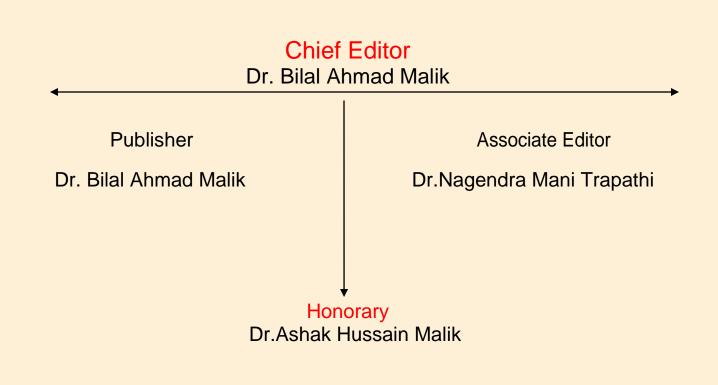# North Asian International Research Journal Consortium

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Comparative study on seroprevalence of Herpes simplex virus type two (HSV-2) IgG antibodies between Indians and Nigerian students of Jodhpur National University, Rajasthan, India.

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ABSTARCT

This study was carried out to compare the seroprevalence of HSV-2 IgG antibodies between Indian and Nigerian students of Jodhpur National University. The prevalence was determined in the sera taken from both of the students. A total of 90 blood samples were collected with equal proportion. The sera samples were subjected for HSV-2 IgG detection using HSV-2 IgG ELISA kit as per manufacturers manual. The prevalence of HSV-2 IgG antibodies among female students was significantly lower (14.4%) than among male students (56.7%), and significantly higher prevalence was detected in students from urban areas (60%) than in those from rural area (11.1%). Comparatively, this study showed that the seroprevalence of HSV-2 IgG antibodies were more prevalent among Nigerian students (48.9%) than in Indian students (22.2%). Herpes simplex virus type two (HSV-2) is a predominantly sexually transmitted virus that is the most common cause of genital ulceration [Corey L, H.H.Hansfield, 2000]. The seroprevalence of HSV-2 IgG antibodies varies considerably Worldwide with age, sex, number of lifetime sexual partners and socio-economic factors [Sauerbrei A, et al, 2000].

Keywords: seroprevalence, Herpes simplex, (HSV-2) IgG, indians, Nigerian.

1.0 INTRODUCION

Herpes simplex virus type 2 (HSV2) infections is the primary cause of genital herpes. It is highly prevalent in human populations in many parts of the world, and is the most common cause of genital ulcer disease worldwide. In developing countries, the major public health importance of HSV2 relates to its potential role in facilitating HIV transmission. HSV2 is highly prevalent in most regions experiencing severe HIV epidemics, with infection rates rising steeply with age to reach levels of 70% or more among adult women and men in some African countries. [Weiss et al, 2004].

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Genital infection with HSV is among the most common sexually transmitted diseases worldwide, with approximately 640,000 new cases diagnosed annually [J. Bras Doencas, 2007]. The epidemiological profile of genital infection by HSV suggests that the risk factors for the acquisition of the virus include age, sex, ethnic group, socioeconomic status, number of sexual partners, age at first intercourse ,and a history of previous sexually transmitted infections(STIs)[Taylor J. et al,2001].

HSV-2 is almost exclusively sexually transmitted, causing infection in the genital oranal area (genital herpes). However, HSV-1 can also be transmitted to the genital area through oral-genital contact to cause genital herpes. Both oral herpes infections and genital herpes infections are mostly asymptomatic but can cause mild symptoms or painful blisters or ulcers at the site of infection .Herpes simplex virus type 2 (HSV2) HSV-2 infection is widespread throughout the world and is almost exclusively sexually transmitted, causing genital herpes. HSV-2 is the main cause of genital herpes, which can also be caused by herpes simplex virus type 1 (HSV-1). Infection with HSV-2 is lifelong and incurable. Scope of the problem Genital herpes caused by HSV-2 is a global issue, and an estimated 417 million people worldwide were living with the infection in 2012. Prevalence of HSV-2 infection was estimated to be highest in Africa (31.5%), followed by the Americas (14.4%). It was also shown to increase with age, though the highest numbers of people newly infected were adolescents. More women are infected with HSV-2 than men; in 2012 it was estimated that 267 million women and 150 million men were living with the infection. This is because sexual transmission of HSV is more efficient from men to women than from women to men.

