedicals, Geneva, Switzerland). Plasma from every participant during each survey was stored at -80°C for future use. Once the participant tested HIV positive, he/she was not retested in the next survey. Individuals who were positive were included in the current study as HIV positive study subjects and those who had a negative HIV test result during survey two were also included as HIV negative.

5.4.3. Inclusion criteria

This study included participants in the EMINI Survey II (2008-2009) with known HIV status, and age between 15 and 49 years.

5.5. Determination of sample size

The sample size was calculated according to the following formula

$$n = [A + B]^2 * [(p1*(1-p1)) + (p2 * (1-p2))]/ [p1-p2]^2$$

Where

n = the sample size required in each group (double this for total sample)

p1 = The proportion of HSV-2 among the HIV-positive women (86%=0.86) in Mulago hospital Uganda[129]

p2 = The proportion of HSV-2 among the HIV-negative women (62.5%=0.625) in Mulago hospital Uganda[129]

p1-p2 = size of difference of clinical importance = 0.235

A = Significance level = when alpha error is 1%

B = Desired power = 0.99

Sample Size = 145 for each study population; i.e. A minimum of 145 HIV positive and 145 HIV negative study participants were required.

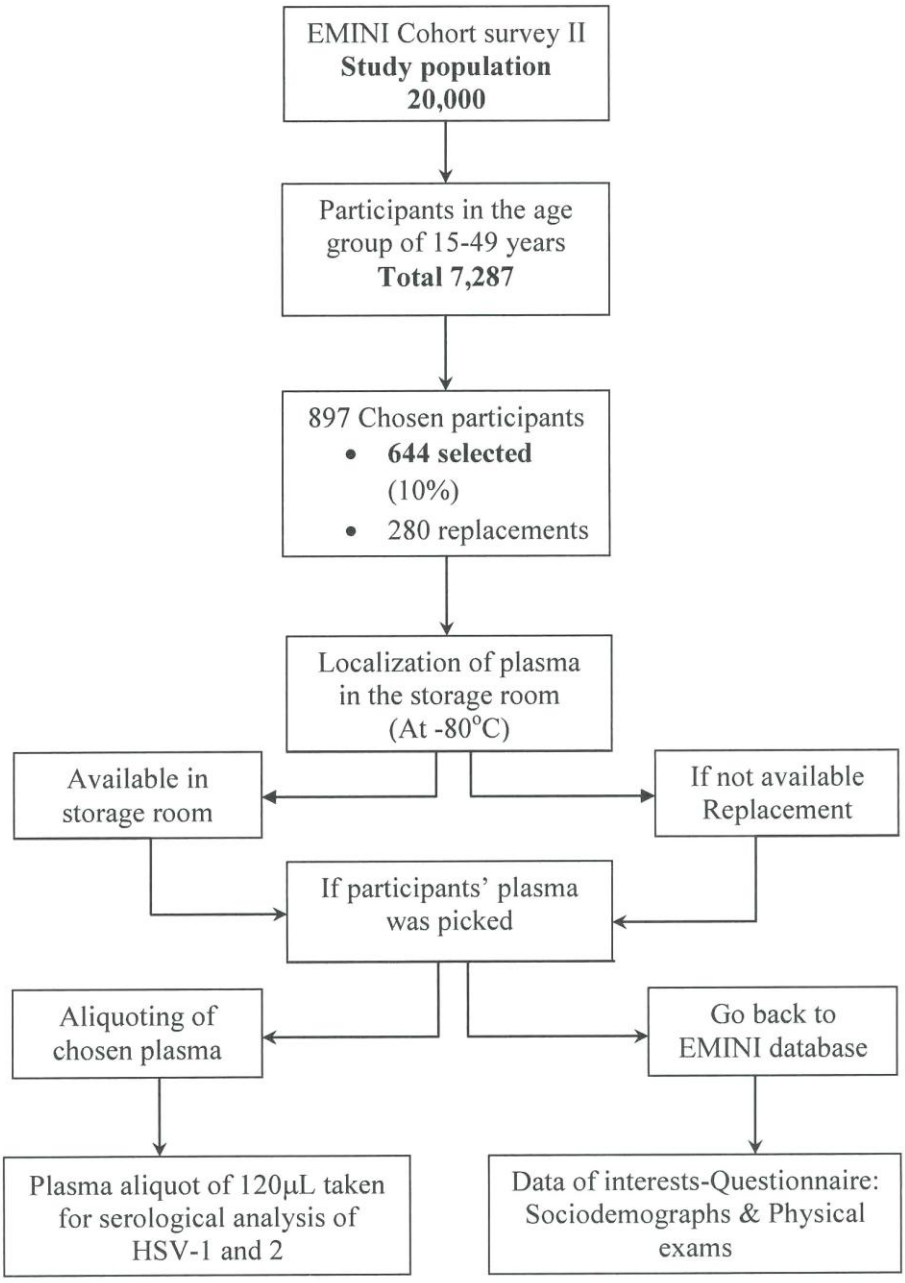
5.6. Sampling method

In EMINI cohort of 20,000 individuals, there are 7,287 participants in the age group 15-49 years old. Randomization of EMINI participants meeting the inclusion criteria was done using Stata 11 after stratification by Age, Sex, and HIV status. Stratification was necessary because otherwise the number of HIV positive participants would have been too low for meaningful comparisons.

Each participant was assigned a random number and 23 participants were initially selected per stratum (overall = 23 participants x 28 strata = 644 participants). Strata with less than 23 participants were filled from higher age strata with the same Sex and HIV combination. For each stratum 10 participants were also selected for replacement in case the originally selected samples could not be found in the storage room (Total replacements were 280).

Following the identification of randomly selected study participants, localization of sera was done. These sera were stored at -80°C. An aliquot of 120uL was taken from each serum of chosen participant together with data of sociodemographic characteristics and physical exams kept in EMINI database.

5.7. Flow chart of recruitment of study participants[1]



[1] Narration in sampling method

5.8. Research Instruments

Responses from EMINI interviews were used to assess the socio-demographic characteristics of participants.

The HSV-2 IgG ELISA (Kalon Biological Ltd, Surrey, UK), which has the highest sensitivity (92.3%) and specificity (97.7%) of commercially available assays when used on African samples[127] was used to examine samples for HSV-2 infection and and Virotech IgG HSV-1 ELISA kit (Enzygnost, Behring, Marburg, Germany) which has sensitivity of 98.6% and specificity of 98.9% was used to screen HSV-1 infection.

5.9. Laboratory Methods

Archived plasma of randomly chosen participants from EMINI cohorts frozen at -80°C were screened for the presence of Herpes Simplex Virus type 1 and 2 (HSV-1 and HSV-2) specific IgG class antibodies using Kalon HSV-2 IgG ELISA and Virotech IgG HSV-1 ELISA kits respectively and results were linked to responses of data obtained from a demographic characteristics and sexual risk behavior questionnaire. Standard operating procedures (see appendix page 79) and data worksheet for each kit were made and followed according to recommendations by respective manufacturers. HSV-1 and HSV-2 ELISA tests were run in series, starting with HSV-2 and followed by HSV-1.

Serological analyses of plasma were done from October to December 2009 at NIMR-MMRP main laboratory, Mbeya, Tanzania. This is the only laboratory in Tanzania that has been accredited by the College of American Pathologists (CAP).

5.10. Data Collection and Management

Data regarding age, gender, socio-demographic status, relevant behaviours of participants, and physical examination were all collected during interviews in the participants' households using PDAs for data capture. They were downloaded into Microsoft Access databases and imported into Stata 11 software where data checking and management was performed. Laboratory test results for HIV, HSV-1 and HSV-2 were recorded on paper forms, double entered into MS Access databases, compared and corrected and then imported into Stata 11 where they were combined with the interview data.

5.11. Data analysis

All analyses were done in Stata 11.0 (Stata Corp., College Station, TX). Univariate and multivariate associations of different factors with HSV-1 and HSV-2 infection were analysed using poisson regression with robust variance estimates.

5.12. Institutional Ethical Clearance

Ethical clearance was sought from Muhimbili University of Health and Allied Sciences, and Mbeya Regional ethical Committees.

5.13. Confidentiality

Confidentiality was observed and no unauthorized persons had access to the collected data. Each subject had been assigned a study identification number, and no individual names were linked with these IDs.

6. RESULTS

A total of 640 subjects from nine wards of Mbeya region were selected for this study. The socio-demographic characteristics of these participants are shown in table 1.

About 50.2% of the participants were men. The majority of the study population (58.4%) had completed primary level of education and 54 participants (8.4%) were still in schools and there were post secondary school subjects. Four hundred and forty eight (70.0%) participants were peasants or involved in fishing. Most of participants were involved in seasonal activities (50.5%) and 12.3% had permanent employment.

There was slightly equal numbers of HIV positive (50.3%) and negative (49.7%) individuals. Three hundred and fifty two participants (55.0%) were married with one spouse, and Christians comprised 77.5% of the study population.

