

Seroprevalence of herpes simplex virus type 1 among people living with HIV in Mbeya, Tanzania

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Abstract

Background Despite the significant decline in the prevalence of HIV in Tanzania, the prevalence rates in the Mbeya, Iringa, and Njombe regions are higher than the national average and have remained stable for years. The current stable HIV prevalence may be driven by factors such as a high incidence of sexually transmitted infections (STIs) and high-risk behaviours. In sub-Saharan Africa, it has previously been observed that up to 50% of HIV cases were attributed to herpes simplex type 2 (HSV-2) among low-risk populations. Because the proportion of sexually transmitted HSV-1 is rising and HSV-1 tends to cause the shedding of oropharyngeal mucosal ulcers, it is essential to study the interaction between HSV-1 and HIV infections. **Methods** We conducted a study in the Mbeya region using the archived blood sera of participants from the recently completed EU-funded EMINI project. A specially designed questionnaire was used to obtain the social and demographic characteristics of the study participants in the database. We tested archived participants' sera for herpes simplex virus type 1 using Virotech HSV-1 (gG1) IgG ELISA (Enzygnost, Behring, Germany). Univariate and multivariate Poisson regression models were used to identify factors associated with HSV-1. **Results** A total of 640 adults were randomly recruited after stratification by HIV status, age, and sex. The overall seroprevalence of HSV-1 in the study population was 92.1%. The extrapolated prevalence estimate of herpes simplex virus type 1 in the general population was 95.0% (96.0% in males versus 94.0% in females). Males and females were equally affected by HSV-1. HSV-1 was less prevalent in HIV-positive individuals than in HIV-negative individuals. **Conclusion** People living with HIV were less likely to be HSV-1 seropositive. Further cohort studies can evaluate whether herpes simplex virus type 1 can reduce the incidence of HIV infection.

Background

In mainland Tanzania, the prevalence of human immunodeficiency virus (HIV) infection among adults aged 15-49 years is 4.7%, and the prevalence among females is twice that among males (6.2% vs. 3.1%). Although there is evidence of a significant decline in the prevalence of HIV in Tanzania, the prevalence rates in the Mbeya, Iringa, and Njombe regions are higher than the national average and are stable. (1)

The current stable HIV prevalence may be driven by a high incidence of sexually transmitted infections (STIs) and high-risk behaviours among patients in sub-Saharan Africa who are undergoing antiretroviral therapy (ART). (2,3) In contrast, individuals undergoing ART in northern Africa tend to adopt protective behaviours more frequently than those who are not undergoing treatment. (4)

HSV-1 predominantly causes orolabial ulcers and is acquired during childhood, while HSV-2 causes genital herpes that are commonly associated with sexual activities. Sufficient evidence has demonstrated an increase in HSV-1 anogenital isolates. (5)

There is some evidence that a large proportion of HIV infections is attributable to HSV-2. (6,7) In sub-Saharan Africa, it has previously been observed that among low-risk populations, 25% (8) to 50% (9) of

HIV-1 cases were attributable to HSV-2. HSV-2 disrupts the epithelial barrier and recruits immune cells, which are targets for HIV in the genital area, thereby facilitating the entry of HIV. (10)

Since the proportion of sexually transmitted HSV-1 is rising (5) and HSV-1 leads to the persistent shedding of oropharyngeal mucosal ulcers, (11) it is essential to study the potential role of HSV-1 as a risk factor for HIV infection.

Methodology

The current study was a population-based cross-sectional study that analysed data and blood from a subset of participants who were recruited into the EMINI (Establishment of the Infrastructure to Evaluate and Monitor the Impact of New Interventions) cohort.

EMINI was a population-based cohort study that was conducted in the Mbeya region between May 2005 and 2009 and included 20,000 people enrolled from 10% of all households in nine randomly selected communities (urban, semi-urban and rural). The overall objective of the EMINI project was to contribute to the general improvement of health by controlling communicable diseases in the Mbeya region.

During each of the annual study visits, a research team visited every participant to conduct a general physical examination, perform interviews (particularly about sexual risk behaviours), and collect several specimens, including blood samples.

The blood specimens collected from the participants were then tested for HIV-1 in the NIMR-MMRC central laboratory at the Mbeya Zonal Referral Hospital in accordance with the existing national HIV testing algorithm. During each survey, plasma from every participant was stored at -80°C for future use.

The HSV-1-specific gG1 IgG assay (Enzygnost, Behring, Marburg, Germany) was used to examine samples for HSV-1. This assay is an ELISA for the semiquantitative and qualitative detection of IgG antibodies against HSV-1 in human serum. Standard operating procedures and data worksheets for this kit were made and followed in accordance with the manufacturer's recommendations.