Genital ulcer disease enhances the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects, and clinical research has shown effects of HSV2 infection on genital HIV shedding. The reciprocal effect of HIV immune suppression on the exacerbation of HSV2 symptoms implies that there is a positive feedback loop, with HIV enhancing HSV2 expression, which in turn may enhance HIV infectiousness and it spread. Accumulating data suggest that HSV2 may be responsible for a substantial proportion of new HIV infections in some parts of Africa. There is an urgent need to consider potential control measures for HSV2 that might be applied in an effort to curb HIV transmission. These might include episodic or suppressive antiviral therapy, for example among high-risk groups, and behavioural interventions designed to reduce herpes transmission. Candidate HSV2 vaccines and vaginal microbicides are also under development.[WHO/UNAIDS/LSHTWORKSHOP (LONDON,2001]. An increasing proportion of genital ulcer cases in Africa are now attributable to HSV2, and the implications for treatment algorithms also need to be considered. Given the increasing awareness of the link between HSV2 and HIV, an international technical workshop was held in February 2001 to review existing knowledge concerning the epidemiology and control of HSV2 in developing countries and its interaction with HIV. The main aim of the workshop was to establish future research and operational priorities for genital herpes control. While the main focus was on developing countries, where the public health burden of HSV2 is greatest, experts from industrialized countries were also invited to share perspectives from these countries, where much previous research has been conducted. HSV-2 is the principle serotype that causes recurrent or subclinical genital infection. [Weiss et al, 2001].

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1.1 SIGNS AND SYMPTOMS

Genital herpes infections often have no symptoms, or mild symptoms that go unrecognised. Most infected people are unaware that they have the infection. Typically, about 10-20%   of people with HSV-2 infection report a prior diagnosis of genital herpes. Those who do have symptoms usually have an outbreak 2 to 10 days, or within 3 weeks, after they get the virus. The first symptoms are   usually   the worst. They are   called primary herpes. Signs and symptoms may include: fever headache, and muscle aches. Three days later, painful blisters and skin ulcers appear where you were infected. Genital herpes is characterised by one or more genital or anal blisters or open sores called ulcers. In addition to genital ulcers, symptoms of new genital herpes infections often include fever, body aches, and swollen lymph nodes. After an initial genital herpes infection with HSV-2, recurrent symptoms are common but often less severe than the first outbreak. The frequency of outbreaks tends to decrease over time. People infected with HSV-2 may experience sensations of mild tingling or shooting pain in the legs, hips, and buttocks before the occurrence of genital ulcer.

1.2 TRANSMISSION

Risk of transmission appears to be greatest during lesional recurrences or prodrome, and patients should be advised to abstain from sexual contact during this time. Transmission can occur in the absence of lesional recurrence as a result of sub-clinical viral shedding. Efficacy of condoms toprevent sexual transmission has not been formally assessed; however, indirect evidence from failed vaccine trials provides strong support for their consistent usage (IIb, B).1, 2 HSV-2 is mainly transmitted during sex, through contact with genital surfaces, skin, sores or fluids of someone infected with the   virus. HSV-2 can be transmitted from skin in the genital or anal area that looks normal and is often transmitted in the absence of   symptoms. In rare circumstances, HSV-2 infection can be transmitted from a mother to her infant during delivery.

1.3 TREATMENT

Antivirals, such as acyclovir, famciclovir, and valacyclovir are the most effective medications available for people infected with HSV. These can help to reduce the severity and frequency of symptoms, but cannot cure the infection.

AIM AND OBJECTIVES:

1.4 AIM:

To study and compare the seroprevalence of herpes simplex virus type two IgG (HSV-2) among Indian and Nigerian students of Jodhpur National University, Rajasthan, India.

