



## Seroprevalence of Anti HSV-2 IgG among Women of Reproductive Age attending General Hospital Dutse

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### Abstract

*Herpes simplex virus type 2 (HSV-2) remains one of the widespread and chronic sexually transmitted infections (STI) globally with highest disease burden in African females. A study to determine the humoral immune response to HSV-2 was carried out with a view to “x-raying” the magnitude of the infection among females of reproductive age in Dutse. A total of 94 clients were recruited for the study and Samples were randomly collected after obtaining their consent. A structured questionnaire was administered to capture demographic information in addition to knowledge, attitude and practices of each respondent. Samples were tested for the presence of IgG antibodies against HSV-2 using enzyme linked immunosorbent assay (ELISA). Results were further subjected to Chi-square test using SPSS version 22.0 where p-values of  $\leq 0.05$  were considered statistically significant. Out of the 94 samples tested, 29 (30.9%) were positive for anti HSV-2 IgG with high Seroprevalence rates among all age groups. However, age group 15-19 recorded the highest Seroprevalence of 46.2%. Similarly, age groups 20-29, 30-39 and  $\geq 40$  recorded 28.8%, 26.3% and 33.3% respectively. Chi-square test showed that Seroprevalence of HSV-2 was not significantly associated with age, marital status or level of education. However, it was strongly associated with number of sexual partners ( $p= 0.048$ ). Higher Seroprevalence was also recorded among women with no educational exposure than those with at least primary or higher level of education. However, Seroprevalence of infection was not statistically associated with level of education  $p= (0.059)$ . Married women recorded a higher Seroprevalence than the singles. The findings of this research indicates a high Seroprevalence of Herpes Simplex - 2 Virus among women of reproductive age which implies a public health problem to the populace of Dutse and Jigawa state at large making it necessary for the government and other stakeholders to properly curtail the situation through public enlightenment, and implementation of other health policies.*

**Key words:** Herpes simplex virus, ELISA, Samples, Seroprevalence, STIs, Dutse.

### INTRODUCTION

Herpes Simplex Virus Type-2 (HSV-2) is a sexually transmitted infection (STI) that is chronic, widespread, and infectious during both its symptomatic and asymptomatic periods (WHO, 2001). The infection is a significant factor for increased risk of acquisition and transmission of human immunodeficiency virus (HIV). A meta-analysis of studies on HSV-2 found that infection with HSV-2 doubled the risk of becoming infected with HIV through transmission during sexual activity (Wald and Link, 2002).

Herpes Simplex Virus Type-2 is the primary cause of genital herpes. It is highly prevalent in human populations in many parts of the world and it is the most common cause of genital

ulcer disease (GUD) worldwide (Kroon, 1994). Seroprevalence in the adult general population in sub-Saharan Africa ranges from 30% to 80% in women and from 10% to 50% in men (Weiss, 2004). The first documented isolation of HSV-2 in Nigeria was done in Ibadan (Oni *et al*, 1994). The HSV2 Seroprevalence rate among female sex workers in Lagos, Nigeria, was 59% (Dada *et al*, 1998). Higher HSV-2 Seroprevalence was reported in a multi-centre study of sex workers in four sub-Saharan African cities (Lagarde *et al.*, 2001).

Newborns of women known to be infected with genital herpes are at risk of neonatal transmission; hence delivery of these babies by caesarean section is desirable (Hayatudeen *et al.*, 2017).

Furthermore, new-borns at greatest risk were born to women exhibiting genital herpes for the first time during the time of delivery (Brown *et al.*, 1991). Testing for Seroprevalence of antibodies to HSV-2 is one way to estimate the Seroprevalence of genital herpes in a population (Kroon, 1994).

There is limited information about risk factors for acquisition and transmission of HSV-2 infection. Several longitudinal studies have identified HIV infection, being female, inconsistent condom use, number of sex partners, frequency of sexual activity, and other sexually transmitted infection (STIs) as predictors of HSV-2 acquisition (Wald, 2004). Evidence from in vivo studies shows that genital shedding of HSV-2 is higher in HIV-positive individuals (Mbopi-Ke'ou *et al.*, 2000) suggesting a potential role that HIV infection plays in HSV-2 transmission.

Female bar, hotel workers and sex workers in resource-poor settings are often at higher risk for STIs because they engage in high-risk behaviours, including accepting money or gifts in exchange for sex (Nagot *et al.*, 2002). Historically, women of child bearing age especially sex workers have been blamed for spreading sexually transmitted diseases including HIV and other infectious diseases like HSV -2 (Sani, *et al.*, 2015). Determining HIV Seroprevalence among this group of highly mobile population has been quite challenging. This population might continue to serve as a core group for the transmission of HIV, HSV-2, and other STIs where the HIV epidemic is generalized (Riedner *et al.*, 2003). Thus, improved knowledge of factors associated with HSV-2 infection in this population is important for the design of interventions.

Most infected people are unaware that they have the infection (Hayatuddeen *et al.*, 2018). Typically about 10-20% of people with HSV-2 infection report a prior diagnosis of genital herpes. Therefore this research provides an insight on Seroprevalence of HSV-2 and its associated risk factors. It can also serve as a useful tool in educating the general populace about the disease with a view to assisting the early diagnosis of the disease.

