ORIGINAL ARTICLE

# SEROPREVALENCE OF HERPES SIMPLEX VIRUS-1 IN HIV POSITIVE PATIENTS ATTENDING A TERTIARY HOSPITAL IN NIGERIA

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## ABSTRACT

**Background:** Herpes Simplex Virus type 1 (HSV1) is the primary cause of cold sores. This study aimed to determine seroprevalence among HIV positive patients attending the Highly Active Antiretroviral Therapy (HAART) clinic of the University of Ilorin Teaching Hospital, Ilorin.

**Methods:** A total of 200 blood samples were collected from consenting adult and were analyzed for HSV 1 antibodies using ELISA. Socio demographic data, socio economic factors, behavioral characteristics and the health status of the subjects were obtained via the administered questionnaire.

**Results:** A seroprevalence of 56.0% (112/200) was obtained from the subjects out of which 87 (43.5%) were from urban and 25 (12.5%) rural (P= 0.354). High seropositivity was noticed amongst subjects of monogamous marriage (34.5%) (P= 0.314). Seropositivity of 24.5% (Christians) and 31.5% (Muslims) was recorded at P= 0.471. Trend of increasing seropositivity was recorded in order of single, married, divorced and widowed (P=0.429). The group with primary level of education had the highest HSV1 prevalence (P= 0.969) while analysis of subject by occupation reveals that the retiree and self-employed had the highest seroprevalence (66.7% and 58.0%) at P= 0.795. Respondent that reported no use of condom and those without an answer had the highest prevalence (P= 0.098) and similarly, subject that gave no answer for frequency of condom usage had the highest prevalence (P= 0.463).

**Conclusion:** Since most of the infected human are asymptomatic to HSV1, adherence to clean hygiene and safe sex should be maintained.

**KEY WORDS:** HSV1, HIV, Seroprevalence, Co-infection

## INTRODUCTION

Herpes simplex virus is a common cause of ulcerative mucocutaneous disease in both immuno-competent and immuno-compromised persons[1]. Herpes simplex virus type 1 is mostly acquired during childhood and causes orolabial ulcers. The hallmarks of HSV infection are periodic and symptomatic reactivation and asymptomatic viral shedding[2]. Infection with HSV is a lifelong condition and the virus becomes permanently latent in the nerve root ganglia corresponding to the site of inoculation[3]. It induces antibody and cell mediated immune response that modulates the severity of the recurrence but these are not sufficient to eradicate the disease[4]. The precise origin of herpes in humans is unidentified however, acknowledgement of its presence is as early as ancient Greece when documented sores that seemed to creep or crawl was made by Hippocrates[1]. Appropriately, the term "herpes" is derived from the Greek word "to creep"[2]. Infection caused by herpes simplex virus is worldwide and >80 of them have been identified as pathogens of human[3, 4]. Infection by HSV is not curable and has been known to persist through life time of the host alternating between latent and active states [5] (http://ezinearticles.com/?What-is-The-History-of-Herpes-and-How-is-it-Spread?&id=591001).

HSV-associated diseases are the most prevalent

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and it's estimated to have affected 60% to 95% of human adults[6]. Several documented factors such as age, race, geographic location, and socioeconomic status (with less industrialized nations having higher seropositivity)[2]. According to CDC, the overall nationwide prevalence of herpes is likely higher than 15.5% due to the increase in genital herpes infection by HSV-1 which is typically acquired in childhood[7]. Herpetic genital ulcers can bleed easily, and when in contact with the mouth, vagina, or rectum during sex, they may increase the risk of sexually transmitted diseases (e.g. HIV)[8]. Amongst people living with HIV, HSV 1 is common with prevalence that approximates to or exceeds those in the general population[9]. Rare but serious complications such as encephalitis, blindness, aseptic meningitis and extra genital lesions development in the buttocks, thigh, groin, finger etc. can result from infections by HSV1&2[10].

To the best of our knowledge, there exist no previous data on the co-infection of these viruses (HSV1 and HIV) in Ilorin, Kwara state, Nigeria. Thus, this research aims to create awareness of the risk factors by providing a baseline data for the prevalence of HSV-1 and its co-infection with HIV in this area.

