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HSV-2 Seropositivity among HIV Patients in Khartoum State

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Abstract

A cross-sectional study for HSV 2 seropositivity among HIV patients was performed at voluntary counseling and testing centers (VCT) Khartoum, Sudan during March to June 2016. 93 individuals were selected to participate in this study. Blood sample from each patient was collected. Samples were tested for antibodies to HSV II (IgM and IgG) using ELISA (EUROIMMUN/ORDER NO. EI 2532-9601-2 G.). The final interpretation of results showed a high prevalence of HSV II infection. HIV patients coinfected with herpes simplex II showed that the infection was higher in male patients as compared to females. The age cohort 35-44 years, patients not on HIV treatment and patients with low educational level indicated high seroprevalence of HSV-II.

Keywords: Herpes simplex virus; HIV; HSV-2 infection; Human antibodies

Introduction

HSV-2 is a common causative agent of ulcers Mucous skin diseases in both immunodeficiency and immune-competent humans [1]. People with HSV-2 are more susceptible to HIV. This may be because HSV-2 causes the genital mucosa Membranes and genital ulcers that may provide Entry and exit of HIV [2]. Recent research on HIV patients infected with STI (concluded that the effects of HIV infection on immunity can increase susceptibility to other STIs especially in immune-compromised individuals [3]. For example, HIV and HSV-2 co-infections are widespread and both infections can be facilitated else. A cohort study was done in Uganda to clarify the reciprocal relationship between HIV and HSV-2 showed that 1.09% of HIV seroconversion rate. The seroconversion of HIV and HSV2 were more associated, about (56%) of HIV and HSV-2 infection occurred in the same time frame.

In 25% of cases, HIV infection exceeds HSV-2 infection, while in 19% of cases HSV 2 infection exceed HIV transmission [4]. HSV-2 are increased concentrations of HIV in blood plasma and genital fluids, also increased HIV shedding and acquisition [5,6].

Material and Methods

- **Study design:** This design of the current study was chosen as cross-sectional study
- **Study population:** Study population comprises of HIV seropositive patients. The variables include age, gender, educational degree, residence, mode of transmission, antiviral treatment, and the stage of HIV infection
- **Study area:** Our study was conducted in Sudan country at Voluntary Counseling and Testing
- Centers (VCT) of Omdurman Teaching Hospital in Khartoum State
- **Sample size:** A total of 93 patients suffered from HIV infection were enrolled in this study
- **Data collection:** A designed questionnaire was used to get informative data about the history of each person. These include age, gender, residence, duration of illness, taking treatment and clinical situation of patients
- **Ethical consideration:** Permission to carry out the study was taken from the ethics committee of the Faculty of Medical Laboratory, Department of Medical Microbiology. A written and signed consent was taken for sample collection by Directorate of Preventive Medicine, Ministry of Health, Khartoum State
- **Specimen collection:** 5 ml of venous blood was collected into EDTA vacutioners under aseptic technique from each patient under study after having his/her written consent to participate in the study. The specimens were centrifuged at 2000 rpm and the plasma separated into plain containers. Serology test was performed using HSV-2 IgG and IgM ELISA test kits (EUROIMMUN/ORDER NO. EI 2532-9601-2 G)
- **Detection of HSV2 antibodies:**

1. **ELISA: Principle of the test:** A semi-quantitative or quantitative ELISA to detect antibodies (IgM, IgG, and IgA) against the HSV-2 specific glycoprotein G2 in serum or plasma was provided. The kit consists of 12 microtiter strips, each strip with 8 break-off reagent wells coated with purified glycoprotein G2. Firstly, patient samples (diluted) are incubated in the coated wells. In positive samples, IgG or IgM antibodies bind specifically to the antigens. The second incubation is carried to detect the bound antibodies using an enzyme-labeled anti-human IgG or IgM (enzyme conjugate) catalyzing a color reaction
2. **Components of the test kit:** Microplate wells coated with antigens, calibrators, and controls, enzyme-conjugated sample buffer, wash buffer, chromogen/substrate solution, stop solution, test instruction, and quality control certificate.
3. **Storage and stability:** Brought the reagents to room temperature approximately 30 minutes before use. Check the expiry date of reagents, stored at +2°C to +8°C and protected from contamination
4. **Test performance (semi-quantitative):** 100 µl of patient samples (serum) were diluted in 900 µl sample buffer and mixed well by