Table 1. Frequency distribution of Socio-demographic characteristics among study participants (n=640)

Demography		Frequency	Percent
HIV status			
	Negative	322	50.3
	Positive	318	49.7
Sex			
	Female	319	49.8
	Male	321	50.2
Age group			
	15-19	77	12.0
	20-24	83	13.0
	25-29	106	16.5
	30-34	101	15.8
	35-39	90	14.1
	40-44	90	14.1
	45-49	93	14.5

(Continues on next page)

Demography		Frequency	Percent
Level of education			
	No formal education	88	13.8
	Uncompleted primary	84	13.1
	Completed primary	374	58.4
	Uncompleted secondary	40	6.3
	Still in school	54	8.4
	Post secondary school	0	0
Marital status			
	Never Married	154	24.1
	Married-One Spouse	352	55.0
	Married-Multiple Spouses	34	5.3
	Separated/Divorced	44	6.9
	Widowed	56	8.7
Religion			
	No religion	107	16.7
	Christian	496	77.5
	Muslim	19	3.0
	Don't know	18	2.8
Employment type			
	Unemployed	45	7.0
	Occasional employment	91	14.2
	Seasonal employment	323	50.5
	Permanent employment	79	12.3
	Self-employed	42	6.5
	Student	54	8.4
	No info	6	0.9

Six hundred and thirty nine sera were tested for HSV-2 and ten specimens had indeterminate results and one sample was missing. While Sera of 629 study subjects were tested for HSV-1, among which 18 sera were indeterminate and 11 participants' sera had already been used up during HSV-2 testing. None of the sera with indeterminate results were retested.

Table 2. Number of valid HSV-1 results and seroprevalence of HSV-1 by HIV Status, Sex and Age among study participants (n=611)

	HIV status								
	Negative			Positive			Total		
Age group	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
15-19	23	24	47	8	20	28	31	44	75
	95.7	95.8	95.7	100	95	96.4	96.8	95.5	96
20-24	23	24	47	14	19	33	37	43	80
	95.7	100	97.9	92.9	89.5	90.9	94.6	95.3	95
25-29	22	23	45	39	19	58	61	42	103
	95.5	95.7	95.6	92.3	84.2	89.7	93.4	90.5	92.2
30-34	23	23	46	28	20	48	51	43	94
	95.7	95.7	95.7	89.3	70	81.3	92.2	83.7	88.3
35-39	23	23	46	19	17	36	42	40	82
	100	95.7	97.8	100	82.4	91.7	100	90	95.1
40-44	22	22	44	23	21	44	45	43	88
	95.5	95.5	95.5	91.3	81	86.4	93.3	88.4	90.9
45-49	23	22	45	23	21	44	46	43	89
	100	90.9	95.6	82.6	81	81.8	91.3	86	88.8
Total	159	161	320	154	137	291	313	298	611
	96.9	95.7	96.3	91.6	83.2	87.6	94.2	89.9	92.1

Out of 629 participants who were tested for Herpes Simplex Virus type 1, only 611 had determinate HSV-1 IgG results, women consisted 298 and males were 313. Within this group, 320 samples had HIV negative results and about 291 were HIV infected.

Overall seroprevalence of HSV-1 infection was 92.1% as shown in table 2. Taken as whole females had a prevalence of 89.9% compared to 94.2% of males. HIV negative females were 95.7% reactive to herpes simplex type 1 virus as compared to 83.2% of HIV positive females. These proportions were slightly lower as compared to prevalence of 96.9% and 91.6% of HIV negative and positive males respectively.

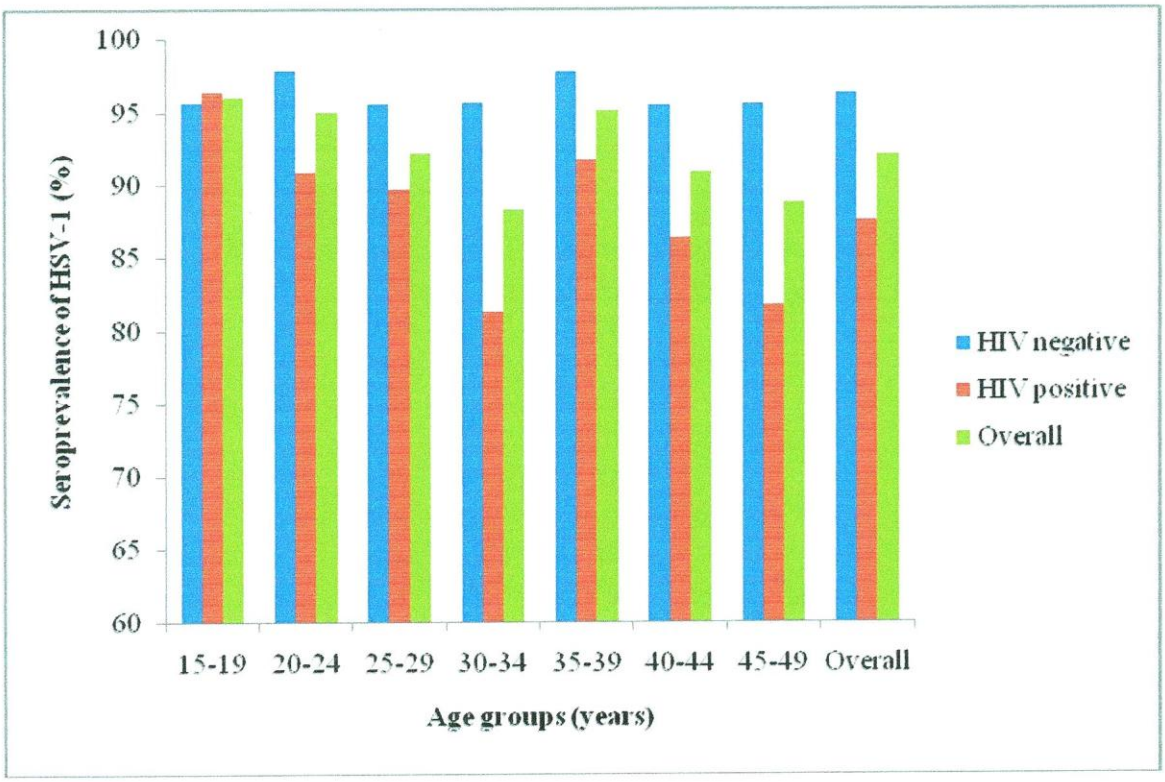


Figure 2. Seroprevalence of Herpes Simplex Virus type 1 by HIV Status and Age among study participants

Figure 2 above shows the seroprevalence of Herpes Simplex Virus type 1 infections among study participants without considering sex. HIV uninfected individuals in the age group of 20-24 years have the highest prevalence of HSV-1 (97.9%). HIV infected individuals in the age group of 30-34 years are least affected (81.3%) with HSV-1. Overall HIV negative individuals had highest prevalence of HSV-1 (> 95%) compared to HIV infected individuals (> 81%). When HIV status is not considered, the overall prevalence is more than 88% and the age group 35-39 being the most affected with prevalence of 95.1%.

Table 3. Number of valid HSV-2 results and seroprevalence of HSV-2 by HIV Status, Sex and Age among study participants (n=629)

	HIV status								
	Negative			Positive			Total		
Age group	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
15-19	23	24	47	8	22	30	31	46	77
	4.3	4.2	4.3	0.0	45.5	33.3	3.2	23.9	15.6
20-24	23	24	47	15	21	36	38	45	83
	8.7	25.0	17	40.0	90.5	69.4	21.1	55.6	39.8
25-29	21	23	44	37	22	59	58	45	103
	14.3	39.1	27.3	70.3	86.4	76.3	50.0	62.2	55.3
30-34	23	21	44	28	23	51	51	44	95
	30.4	52.4	40.9	78.6	95.7	86.3	56.9	75.0	65.3
35-39	23	23	46	20	23	43	43	46	89
	39.1	43.5	41.3	90.0	87.0	88.4	62.8	65.2	64
40-44	22	22	44	24	22	46	46	44	90
	40.9	63.6	52.3	91.7	95.5	93.5	67.4	79.5	73.3
45-49	23	23	46	23	23	46	46	46	92
	60.9	82.6	71.7	78.3	100.0	89.1	69.6	91.3	80.4
TOTAL	158	160	318	155	156	311	313	316	629
	28.5	43.6	36.2	72.3	85.9	79.1	50.2	64.6	57.4

As seen in table 3 the overall 57.4% of samples were reactive to HSV type 2 specific IgG, 64.6% of women had positive HSV-2 results compared to only 50.2% of males. Fewer HIV negative individuals (36.2%) compared to 79.1% of HIV positive study subjects had reactive HSV-2 test. In the HIV negative population, 43.6% of females compared to 28.5% of males were reactive to HSV-2 while in the HIV positive group 85.9% of females compared to 72.3% of males had Herpes Simplex type 2 IgG antibodies. Notably the overall seroprevalence is lower in the younger age group compared to adults.