## MATERIALS AND METHODS

### Study Area

The study was conducted in Dutse capital of Jigawa state North-Western Nigeria. The state shares border with Bauchi, Yobe and Kano state. The global location of Dutse is between latitude  $11^{\circ} 42' 04''$  North and longitude  $9^{\circ} 20' 31''$  East of the Equator. Dutse has an estimated population of 153,000 (FGN, 2007).

Dutse is predominantly inhabited by the Muslim Hausa and Fulani Tribes. Being a purely agrarian Sahel State, the major economic activities in Dutse are farming and cattle rearing.

### Study Design

The study was a descriptive cross sectional study in which structured questionnaire was administered to the clients and blood samples were collected from the consented and analysed.

### Study Population

The study population comprised of women of reproductive age 15-45 years attending General Hospital Dutse.

### Ethical Consideration

Ethical approval was obtained from the research and ethical committee of Jigawa State Ministry of Health following a Letter of introduction from the Department of Microbiology and Biotechnology; Federal University Dutse.

### Inclusion Criteria

The study included only women of reproductive age >15 years attending General Hospital Dutse. Those below the age of 15 years and those not willing to participate were excluded.

### Sample Size

The sample size for this study was determined using the formula  $n = \frac{Z^2 Pq}{d^2}$  described by Hayatuddeen *et al.* (2016) and was found to be 94 considering a previous prevalence of HSV-2 in Jos, Nigeria (Agabi *et al.*, 2010).

### Sample collection

Five millilitres (5ml) of whole blood was aseptically collected from clients in plain plastic vacutainer tubes from brachiocephalic vein in the antecubital fossa of the upper arm. The blood samples collected were centrifuged at 1500rpm for 10 minutes. Using a micropipette, each serum sample was transferred to sterile labelled tubes and stored at  $-20^{\circ}\text{C}$  until ready for use (Hayatuddeen *et al.*, 2016).

### Serological Assay for HSV-2 IgG.

A commercially available HSV-2 antibody ELISA catalogue enzyme-linked immune assay for the detection of HSV-2 IgG antibody was employed. All the reagents and the micro titre ELISA plates were brought to room temperature for 30 minutes before use.

A micropipette was used to dispense 100 $\mu$ l of the negative and positive control into well 1 and 2 respectively. 100 $\mu$ l of the samples were added to their appropriate wells and covered with adhesive strip and incubated for 30 minutes at 37°C. The wells were then washed vigorously with distilled water and excess wash buffer was removed after the last washing by slapping the plates on a clean absorbent tissue. Two (2) drops of reagent 1 (blue solution) was added to each well and incubated at room temperature for 5 minutes. The wells were washed as above and two (2) drops of reagent 2 (red solution) was then added to each well and incubated for 5 minutes at room temperature and the washed. Two (2) drops of chromogen was added to each well and incubated for 5 minutes at room temperature. Finally, 2 drops of stock solution was then added to each well and mixed gently by tapping the side of the plate with the index finger. The wells with intense yellow colour were considered positive while those that did not develop the yellow colour were regarded as negative.

#### Data Analysis

Data obtained from the questionnaire and laboratory analysis were subjected to chi square analysis using SPSS version 22.0.

### RESULTS AND DISCUSSION

In the present study, HSV-2 infection among women of reproductive age attending General Hospital Dutse was found to be 30.9% which is slightly higher than that of Diawara *et al.*, (2008). Based on the findings of this research the Seroprevalence was found to be higher among females between the ages of 15-19 years because they are more sexually active even though, there was no significant difference based on the P value (0.629) therefore, age group might not be a risk factor for HSV-2 infection. The results show that sexual behaviour is a contributing factor to HSV-2 infection because those subjects that have at least a sexual partner have a higher Seroprevalence of 33.7% while it was not found in those who have not started any sexual activity. This might be due to the fact that exposure to the virus is higher in this group than those who are not engaged in any sexual

activity and with a P value of 0.048, number of sexual partners is therefore a risk factor for HSV-2 infection.

The results also show that there was no significance difference between level of education and HSV-2 infection as indicated in the tables, implying that Seroprevalence of the disease is not influenced by the educational background of the clients. The infection was however found to be more prevalent among married women compared to unmarried (single) and divorced. This could be attributed to the polygamous nature of Dutse and indeed North Western Nigeria which provides a possibility of STI transmission among spouses if the husband is unfaithful, that could also lower the immunity of the susceptible individuals in addition to pregnancy and other immunological factors. However, with a P value of 0.430 the marital status is not a risk factor of HSV-2 infection. However, the findings have shown no statistical significance as the Seroprevalence of the disease is not influenced by the marital status of the patient.