vortexing. Add 100 µl of each calibrator, positive and negative controls and diluted into the individual microplate wells according to the pipetting protocol, then incubated for around 30 minutes at normal room temperature and washed manually three times using 300 µl of working strength wash buffer for each wash. Add 200 µl of the enzyme into each of the microplate wells, incubated for around 30 minutes at normal room temperature, and washed three times. After that 100 µl quantity of chromogen/ substrate solution was pipetted into each of the microplate wells, incubated for 15 minutes at room temperature and protected from direct sunlight. Then add 100 µl of stop solution was into each of the microplate wells and incubated for 15 minutes at room temperature. Finally, the results were measured by automated analysis device software

5. **Calculation of results:** The cut-off recommended by EUROIMMUN is the extinction value of the calibrator defines the upper limit of the reference range of non-infected persons. The values above the indicated cut-off are to be considered as positive, those below as negative. The calibration is performed in relative units (RU)/ml
6. The results were evaluated semi quantitatively, a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2 as represented below:

$$Ratio = \frac{Extinction\ of\ the\ control\ or\ patient\ sample}{Extinction\ of\ calibrator\ 2}$$

The recommends of interpreting results by EUROIMMUN as follows:

Ratio<0.8: negative; Ratio ≥ 0.8 to <1.1: borderline; Ratio ≥ 1.1: positive

Standard characteristics

- **Purity:** The microplate wells were coated with purified glycoprotein G2 isolated from HSV-2. The purity of the preparation was verified by SDS polyacrylamide gel electrophoresis. Glycoprotein G2 (molecular weight 108 kDa) is a membrane protein which is only present in HSV-2 but not in HSV-1
- **Linearity:** The linearity of the Anti-HSV-2 (gG2) ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R2 for all sera was >0.95. The Anti-HSV-2 (gG2) ELISA (IgG) is linear at least in the tested concentration range (3 RU/ml to 200 RU/ml)
- **Detection limit:** The mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer is defined as a lower detection limit. The detection limit of the HSV-2 IgM ELISA is a ratio 0.05, while HSV-2 IgG ELISA is 1.4 RU/ml
- **Cross-reactivity:** The specificity of the ELISA are ensures by the quality of the antigen used. In particular problems such as anti-HSV-1 antibodies that often cross-react with anti-HSV-2 antibodies causing false positive results do not occur with this ELISA.
- **Interference:** If samples are lipaemic and icteric and Haemolytic that will not influence the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.
- **Specificity and sensitivity:** A total of 93 clinically characterized patient samples (Lab quality inter laboratory test, Finland) were examined with the EUROIMMUN Anti- HSV-2 (gG2) (IgG) ELISA. The test showed specificity and a sensitivity of 100% each. The values for 4 of the samples were borderline that were not included in the calculation. 44 clinically characterized patient samples (Interlaboratory test samples of the INSTAND, Germany) were examined with the EUROIMMUN Anti-HSV-2 (gG2) IgM ELISA. The test shows a specificity of 100%. Sensitivity and specificity of the Anti-HSV-2 (gG2) ELISA was tested with respect to another commercial ELISA using 88 samples. The sensitivity with respect to the other test amounted to 100% with a specificity of 94.7%.
- **Reference range:** The levels of anti-HSV-2 antibodies (IgG) were tested with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, the blood donors showed 9.6% anti-HSV-2 positive (IgG), which reflects the known percentage of infections in adults. A total of 300 healthy blood donors were tested levels of anti-HSV-2 antibodies (IgM). With a cut-off of ratio 1.0, all blood donors were anti-HSV- 2 negative (IgM).
- **Statistical analysis:** Analyses were accomplished using a computer application for Statistics program (SPSS 13.0, SPSS Inc. Chicago, IL, USA). The frequencies were analyzed using the Chi-Square test. The relationship between CMV positive and data of the questionnaire was tested by Pearson analysis. The p-value<0.05 was considered statistically significant.

