Quest Journals
Journal of Medical and Dental Science Research
Volume 5~ Issue 5 (2018) pp: 08-14
ISSN(Online) :2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Survey of Immunoglobulin G and M of Herpes Simplex Virus Type 2 among HIVPatients in some selected Hospitals of Katsina State

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ABSTRACT
Herpes simplex viruses (HSV) are large double-stranded DNA virus that are proficient in promoting cell death upon infection and in establishing latency in sensory ganglia and replicating in epithelial cells during primary and recurrent infection. Herpes Simplex Virus type 2 (HSV-2) is a significant public health problem being it one of the most prevalent sexually transmitted infections (STIs) worldwide and the leading cause of genital ulcerative disease (GUD) that is common both in industrialized and developing countries. The objective of this study was to survey Immunoglobulin G and M of Herpes Simplex Virus type 2 among HIV positives patients in Katsina State, by determining the sociodemorgraphic and risk factors associated with HSV-2 infection. A cross-sectional serological survey enrolling 125 HIV positive participants attending public health care settings in six local government of Katsina State was conducted. Serum samples were obtained from randomly selected subjects. Samples were tested using an IgG and IgM HSV-2 specific commercial enzyme-linked immunosorbent assay kit. The overall prevalence of HSV-2 IgG is 74.4% and 40.0% for IgM ranging from 81.8% in Katsina, 81.3% in Daura, 69.2% in Malumfashi, 64.3% in Baure, 63.2% in Funtua and 82.4% in Dutsinma for IgG respectively while IgM recorded prevalence rate of 23.5% in Dutsinma, 36.4% in Katsina, 42.9% in Baure, 62.5% in Daura, 36.8% in Funtua and 42.3% in Malumfashi. HSV-2 prevalence increased with age and HIV positivity. These results demonstrate a high prevalence of Herpes type 2 positivity among the participants. We recommend improved education regarding Herpes type 2 among the populace and increasing routine testing for Herpes type 2 antibodies to prevent HSV-2 related morbidity and mortality, particularly in immunocompromised patients.
***KEYWORDS**: Herpes, GUD, prevalence, HIV, ELISA.*

Received 1st July, 2018; **A**ccepted 10 July, 2018 © The author(s) 2018. **P**ublished with open access at **www.questjournals.org**

I. INTRODUCTION

Herpes simplex viruses (HSV) are large double-stranded DNA virus belonging to *Alphaherpesvirus* subfamily of *Herpesviruses* surrounded by an envelope of lipid glycoprotein (Kahsay *et al.*, 2015). Once the virus has contact with the mucous membranes or skin wounds, it begins to replicate (Akhtar *et al.*, 2009).The virus is then transported within nerve cells to their roots where it remains inactive (latent*)* and persists for the whole life of the infected person (Wald, 2008). During inactive periods or latent state, the virus cannot be transmitted to another person (Rajagopal *et al.*, 2014).

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). Primary HSV-2 infections are usually symptomatic but may be asymptomaticwhen there are no pre-existing antibodies against HSV-2 (Tronstein *et al.*, 2011). Recurrence 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).

Herpes Simplex Virus Type-2 is sexually transmitted that is horizontally transmitted (CDC, 2013) and it can also be transmitted through oral and anal sex (Schiffer *et al.*, 2014). Herpes simplex virus type-2 is

periodically shed in the human genital tract, most often asymptomatic (Phipps, 2015). Herpes simplex virus type-2 may also be transmitted vertically during childbirth from mother to its child (Shannon *et al.,* 2015).

Herpes Simplex Virus type-2 is a significant public health problem being one of the most prevalent sexually transmitted infections (STIs) worldwide and the leading cause of genital ulcerative disease (GUD) that is common both in industrialized and developing countries (Bradley *et al.,* 2014). Majority of the infections are asymptomatic and this enhances HSV-2 transmission because asymptomatic individuals shed the virus and transmit the disease (Phipps, 2015).