The result is in agreement with the results obtained by Diawara *et al.*, (2008) which shows lower HSV-2 Seroprevalence of 20.7%, and 26% found among pregnant women in Tanzania and Senegal, all of sub-Saharan Africa region. It also agrees with the report of HSV-2 Seroprevalence among adult female population in sub-Saharan Africa which ranges from 30% to 80% (Weiss, 2004). The result obtained from this study is much higher than the HSV-2 Seroprevalence of 7.5% and 9% reported among pregnant women in India and USA respectively (Rathore, 2010). However, it is lower than the HSV-2 Seroprevalence reported in a multi-Centre study in four sub-Saharan Africa cities: 90.0% in Cotonu, Benin Republic; 84.1% in Yaounde, Cameroun; 93.9% in Kisumu, Kenya; and 87.7% in Ndola, Zambia, as of June 1997 to March 1998 (Weiss, 2004), as well as 69.0% in Mwanza City, Tanzania (Vallely *et al.*, 2010). Our findings are also lower than the Seroprevalence of HSV-2 infection in Nigeria, earlier reported (59.0%) among commercial sex workers (Dada 1998) and 87.0% among clients attending the sexually transmitted infections Clinic in Jos (Agabi *et al.*, 2010).

Table 1: Seroprevalence of HSV-2 in relation to age group.

Age group (years)	Number examined	IgG Positive (%)	IgG negative	$\chi^2$ value	P-value
15-19	13	6(6.38)	7(7.45)	1.734	0.629
20-29	59	17(18.10)	42(44.68)		
30-39	19	5(5.32)	14(14.90)		
$\geq$ 40	3	1(1.10)	2(2.13)		
<b>Total</b>	<b>94</b>	<b>29(30.85)</b>	<b>65(69.15)</b>		

Table 2: Socio-demographic and risk factor distribution HSV-2

Parameters	No. Examined	IgG Positive (%)	IgG Negative	P-value
<b>Educational Level</b>				
Illiterate	52	19(20.21)	33(35.11)	0.591
Primary	5	2(2.13)	3(3.19)	
Secondary	12	2(2.13)	10(10.64)	
Tertiary	3	1(1.06)	2(2.13)	
Informal	22	5(5.32)	17(18.09)	
<b>Total</b>	<b>94</b>	<b>29(30.85)</b>	<b>65(69.15)</b>	
<b>Marital Status</b>				
Single	12	3(3.19)	9(9.58)	0.430
Married	79	26(27.66)	53(56.39)	
Divorced	3	0(0)	3(3.19)	
<b>Total</b>	<b>94</b>	<b>29(30.85)</b>	<b>65(69.15)</b>	
<b>Number of Sexual Partners</b>				
No partner	8	0(0)	8(8.50)	0.048
One partner	86	29(30.85)	57(60.64)	
<b>Total</b>	<b>94</b>	<b>29(30.85)</b>	<b>65(69.15)</b>	

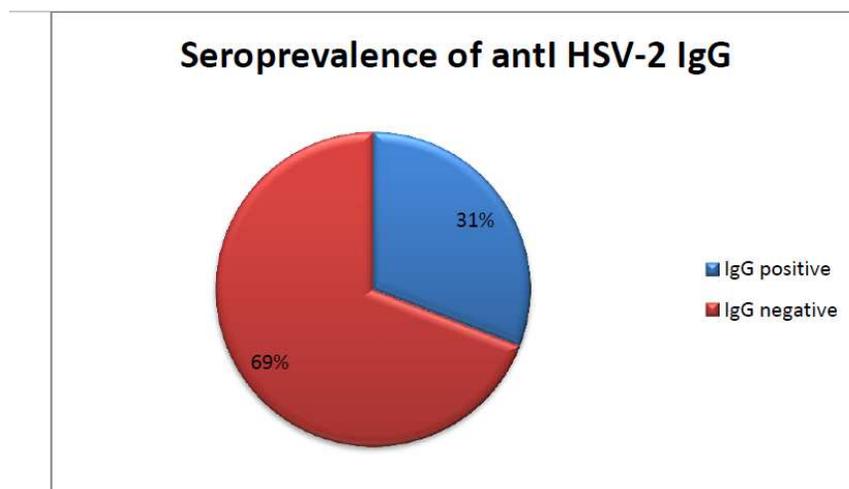


Figure 1: Overall Prevalence of anti HSV-2 IgG

**CONCLUSION**

The findings of this research indicates a high Seroprevalence of Herpes Simplex - 2 Virus among women of reproductive age which implies a public health problem to the populace of Dutse and Jigawa state at large making it necessary for the government and other stakeholders to properly curtail the situation through public enlightenment, and implementation of other health policies.

**Recommendations**

The following recommendations were made based on the findings of the present study:

1. It is recommended that health authorities should urgently recognize the importance of early diagnosis of HSV-2 infection especially among females and the most at risk individuals.
2. The need for use of condoms where necessary cannot be overemphasized.

3. Great efforts and funding are currently invested in research and development of several viral vaccines, this study shows that there is equal need to develop an HSV-2 vaccine so as to curtail the spread of HSV-2 and HIV.
4. There is also the need for public enlightenment among the general public on basic facts, mode of transmission, prevention, management and control of HSV-2. This could be through focus group discussions, town hall meetings, sensitization seminars, workshops and other programs.
5. Government, well-meaning individuals, non - Governmental organizations should assist in the supply of consumables, man power need in health facilities etc., to achieve ultimate goal of health for all.

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