Results

In the present study, a total of 93 HIV patients were studied for the presence of antibodies to HSV-2 (IgG and IgM) by using ELISA. Serum samples were collected from 49 (52.7%) males and 44 (47.3%) females, who attending to VCT center of Omdurman Teaching Hospital in Khartoum State.

Seroprevalence of HSV-2 related with gender

HSV-2 IgG prevalence was higher than the prevalence of HSV-2 IgM in HIV infected. Anti HSV-2 IgG were detected as positive in 44 (47.3%) individuals, 23 (24.7%) males and 21 (22.6%) females, and the IgM antibodies were detected in 3 individuals (3.2%), all of them are males that also were shown in Table 1.

Variable	Study group	Total No. (%)	HSV-2 positive		p-value	
			IgG (%)	IgM (%)	IgG	IgM
Status	HIV seropositive individuals	93 (100%)	44 (47.3%)	3 (3.2%)		

Gender	Male/Female	49 (52.7%) 44 (47.3%)	23 (24.7%) 21 (22.6%)	3 (3.2%) 0 (0%)	1.000	0.244

Table 1: Seroprevalence of HSV-2 IgG and IgM in relation to the gender of HIV patients.

Seroprevalence of HSV-2 related with age (years)

HSV-2 IgG Prevalence by age groups showed that those within the age group 35-44 had the highest prevalence (18.3%), followed by those within the age group 25-34 (13%) and the age group of 45-54 years (8.61%), while the lowest prevalence was recorded among the age group 55- 64 (4.3%) and 15-24 (3.2%).

Seroprevalence of HSV-2 and it is an association with HIV mode of transmission. In the present study, found that HIV sexually infected patients had the higher prevalence of HSV-2 (45.2% IgG and 3.2% IgM) than those who infected with HIV by blood transfusion (2.1% IgG and 0% IgM) as shown in Table 2.

Variable	**Study group**	**Total No. (%)**	**HSV-2 positive frequency (%)**		**p-value**	
			IgG (%)	IgM (%)	IgG	IgM
Status	HIV seropositive individuals	93 (100%)	44 (47.3%)	3 (3.2%)		
HIV-transmission	Sex blood	87 (93.5%) 6 (6.45%)	42 (45.2%) 2 (2.1%)	3 (3.2%) 0 (0%)	0.680	1.000

Table 2: Prevalence of HSV-2 IgG and IgM seropositivity in HIV patients according to the HIV acquired ways.

Seroprevalence of HSV-2 associated with the residence

The prevalence of HSV-2 seropositivity was shown higher in the rural group (42% IgG and 3.2% IgM) than the urban group (5.3% IgG and 0% IgM).

Seroprevalence of HSV-2 in the various stages of HIV infection

The study group also divided according to the stages of HIV infection into four classes, HSV-2 IgG was higher in stage one (20.4%), followed by stage two (18.3%), then stage three (8.6%), and negative (0%) in stage four (the last stage). 2.1% and 1.1% was detected positive to HSV-2 IgM in stage two and stage one respectively as shown in Table 3.

Variables	**Study group**	**Total No. (%)**	**HSV-2 positive**		**p-value**	
			IgG (%)	IgM (%)	IgG	IgM
Status	HIV seropositive individuals	93 (100%)	44 (47.3%)	3 (3.2%)		
HIV stages	1	32 (34.4%)	19 (20.4%)	0 (0%)	0.091	0.596
	2	37 (39.7%)	17 (18.3%)	2 (2.1%)		
	3	21 (22.5%)	8 (8.6%)	1 (1.1%)		
	4	3 (3.2%)	0 (0%)	0 (0%)		

Table 3: Prevalence of HSV-2 IgG and IgM seropositivity in relation to HIV different stages among HIV patients.