The virus annually affects an estimate of 400 million persons in the reproductive age range worldwide (Looker *et al.,* 2015).Herpes Simplex Virus Type-2 infection is particularly devastating when it occurs in immunocompromised patients and, unfortunately, coinfection is common (Schiffer *et al*., 2014). Of major public health importance is the interaction of HSV-2 with human immunodeficiency virus (De Baetselier *et al.,* 2015). HSV-2 infection increases the risk of acquiring HIV infection by two-to-three folds, HIV transmission on a per-sexual act basis by up to five folds, and may account for 40–60% of new HIV infections in high HSV-2 prevalence populations (Edith *et al.,* 2014). Patients with HIV may have more frequent, severe and prolonged episodes of recurrences of genital herpes especially in those with a low CD4 count <200/μL (Dellar *et al.,* 2015). There is a higher rate of subclinical shedding of HSV-2 (i.e. in the absence of obvious genital lesions) in those infected with HIV (Schiffer *et al*., 2014). Human Immunodeficiency Virus (HIV) also increases the risk of acquisition and transmission of HSV-2. Herpes Simplex Virus Type-2 infection actually defines AIDS in the case of chronic ulcers of more than one month duration, bronchitis, pneumonitis or oesophagitis (Kalu, 2014).

In Nigeria, genital herpes has been reported to be associated with considerable morbidity and mortality (Duru *et al.,* 2014) such that infection in neonate with the virus is rare, but has a high risk of mortality and morbidity in Nigeria (Kalu, 2014) and there higher prevalence of HSV-2 infection in Nigeria (Agabi *et al.,* 2010).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.

II. METHODOLOGY

Study Area

The study was carried out in Katsina State located at the extreme northern margin of Nigeria, the state covers a total area of about 23,938sqkm (3,370sq) with a total population of 5,801,584 people, going by 2006 census (FGN 2007). The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. Katsina State has predominantly Hausa-Fulani indigenes. About 75% of the people are farmers and others are traders and livestock owners. The state has thirty four (34) local governments' areas (LGAs). The LGAs are divided into three (3) senatorial zones according to their geographical locations, namely; Funtua zone (South), Katsina zone (Central), and Daura zone (North) (Dauda *et al.,* 2011).

Study Design

The study was a descriptive cross-sectional and experimental study in which structured questionnaire was administer to the participants and blood samples were collected from those consented and analysed.

Study Population

The study population comprised of male and female coming for HIV screening from six hospitals were conveniently selected for the study. The hospitals selected for the study were; General Hospital Funtua Children's and Maternity Hospital Malumfashi, General Hospital Dutsinma, General Hospital Katsina, General Hospital Daura and General Hospital Baure.

Ethical Approval

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

Blood Sample Collection

A total of 125 blood samples were collected aseptically using 5ml syringe from patients who gave consent by the laboratory technologist in the selected Hospitals of Katsina State. The blood was allowed to clot for 30 minutes and centrifuged at 1000rmp 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 Antibodies

The serum sample were analyzed using HSV-2 IgG and IgM specific ELISA kits manufactured by Diagnostic Automation / Cortez Diagnostics Inc, USA. The manufacturer's instruction were strictly followed.

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III. RESULTS

Of the 125 sera analysed, 74.4% (93/125) were positive for IgG and 40.0% (50/125) were positive for IgM while no statistical significant association observed with HSV-2 infection ($^{IgG}\chi^2$ = 7.843, df= 3, p= 0.432; $^{IgM}\chi^2$= 62.45, df= 3, p= 0.07) as shown in Table 1.