We linked the assay results to each participant's demographic characteristics and their responses to the sexual risk behaviour questionnaire. Data regarding age, sex, sociodemographic status, appropriate behaviours of participants, and physical characteristics were all collected during interviews in the participants' households using PDAs. The data were downloaded into Microsoft Access databases and imported into Stata 16.0 (StataCorp, College Station, Texas 77845 USA) statistical software, which was used for data analyses.

Overall, 7,287 participants in the EMINI cohort were between the age of 15 and 49 years old. Participants meeting the inclusion criteria were randomized using Stata 16 after being stratified 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 fewer 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 selected initial samples could not be found (total number of replacements was 280). Responses from EMINI interviews were used to assess the socio-demographic characteristics of participants.

We performed all analyses in Stata. Univariate and multivariate associations of different factors with HSV-1 infection were analysed using Poisson regression with robust variance estimates.

A stratum (plural strata) refers to a subset (part) of the population that is being sampled.

Results

We randomly selected a total of 640 subjects from nine wards of the Mbeya region, and the sample has an equal distribution of sex and HIV status. The majority of the study population had completed a primary level of education (58.4%), were farmers (70.0%), were involved in seasonal activities (50.5%), were married with one spouse (55.0%), and were Christians (77.5%). Some of the socio-demographic characteristics of these participants are shown in table 1.

Six hundred and twenty-nine sera were tested for HSV-1; 18 sera were indeterminate, and in 11 participants, the volume of sera was insufficient to run the test. None of the sera with indeterminate results were retested. From 611 participant sera tested for herpes simplex virus type 1, 51.2% were males, and 52.4% of sera came from HIV-negative individuals.

The overall prevalence of HSV-1 infection was 92.1%, with males and females having a prevalence of 94.2% and 89.9%, respectively. A total of 95.7% of HIV-negative females and 83.2% of HIV-positive females were reactive to herpes simplex virus type 1. These proportions were slightly lower than the prevalence of 96.9% and 91.6% among HIV-negative and HIV-positive males, respectively.

We stratified the study participants by HIV status, sex, and age to have enough participants in each stratum. Approximately 50% of study subjects were infected with HIV; the prevalence of HIV infection among the general population of Mbeya was 9.3%. The unadjusted seroprevalence did not interfere with the analysis of the seroprevalence of HSV infections in HIV-infected and uninfected individuals; however, the unadjusted data did not represent the overall prevalence in the general population. After extrapolating the current results to the age, sex and HIV status distribution of the EMINI study population, the prevalence estimate of herpes simplex virus type 1 in the general population was 95.0% (96.0% in males versus 94.0% in females).

A chi-square test of independence was performed to examine the relationship between sex and HSV-1 seropositivity. The relation between these variables was significant, $X^2(1, N = 611) = 3.929, p = .047461$. Men were more likely than women to be HSV-1 positive (Table 2).

The correlation between HSV-1 and HIV status was tested using chi-square and Z score tests. A chi-square test of independence showed that there was a significant association between HSV-1 seropositivity and HIV status, $X^2(1, N = 611) = 15.6482, p = .000076$; these findings were consistent with additional analyses, with a Z score of -3.9558 and a p-value of .00004. The result suggests that either disease, namely, HIV, or HSV-1, would be protective against the other.

Poisson regression analysis with robust variance estimates showed that there was no significant change in the trend of seroprevalence of herpes simplex virus type 1 over age by HIV status.

To assess the univariate association of sex and HIV status with HSV-1 infection, Poisson regression analysis with robust variance estimates for stratified variables was used (Table 3). There was no significant difference in HSV infections between females and males. People with HIV were less likely to have HSV-1 infections. When we included the same variables in a multivariate model, the results revealed a 7% decrease in the prevalence of HSV-1 among HIV-positive patients.

Discussion

This study presents the first report of the prevalence of herpes simplex virus type 1 in the general population of Tanzania based on their HIV status. The unadjusted seroprevalence of herpes simplex virus type 1 infections in this study population was 92.1%, and the prevalence estimate for the general population was 95.0%. The rate in our study is higher than the prevalence rates in the following countries: the United States of America, 67%; (12) Israel, 59.8%; (13) and Switzerland, 80%. (14) The estimate from our study is, however, comparable to some regions in Syria, (15) rural Uganda (91.2%) (16) and the urban region of Tanzania (92%). (17)

In contrast to other studies where the seroprevalence of HSV-1 was significantly higher in females (18–20), this study shows that men are more often infected than women, although this association was no longer significant after adjusting for other factors in the multivariate model (PR 1.04, p-value=.110).

Surprisingly, the present study shows a lower prevalence of herpes simplex virus type 1 infections among HIV-positive individuals compared to HIV-negative individuals (87.6% vs. 96.3%). In univariate and multivariate analyses, HIV positivity was associated with lower HSV-1 seropositivity.