Seroprevalence of HSV-2 related to the educational levels of HIV patients

The seroprevalence of HSV-2 IgG was found to be highest among those who are illiterates (15%), followed by those with primary education and intermediate education (13% of both), and the lowest prevalence in those with university education (6.4%), while the prevalence of IgM was appeared the same in the whole levels (1.1%) except in the intermediate group which was zero (0%).

Seroprevalence of HSV-2 related to different HIV clinical situations

The major of HIV seropositive individuals in this study had many clinical situations. Among nine (9.7%) pregnant women, 4 (4.3%) was detected as HSV-2 IgG positive, while IgM was negative (0%), in four (4.3%) of HIV patients with skin rash situation, there were found three (3.2%) positive persons to HSV-2 IgG, but IgM was also negative (0%) and One person (1.1%) out of two (2.2%) with Varicella zoster infection was shown positive to HSV-2 IgG antibodies as shown in Table 4.

Variables	Study group	Total No. (%)	HSV-2 positive frequency (%)		p-value	
			IgG (%)	IgM (%)	IgG	IgM
Status	HIV seropositive individuals	93 (100%)	44 (47.3%)	3 (3.2%)		
Pregnant	Yes/No	9 (9.7%) 84 (90.3%)	4 (4.3%) 40 (43%)	0 (0%) 3 (3.2%)	1.000	1.000
Skin rash	Present/Absent	4 (4.3%) 89 (95.6%)	3 (3.2%) 41 (44.1%)	0 (0%) 3 (3.2%)	0.341	1.000
V. zoster	Present/Absent	2 (2.2%) 91 (97.8%)	1 (1.1%) 43 (46.2%)	0 (0%) 3 (3.2%)	1.000	1.000

Table 4: Prevalence of HSV-2 IgG and IgM seropositivity in relation to pregnant, skin rash and Varicella zoster among HIV patients.

Seroprevalence of HSV-2 in patients those taken antiviral treatment and those not on treatment

Those without antiviral treatment showed a higher prevalence of HSV-2 (27.9% IgG and 2.1% IgM) than in others with treatment (19.3% IgG and 1.1% IgM) as shown in Table 5.

Variables	Study group	Total No. (%)	HSV-2 positive frequency (%)		p-value	
			IgG (%)	IgM (%)	IgG	IgM
Status	HIV seropositive individuals	93 (100%)	44 (47.3%)	3 (3.2%)		
Treatment	Yes/No	51 (54.8%) 42 (45.2%)	18 (19.3%) 26 (27.9%)	1 (1.1%) 2 (2.1%)	0.013	0.587

Table 5: Prevalence of HSV-2 IgG and IgM seropositivity in HIV infected patients those taken antiviral treatment and others without treatment. Note: In all our findings, there was no statistical significance (p-value>0.05).

Discussion

In this study, a total of 93 HIV-infected patients, 44 (47.3%) were HSV-2 IgG positive, 18.3% of them were in the age group of 35-44 years among 23 (24.7%) males and 21 (22.6%) females, while three persons just (3.2%) were positive for IgM and all are males above 44 years. Is the fact that acquisition of HIV and HSV-2 is more in females than males like studies from India, the UK and Kenya [7-9].

Studies showed that females are more susceptible to HSV-2 infection biologically the virus transmitted from male to female is more common than from female to male. also, the female genitalia has plenty of soft tissue that is exposed to skin contact, which is presumably more susceptible to any virus or STD [8]. Our study showed a higher prevalence of HSV-2 among males (24.7% IgG and 3.2% IgM) than females (22.6% IgG and 0% IgM) contrasts previous reports, probably because of the difference in sample size between males 49 (52.7%) and females 44 (47.3%) participated in this study. Similar results to our findings were also reported by Flavia and his colleague in Brasil [10].