1.5 OBJECTIVES:

- ➤ To determine the seroprevalence of the virus.
- ➤ - To compare the seroprevalence of the virus among the Indians and the Nigerian students' of Jodhpur National University.
- ➤ To determine the most vulnerable group among the two races.

2.0 MATERIALS AND METHODS

2.1 STUDY AREA (SAMPLE SITE):

The research was conducted at the laboratory of Microbiology Department, Jodhpur National University, Jodhpur, Rajasthan, India.

2.2 STUDY POPULATION

Study participants, consisting male and female students, at recruitment, all volunteers were interviewed, counseled and educated about the HSV2 prevalence study prior to enrolment. Acceptance of testing was an enrolment criterion. All volunteers gave informed consent for storage of their blood samples. A survey questionnaire was administered to each parturient for collection of data on socio-demographic characteristics and HSV2 vaccination.

2.3 SAMPLE SIZE:

Ninety blood (90) samples were collected from apparently healthy Nigerian as well as Indian students of Jodhpur National University.

2.4 SAMPLE COLLECTION:

Specimen of 5 ml venous blood were collected from all the enrolled study participants in dry tubes and immediately kept at +4°C. Sera samples were separated on the same day and stored at -20°C freezer in aliquots until analyzed. Sera samples were subjected for HSV2 IgG detection using HSV2 IgG ELISA kit as per manufacture's manual. .

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2.5 ASSAY PROCEDURE:

All specimens and kit reagents were brought to room temperature (18-26C) and gently mixed.

➢ Desired numbers of coated strips are placed into the holder.
➢ As negative control, positive control, and calibrator are ready to use, 1:21 dilution of test sample was prepared by adding 10µl of the sample to 200µl of sample diluent and mixed well.
➢ 100µl of diluted sera, calibrator and controls are dispensed into the appropriate wells. For the reagent blank, 100µl sample diluent was dispensed in 1A well position. The holder was tapped to remove air bubbles from the liquid and mixed well, and then incubated for 20 minutes at room temperature.
➢ Liquid was removed from all wells and washed well three times with 300µl of 1X wash buffer and then blotted on absorbance paper or paper towel.
➢ 100µl of enzyme conjugate was dispensed to each well and incubated for 20 minutes at room temperature.
➢ The enzyme conjugate was removed from all wells. Washed well three times with 300µl of 1X wash buffer and then blotted on absorbance paper.
➢ 100µl of HSV-2 was dispensed and incubated for 10 minutes at room temperature.
➢ 100µl of stop solution was added.
➢ Optical Density (O.D.) was read at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600-650 nm.

2.6 CALCULATION OF RESULTS:

❖ Check   calibrator factor (CF) value on the calibrator bottle. This value might vary from lotto lot. Make sure you check the value on every kit.
❖ Calculate cut-off value: calibrator OD x Calibrator Factor (CF).
❖ Calculate the Ab (Antibody) index of each determination by dividing the mean values of each sample by cut-off value.

2.7 QUALITY CONTROL:

The test run may be considered valid provided the following criteria are met:
✓ The O.D. of the Calibrator should be greater than 0.250.
✓ The Ab index for Negative control should be less than 0.9.
✓ The Ab index for positive control should be greater than 1.2.

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2.8 INTERPRETATION:

The following is intended as a guide to interpretation of HSV-2 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample population encountered.

Antibody Index Interpretation

<0.9   No detectable IgG antibody to HSV-2 by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.

>1.1   Detectable IgG antibody to HSV-2 by ELISA.

3.0 RESULT:

3.1 TABLE: 1 SHOWING PREVALENCE OF IgG ANTIBODIES AGAINST HSV-2 IN ALL THE SAMPLES TESTED

Samples number	Positive	Negetive
1	+	
2	+	
3	+	
4	+	
5	+	
6	+	
7	+	
8	+	
9	+	
10	+	
11	+	
12	+	
13	+	
14	+	
15	+	
16	+	
17		_
18	+	
19	+	
20	+	
21	+	
22	+	