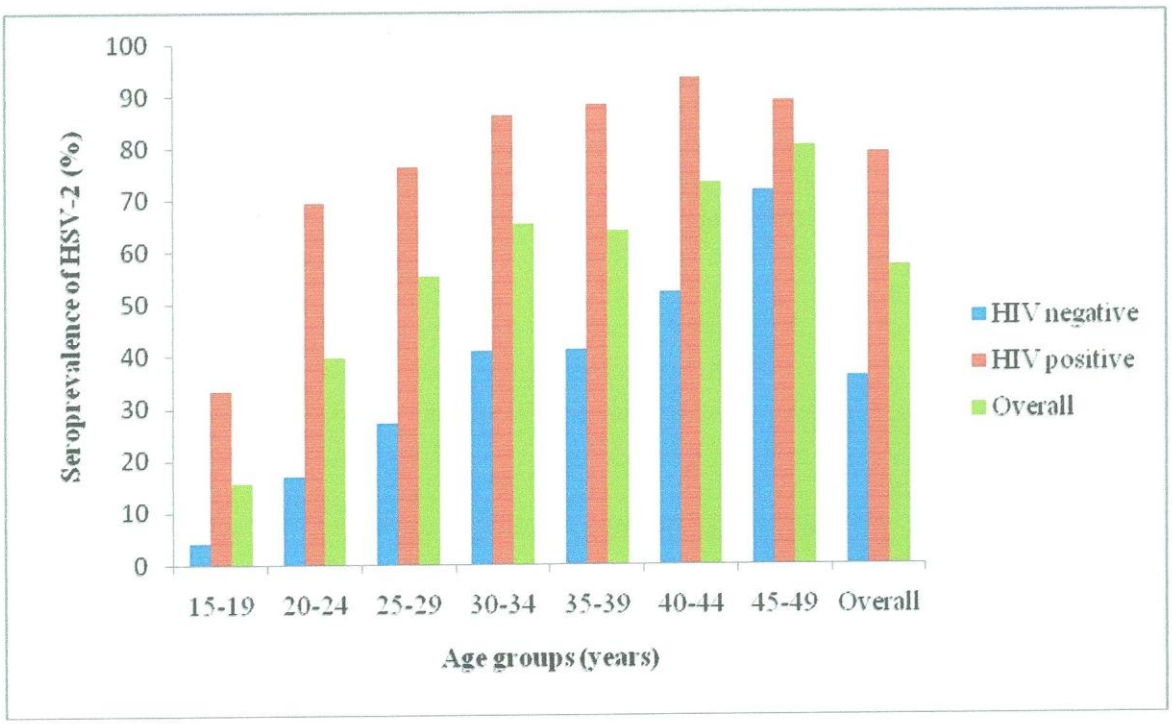


Figure 3. Seroprevalence of Herpes Simplex Virus type 2 by HIV Status and Age among study participants

The chart above, (figure 3) is a simplification of data shown in table 3 that shows the seroprevalence of Herpes Simplex Virus type 2 infections among study participants without considering sex. HIV infected individuals in the age group of 40-44 years have the highest prevalence of HSV-2 (93.5%) compared to only 33.3% of HIV infected individuals in the age group of 15-49 years. The prevalence is also highest (71.7%) among HIV negative in the age group of 45-49 years compared to only 3.3% of HIV negatives in the age group of 15-19 years. When overall prevalence of HSV-2 infections is considered among different age groups, data shows individuals between 45 and 49 being more affected with seroprevalence of 80.4% compared to only 57.4% in this study population.

Table 4. Increase of HSV-1 prevalence with age - Results of a poisson regression test with robust variance estimates.

	Number of Observations	Prevalence ratios	p-value	95% CI
a) P-values for trend of HSV-1 over age by HIV infection status and Sex				
HIV negative females	161	0.99	0.16	0.97-1.01
HIV negative males	159	1.01	0.34	0.99-1.02
HIVpositive females	137	0.97	0.13	0.94-1.01
HIV positive males	154	0.98	0.25	0.95-1.01
b) P-values for trend of HSV-1 over age by HIV infection status, regardless of Sex				
HIV negative	320	1.00	0.84	0.99-1.01
HIV positive	291	0.98	0.08	0.96-1.00
c) P-value for trend of HSV-1 over age regardless of HIV status and Sex				
	611	0.99	0.82	0.98-1.00

Table 4 shows the trend of seroprevalence of Herpes Simplex Virus type 1 infections by age. All the prevalence ratios are less than one and all the p-values are greater than 0.05. These findings suggest that a Herpes Simplex Virus type 1 infection doesn't have a significant change in trend of prevalence by age.

Table 5. Increase of HSV-2 prevalence with age - Results of a poisson regression test with robust variance estimates.

	Number of Observations	Prevalence ratios	p-value	95% CI
a) P-values for trend of HSV-1 over age by HIV infection status and Sex				
HIV negative females	160	1.31	<0.001	1.21-1.43
HIV negative males	158	1.43	<0.001	1.26-1.63
HIV positive females	156	1.08	<0.001	1.04-1.12
HIV positive males	155	1.14	<0.001	1.08-1.21
b) P-values for trend of HSV-1 over age by HIV infection status, regardless of Sex				
HIV negative	318	1.36	<0.001	1.26-1.46
HIV positive	311	1.10	<0.001	1.06-1.14
c) P-value for trend of HSV-1 over age regardless of HIV status and Sex				
	635	1.18	<0.001	1.14-1.22

Table 5 shows prevalence ratios for the trend of Herpes Simplex Virus type 2 infections over age. All the prevalence ratios are positive and greater than one and all their p-values are less than 0.001 and the confidence intervals are greater than one; meaning there is a strong and significant trend that HSV-2 prevalence increases with age. These prevalence ratios indicate how much the probability of being HSV-2 seropositive increases with every 5 years of age.

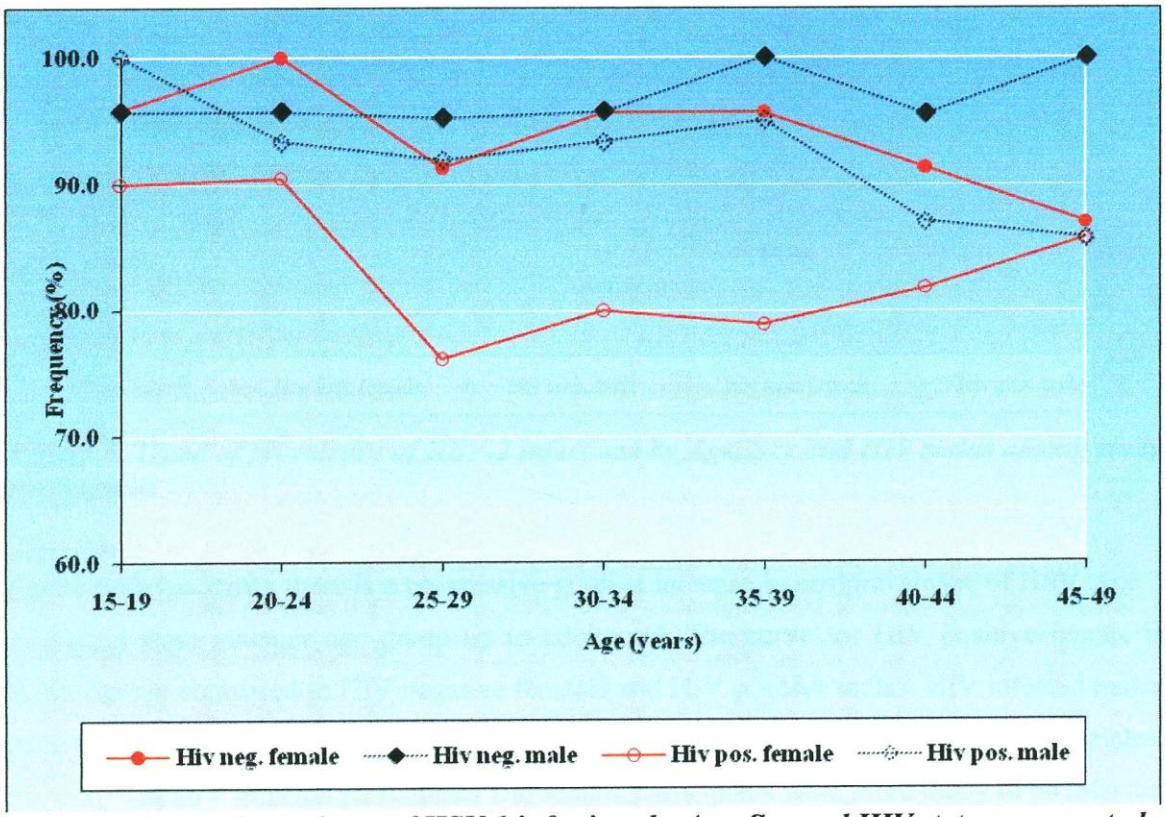


Figure 4. Trend of prevalence of HSV-1 infections by Age, Sex and HIV status among study participants

When presented in a line graph as in figure 4, there is no significant change in trend of HSV-1 from 15 years old up to 49 years. Surprisingly individuals with HIV infections had lower HSV-1 infection relative to individuals without HIV infections; and HIV positive women had the lowest prevalences compared to fellow HIV negative females and also males.

