UJMR, Volume 2 Number 1 June, 2017 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.1721.027
Received: 29th Sept, 2016 **Accepted:** 5th Jan, 2017

Seroprevalence of Immunoglobulins G and M Associated With Herpes Simplex Virus Type 2 among Apparently Healthy Individuals in Katsina State, Nigeria

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Abstract
Infection with Herpes Simplex Virus type 2 (HSV-2) is a significant public health problem being the leading cause of genital ulcerative disease. This study was aimed to evaluate the seroprevalence of herpes simplex virus type 2 among healthy individuals in Katsina State, and to determine the socio demographic and risk factors associated with HSV-2 infection in the study area. A cross-sectional serological survey enrolling 113 apparently healthy individuals from six Local Government Areas of Katsina State was conducted, to evaluate HSV-2 seroprevalence. Serum samples were obtained from randomly selected subjects. Samples were analysed using Immunoglobulins G (IgG) and M (IgM) HSV-2 specific commercial enzyme-linked immunosorbent assay kits. The overall seroprevalence of HSV-2 IgG and IgM was 71.7% and 32.7% respectively. HSV-2 seroprevalence increased with age. The results obtained demonstrated a high prevalence of HSV-2 seropositivity among apparently healthy individuals. We recommend educational awareness of Herpes type 2 infections among the populace and routine testing for Herpes type 2 antibodies in hospital laboratories to prevent HSV-2 related morbidity and mortality, particularly in apparently healthy individuals.
Keywords: HSV-2, Seroprevalence, IgG, IgM, Katsina, Nigeria

INTRODUCTION

Herpes Simplex Virus Type 2 (HVS-2) is a common human pathogen that can cause primary and recurrent infections of mucous membranes (Gupta *et al.*, 2007; Clara *et al.*, 2011). Primary HSV-2 infections are usually symptomatic but may be asymptomatic when there are no pre-existing antibodies against HSV-2 (Tronstein *et al.*, 2011). Recurrent infection takes place when HSV-2 reactivates in sacral ganglia, and is transported in the peripheral nerves back to the mucosal or skin surface (Barnabas *et al.*, 2011). Recurrence may be triggered by physical or emotional stress, fever, ultraviolet light and tissue damage (CDC, 2013).

Genital herpes may be caused by either herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) but, globally, majority of cases are caused by HSV-2 (Aminu *et al.*, 2014); infection is common in both the industrialized and developing worlds, and HSV-2 uncommonly causes infection by non-sexual means (Barnabas *et al.*, 2011). The ability of the virus to successfully avoid clearance by the immune system by entering a non-replicating state known as latency leads to lifelong infection (Agabi *et al.*, 2010), although whether latency always accompanies infection is unclear. Periodic reactivation from latency is possible and leads to viral shedding from the site of the initial infection (Biraro *et al.*, 2013).

Majority of persons with genital herpes do not know they have the disease and infection and reactivation are typically "asymptomatic" (Looker *et al.*, 2015). Despite the typically asymptomatic nature of genital herpes, which facilitates its spread in the population, and means it is a useful marker of sexual behavior (Luseno *et al.*, 2014), genital herpes is associated with considerable morbidity and even mortality. Genital lesions due to herpes are often very painful, and can lead to substantial psychological morbidity (CDC, 2013).

The virus annually affects an estimate of 417 million persons in the reproductive age range worldwide (Biraro *et al.*, 2013; Looker *et al.*, 2015; WHO, 2015). Herpes Simplex Virus Type-2 infections is widely distributed in European countries, although there are large differences in the percentage of the population exposed to the virus with Bulgaria has a higher seroprevalence value of 23.9% (Luseno *et al.*, 2014; Looker *et al.*, 2015).

In Nigeria, genital herpes has been reported to be associated with considerable morbidity and mortality (Duru *et al.*, 2014; Okonko *et al.*, 2015) such that infection in neonate with the virus is rare, but has a high risk of mortality and morbidity in Nigeria (Kalu *et al.*, 2014)

and there is higher prevalence of HSV-2 infection in Nigeria (Mawak *et al.*, 2009; Agabi *et al.*, 2010; Aminu *et al.*, 2014; Looker *et al.*, 2015). Since there is high prevalence of HSV-2 infection in Nigeria and other developing countries, there is need to educate people on the prevalence of HSV-2 infection in the population, the importance of routine HSV-2 screening, and how to help partners to make responsible choices regarding their sexual practices.

There is paucity of data on the seroprevalence of HSV-2 in Northern Nigeria and in Katsina State in particular, hence this study aimed to provide information with regards to the disease burden and need for improved educational awareness in Katsina State and Nigeria as a whole.