## METHODS

This is a prospective study involving human participants. The ethical approval for the research was obtained from the ethical board of University of Ilorin Teaching Hospital (PAN/2013/07/1231) and the recruited respondent included only consenting adult patients (via signed informed consent form) with HIV and attending the Highly Active Antiretroviral clinic of the UITH.

Study Area: The study was carried out in the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara state Nigeria. It is a 520 bedded tertiary hospital serving as a referral center for all primary and secondary healthcare facilities in Kwara state and adjoining states like Kogi, Niger, Oyo and Osun states. Ilorin Kwara state is located on latitude 8.5oN and longitude 4.55oE (Google Map, 2011).

Sample Collection: Blood sample of 5ml was drawn intravenously from each consenting patients via a 21 gauge needle in to plain and pre-labeled sample bottles. This was immediately followed by serum separation and then stored at 4°C.

Serology: The ELISA (Enzyme Linked Immuno-Assay) method was used according to manufacturer's instruction (Dia pro Diagnostic Bioprobes). All the serum samples were diluted 1:100 via the provided diluents. 100ul of diluted serum samples was inoculated in to the wells and seronegatives was used as controls. Incubation of the micro-plate was at 37oC for 60mins followed by washing which was done 5 times using 350ul of washing solution provided. 100ul of enzyme conjugate was added to wells except that of blank, micro-plate was incubated at same condition and washing was repeated. 100µl of chromogen/substrate mixture was added to all the wells before the third incubation at 18-24oC for 20mins. Change of colour was observed at 450nm using the micro-well plate reader after the addition of 100µl of sulphuric acid to all the wells.

Data Analysis: Data was analyzed using Statistical package for the Social Science (SPSS) version at significance level of $P<0.05$ and 95% C.I (confidence interval).

## RESULTS

The assay results for HSV1 revealed that 112 (56.0%) of the respondent were positive for HSV. Result from CD4 count showed that 119 (59.5%) had high CD4 count, while 81 (40.5%) had low CD4 count. 69 (34.5%) of respondent from monogamous marriage and 43 (21.5%) from polygamous were HSV1 positive (P= 0.314), while 44 (22.0%) and 37 (18.5%) from monogamous and polygamous marriage, respectively, had high CD4 count (P= 0.322).

Most of the subjects (160 (80.0%) resides in the urban area, while 40 (20.0%) resides in the rural area. Of the 112 (56.0%) HSV1 positive subjects, 87 (43.5%) and 25 (12.5%) were from urban and rural respectively (P= 0.354), while based on CD4 counts, respondent from urban area had high count of 65 (32.5%) and a low count of 95 (47.5%), while respondent from the rural area had high and low count of 16 (8.0%) and 24 (12%) respectively (P= 0.943).

A total of 21 (10.5%) respondent reported to have other disease as at time of recruitment and 13 (6.5%) of them were positive for HSV1, while 8 (4.0%) were not. On the other hand, out of 179 (89.5%) respondent that reported had no other disease as far as they know, 99 (49.5%) tested positive to HSV1 screening while 80 (40.0%) were negative (P= 0.564). High and Low CD4 count of 71 (35.5%) and 108 (54.0%) respectively were recorded for respondent without any other disease while 10 (5.0%) and 11 (5.5%) respectively was recorded for respondent that reported having other diseases at the period of sample collection (P= 0.482).

Table 1 and 2 shows HSV1 and CD4 count status of participant in relation to the demographic data and risk factors while Figure 1 represent the results of the HSV1 assay for respondent with respect to their responses on history of blood transfusion (P= 0.314), tribal mark (P= 0.269) and tattoos (P= 0.245). Result from the CD4 count revealed that 21 (10.5%) and 60 (30.0%) of 81 (40.5%) respondent with high CD4 count indicated Yes and No respectively to history of blood transfusion, while 48 (24.0%) and 71 (35.5%) of 119 (59.5%) with low CD4 count were recorded for respondent with and without blood transfusion

(P= 0.035).