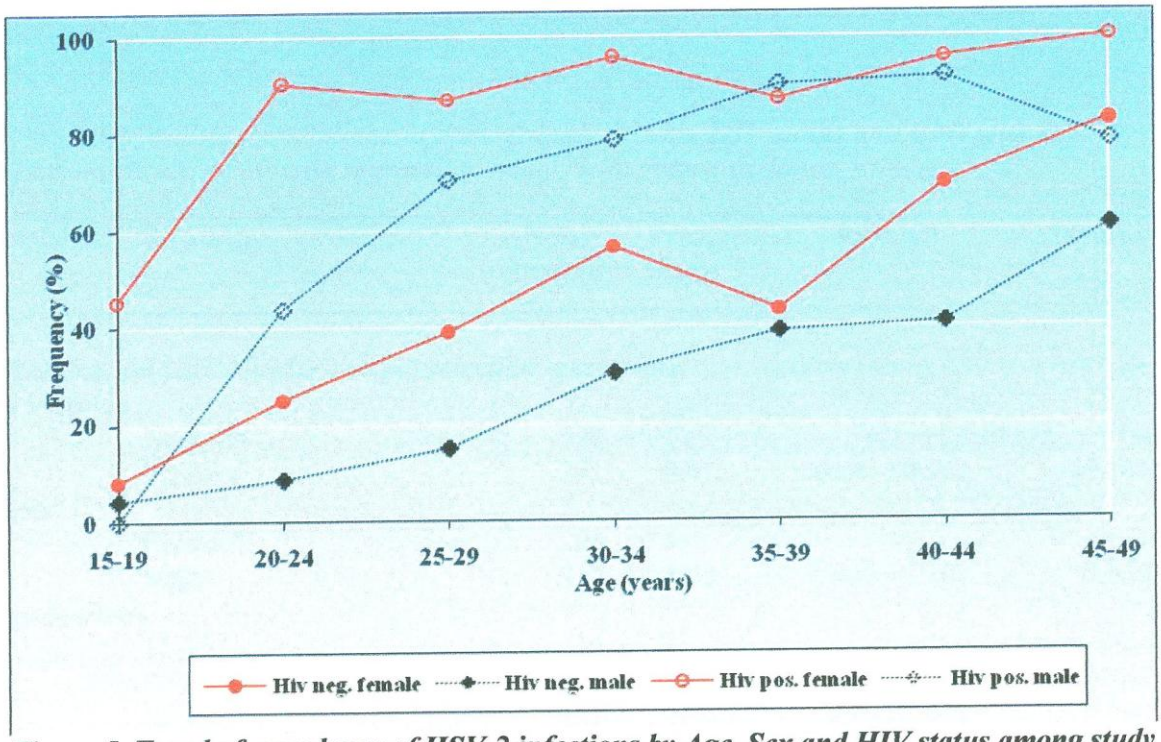


Figure 5. Trend of prevalence of HSV-2 infections by Age, Sex and HIV status among study participants

Figure 5 above shows there is a progressive gradual increase in seroprevalence of HSV type 2 infections from younger age group up to adulthood. The curve for HIV positive female is much steeper compared to HIV negative females and HIV positive males. HIV infected males have also occupied a large area under the curve compared to HIV negative male and females, showing that HIV infected participants and female participants were more likely to be infected with HSV-2 and that prevalence in all groups increased with age.

The univariate univariate model (table 6) for HSV-1 infection shows that being HIV positive is associated with a decrease of HSV-1 infection of about 10% (p-value<0.001), however, sex and age have no role. When all other variables are adjusted for Gender, Age and HIV status, there are no effects of being HSV-1 positive by level of education, marital status or employment type but being a Muslim was associated with an increase of prevalence of HSV-1 by a factor of 1.16 (p-value<0.001).

Table 6. Univariate associations of demographic factors, HIV status and BMI with HSV-1 infection, results of poisson regression models with robust variance estimates.

Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Stratification variables for sample selection, univariate					
HIV status					
	Negative*	320	1	--	--
	Positive	291	0.91	(0.87 - 0.96)	<0.001
Sex					
	Female*	298	1	--	--
	Male	313	1.05	(1.00 - 1.10)	0.050
Age in years					
	15-19*	75	1	--	--
	20-24	80	0.99	(0.92 - 1.06)	0.764
	25-29	103	0.96	(0.89 - 1.03)	0.280
	30-34	94	0.92	(0.84 - 1.00)	0.059
	35-39	82	0.99	(0.93 - 1.06)	0.789
	40-44	88	0.95	(0.87 - 1.03)	0.186
	45-49	89	0.92	(0.85 - 1.01)	0.078
Socio-demographic variables adjusted only for Sex, HIV status and Age					
Education level					
	No formal education*	80	1	--	--
	Uncompleted primary	79	0.99	(0.90 - 1.09)	0.857
	Completed primary	361	1.00	(0.93 - 1.09)	0.949
	Uncompleted secondary	37	0.97	(0.86 - 1.11)	0.696
	Still a Student	54	1.00	(0.90 - 1.11)	0.975
Marital status					
	Never Married*	151	1	--	--
	Married-One Spouse	335	0.98	(0.93 - 1.05)	0.625
	Married-Multiple Spouses	31	0.92	(0.78 - 1.08)	0.312
	Separated/Divorced	43	0.98	(0.87 - 1.10)	0.689
	Widowed	51	1.02	(0.91 - 1.15)	0.716
Religion					
	No Religion*	99	1	--	--
	Christian	475	1.05	(0.98 - 1.14)	0.171
	Muslim	19	1.16	(1.07 - 1.26)	<0.001
	No response or N/A	18	1.00	(0.87 - 1.15)	0.980

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Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Employment type					
	Unemployed*	41	1	--	--
	Occasional employment	88	0.95	(0.86 - 1.05)	0.294
	Seasonal employment	307	0.97	(0.90 - 1.04)	0.382
	Permanent employment	73	0.96	(0.87 - 1.07)	0.477
	Self-employed	42	1.04	(0.96 - 1.13)	0.364
	Student	54	0.96	(0.87 - 1.06)	0.399
	No response or N/A	6	0.88	(0.60 - 1.29)	0.509
Socio-demographic and behavioural variables adjusted for Sex, HIV status and Age					
Had blood transfusion last year					
	No*	598	1	--	--
	Yes	10	1.09	(1.05 - 1.14)	<0.001
	No response or N/A	3	1.07	(0.99 - 1.15)	0.072
Person ever had sex					
	No*	64	1	--	--
	Yes	530	1.12	(1.00 - 1.26)	0.051
	No response or N/A	17	1.16	(0.99 - 1.36)	0.069
Age at first sexual intercourse					
	Never had Sex*	64	1	--	--
	<16	51	1.08	(0.94 - 1.23)	0.278
	16-19	325	1.13	(1.00 - 1.28)	0.041
	≥20	154	1.14	(1.00 - 1.29)	0.053
	No response or N/A	17	1.16	(0.99 - 1.37)	0.061
Number of sex-partners last year					
	None*	106	1	--	--
	1	280	1.06	(0.97 - 1.16)	0.181
	≥2	115	1.05	(0.95 - 1.16)	0.346
	No response or N/A	110	1.05	(0.96 - 1.16)	0.288
Ever used condom last year					
	No*	483	1	--	--
	Yes	126	0.96	(0.89 - 1.02)	0.184
	No response or N/A	2	1.06	(0.96 - 1.16)	0.245
Number of children					
	None*	120	1	--	--
	1	87	1.02	(0.95 - 1.11)	0.579
	2-3	167	1.01	(0.93 - 1.10)	0.835
	≥4	200	1.01	(0.92 - 1.11)	0.880
	No response or N/A	37	1.07	(0.99 - 1.16)	0.080

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Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Spouse lives in household					
	No*	32	1	--	--
	Yes	334	1.08	(0.93 - 1.25)	0.313
	No response or N/A	245	1.09	(0.94 - 1.27)	0.239
Number of spouses living in household					
	1*	326	1	--	--
	2	7	0.76	(0.48 - 1.21)	0.253
	3	1	0.00	--	--
	No response or N/A	277	0.98	(0.93 - 1.04)	0.558
Area of residence for most of life					
	Rural Area*	519	1	--	--
	Town/City	74	1.03	(0.98 - 1.10)	0.264
	No response or N/A	18	0.96	(0.84 - 1.09)	0.490
Nights away from home last month					
	0*	535	1	--	--
	≥1	75	0.94	(0.86 - 1.02)	0.148
	No response or N/A	1	1.12	(1.05 - 1.20)	<0.001
Nights away from home last year					
	0*	292	1	--	--
	≥1	266	1.00	(0.95 - 1.05)	0.844
	No response or N/A	53	1.03	(0.96 - 1.12)	0.420
Circumcised (males only)					
	No*	258	1	--	--
	Yes	55	1.08	(1.04 - 1.12)	<0.001
Health and Anthropometric variables adjusted for Sex, HivStatus and Age					
HSV-2 Status					
	Negative*	265	1	--	--
	Indeterminate	10	0.97	(0.78 - 1.19)	0.751
	Positive	335	0.95	(0.90 - 0.99)	0.012
Hypertensive					
	No*	557	1	--	--
	Yes	53	1.07	(1.00 - 1.14)	0.053
	No response or N/A	1	1.04	(0.99 - 1.10)	0.121
Body mass index					
	<18.50*	48	1	--	--
	18.50-24.99	448	0.99	(0.92 - 1.07)	0.833
	25.00-29.99	85	1.00	(0.90 - 1.10)	0.965
	≥30.00	26	0.91	(0.76 - 1.08)	0.285
	No response or N/A	4	0.81	(0.48 - 1.36)	0.428

Probability of being seropositive for HSV-1 also increases by 9% (p-value<0.001) if there is a history of blood transfusion, also circumcision by 1.08 (p-value<0.001), and age at first sexual intercourse in the age of 16 up to 19 years by a factor of 1.13 (p-value=0.041). Being HSV-2 positive decreases the chance of being HSV-1 positive by a factor of 5% (p-value=0.012). Also the prevalence increase in individual with elevated blood pressure by 7% (p-value=0.053). However prevalence of HSV-1 is not associated with the number of sexual partners, using condoms, number of children, number of spouses or living with a spouse, area of residence for most of life, nights away from home, fever or body mass index.