23	+	
24	+	
25	+	
26	+	
27	+	
28	+	
29	+	
30	+	
31	+	
32	+	
33	+	
34	+	
35	+	
36	+	
37	+	
38	+	
39	+	
40	+	
41	+	
42	+	
43	+	
44	+	
45	+	
46	+	
47		−
48	+	
49		−
50	+	
51	+	
52	+	
53	+	
54		−
55		−
56	+	
57		−
58	+	
59		−
60		−

61		_
62		_
63		_
64	+	
65	+	
66		_
67		_
68		_
69		_
70	+	
71	+	
72		_
73		_
74	+	
75	+	
76		_
77	+	
78	+	
79	+	
80	+	
81		_
82		_
83	+	
84		_
85		_
86		_
87		_
88	+	
89		_
90		_
TOTAL	64 = 71.11%	26 = 28.9%

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3.2 TABLE 2 SHOWING PREVALENCE OF IgG ANTIBODIES AGAINST HSV-2 IN RELATION TO GENDER

Gender	Number of subjects Tested	Seropositive	% Prevalence
Male	59	51	86.4%
Female	31	13	41.9%
Total	90	64	71.1%

3.3 TABLE 3 SHOWING PREVALENCE OF IgG ANTIBODIES AGAINST HSV-2 IN RELATION TO AGE GROUP

Age	Number of subjects Tested	Seropositive	% Prevalence
17-22	45	20	44.4%
23-28	32	31	96.9%
29-34	13	13	1OO%
Total	90	64	71.1%

3.4 TABLE 4: SHOWING PREVALENCE OF IgG ANTIBODIES AGAINST   HSV-2   IN RELATION TO NATIONALITY

Nationality	Number of subjects Tested	Seropositive	% Prevalence
Nigerian	45	44	97.8%
Indian	45	20	44.4%
Total	90	64	71.1%

3.5 TABLE 5 SHOWING PREVALENCE OF IgG ANTIBODIES   AGAINST   HSV-2 IN RELATION TO AREA LIVE BY THE SUBJECTS

Area live	Number of subjects Tested	Seropositive	% Prevalence
Urban	77	54	70.1%
Rural	13	10	76.9%
Total	90	64	71.1

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3.6 DISCUSSION

The study of Seroprevalence of HSV-2 IgG antibodies is vital for a better understanding of the public health importance of disease due to HSV. In this research, comparative study on seroprevalence of HSV-2 IgG antibodies between apparently healthy Nigerian and Indian students of Jodhpur National University was carried out. And Of the total number of ninety (90) samples tested, 64/90 (71.1%) were seropositive for herpes simplex virus type 2 (HSV-2) IgG antibodies. The distribution of HSV-2 IgG antibodies according to gender shows that, of the 90 samples tested, 59 males were tested and 51/59 (86.4%) were observed to be seropositive. Of the 31 females tested, 13/31(41.9%) were observed to be positive for anti-HSV-2 (Table 2).

The seroprevalence of HSV-2 in various age groups shows that, age group between 29-34 had the highest seroprevalence of 100% followed by group   23-28 with a seroprevalence of 96.9%, then followed by group 17-22 with a seroprevalence of 44.4% and happens to be the group with least seroprevalence. However, this study shows a significant variation in seroprevalence among various age groups. Up to 90% of persons seropositive for HSV-2 antibody have not been diagnosed with genital herpes. Many have mild or unrecognised disease and probably most, if not all shed virus from genital area intermittently. HSV-2 antibodies are not routinely detected until puberty, and then seroprevalence increases with increase in age in some Countries, and is higher in women than in men in all age groups and varies by race/ethnicity.[Centre for disease control,2011]. Surprisingly, in this study the seroprevalence of HSV-2 antibodies among female students was lower than among males, though the number of males tested was higher than that of females. The variation might reflect regional sex-specific differences in exposure to the virus. Since previous infection with HSV-1 may protect against HSV-2 infection or attenuate the severity of the disease. Lower antibody prevalence among females may result in a higher number of primary HSV-2 infections that are mostly localised in genital tract in sexually active individuals. [Davidovici BB, 2006]. However, the high prevalence of HSV-2 IgG antibodies in males in this research may probably be due to start of sexual activities or acquired in response to intrauterine or neonatal infections. Such infections may be clinically unapparent or not recognised clinically [MarquSez L, 2006]. It should not be forgotten, however, that the low HSV-2 IgG prevalence might also be caused, at least partially by false-positive test result because of the limited specificity of HSV-2 antibody test [Chiarini F, et al.2009].