Tribal mark analysis based on result from low and high CD4 count had 59 (29.5%) and 43 (21.5%) respectively for those with tribal mark while others without tribal mark had 60 (30.0) for low CD4 count and 38 (19.0%) for high CD4 count (P= 0.626).
88 (44.0%) respondent without tattoos and 31 (15.5%) with tattoos had low CD4 count while those with high CD4 count was 20 (10.0%) with tattoos and 61 (30.5%) without tattoos (0.829).

**Table 1:** HSV1 and CD4 count status of participant in relation to risk factors and demographic data

| | | HSV -1 | | CD4 | | Total (%) | P value |
|---|---|---|---|---|---|---|---|
| | | Positive (%) | Negative (%) | Low (%) | High (%) | | HSV1 CD4 |
| **Age** | 21-30 | 9 (4.5) | 13 (6.5) | 14 (7.0) | 8 (4.0) | 22 (11.0) | 0.479, 0.949 |
| | 31-40 | 52 (26.0) | 33 (16.5) | 50 (25.0) | 35 (17.5) | 85 (42.5) | |
| | 41-50 | 32 (16.0) | 27 (13.5) | 35 (17.5) | 24 (12.0) | 59 (29.5) | |
| | 51-60 | 16 (8.0) | 11 (5.5) | 15 (7.5) | 12 (6.0) | 27 (13.5) | |
| | 61-70 | 3 (1.5) | 3 (1.5) | 4 (2.0) | 2 (1.0) | 6 (3.0) | |
| | 71-80 | 0 (0.0) | 1 (0.5) | 1 (0.5) | 0 (0.0) | 1 (0.5) | |
| **Marital status** | Single | 7 (3.5) | 11 (5.5) | 12 (6.0) | 6 (3.0) | 18 (9.0) | 0.429, 0.898 |
| | Married | 76 (38.0) | 59 (29.5) | 80 (40.0) | 55 (27.5) | 135 (67.5) | |
| | Divorced | 8 (4.0) | 5 (2.5) | 8 (4.0) | 5 (2.5) | 13 (6.5) | |
| | Widowed | 21 (10.5) | 13 (6.5) | 19 (9.5) | 15 (7.5) | 34 (17.0) | |
| **Level of Education** | None | 21 (10.5) | 17 (8.5) | 21 (10.5) | 17 (8.5) | 38 (19.0) | 0.969, 0.208 |
| | Primary | 35 (17.5) | 25 (12.5) | 41 (20.5) | 19 (9.5) | 60 (30.0) | |
| | Secondary | 22 (11.0) | 17 (8.5) | 25 (12.5) | 14 (7.0) | 39 (19.5) | |
| | Tertiary | 34 (17.0) | 29 (14.5) | 32 (16.0) | 31 (15.5) | 63 (31.5) | |
| **Occupation** | Unemployec | 6 (3.0) | 5 (2.5) | 7 (3.5) | 4 (2.0) | 11 (5.5) | 0.795, 0.334 |
| | Student | 3 (1.5) | 5 (2.5) | 6 (3.0) | 2(1.0) | 8 (4.0) | |
| | Civil servants | 21 (10.5) | 19 (9.5) | 19 (9.5) | 21 (10.5) | 40 (20.0) | |
| | Self employed | 80 (40.0) | 58 (29.0) | 86 (43.0) | 52 (26.0) | 138 (69.0) | |
| | Retiree | 2 (1.0) | 1 (0.5) | 1 (0.5) | 2 (1.0) | 3 (1.5) | |
| **Religion** | Islam | 63 (31.5) | 45 (22.5) | 60 (30.0) | 48 (24.0) | 108 (54.0) | 0.471, 0.218 |
| | Christianity | 49 (24.5) | 43 (21.5) | 59 (29.5) | 33 (16.5) | 92 (46.0) | |