A study reported seropositivity of HSV-2 in HIV-infected patients are 47% in Kolkata by Cohen and his colleague, 49% in Andhra Pradesh by Auvert and his colleague and 48.4% in Delhi by Jindal and his colleague, which were in line to our observations. This represents a much lower prevalence compared to the work done by Wang and his colleague and Kapi ga and his colleague in which 59.0% in commercial sex workers and 87.0% of patients attending the STI clinic in Jos tested positive to HSV-2 respectively [11-15]. The relatively low prevalence of HSV- 2 in the present study can be attributed to the differences between the study population and above-mentioned studies and our study. While their study population was among the high-risk group (commercial sex workers) and STI clinic attendees, this study was among the general population who accepted voluntary counseling and testing for HIV. Also, the higher rates were observed in other

countries, for example, 55% in the UK, 87% in South Africa and 86% in Uganda [11,16,17]. These results confirm the strong association of HSV-2 infection with HIV. It has been confirmed that genital herpes caused by HSV-2 has been associated with two-to three-fold increased risk of HIV acquisition and transmission. HSV-2 seroprevalence rates are higher in HIV-positive patients than in HIV negative patients.

In the present study, the highest numbers of HSV-2 seropositive patients were in 35-44 age group, which gradually increased in the 25-34 years age group, peaked at 35-44 years, and then decreased with further increase in age. The increase in the prevalence at each higher level of age may be related to increasing years of sexual activity. A similar association of HSV-2 prevalence and age was reported by Gita Ramjee and his colleague in South Africa [17]. The same results were observed in the study of Anuradha and his colleague in India and Nilanjan Chakraborty and his colleague which shows that the increased level of sexual activity is the important contributing factor for the highest seroprevalence of HSV2 [18,19]. Similar observations were also reported from the WHO by Strachan and his colleague [20].

A review done in 2004 which showed that there is a common finding among seroepidemiological surveys that socio-economic, religious and educational status has no significant effect on the prevalence of HSV-2 infection, this not agreed by our study in which persons who are illiterates had a higher HSV-2 seropositivity (15% IgG and 1.1% IgM), followed by those with primary education (13% IgG and 1.1% IgM), and intermediate education (13% IgG and 0% IgM), and this may be due to the large illiterates samples (33.3%), while the lowest prevalence was found in those with university education (6.4% IgG and 1.1% IgM), also this probably due to the few samples from them (14%) [21]. But we must note that there was a consistently high prevalence of HSV-2 infection related to religious and educational status in Sudan because it is an Islamic country.

Other studies reported that the seroprevalence of HSV-2 was 51% in persons with primary education, and 60.6% in illiterates as conducted by Flavia and his colleague, Kapiga and his colleague respectively [10,15]. These results were in line with our findings. The prevalence of antibodies to HSV-2 in our study was shown higher among individuals with sexual activities. A similar association was reported by Cowan and his colleague by Gwanzura and his colleague, Arama and his colleague and Flavia and his colleague [10,22-24]. Promiscuous behavior is one of the risk factors for acquiring and transmission both HIV and HSV-2 [25-27].

Conclusion

The present study showed higher seropositivity of HSV2 in rural than urban residence group, and this may be due to the difference in sample size between two groups in the present study. Other study conducted by Arama and his colleague, showing the difference in the seroprevalence in rural and urban areas was scanty. A slight increase in seropositivity (48.71%) showed in the urban group compared to the rural group (48.07%). Also, our findings showed that the prevalence of HSV-2 in HIV patients who have taken antiviral treatment was lower as compared to those lacking treatment. Similar results were shown in a study conducted by CDC and this may be related to that initiation of suppressive therapy in patients co-infected with HIV and HSV-2 would benefit by reducing herpes transmission and herpes reactivation and also through salutary effects on HIV. In this study, there was a relation between HSV-2 infection and pregnancy. This result was similar to those reported in Tanzania (33%). This high rate reflects that many pregnant women have already been in contact with the virus HSV-2 without having the ability to date the time of infection. As subclinical forms are found more, many patients carry antibodies without any memory of initial herpes. The risk transmission higher case herpes is in of lesions during primary infection or reactivation.

One of the limitations of the study is that it is a cross-sectional study and is based on HIV-positive patients attending the VCT center of Omdurman teaching hospital. This might not be is a presentation of the population.

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