MATERIAL AND METHODS

Study Design

The study was a descriptive cross-sectional and experimental study in which structured questionnaire was administered to obtain information on socio-demographic and risk factors to apparently healthy individuals who served as volunteers, and blood samples were collected from those who consented.

Inclusion Criteria

This includes any apparently healthy individuals from the study area that gave their consent.

Ethical Approval

Ethical approval was obtained from the Ethical Committee of Hospital Services Management Board, Katsina State.

Blood Sample Collection

A total of 113 blood samples were collected aseptically using 5ml syringe from participants who gave consent in the study area. The blood was allowed to clot for 30 minutes and centrifuged at 1000rpm for 10 minutes. The serum was carefully removed with a transfer pipette and transferred aseptically to a sterile labeled serum storage screw-capped container and stored at -20^{o}C in a freezer until analyzed.

Serological Assay for HSV-2 IgG and IgM

The serum samples were analyzed using HSV-2 IgG and IgM specific ELISA kits (Diagnostic Automation/Cortez Diagnostics Inc, USA). The samples were analyzed according to manufacturer's instruction.

Data analysis and presentation

Analysis of HSV-2 infection among the patients according to socio-demographic and risk factors studied was done using GraphPad Statistical Software. Chi-square test was used and P values < 0.05 were considered statistically significant.

RESULTS

Of the 113 sera analysed, 71.7% (81/113) were seropositive for IgG and 32.7% (37/113) were seropositive for IgM (Figure 1).

Analysis of the results by sex showed that there was no significant association between sex of the participants and HSV-2 seroprevalence ($^{IgG}x^2$= 0.150, df= 1, p=0.314; $^{IgM}x^2$= 1.016, df= 1, p= 0.698), however, females had higher IgG and IgM seroprevalence compared to males (Table 1.0). Females participants were two times more likely to be infected than males (OR=2.017, 95% C.I=0.397-5.730).

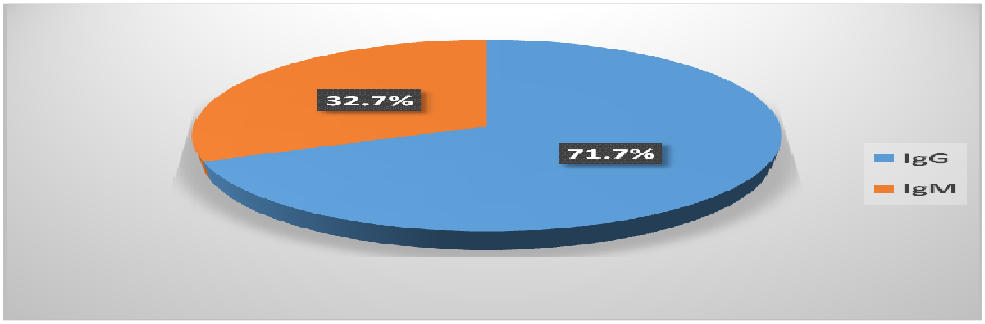

Figure 1: Overall Seroprevalence of HSV-2 in the study area

Table 1: Seroprevalence of HSV-2 Infection in Relation to Sex in Katsina State

Gender	IgG Positive	IgM Positive	P-value
Male	30 (54.9)	61 (43.4)	0.314^{IgG}
Female	83 (73.5)	52 (46.1)	0.698^{IgM}
Total	**81 (71.7)**	**37(32.7)**	

($^{IgG}x^2$= 0.150, df= 1, p=0.314; $^{IgM}x^2$= 1.016, df= 1, p= 0.698)

Analysis of the result by age group showed that higher IgG seroprevalence was recorded among participants in aged group 41-50 years (38.3%), while participants in aged group 51 years above had the lower IgG seroprevalence of (14.8%). The association observed between age of the participants and IgG seroprevalence was statistically not significant ($^{IgG}x^2$= 10.386, df= 8, p= 0.239) however, there was significant association observed between IgM seroprevalence and age of the participants ($^{IgM}x^2$=12.510, df= 4, p=0.023) (Table 2.0).

The result was analysed according to socio-demographic factors as shown in Table 3. A higher seroprevalence of IgG 56 (83.6%) was detected among those with high socioeconomic status while higher seroprevalence of IgM 18 (39.1%) was detected among those with low socioeconomic status, there is no statistically significant association between socio-economic background and HSV-2 infection (^{IgG}P= 0.432, ^{IgM}P= 0.768).