**Table 2:** HSV1 and CD4 count status of participant in relation to risk factors.

| | | HSV -1 | | CD4 | | Total (%) | P value |
|---|---|---|---|---|---|---|---|
| | | Positive (%) | Negative (%) | Low (%) | High (%) | | HSV1 CD4 |
| **Sharing of Items** | Yes | 36 (18.0) | 32 (16.0) | 48 (24.0) | 20 (10.0) | 68 (34.0) | 0.532, 0.022 |
| | No | 76 (38.0) | 56 (28.0) | 71 (35.5) | 61 (30.5) | 132 (66.0) | |
| **Sexual partner** | Yes | 24 (12.0) | 20 (10.0) | 31 (15.5) | 13 (6.5) | 44 (22.0) | 0.662, 0.126 |
| | No | 87 (43.5) | 68 (34.0) | 88 (44.0) | 67 (33.5) | 155 (77.5) | |
| | Na | 1 (0.5) | 0 (0.0) | 0 (0.0) | 1 (0.5) | 1 (0.5) | |
| **Use of condom** | Yes | 55 (27.6) | 53 (26.6) | 66 (33.2) | 42 (21.1) | 108 (54.3) | 0.098, 0.359 |
| | No | 38 (19.1) | 29 (14.6) | 41 (20.6) | 26 (13.1) | 67 (33.7) | |
| | Na | 18 (9.0) | 6 (3.0) | 11 (5.5) | 13 (6.5) | 24 (12.1) | |
| **Condom usage frequency** | All times | 27 (13.5) | 25 (12.5) | 31 (15.5) | 21 (10.5) | 52 (26.0) | 0.463, 0.819 |
| | Most times | 12 (6.0) | 11 (5.5) | 13 (6.5) | 10 (5.0) | 23 (11.5) | |
| | Sometimes | 16 (8.0) | 17 (8.5) | 22 (11.0) | 11 (5.5) | 33 (16.5) | |
| | Na | 57 (28.5) | 35 (17.5) | 53 (26.5) | 39 (19.5) | 92 (46.0) | |