When several variables (table 7) were included into the same model, a multivariate association of HSV-1 is shown by a decrease in prevalence of HSV-1 by 0.093 (p-value=0.021) and 0.94 (p-value=0.034) in HIV and HSV-2 positive individuals respectively but also by 0.87 if you are in the age group of 30-34 years and 45-49 years; however, there is an increase of factor of 1.13 (p-value=0.006) if you are a Muslim, up to 1.14 (p-value=0.036) if age at first sexual debut is 16 years and above and 1.08 (p-value=0.031) if hypertensive.

Table 7. Mutivariate associations of demographic factors, HIV status and BMI with HSV-1 infection among study participants. *The below model includes all variables where the multivariate p-values for at least of the strata reached significance ($p<0.05$)*

Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
HIV status					
	Negative*	320	1	--	--
	Positive	291	0.93	(0.89 - 0.98)	0.004
HSV-2 Status					
	Negative*	265	1	--	--
	Indeterminate	11	0.98	(0.81 - 1.19)	0.862
	Positive	335	0.94	(0.90 - 0.98)	0.004
Sex					
	Female*	298	1	--	--
	Male	313	1.04	(0.99 - 1.10)	0.110
Age in years					
	15-19*	75	1	--	--
	20-24	80	0.94	(0.85 - 1.04)	0.250
	25-29	103	0.92	(0.82 - 1.02)	0.128
	30-34	94	0.87	(0.77 - 0.98)	0.021
	35-39	82	0.93	(0.84 - 1.03)	0.172
	40-44	88	0.89	(0.79 - 1.00)	0.059
	45-49	89	0.87	(0.77 - 0.99)	0.034
Religion					
	No Religion*	99	1	--	--
	Christian	475	1.05	(0.97 - 1.13)	0.211
	Muslim	19	1.13	(1.04 - 1.24)	0.006
	No response or N/A	18	1.04	(0.90 - 1.20)	0.607
Age at first sexual intercourse					
	Never had Sex*	64	1	--	--
	<16	51	1.09	(0.95 - 1.24)	0.223
	16-19	325	1.14	(1.01 - 1.30)	0.036
	≥20	154	1.15	(1.01 - 1.31)	0.041
	No response or N/A	17	1.19	(1.01 - 1.39)	0.036
Circumcised (males only)					
	No*	556	1	--	--
	Yes	55	1.06	(1.01 - 1.10)	0.011
Hypertensive					
	No*	557	1	--	--
	Yes	53	1.08	(1.01 - 1.15)	0.031
	No response or N/A	1	1.12	(1.01 - 1.25)	0.037

Table 8 shows association of various factors with HSV-2 infection using Poisson regression with robust variance estimates for stratification variables in univariate and multivariate models.

In a univariate model, it was found being HIV positive increases the chance of having Herpes Simplex type 2 infections about two-folds ($p<0.001$). Increasing age is significantly associated with increased probability of having Herpes Simplex Virus infections 2 to 5 times (p-value < 0.001). Those who have had sex had four folds higher chances of being reactive to Herpes Simplex Virus infections type 2 (p-value=0.005). Those who started sexual intercourse before the age of 16 and those who debuted at 20 years and above have higher chances of being HSV-2 positive by more than 4.6 (p-value=0.003) and 4 (p-value=0.007) times respectively compared to individuals who never had sexual intercourse in their life. Also the number of sexual partners increases the chance of being HSV-2 positive by 1.88 (p-value<0.001) if one two or more number of sex partners for the last year.

Participants having one or more kid slightly had increased chance of being HSV-2 seropositive (p-value=0.007), however factors such as marital status, site of residence, religion, tribe, being HSV-1 positive, circumcision, night away from home last year or month, number of spouse living in the household, coming from town or rural areas, fever, history of blood transfusion and hypertension does not increase the chance of being HSV-2 seropositive; however, still being a student decreases the chance of being HSV-2 seropositive by a factor of 0.15 (p-value=0.007). However obese participants were 1.9 times more likely to have HSV-2 IgG antibodies (p-value = 0.001)

Table 8. Univariate associations of demographic factors, HIV status and BMI with HSV-2 infection, results of poisson regression models with robust variance estimates.

Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Stratification variables for sample selection, Univariate					
HIV status					
	Negative.*	318	1	--	--
	Positive	311	2.19	(1.87 - 2.56)	<0.001
Sex					
	Female*	316	1	--	--
	Male	313	0.78	(0.68 - 0.89)	<0.001
Age in years					
	15-19*	77	1	--	--
	20-24	83	2.55	(1.42 - 4.57)	0.002
	25-29	103	3.55	(2.05 - 6.15)	<0.001
	30-34	95	4.19	(2.44 - 7.19)	<0.001
	35-39	89	4.11	(2.39 - 7.07)	<0.001
	40-44	90	4.71	(2.76 - 8.03)	<0.001
	45-49	92	5.16	(3.04 - 8.77)	<0.001
Socio-demographic variables adjusted only for Sex, HIV status and Age					
Education level					
	No formal education*	87	1	--	--
	Uncompleted primary	83	0.87	(0.71 - 1.06)	0.170
	Completed primary	365	1.01	(0.88 - 1.15)	0.935
	Uncompleted secondary.	40	1.00	(0.80 - 1.25)	0.997
	Still a student	54	0.15	(0.04 - 0.60)	0.007
Marital status					
	Never Married*	152	1	--	--
	Married-One Spouse	344	1.17	(0.91 - 1.52)	0.219
	Married-Multiple Spouses	33	1.21	(0.90 - 1.63)	0.212
	Separated/Divorced	44	1.17	(0.87 - 1.58)	0.298
	Widowed	56	1.05	(0.79 - 1.40)	0.715
Religion					
	No religion*	105	1	--	--
	Christian	487	0.93	(0.80 - 1.07)	0.293
	Muslim	19	0.99	(0.76 - 1.30)	0.947
	No response or N/A	18	0.00	--	--

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Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Employment type					
	Unemployed*	44	1	--	--
	Occasional employment	91	1.17	(0.91 - 1.49)	0.218
	Seasonal employment	315	1.11	(0.90 - 1.38)	0.324
	Permanent employment	77	1.18	(0.94 - 1.48)	0.161
	Self-employed	42	0.95	(0.70 - 1.30)	0.761
	Student	54	0.17	(0.04 - 0.66)	0.011
	No response or N/A	6	0.84	(0.29 - 2.45)	0.747
Socio-demographic and behavioural variables adjusted for Sex, HIV status and Age					
Had blood transfusion last year					
	No*	616	1	--	--
	Yes	10	1.17	(0.75 - 1.81)	0.483
	No response or N/A	3	1.32	(0.63 - 2.78)	0.463
Person ever had sex					
	No*	63	1	--	--
	Yes	544	4.20	(1.53 - 11.51)	0.005
	No response or N/A	22	4.55	(1.62 - 12.76)	0.004
Age at first sexual intercourse					
	Never had Sex*	63	1	--	--
	<16	56	4.56	(1.66 - 12.53)	0.003
	16-19	328	4.11	(1.50 - 11.28)	0.006
	≥20	160	4.01	(1.46 - 11.03)	0.007
	No response or N/A	22	4.49	(1.60 - 12.60)	0.004
Number of sex-partners last year					
	None*	105	1	--	--
	1	288	1.58	(1.26 - 1.98)	<0.001
	≥2	116	1.88	(1.45 - 2.44)	<0.001
	No response or N/A	120	1.64	(1.28 - 2.10)	<0.001
Ever used condom last year					
	No*	496	1	--	--
	Yes	130	1.24	(1.09 - 1.42)	0.001
	No response or N/A	3	2.07	(0.97 - 4.44)	0.061
Number of children					
	None*	122	1	--	--
	1	90	1.32	(1.02 - 1.72)	0.036
	2-3	173	1.38	(1.09 - 1.73)	0.007
	≥4	204	1.18	(0.93 - 1.50)	0.162
	No response or N/A	40	1.27	(0.87 - 1.84)	0.208