Analysis of the result by age group is shown in Table 2, Higher IgG prevalence was recorded among participants in aged group 41-50 years (88.6%: 31/35), while participants in aged group 51years above had the lower IgG prevalence of (52.1%: 12/23). The association observed between age of the participants and previous HSV-2 infection was statistically not significant ($^{IgG}\chi^2$= 10.386, df= 8, p= 0.239). There is significant association observed between current HSV-2 infection and age of the participants ($^{IgM}\chi^2$=12.510, df= 4, p=0.023). Generally prevalence of HSV-2 infection is higher in participants of higher age groups than those in lower age groups.

Analysis of HSV-2 infection by hospital showed that higher IgG prevalence of (82.4%: 14/17) in General Hospital Dutsinma, While lower IgG prevalence was detected in General Hospital Funtua (63.2%: 12/19). The association between previous HSV-2 infection and hospitals observed was statistically significant ($^{IgG}\chi^2$= 25.415, df= 5, p= 0.003). General Hospital Daura had higher IgM prevalence rate (62.5%: 10/16), while the least prevalence of IgM was detected in General Hospital Dutsinma (23.5%: 4/88).There is no statistically significant association observed between current HSV-2 infection and hospital studied ($^{IgM}\chi^2$= 8.646, df= 5, p= 0.124) (Table 3).

Analysis of result by clinical symptoms are shown on Table 4. None of the symptoms observed was significantly associated with HSV-2 infection, but IgG prevalence rate is higher in the participants without blister (85.3%: 81/95) compared to those participants with blister. Furthermore participants without blister had higher IgM prevalence rate (41.1%: 39/95) compared to participants with blister odd ratio shows that those without blister are more likely to be infected with the virus (OR=5.367, 95% C.I=2.204-3.730).

The analysis of result according to muscle ache shows that (78.6%: 66/84) of the participants without muscle ache had higher IgG prevalence rate compared to participants with muscle ache, while participants with muscle ache had higher IgM prevalence rate (42.9%: 36/84) compared to participants without muscle ache but odd ratio indicate that participants with muscle ache are more likely to be infected with the virus (OR=4.009, 95% C.I= 6.970-8.472).

However, participants with burning sensation had lower IgG prevalence rate (78.4%: 80/102) compared to participants without burning sensation, while participants without burning sensation had lower IgM prevalence rate (13.0%: 3/23) compared to the participants with burning sensation but odd ratio indicate that participants without burning sensation are more likely to be infected with the virus (OR=2.734, 95% C.I= 0.397-4.030).

However, participants without vaginal discharged had higher IgG prevalence rate (94.8%: 74/78) compared to participants with vaginal discharged, while participants without vaginal discharged had higher IgM prevalence rate (41.0%: 32/78) compared to participants with vaginal discharged odd ratio indicate that participants with vaginal discharged are more likely to be infected by the virus (OR= 2.117, 95% C.I=1.736-2.730).

Analysis further also reveals that participants without adenopathy had higher IgG prevalence rate (76.2%: 77/101) compared to participants with adenopathy, while participants without adenopathy had higher IgM prevalence rate (43.6%: 44/101/114) compared to participants without adenopathy but odd ratio indicate that participants with adenopathy are more likely to be infected with the virus (OR= 3.142, 95% C.I=1.201-4.110).

Analysis of the result according to the risk factors is shown in Table 5. Majority (82.7%: 62/75) participants that agreed to be involved in sexual activity had higher IgG prevalence compared to participants that agreed not to be involved, while those that claimed to have involved in sexual activity had higher IgM prevalence (48.0%: 36/75) compared to those that claimed to have involved in sexual activity. There is no statistical significant association observed between sexual activity of the participants and HSV-2 infection ($^{IgG}\chi^2$= 0.033, df= 1, p= 0.856; $^{IgM}\chi^2$= 0.325, df= 1, p= 0.569) but odd ratio shows those claimed involved in sexual activity are tree times more likely to be infected with the virus (OR=3.567, 95% C.I=1.234-4.730).