However, higher IgG seroprevalence rate 22 (84.6%) was recorded among those participants with tertiary level of education, while those with primary school level had the lower IgG seroprevalence of 13 (39.4%). Similarly, participants with tertiary level of education had higher IgM seroprevalence 17 (65.4%), while those with none level had the lower IgM seroprevalence rate of 2 (16.7%). Similarly, no statistical significant association was observed between HSV-2 infection and educational level of the participants (^{IgG}P= 0.211, ^{IgM}P= 0.413).

Analysis of result by occupation showed un-employed participants had higher IgG seroprevalence rate 36 (69.2%), while lower IgG seroprevalence was detected among civil servants 5 (31.3%), also higher IgM seroprevalence rate 28 (53.8%) was seen among un-employed and lower IgM seroprevalence rate was recorded among civil servant 3 (18.8%). There was no statistical significant association observed between HSV-2 infection and the occupation (^{IgG}P= 0.621, ^{IgM}P= 0.509).

Table 2: Seroprevalence of HSV-2 Infection in Relation to Participants' Age in Katsina State

Age group (Years)	IgG Positive (%)	IgM Positive (%)	P-value
10-20	13 (16.0)	12 (14.8)	
21-30	14 (17.3)	07 (18.9)	^{IgG}P=0.239
31-40	13 (16.0)	10 (27.0)	^{IgM}P=0.023
41-50	31 (38.3)	05 (13.5)	
51-above	12 (14.8)	03 (8.1)	
Total	**81 (71.7)**	**37 (32.7)**	

Analysis of result by marital status of the participant's showed that married participants had higher IgG seroprevalence rate 49 (75.4%), while lower IgG seroprevalence rate was detected among single participants. On the other hand also, married participants had higher IgM seroprevalence rate 29 (44.6%), lower IgM seroprevalence rate of 14 (29.1%) was seen among single participants. There was statistical significant association observed between HSV-2 infection and marital status of the participants ($^{IgG}x^2$= 2.601, df= 3, p= 0.046; $^{IgM}x^2$= 5.406, df= 3, p= 0.013).

TABLE 3.0: Seroprevalence of HSV-2 infection in Relation to Sociodemographic Factors

Factor	N0. Tested	IgG Positive (%)	P-value	IgM Positive (%)	P-value	OR
Socio Economic Status						
Low	46	25 (54.3)	0.432	18 (39.1)	0.768	
High	67	56 (83.6)		26 (38.8)		2.324
Education						
None	12	7 (58.3)	0.211	2 (16.7)	0.413	
Primary	33	13 (39.4)		6 (18.2)		
Secondary	42	31 (73.8)		13 (30.9)		
Tertiary	26	22 (84.6)		17 (65.4)		
Occupation						
Civil servant	16	5 (31.3)	0.621	3 (18.8)	0.509	
Self-employed	46	21 (45.7)		11 (23.9)		
Unemployed	52	36 (69.2)		28 (53.8)		
Marital Status						
Single	48	32 (66.7)	0.876	14 (29.1)	0.611	3.111
Married	65	49 (75.4)		29 (44.6)		

Analysis of the result according to the risk factors is shown in Table 4. Majority of the participants (82.7%) that agreed to be involved in sexual activity had higher IgG seroprevalence compared to participants that agreed not to be involved, similarly, those that claimed to have involved in sexual activity had higher IgM seroprevalence (48.0%: 36/75) compared to those that claimed not to have been involved in sexual activity. There was no statistical significant association observed between sexual activity of the participants and HSV-2 infection ($^{IgG}x^2$= 0.033, df= 1, p= 0.856; $^{IgM}x^2$= 0.325, df= 1, p= 0.569) but odd ratio shows that those that agreed to be involved in sexual activity are three times more likely to be infected with the virus than those that did not (OR=3.567, 95% C.I=1.234-4.730).

Participants that had single sexual partner had a lower IgG and IgM seroprevalence (50.0% and 47.1%) compared to those claimed to have more than one sexual partner with IgG and IgM seroprevalence of 64.4% and 62.2% respectively. There was statistically significant association between IgG and IgM seroprevalence and the number of sexual partners ($^{IgG}x^2$= 0.433, df= 1, p= 0.511; $^{IgM}x^2$= 1.231, df= 1, p= 0.367) but odd ratio shows that those with more than one sexual partner are two times more likely to be infected with the virus than those with single sex partner (OR=2.789, 95% C.I=3.970-5.412).

Further analysis showed that participants that used protection had lower IgG seroprevalence rate of 46.1% compared to those who did not agreed to use protection, while IgM seroprevalence was higher among those participants that agreed to use protection with 82% prevalence rate compared to those participants did not agreed to use protection. There was statistical significant association observed between IgG seroprevalence and use of protection ($^{IgG}x^2$= 1.116, df= 1, p= 0.034; $^{IgM}x^2$= 0.154, df= 1, p= 0.395).