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Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Spouse lives in household					
	No*	33	1	--	--
	Yes	344	1.00	(0.80 - 1.26)	0.996
	No response or N/A	252	0.91	(0.71 - 1.15)	0.417
Number of spouses living in household					
	1*	336	1	--	--
	2	7	1.11	(0.79 - 1.58)	0.548
	3	1	1.06	(0.91 - 1.23)	0.470
	No response or N/A	285	0.93	(0.83 - 1.05)	0.246
Area of residence for most of life					
	Rural Area*	531	1	--	--
	Town/City	80	0.96	(0.81 - 1.15)	0.671
	No response or N/A	18	0.00	--	--
Nights away from home last month					
	0*	546	1	--	--
	≥1	81	1.03	(0.89 - 1.19)	0.704
	No response or N/A	2	1.87	(0.87 - 3.99)	0.108
Nights away from home last year					
	0*	298	1	--	--
	≥1	275	1.08	(0.96 - 1.22)	0.211
	No response or N/A	56	0.99	(0.79 - 1.24)	0.951
Circumcised (males only)					
	No*	259	1	--	--
	Yes	54	1.05	(0.84 - 1.32)	0.658
	No response or N/A	316	1.37	(1.21 - 1.55)	<0.001
Health and Anthropometric variables adjusted for Sex, HIV Status and Age					
HSV-1 status					
	Negative*	47	1	--	--
	Indeterminate	18	0.98	(0.80 - 1.21)	0.870
	Positive	553	0.88	(0.77 - 1.00)	0.058
	No response or N/A	11	0.99	(0.72 - 1.36)	0.945
Body temperature above 37 Celsius					
	No*	482	1	--	--
	Yes	146	0.97	(0.85 - 1.10)	0.628
	No response or N/A	1	3.34	(2.60 - 4.28)	<0.001

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Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
Hypertensive					
	No*	573	1	--	--
	Yes	55	1.12	(0.95 - 1.32)	0.164
	No response or N/A	1	3.37	(2.63 - 4.32)	<0.001
Body Mass Index					
	<18.50*	50	1	--	--
	18.5-24.99	460	1.50	(1.13 - 2.01)	0.006
	25.00-29.99	88	1.49	(1.09 - 2.05)	0.013
	≥30	27	1.93	(1.32 - 2.82)	0.001
	No response or N/A	4	1.99	(1.09 - 3.66)	0.026

In a multivariate poisson regression with robust variance estimates (table 9), being HIV positives were still about twice as likely to be HSV-2 infected than negatives (p-value≤0.001) and increasing age was still significantly related to increasing HSV-2 infection. Having two up to three children increases the chance by 1.29 (p=0.028) while probability increases by 1.32 to 1.61 when you have one sexual partners and two or more sexual partners respectively (p-values=0.010 and <0.001 respectively). Participants with body mass index of 30kg/m^2 and above shows an increased likelihood of being HSV-2 positive by 1.67 (p-value=0.01). Meanwhile being a male and still being a student decreases the chance of being HSV-2 seropositive by 28% (p-value≤0.001) and 22% (p-value=0.033) respectively.

Table 9. Multivariate associations of demographic factors, HIV status and BMI with HSV-2 infection among study participants. *The below model includes all variables where the multivariate p-value for at least one of the strata reached significance ($p<0.05$)*

Variable	Stratum	N in stratum	Prev. Ratio	95% CI	P-value
HIV status					
	Negative*	318	1	--	--
	Positive	311	2.05	(1.78 - 2.35)	<0.001
Sex					
	Female*	316	1	--	--
	Male	313	0.72	(0.62 - 0.83)	<0.001
Age in years					
	15-19*	77	1	--	--
	20-24	83	1.50	(0.95 - 2.37)	0.083
	25-29	103	1.77	(1.14 - 2.76)	0.011
	30-34	95	2.10	(1.36 - 3.25)	0.001
	35-39	89	2.25	(1.44 - 3.49)	<0.001
	40-44	90	2.70	(1.74 - 4.19)	<0.001
	45-49	92	2.87	(1.84 - 4.48)	<0.001
Participant is still student					
	No*	575	1	--	--
	Yes	54	0.22	(0.06 - 0.88)	0.033
Number of children					
	None*	122	1	--	--
	1	90	1.19	(0.92 - 1.52)	0.181
	2-3	173	1.29	(1.03 - 1.61)	0.028
	≥4	204	1.11	(0.89 - 1.40)	0.348
	No response or N/A	40	1.19	(0.82 - 1.74)	0.364
Number of sex-partners last year					
	None*	105	1	--	--
	1	288	1.32	(1.07 - 1.63)	0.010
	≥2	116	1.61	(1.26 - 2.07)	<0.001
	No response or N/A	120	1.36	(1.07 - 1.71)	0.011
Body Mass Index					
	<18.50*	50	1	--	--
	18.50-24.99	460	1.36	(1.04 - 1.78)	0.024
	25.00-29.99	88	1.33	(0.99 - 1.77)	0.059
	≥30	27	1.67	(1.15 - 2.43)	0.007
	No response or N/A	4	2.03	(1.15 - 3.58)	0.015

7. DISCUSSION

This study presents probably the first report of prevalence of Herpes Simplex Virus type 1 and 2 infections among HIV affected individuals in Tanzania. Stratification of study participants by HIV status, sex and age was done in order to have enough participants in each stratum. About 50% of study subjects were HIV infected, contrary to 9.3% of prevalence of HIV infection in the general population of Mbeya. This did not interfere with the analysis for the seroprevalence of HSV infections in HIV infected and even in uninfected individuals; however, it does not represent the overall prevalence in the general population. In order to calculate the prevalence of Herpes Simplex Virus infections in the general population extrapolation of these research findings was possible after standardizing current results to the age, sex and HIV status distribution of the EMINI study population.

Figure 6 shows the estimate for the overall prevalence of HSV-1 and HSV-2 in EMINI study which was 95.1% and 34.4% respectively. Among HIV positive participants the prevalence of HSV-2 is 82.1% (vs. 27.4% in HIV negative) while HSV-1 prevalence was 96.3% in HIV negative individuals compared to 87.4% in HIV positive individuals. Since EMINI is a population based study conducted at various sites in Mbeya region with diverse conditions, it is likely that the prevalence in the population between 15 and 50 years in Mbeya region are similar.

In this cross sectional study that used stratified random selection of 640 subjects, it has been found that the overall seroprevalence of Herpes Simplex Virus type 2 infections is 57.4% which translates into a population prevalence of 34.4%. This proportion is similar to that from the Carribean Island population (34%)[4] and lower than that found in the Rakai study in Uganda, where 58.2% of study population had serological evidence of HSV-2 infection[130], and differs slightly with findings among fishermen in Kisumu, Kenya where the seroprevalence was 63.9%[131], in a rural Ugandan population (67.9%) [12]. This result is consistent with other study from Moshi Tanzania where the prevalence was 56.3%[132], however, it was lower compared to one recent study from Dar es Salaam[16]. Differences in the

prevalences could be explained by the fact that this study population was highly selected but still results are comparable with other studies done in the general population of some areas.

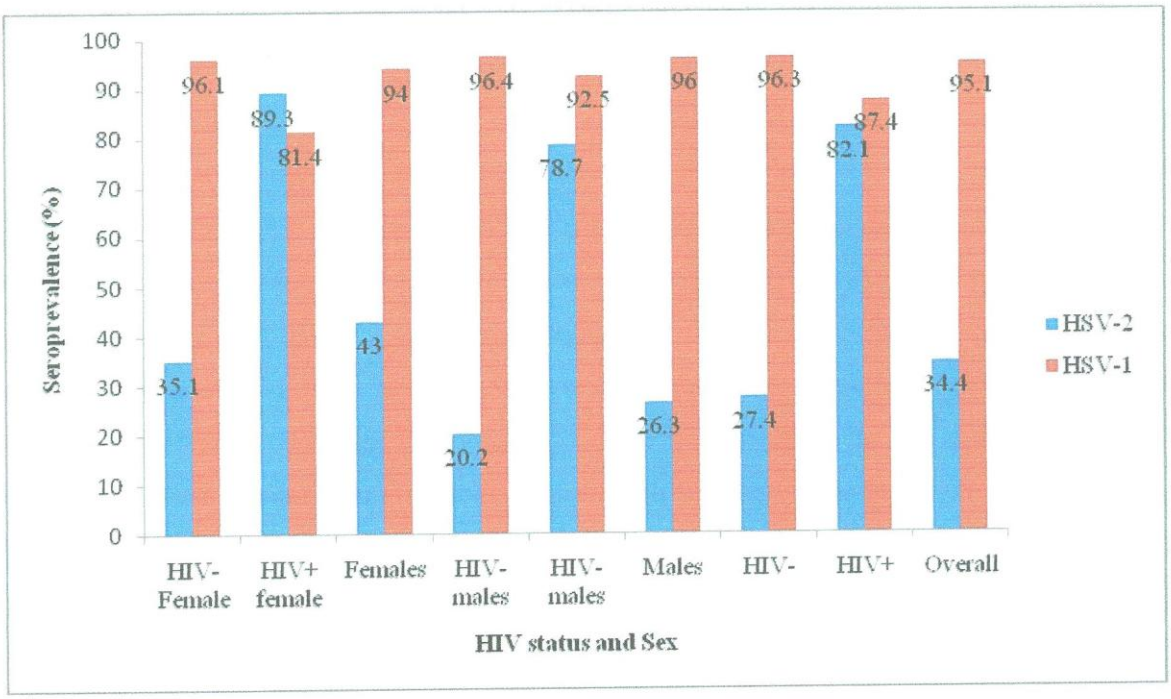


Figure 6. Extrapolated estimate of HSV-1 and HSV-2 prevalence in persons from 15 to 49 years of age in the general population of Mbeya region.