The seroprevalence of HSV-2 IgG antibodies on the basis of marital status show a wide difference between married and single students. Of the total of 87 single students tested, 61/87 (70.1 %) were found to be seropositive, while all the 3/3 (100%) married students tested were also seropositive. Epidemiological studies have shown an association between number of sexual partners and HSV-2 seroprevalence.[Fleming DT, 1997]. However, the seroprevalence of HSV-2 in relation to area lived by the students shows that 54/77(70.1%) of 77 students lived in urban areas had HSV-2 antibodies, while of the 13 students that lived in rural area, 10/13 (76%) were observed to be seropositive. Previous studies show that HSV-2 IgG is more prevalent in cities than in rural areas [C.D.C herpes, 2010].

The seroprevalence of HSV-2 IgG antibodies in relation to nationality which is the main aim of the study shows that, a total of 90 samples, 45 from Nigerians and 45 Indians were tested. Of the 45 samples of Nigerian

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students tested, 44/45(97.8%) were observed to be seropositive. On the other hand, 20/45(44.4%) samples out of the 45 Indian students tested were found to be seropositive. Comparatively, this shows that herpes simplex virus type two IgG is more prevalent in Nigerian students than in their Indian counterparts. Overall results shows that, of the total number of 90 samples tested, 64/90(71,1%) were seropositive. Nigerian students accounts for 44(48.9%) seroprevalence of HSV-2 antibodies, while Indian students accounts for only 20(22.2%) seropositive. These differences in seroprevalence between Nigerian and Indian students is related to the facts that HSV-2 seroprevalence varies considerably worldwide with age, sex, number of lifetime sexual partners and socio-economic status. [Sauerbrei A, et al.2000]. Seroprevalence of HSV-2 IgG antibodies are also varies by race and ethnicity, and it's believed to be higher in larger cities than in rural areas. In U.S.A, the current prevalence of genital herpes caused by HSV-2 is roughly one in four or five adults, with approximately 50 million people infected, and estimated 0.5 million new genital herpes infection occurring each year. [Schartz M R, 2007].

HSV-2 is more common in Sub-saharan Africa than in Europe or the North America. Up to 80% of women and 53% of men in sub-saharan Africa are seropositive for HSV-2. These are the highest levels of HSV-2 infection in the world; also exact levels vary from Country to Country in this Continent. In most African Countries HSV-2 prevalence increases with age. [WeissH, 2004]. In India, about 16.6% people are seropositive for HSV-2. Men are more likely to be infected than women, and increasing seroprevalence of this virus is associated with an increasing age.[Center for Disease Control, herpes,2011]. This study demonstrated that the serologic evidence of HSV-2 prevalence is more common in the population of Nigerian students than in their Indian counterparts, its also more common in male students than in female students.

4. CONCLUSION

In this study, we analysed the sera of 90 subjects comprising both Nigerian and Indian students of different sexes, age groups, locality and socio-economic status, and seroprevalence of HSV-2 IgG antibodies were determined and compared among them. The greater proportion of HSV-2 IgG antibodies obtained in Nigerian students than in Indian students may be due their variation in age, socio-economic status and exposure to sexual activities. Previous infection with HSV-2 and neonatal infection can also contribute to high prevalence its IgG in the sera of the patients. However, the low prevalence of HSV-2 IgG antibodies in females may be due to limited number of sexual partners or by a possible cross-immunity conferred by prior infection with HSV-1. Other studies reported that primary infection of the genital tract with herpes is mainly due to HSV-1, which could explain high prevalence of its detection. Moreover, the prevalence and transmission of Herpes simplex virus type two (HSV-2) can drastically be reduced by abstaining from sexual activity whilst experiencing its symptoms. Treatment and limiting the number of sexual partners can help reduce the prevalence.

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