However, participants claimed to have single sexual partner had a lower IgG prevalence rate (69.2%: 54/78) compared to those claimed to have morethan one sexual partner, while participants with morethan one sexual partner had the higher IgM prevalence rate (59.6%: 28/47) compared those claimed to have single sexual partner.The association observed between sexual activity of the participants and HSV-2 infection was not statistically significant ($^{IgG}\chi^2$= 0.433, df= 1, p= 0.511; $^{IgM}\chi^2$= 1.231, df= 1, p= 0.267) but odd ratio shows that those with morethan one sexual partner are more likely to be infected with the virus (OR=2.789, 95% C.I=3.970-5.412).

Further analysis showed that participants that agreed to use protection had lower IgG prevalence rate (47.8%: 11/23) compared to those who did not agreed to use protection, while participants that use protection

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had lower IgM prevalence (39.1%: 9/23) compared to those participants did not agreed to use protection. There is no statistical significant association observed between HSV-2 infection and use of protection ($^{IgG}\chi^2$= 1.116, df= 1, p= 0.734; $^{IgM}\chi^2$= 0.154, df= 1, p= 0.695) but odd ratio confirm the association was not statistically significant (OR=2.654, 95% C.I=0.121-3.000).

The IgG prevalence among cancer patient's participants is higher 100% compared to participants that are non-cancer participants, while participants that are non-cancer patients had lower IgM prevalence rate. There is statistical significant association observed between cancer status of the participants and previous HSV-2 infection and no statistical significant association observed between cancer status of the participants and current HSV-2 infection ($^{IgG}\chi^2$= 3.321, df= 1, p= 0.022; $^{IgM}\chi^2$= 0.156, df= 1, p= 0.563). Odd Ratio indicate that cancer participants are more likely to be infected with the virus (OR=5.734, 95% C.I=0.397-8.730).

The result was analysed according to socio-demographic factors as shown on Table 6. A higher prevalence of (79.7%: 63/79) and (40.5%: 32/79) for IgG and IgM was detected among those with low socioeconomic status, while those with high socioeconomic status had lower prevalence rate. There is no statistically significant association between socio-economic background and HSV-2 infection ($^{IgG}\chi^2$= 3.341, df= 1, p= 0.063; $^{IgM}\chi^2$= 2.219, df= 1, p= 0.136) and odd ratio confirm those with high socioeconomic status are two times more likely to be infected with the virus (OR=2.007, 95% C.I=0.321-5.210).

However, higher IgG prevalence rate (82.4%: 28/34) was recorded among those participants with primary level of education, while those with secondary school level had the lower IgG prevalence. Those participants with secondary level of education had higher IgM prevalence (52.2%: 24/46), while those with none level had the lower IgM prevalence rate. Similarly, no statistical significant association was observed between HSV-2 infection and educational level of the participants ($^{IgG}\chi^2$= 1.847, df= 3, p= 0.605; $^{IgM}\chi^2$= 1.654, df= 3, p= 0.647).

Analysis of result by occupation showed that self-employed had higher IgG prevalence rate (80.6%: 29/36), while lower IgG prevalence was detected among farmers, while higher IgM prevalence rate (60.0%: 6/12) was seen among self-employed and lower IgM prevalence rate was recorded among civil servant (20.0%: 7/35). There is no statistical significant association observed between HSV-2 infection and the occupation ($^{IgG}\chi^2$= 6.791, df= 3, p= 0.079; $^{IgM}\chi^2$= 2.234, df= 3, p= 0.525).