In this study females had a higher prevalence of Herpes Simplex Virus type 2 infections compared to men (64.6% vs 50.2%). This is lower compared to results from Rakai among men aged 15-49 years (33.76%)[133] and similar to prevalence of 64.5% that was reported among females in rural Zimbabwe[134] and relatively similar to studies from Uganda (69.3% vs 32.2%)[130] and Mwanza(75% vs 60%)[17]. Yet this study found seroprevalence of HSV-2 among females to be lower compared to the study that was done in Mbeya region among female bar workers, where the prevalence was 87%[18]; however, these bar workers had higher rate (68%) of HIV infections compared to 50% in this study population.

A study done in Dar es Salaam, Tanzania, among adolescents aged 17 to 20 years, showed a HSV-2 prevalence of 40% [14] whereas in this study only 15.5% of children in the age between 15 and less than 20 years were reactive to HSV-2. One reason for this may be that this age-stratum in the index study included younger participants; however, this is unlikely to explain the whole difference.

Taken as a whole, having higher prevalence of HSV-2 among females could be one of the factors that increase women's susceptibility to HIV infections[135]. But this also reflects the current prevalence of HIV in Tanzania where more women are affected than men. The question is why HSV-2 is more common in women. Some investigators have suggested that anatomical difference between females and males[136], differences in the pattern of mixing between the two genders may also expose women to higher prevalence of infections at younger ages[137] and also a tendency of young women to form partnership with older men[138]. Anderson et al described differences in the distribution of sexual risk behaviours between men and women being a contributing factor[139].

The unadjusted seroprevalence of Herpes simplex virus type 1 infections in this study population was 92.1%, the prevalence estimate for the general population is 95.1%. This is higher compared to that from United states of America where it is estimated at 67%[140], Israel 59.8% [141] and 80% from Switzerland[142]. It is however comparable to studies in Syria(more than 95%)[143], rural Uganda (91.2%)[12] and in urban region of Tanzania (92%)[14].

The index study further demonstrates that seroprevalence of HSV-1 among males is relatively higher compared to that of females (94.2% vs 89.9%), the prevalence estimate in the general population was 96.0% in males versus 94.0% in females. even though there is no statistical significance and the reverse being true this is much higher compared to other studies including from Japan (55.4% vs 63.3%)[144], but agrees with a study from Romania (91.1%) in each sex[145] and the study that was done in Brazil, Estonia, Morocco, and Srilanka where the prevalences ranged from 78.5%-93.6% in males and 75.5%-97.8% in females[146].

In agreement with the research findings of other investigators[55, 147-149] in their observation and cohort studies, HIV was strongly related to HSV-2 seropositivity in a univariate and multivariate model. Generally the prevalence of Herpes Simplex Virus type 2 infections was higher among HIV positive study subjects compared to HIV negative individuals (79.1% vs 36.2%). These prevalences were relatively similar to that of South Africa where HIV positive individuals had a prevalence of 71.6% compared 19.6% of HIV negative[147] and also agrees with recent findings from India (49% vs 22%) [148] and Brazil[149]. This result probably relates to level of poverty and limited access to health.

Unexpectedly, the present study shows a lower prevalence of herpes simplex type 1 infections among HIV positive individuals compared to HIV negatives (87.6% vs 96.3%), however, relatively lower to findings in the HIV infected German population where the prevalence was 91.1% [150]. Also this study has shown that 95.6% of non HIV infected females compared to 83.2% of HIV infected were seropositive for HSV-1 while 96.9% of males who HIV positive, were HSV-1 seropositive compared to 91.6%. In a multivariate analysis HIV positive individual had lower HSV-1 positivity with a prevalence ratio of 0.93 (p-value < 0.05) this is quite contrary to other reports[151, 152]. Since HSV-1 is more prevalent in males, probably it provides some degree of cross protection to HSV-2 which is less prevalent in males as some literatures have already reported that HSV-1 infection might render a certain protection against an HSV-2 infection[7].

According to the uni- and multivariate analysis of predictors of HSV-2, the present study confirms findings from other studies. In this study HIV seropositivity was associated with twice increased likelihood of being HSV-2 positive, and this agree with several studies[16, 18, 132, 148].

Like other research findings this study has also shown that the seroprevalence of HSV-2 significantly increases with age[141, 142, 145, 153], women are more infected than men[135, 145, 153], depend on number of sexual partners and coitarchal age[130, 145, 149].

However, the following factors, some of which were associated with HSV-2 infection in other studies did not show an association in this current study: level of education[145], marital status[142], type of employment, blood transfusion, condom use, and number of children and religion[132] were not predicters of HSV-2 seropositivity.

Participants who reported to be circumcised were not protected against HSV-2 like in other studies[154], this is different to many other studies which have shown there is a protective effect of male circumcision on HSV-2 acquisation by males[131, 155, 156]. This could be explained by the fact that societies in mainland do circumcision at a later age following sexual maturity.

In a multivariate analysis, prevalence of HSV-2 infections was independently and significantly shown to be associated with overweight and obesity (PR 1.67; $p<0.05$) which contradicts findings from the study which showed being obese or overweight was not associated with HSV-2 seropositivity[157]. HSV-2 was not significantly associated with essential hypertension as it has been reported in other studies[158], although there was a non-significant association in the same direction visible in these data.

Contrary to other studies where seroprevalence of HSV-1 was significantly higher in females[141], these data show that men are more often infected than women, although this association was not significant anymore when adjusted for other factors in the mutivariate model (PR 1.04, p-value=0.05)[152]. In this study it was found that HSV-1 is less prevalent among HIV infected individuals. This finding is surprising and difficult to explain. More research is required into this area.

In the present study, HSV-1 seropositivity was negatively associated with HSV-2 seropositivity both in the univariate and the mutivariate (PR=0.94, p-value=0.004) models. This is contrary to a study done in Israel where the seroprevalence was significantly high among subjects seropositive for HSV-2[141, 142, 144].

Some studies have found that only 17% of children had HSV-1 antibody at the age of 8 years and concluded that HSV-1 infectionis becoming acquired later in life[159]. However, in the current study the highest prevalence (96%) of HSV-1 was found in the age group of 15-19 years, which does not real support this hypothesis. In Israel HSV-1 reached 50% seropositivity at the age of 14 years, although this is lower than in the index study group, they also found the prevalence to be stable over various age groups like in this study[160].

Like in previous works this study has demonstrated the role of age at first sexual intercourse[152, 161] from 16 years and to be associated with HSV-1 seropositivity. Meanwhile under mutivariate models, being a Muslim (PR 1.13, p-value<0.05) and hypertension (PR 1.08, p<0.05) were independently associated with HSV-1 seropositivity.

Participants in the age group of 30-34 years (PR 0.87, p=0.021) and 45-49 years (PR 0.87, p=0.034) were relative less likely to be HSV-1 seropositive than others. Suprisingly, those who are circumcised were more affected with HSV-1 (PR 1.06, p-value<0.05), however, the age interval of circumcision and sexual debut was not sought off.

In contrast to other studies sex[146], age[146, 160, 162], level of education[142], marital status, ever having had sex, number of sexual partners, condom use, number of spouses, area of residence most of lifetime and body mass index had no influence on the prevalence.

The prevalence of HSV-1 is not affected by age contrary to HSV-2, this can be explained by the fact that HSV-2 is more of a sexually transmitted infections and individuals starts to acquire it when are practicing sex whilst HSV-1 is acquired during childhood[159].

In this study it has been shown HSV type 1 and HSV- type 2 are associated with hypertension and overweight or obesity respectively. Association of herpes simplex infections with cardiovascular morbidities have been under research for some time now. Some studies found significant association of herpes simplex type 1 with metabolic syndromes[163] and type 2 diabetes mellitus and dyslipidemia[164, 165] however, the underlying pathogenesis is still unclear. Some investigators propose that HSV infection leads to lipid accumulation. When it affect the endothelial cells, leukocytes will be attracted with subsequent inflammatory damage; it also activates procoagulant changes on the endothelium with increased thrombin generation and platelet adhesion, and changes its interaction with extracellular matrix proteins[166].

Herpes Simplex Virus infections have also been linked with coronary heart diseases[167-170] whereby HSV-heat shock protein 60 IgG antibodies seems to have played an important role[168] and Herpes Simplex has also been implicated in atherosclerosis through endothelial dysfunction[171]. Some studies have shown Herpes Simplex Virus infected monocytes are related to the incidence of human coronary atherosclerosis[159, 172].