TABLE 4.0: Seroprevalence of HSV-2 Infection in Relation to Possible Risk Factors

Variables	Number Tested	IgG			IgM			
		Positive (%)	x^2	P-value	Positive (%)	x^2	P-value	OR
Sexual Activity								
Yes	75	62 (82.7)	0.033	0.000	36 (48.0)	0.325	0.569	3.567
No	38	11 (28.9)			14 (36.8)			
Partners								
1	68	34 (50.0)	0.433	0.011	32 (47.1)	1.231	0.367	2.789
>1	45	29 (64.4)			28 (62.2)			
Use of protection								
Yes	13	06 (46.1)	1.116	0.034	09 (69.2)	0.154	0.395	2.654
No	100	82 (82.1)			41 (41.0)			

DISCUSSION

In the present study, there was a high IgG seroprevalence of 71.7% in the study area. This higher seroprevalence implies that the virus is highly endemic in the communities of the participants. This implies that these individuals have been infected with the virus and had developed antibodies hence carriers of the virus. Infection with HSV-2 appears to be silent and unnoticed in the study area and knowledge of the mode of transmission of the virus also appears to be limited and hence facilitates the spread of the virus in the population. This might be because many of the lesions might be mild and insufficiently troublesome for those positive patients to seek for medical attention under conditions where medical facilities are scarce and home treatment of self-limiting conditions (CDC, 2013).

While IgM seroprevalence of 32.7% is also similar to 43.57% reported in turkey (Duran *et al.*, 2004). Higher seroprevalence of 87.0% was reported by Agabi *et al*. (2010). On the other hand, lower seroprevalence has been reported from North America of 17.9% (Looker *et al.*, 2008), 13.8% in Turkey (Duran *et al*., 2014).

To our knowledge, no study has previously estimated the seroprevalence of HSV-2 among apparently healthy individual in Nigeria. The higher seroprevalence value among apparently healthy individuals reported in this study (71.7%) is similar with 73.8% in Enugu Nigeria (Ojinmah *et al.,* 2012) and 73.9% reported in Iraq (Weiss *et al.,* 2004). However, it was higher than that of 24% reported in Japan among healthy individuals (Hashido *et al.*, 1998) but shows a strong consistent with the German study that recorded 73.3% among healthy individuals as reported by (Wutzler *et al.*, 2000).

However, there was no significant difference observed between male and female

participants, but seroprevalence was higher in female than male probably due to disparity between genders may also reflects anatomical differences in susceptibility to infection and greater efficiency of transmission from men to women (Bradley *et al.,* 2014).

Similar to other studies (Agabi *et al.,* 2010; Wald *et al.,* 2002), in this study there is a clearly significant association between increasing seroprevalence with increasing age. This can be explained by the fact that herpes virus infections persists for life, the seroprevalence increases with age through the sexual active years.

It is interesting to note that there was a consistently high seroprevalence of HSV-2 infection irrespective of educational status. This agrees with a review conducted in 2004 (Weiss *et al.,* 2004). However, high seroprevalence recorded among those participants that did not go to school which agrees with (Obeid *et al.,* 2007; Tidemam *et al.,* 2001) that shows high seroprevalence in less educated people.

The IgG seroprevalence was not significantly associated with the occupation, although higher value was recorded among civil servant. This agreed with Rathore *et al*. (2010) and Biswas *et al*. (2011) that seroprevalence is higher among working class than those that are not working.

Also, in this study, there was a higher seroprevalence among married compared to single individuals which contradict Duru *et al*. (2014) that reported higher seroprevalence in singles compared to married. This may be likely due active sexual life and multiple sex partners and probably extramarital affairs among the married individuals.

In relation to number of sex partners, those with more than one sex partner had higher seroprevalence. Previous studies by Aminu *et al*, (2014), Agabi *et al,* (2010) and Duru *et al*, (2014) have made similar observations. Although this has not attained statistical level of significance underlines the urgent need for reduction in number of sexual partners.

CONCLUSION

Herpes Simplex Virus type 2 IgG and IgM antibodies were detected in 71.7% and 32.7% respectively of the apparently healthy individuals with no visible signs and symptom of the infection in the study area. The results highlight the potential public health impact of HSV-2 in Nigeria where anti-HSV-2 testing is not generally performed in all populations.

Recommendations

In view of the high seroprevalence of HSV-2 obtained in this study, there is need for public enlightenment on genital herpes, its mode of transmission and public health significance. Healthy individuals should be screened for HSV-2 antibodies and further studies with larger sample size are recommended. Since infection with HSV-2 is life-long and has no known cure, primary prevention remains the mainstay of its control.

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