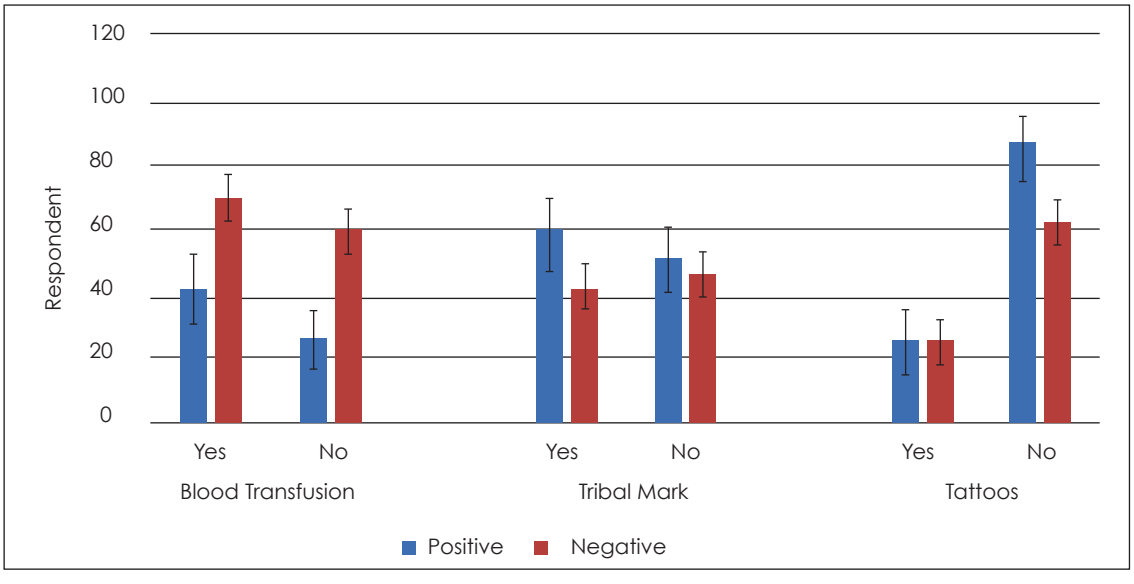


**Fig 1:** HSV1 status of respondent by blood transfusion, tribal mark and tattoo. (95% C.I)

## Discussion

Advancement in scientific research has led to the introduction of blood serum based assays aimed at detecting the glycoproteins of herpes virus. This research has employed the use of type- specific ELISAs which has been found to be most suitable and highly sensitive for detection of HSV1 antibodies and also effective for the screening of a large sample size[11, 12]. The prevalence of HSV1 is above average in this location, this was deduced from the resulting evidence by this research were 56.0% of the sample population was positive. Similar prevalence result of 67% was obtained by Malkin et al. 2002[13] which had larger sample size for the prevalence study. Prevalence of the co-infection and that of a single infection (HSV1 or HIV) cannot be significantly separated since all respondent were confirmed HIV positive patients and thus implies that 56.0% of the HIV positive patients are co-infected with HSV1. In line with this research is another by Shivaswamy et al. 2005[14] on the prevalence of HSV1 and 2 in STD patients where a result of 82.9% was obtained. This also confirms that individual with a form of STD (e.g. HIV) is at higher risk of HSV infection and vice versa. Venkateshwaran et al. 2011[15] also reported high prevalence of HSV1 in HIV positive respondent.

The least age range of respondent was from 21 to 30 but highest positivity to HSV1 was recorded amidst the 31 to 40 age group, and this also coincides with the group having highest CD4 count (Table 1). However, this deduction was not statistically significant (P>0.05). Stability in prevalence within 31 to 70 age of respondent could be due to early acquisition of the virus and most of its risk factors (such as active sexual age with possibility of less protection, contact with broken skin or lesions and contact with fomite which is less common [16]) are more pronounce. This deduction is also in line to Malkin et al. 2002[13] and Johnson et al. 1989.[17] The trend in the prevalence was noticed to increase across the marital status of respondent i.e. single (38.8%), married (56.3%), divorced (61.5), widowed (61.7%) (Table 1) and this correspond to Shivaswamy et al. 2005[14], Malkin et al. 2002[13] HSV1 positivity was found to be high amidst the groups with primary and tertiary educational levels at P>0.05. Majority of the participants claim to be self-employed and the indicated trade included trader, driver, artisan, farmer etc. Prevalence of 57.9% was recorded amidst the self-employed (Table 1) for HSV1 while the highest was within the retired (66.6%) however it is not found to be statistically significant.

The prevalence difference between respondent that reported more than one sexual partner with those that did not was 1.63% but higher prevalence was noticed amidst subjects with partners not more than one (Table 2). These could be due to discrepancies that are usually associated with questionnaires where the level of transparency of the answers is always based on subject response. The result obtained is contrary to the study of Xu et al. 2006[11] who revealed that HSV 1 seroprevalence was generally greater among those with more life time sex partners (Table 2). However, the result also revealed that subjects that uses condom had the least prevalence compared to others that didn't or indicated no answer. It could be deduced that having more than one sexual partner is not a greater risk factor when compared with intercourse without adequate protection with multiple partner. The subject that gave no answer as regard the frequency of condom usage had highest seropositivity and this was followed by those that reported to use at most times but not all times (Table 2). This result may be attributed to other route of HSV1 transmission which is not mostly through sexual route as compared to HSV2. Additionally, infection may have occurred at earlier period in life before subject adherence to condom usage (Johnson et al. 1989 [17]; Malkin et al. 2002 [13]) However, report by Martin et al. 2009[18] shows that consistent and correct use of condom can reduce HSV transmission. Furthermore, another study by Pereira et al. 2012[19] suggests that genital lesions by HSV1 is on the rise and may even exceed the one caused by HSV2. The blood transfusion status and tattoos of respondent were not significant to the acquisition of HSV1 status while tribal mark was significant (Figure 1). This could be due to the low awareness and hygiene level of the traditional mode of inscription that is common within the locality.

## CONCLUSION

Specific information regarding which of the infections (HIV or HSV1) was primary in the subjects prior to this research can't be deduced since both infections compromise the immune system in one way or another, thereby creating an opportunity for secondary infection. However, this research reveals that the prevalence of HSV1 infection is significant and although most of the risk factors were not statistically significant but nonetheless adherence to clean hygiene, sexual protection among others should be maintained at all time since most carrier or infected individual are asymptomatic.

## Acknowledgements

The Authors wish to acknowledge the assistance of the staff of the HAART clinic, staff of Department Hematology and blood bank, and also of the Department of Microbiology and Parasitology, UITH for their supports and technical assistance.

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