This is the first study to report low seroprevalence of HSV-1 in HIV-infected individuals. It is difficult to explain this result, but it might not be related to regular condom use in HIV-positive individuals, which was reported to be low among individuals on antiretroviral drugs in sub-Saharan Africa. (2,3) In the same participants' sera, the seroprevalence of HSV-2 IgG was higher in HIV-positive individuals (unpublished

report). Another study has also reported that HSV-1 infection is not transmitted sexually in HIV-infected individuals. (21)

The findings support the observed negative interaction between HSV-1 and HIV. Calistri et al. (22) used the CD4 human lymphoblastoid cell line CEM that was infected chronically by HSV-1, CEM_{HSV}, and superinfected the cells with HIV-1, and they found that HIV-1 growth was inhibited, probably due to HSV-1 failing to induce HIV-1 gene expression through the transactivation of long terminal repeat (LTR).

Similarly, some studies have found that membrane-bound HSV-1 glycoprotein D is required for inhibiting the release of infectious HIV-1 (23), and another study found that several HSV-1 proteins, such as glycoprotein M, US3, and UL24, potentially restricted the replication of HIV-1 (24).

Conclusions

The prevalence of HSV-1 in this population was high. People living with HIV were less likely to be HSV-1 positive. Further cohort studies can evaluate whether herpes simplex virus type 1 can reduce the incidence of HIV infection.

Abbreviations

HIV - Human Immunodeficiency Virus

AIDS - Acquired Immune Deficiency Syndrome

HSV-1 - Herpes Simplex Virus Type 1

EU - European Union

EMINI - Establishment of the Infrastructure to Evaluate and Monitor the Impact of New Interventions

ELISA - Enzyme Linked Immunosorbent Assay

gG1 - Glycoprotein G 1

NIMR - National Institute for Medical Research

MMRC - Mbeya Medical Research Center

PDA's - Personal Digital Assistants

TX - Texas

MMReC - Mbeya Medical Research and Ethics Committee

CI - Confidence Interval

Declarations

Ethics approval and consent to participate

We sought ethical clearance from the Muhimbili University of Health and Allied Sciences and Mbeya Medical Research and Ethics Committees (MMReC). We observed confidentiality, and no unauthorized persons had access to the collected data. Each subject was assigned a study identification number that was not linked to individual names.

Consent for publication

Not applicable

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

IHM and AAN analysed and interpreted the research findings. IHM and AAN performed the serological testing and were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Not applicable

Study limitations

This study shows that both types of HSV-1 infections are less common in HIV-infected individuals. Limited by its cross-sectional design, the analysis of possible risk factors suggests that inferences from its findings should be made cautiously regarding the possible observed association.

Strength of the study:

The strength of this study is the use of statistical methods tailored to the high prevalence of herpes simplex virus type 1 infection. The use of Poisson regression models with robust variance estimates allows for the direct estimation of prevalence ratios, which are more interpretable than odds ratios in high prevalence situations. (25)

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Tables

Table 1. Frequency distribution of sociodemographic characteristics (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
Level of education	No formal education	88	26.9
	Completed primary	374	58.4
	After primary	94	14.7
Marital status	Never married	154	24.1
	Married-one spouse	352	55.0
	Others	134	20.9
Religion	No religion	107	16.7
	Christian	496	77.5
	Others	36	5.8

Table 2. Prevalence of herpes simplex virus type 1 by sex and HIV status

	Male (Expected total) [X ²]	Female (Expected total) [X ²]	Total
HSV-1			
Positive	295 (288.41) [0.15]	268 (274.59) [0.16]	563
Negative	18 (24.59) [1.77]	30 (23.41) [1.85]	48
	313	298	611
	HIV Positive	HIV Negative	
Positive	255 (268.14) [0.64]	308 (294.86) [0.59]	563
Negative	36 (22.86) [7.55]	12 (25.14) [6.87]	48
	291	320	611

The chi-square statistic is 3.929. The p-value is .047461. This result is significant at $p < .05$.

Table 3. Univariate and multivariate association of various factors with HSV-1 infection

Variable	Stratum	Number in stratum	Prevalence ratio	95% CI	P-value
Univariate					
Sex					
	Female*	298	1	--	--
	Male	313	1.05	(1.00 to 1.10)	0.050
HIV status					
	Negative	320	1	--	--
	Positive	291	0.91	(0.87 to 0.96)	<0.001
Multivariate					
Sex					
	Female*	298	1	--	--
	Male	313	1.04	(0.99 to 1.10)	0.110
HIV status					
	Negative*	320	1	--	--
	Positive	291	0.93	(0.89 to 0.98)	0.004

Results of Poisson regression with robust variance estimates for stratified variables. Univariate and multivariate models for sex, age, and HIV status, all other variables adjusted for sex, age, and HIV status.