Furthermore, analysis of result by marital status of the participant's showed that married had higher IgG prevalence rate (83.8%: 57/68), while lower IgG prevalence rate was detected among divorced. In other hands married participants had higher IgM prevalence rate (50.0%: 34/68), lower IgM prevalence rate of was seen among widows. There was statistical significant association observed between HSV-2 infection and marital status of the participants ($^{IgG}\chi^2$= 2.601, df= 3, p= 0.046; $^{IgM}\chi^2$= 5.406, df= 3, p= 0.013)

TABLE 1: Prevalence of Immunoglobulin G and M among the HIV positives participants

Test	**Total**	**Positive (%)**	Negative (%)	χ^2	**P-value**
IgG	125	93 (74.4)	32 (25.6)	7.843	0.432
IgM	125	50 (40.0)	75 (60.0)	62.45	0.07

TABLE 2: Prevalence of Immunoglobulin G and M in relation to participants' age in Katsina State

Age groups (Years)	**Total**	**IgG**			**IgM**		
		Positive (%)	χ^2	**P-value**	**Positive (%)**	χ^2	**P-value**
10 – 20	14	10 (71.4)	10.39	0.239	01 (7.14)	12.51	0.023
21 – 30	21	13 (61.9)			03 (14.3)		
31 – 40	32	27 (84.3)			19 (59.4)		
41 – 50	35	31 (88.6)			14 (40.0)		
51 and above	23	12 (52.1)			13 (56.5)		
Total	**125**	**93(74.4)**			**50(40.0)**		

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TABLE 3: Prevalence of Immunoglobulin G and M by Hospital in Katsina State, Nigeria.

HospitalTotal		IgG			IgM		
		Positive (%)	χ^2	P-value	Positive (%)	χ^2	P-value
GH Dutsinma	17	14 (82.4)	25.415	0.003	04 (23.5)	8.646	0.124
GH Katsina	33	27 (81.8)			12 (36.4)		
GH Baure	14	09 (64.3)			06 (42.9)		
GH Daura	16	13 (81.3)			10 (62.5)		
GH Funtua	19	12 (63.2)			07 (36.8)		
MCH Malumfashi	26	18 (69.2)			11 (42.3)		
Total	**125**	**93 (74.4)**			**50 (40.0)**		

Keywords:
GH= General Hospital
MCH= Maternal and Children Hospital

TABLE 4: Prevalence of Immunoglobulin G and M in relation to Symptoms Observed.

Factor	Total	IgG			IgM			
		Positive (%)	χ^2	P-value	Positive (%)	χ^2	P-value	OR
Blister			2.538	0.111		6.154	0.213	5.367
Yes	30	12 (40.0)			11 (36.7)			
No	95	81(85.3)			39 (41.1)			
Muscleache			1.389	0.239		2.037	0.154	4.009
Yes	41	27 (65.9)			14 (34.1)			
No	84	66 (78.6)			36 (42.9)			
Burningsensation			21.281	0.466		8.234	1.843	1.734
Yes	23	13 (56.5)			03 (13.0)			
No	102	80 (78.4)			47 (46.1)			
Vaginaldischarge			1.842	0.856		0.033	0.169	2.117
Yes	47	19 (40.4)			18 (38.3)			
No	78	74 (94.8)			32 (41.0)			
Adenopathy			0.362	0.547		2.466	0.093	3.142
Yes	24	16 (66.7)			06 (25.0)			
No	101	77 (76.2)			44 (43.6)			

Keywords: n= Number, (%) =Percentage, (P<0.05)

TABLE 5: Prevalence of Immunoglobulin G and M in relation to risk factors in Katsina State.

Variables	Number	IgG			IgM			
		Positive (%)	χ^2	P-value	Positive (%)	χ^2	P-value	OR
Sexual Activity			0.033	0.856		0.325	0.569	3.567
Yes	75	62 (82.7)			36 (48.0)			
No	50	31 (62.0)			14 (28.0)			
Partners			0.433	0.511		1.231	0.267	2.789
1	78	54 (69.2)			22 (28.2)			
>1	47	39 (82.9)			28 (59.6)			
Use of protection			1.116	0.734		0.154	0.695	2.654
Yes	23	11 (47.8)			09 (39.1)			
No	102	82 (80.4)			41 (40.2)			
Cancer			3.567	0.042		3.567	0.231	3.117
Yes	11	11 (100)			09 (81.8)			
No	114	81 (71.1)			41 (35.9)			

Key: n= Number, (%) =Percentage, (P<0.05).