8. STUDY LIMITATIONS

This study shows that both types of HSV infections are very common in this cohort. Limited by its cross sectional design, the analysis of possible risk factors suggests that inferences from its finding should be made cautiously regarding possible observed association while validity of self reported data on sexual behavior obtained by interview using a structured questionnaire might have underreported sexual behavior.

8.1. Strength of the study

The strength of the index study profits from the use of statistical methods, tailored at the high prevalence of Herpes simplex viruses infection. The use of Poisson regression models with robust variance estimates allows for the direct estimation of prevalence ratios which in high prevalence situations are more interpretable than odds ratios[173, 174].

9. CONCLUSIONAND RECOMMENDATION

The prevalence of Herpes Simplex Virusnfections in this study population is higher than expected. Considering recent results th show its association with the pathogenesis, transmission, and morbidity of HIV, contrcand treatment of this disease urgently needs to be considered. High seroprevalence of HSV-is seen more among females and this coincides with higher number of HIV in infection in tl group.

Available options for control of Herpes nplex virus in this population will include education, suppressive treatment, preventing uisition of other STIs and use of condoms.

Meanwhile, recent association of Herpes Sim Virus infections with cardiovascular disease should be meticulously studied in prospective lies.

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Appendix A: Questionnaire and Physical exam checklist

I. Demography and sexual risk factors

1. HIV status
2. Sex of participant
3. Age in years
4. Area of residence
5. Where have you lived most of your life? Rural Area / Town / City
6. What is your tribe?
7. What is your denomination?
8. What is your highest level of education?
9. What is your current main occupation?
10. What type of work do you do?
11. What is your present marital status?
12. Have you been married before this present marriage?
13. Does your spouse or permanent partner also live in this household?
14. With how many spouses and/or permanent partners do you live in this household?
15. How many nights did you spend outside your community last months?
16. Are you circumcised?
17. At what age were you circumcised?
18. Have you ever had sex?
19. At what age did you first have sexual intercourse?
20. How many people have you had sex with during the last year?
21. How often do you normally use condoms?
22. How many children do you have?
23. Did you ever get a blood transfusion?
24. Do you have any of the following symptoms at present? Did you have any of them in the past?
    a. Painful/painless genital ulcers
    b. Painless genital ulcers
    c. Abnormal genital discharge
    d. Genital warts
    e. Blisters on genitals
    f. Painful urination

II. Physical Examination

1. Weight (in kg)
2. Height (in metres)
3. Body Mass Index (kg/m^2)
4. Temperature (^0C)
5. Systolic blood pressure (mm Hg) if $\geq$ 140mmHg = Hypertensive
6. Diastolic blood pressure mm HG $\geq$ 90mmHg = Hypertensive

Appendix B: Kalon and Virotech Assays procedures

I. Kalon HSV-2 ELISA Assay Procedure

1. Determine the number of tests to be done.
2. Select the number of microwell strips required (remember to consider additional 4 wells for the controls and calibrator)
3. Prepare the required volume of WORKING STRENGTH TRACER by mixing TRACER CONCENTRATE and ASSAY DILUENT 1 to 40, e.g. add 250µL Tracer Concentrate to 10mL Assay Diluent.
4. Prepare the required volume of WORKING STRENGTH WASH SOLUTION by diluting WASH CONCENTRATE 1 in 40, e.g. dilute 25ml WASH CONCENTRATE to 1L with purified water.
5. Prepare the required volume of WORKING SUBSTRATE SOLUTION by mixing TMB CHROMOGEN and SUBSTRATE SOLUTION 1 to 40; e.g. add 250µL TMB Chromogen to 10mL substrate solution.

Note:

The substrate solution is colour coded pink as an aid to dispensing. If a blue colour is observed prior to use it means the solution has been contaminated, therefore a fresh dilution should be made.

PRIMARY INCUBATION:

6. Dispense 200µL of ASSAY DILUENT into each well.
7. Dispense 10µL of the KIT CALIBRATOR and the two KIT CONTROLS into their designated microwells

   Note: The cut - off Calibrator should be run in duplicate.
8. Mix each well by pumping the diluted serum in and out of micropipette five times.
9. Dispense 10µL of each test specimen into their designated microwells and mix thoroughly as in step 7.

Note:

Step 6 and 7 should be completed within a combined time of no more than 5 minutes. It is advised that test sera be dispensed into an empty/plane microtitre plate or microtubules (about 40 to50µL) prior to step 6 to 8, and use of a multichannel pipette for dispensing and mixing the sera and diluent, in order to meet the recommended time limit.

10. Seal the plate in the plastic bag and incubate at 37°C for 30minutes.
11. Wash the microwells.
12. If no automated washer, fill and aspirate the microtitre wells with 350µL of fresh WORKING STRENGTH WASH SOLUTION a total of 4 times. Tap out residual wash solution on paper towelling ready for the next step.

SECONDARY INCUBATION

13. Dispense 100µL of WORKING STRENGTH TRACER
14. Seal the plate in the plastic bag and incubate at 37°C for 30minutes.
15. Wash the microwells as before.

ENZYME INCUBATION

16. Dispense 100µL WORKING STRENGTH SUBSTRATE
17. Incubate the plate uncovered at 18 to 25°C for 30 minutes.
18. Dispense 100µL of stop solution into each microwell.

ASSAY COMPLETION

1. Turn on plate reader Tecan Sunrise Absorbance Reader
2. Open HSV2 - Kalon ELISA protocol in the Magellan software for Tecan Sunrise
3. Read the test plate within 30 minutes of addition of the stop solution, using the 450 nm filter.
4. Wipe the bottom of the plate with a Kim-wipe to remove any dust or condensation
5. Place the plate in the drawer of the reader with well A1 at the upper left corner. Record time the plate was read on the worksheet.
6. Discard the plate in the biohazard trash. Clean work area and turn off the reader. Return reagent to fridge.
7. **SAVE PLATE DATA on server O:\Laboratory\Elisa\HSV2 Kalon**
    a. Save plate as the date (i.e. 23-3-09) and the number i.e. #3 after the date.
    b. **Measurements, Records and validity of runs**
        i. **Validity of run:** If Positive Control OD is >0.8, and the ratio of the cut off calibrator OD to the Negative Control OD is >2.0.
        ii. **Interpretation of results:**
            a. OD > Cut off OD × 1.1 is Positive.
            b. OD < Cut off OD × 0.9 is Negative.
            c. Equivocal results will not be repeated.
        iii. Supervisory chain must be informed of all invalid/failed runs.
        iv. If an assay is invalid, a failed run form must be completed and attached to the report and the assay will not be repeated.
        v. The final results and worksheet should be verified by the NIMR MMRP lab supervisor and/or Lab manager.

II. Virotech HSV-1 ELISA Assay Procedure

1. Fill out Virotech ELISA worksheet before beginning assay. Record kit and reagent lot and expiration date information. Record all specimens to be tested. Specimens are run in singleton.
2. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG-positive, negative and cut-off controls as well as diluted patient sera. For cut-off control a double insertion is highly recommended. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
3. After pipetting start incubation for 30 min. at 37°C (with cover).
4. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well (Columbus washer program HSV1 Virotech) Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
5. Pipette 100µl of ready to use conjugate into each well.
6. Incubation of conjugates: 30 min. at 37°C (with cover). Stop conjugate incubation by washing 4 times (please refer to point 4 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark). Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well.
9. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Setyour photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping soution!

I. Reading of the plate:

- **a.** Turn on plate reader Tecan Sunrise Absorbance Reader
- **b.** Open HSV1 Virotech protocol in the Magellan software for Tecan Sunrise

c. Read the test plate within 60 minutes of addition of the stop solution, using the 450 nm filter.
d. Wipe the bottom of the plate with a Kim-wipe to remove any dust or condensation.
e. Place the plate in the drawer of the reader with well A1 at the upper left corner. Record time the plate was read on the worksheet.
f. Discard the plate in the biohazard trash. Clean work area and turn off the reader. Return reagent to fridge.
g. **SAVE PLATE DATA on server O:\Laboratory\Elisa\HSV1Virotech.** Save plate as the date (i.e. 23-3-09) and the number i.e. #3 after the date.

II. Measurements, Records and validity of runs

Copy raw data into HSV-1 excel template. The ready to use controls serve for a semi-quantitative determination of specific IgG-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

Validity of run

a) OD-values

The OD of the blank should be < 0.1.The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)

The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

Calculation of the Virotech Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$\text{VE (Positive control)} = \frac{\text{OD (positive control)}}{\text{OD (cut off control)}}$$

$$\text{VE (Patient serum)} = \frac{\text{OD (patient serum)}}{\text{OD (cut off control)}}$$

Interpretation Scheme IgG VE - Antibodies

VE	Antibodies
< 0.9	Negative
0.9 – 1.1	Borderline
> 1.1	Positive

a. If the measured values are above the defined borderline range, they are considered to be positive.
b. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline and not retested.
c. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
d. Supervisory chain must be informed of all invalid / failed runs. If an assay is invalid, a failed run form must be completed and attached to the report and the assay will not be repeated. The CA log must be filled out as well.