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	Tested	Positive(%)	χ^2	P-value	Positive (%)	χ^2	P-value
Socioeconomic							
High	46	25 (54.3)	3.341	0.063	18 (39.1)	2.219	0.136
Low	79	63 (79.7)			32 (40.5)		
Education							
None	19	13 (68.4)	1.847	0.605	05 (26.3)	1.654	0.647
Primary	34	28 (82.4)			13 (38.2)		
Secondary	46	36 (78.3)			24 (52.2)		
Tertiary	26	16 (61.5)			08 (30.8)		
Occupation							
Civil Servant	35	26 (74.3)	6.791	0.079	07 (20.0)	2.234	0.525
Self Employed	36	29 (80.6)			19 (52.3)		
Unemployed	42	32 (76.2)			20 (47.6)		
Farmer	12	06 (50.0)			04 (33.3)		
MaritalStatus							
Single	17	11 (64.7)	2.601	0.046	08 (47.1)	5.406	0.014
Married	68	57 (83.8)			34 (50.0)		
Widow	25	16 (64.0)			05 (20.0)		
Divorced	15	09 (60.0)			03 (20.0)		

Table 6:Prevalence of Immunoglobulin G and M in relation to sociodemorgraphic factors
Keywords: n= Number, (%) =Percentage

IV. DISCUSSION

On the other hand IgG prevalence in this study (74.4%) in HIV positives participants is lower than 82.0% in Germany (Saurbrei *et al.,* 2005), 97.2% in portacourt Nigeria (Ojinmah *et al.,* 2012) but higher than 20.7% in Tanzania (Yahya-malima *et al.,* 2008), 51.1% in Zimbabwe (Kurewa *et al.,* 2010), 44.3% in Benin, Nigeria (Kalu, 2014), 63.8% in Kampala, Uganda (Edith *et al.,* 2014). This higher IgG prevalence in this study could be due to sexual activity by the pregnant women involved in this study. It also confirm HIV positivity as a one of the predisposing factor that may activate the virus from its latent state.

In contrast to this finding of 40.0% IgM prevalence among HIV positives participants lower value had been reported 23.6% in Kisumu, Kenya (Ondondo *et al.,* 2014), but higher Prevalence rate of 97.2% in Portacourt, Nigeria (Ojinmah *et al.,* 2012), 54.3% in Zari, Nigeria (Aminu *et al.,* 2014) and somewhat similar to 44.3% in Benin by (Kalu, 2014), 41.1% in South Africa (De Baetselier *et al.,* 2015). This indicate a higher incidence of HSV-2 infection among HIV positives participants emphasizing the possible risk of neonatal infection. This could be due to the fact that HIV positivity may activate the latent HSV-2 infection and possibly lead to neonatal infection. Furthermore, the finding showed that HSV-2 infection is not significantly associated with duration of pregnancy which is consistent with (Dellar *et al.,* 2015), and not consistent with (Uddin *et al.,* 2015).

V. CONCLUSION

Conclusively, Overall prevalence 74.4% for IgG and 40.0% for IgM of HSV-2 infection appears to be relatively very high in the study area and it can leads to serious complication associated with HSV-2 infection. This indicate a higher prevalence of HSV-2 infection among HIV positives participants emphasizing the possible risk of transmission to seronegative individuals. This implies that the virus is highly endemic in the communities where these hospitals are situated. This could be due to the fact that HIV positivity may activate the latent HSV-2 infection and possibly lead to transmissionto seronegative individuals.

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Hayatudeen M.R"Survey of Immunoglobulin G and M of Herpes Simplex Virus Type 2 among HIV Patients in some selected Hospitals of Katsina State." Quest Journals Journal of Medical and Dental Science Research 5.5 (2018):08-14.

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