Original Article

Herpes Simplex Virus type 2 Infection among Females in Enugu, Enugu State

[1]OJINMAH UR, FMCP; [2]NNORUKA EN, FMCP [1]OZOH GAO FWACP;
[1]ONYEKONWU CL FMCP. [3]AGUWA EN, FMCP

[1] Consultant physician/ Dermatologist, College of Medicine, University of Nigeria, Enugu Campus.
[2]Professor of Medicine/Dermatology [Deceased] .
[3] Department of Community Medicine, University of Nigeria Teaching Hospital Ituku/Ozalla, Enugu state.

ABSTRACT

BACKGROUND: Herpes simplex virus type 2 infection is life long with manifestation in a small proportion of those infected. It has presented public health concern because of its progressively increasing prevalence which some authorities say is of epidemic proportion in developing countries. Herpes simplex virus type 2 has recently been found to have synergistic effect with human immunodeficiency virus (HIV) and co-infection of the two presents more severe burden to the immunity of the victim. This leads to much morbidity and mortality with negative economic impact. In this study, we set out to determine antibody prevalence of HSV-2 in women attending skin and ante natal care [ANC] clinics in University of Nigeria Teaching Hospital [UNTH] and Enugu State University Teaching Hospital [ESUTH].

MATERIALS AND METHODS: 180 female patients/clients attending skin and ANC clinics in UNTH and ESUTH were enrolled in this descriptive study. Sociodemographic data and medical history were collected using a questionnaire. They were also examined for signs of HSV 2 manifestation, other STIs and WHO staging if HIV positive. Samples were also taken for serology, Gram staining and CD4 T-lymphocyte count.

RESULTS: The prevalence of HSV 2 was found to be 77.8% (n=137) and 14.6% (n=20) had clinical diagnosis of HSV-2.The HIV and HSV-2 co-infection rate was 5.11% (n=9) and out of 11 that were HIV positive, 9 (81.82%) were HSV-2 antibody positive. The HIV prevalence in this study was 6.3%.

CONCLUSION: Herpes simplex virus type 2 infection is common among females in Enugu, Enugu State and is commoner among those with HIV infection.

KEY WORDS: HSV 2 Infection, HIV, Gonorrhoea, Syphilis, Females, Enugu

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INTRODUCTION

About 100 strains/types of herpes viruses have been identified and among these, at least eight are known to infect humans [1,2]. The human herpes viruses include herpes simplex virus types 1 & 2 (HSV -1 &. HSV - 2), Varicella zoster virus (VZV), Epstein - Barr virus (EBV), cytomegalovirus (CMV), human herpes virus type 6 (HHV-6), human herpes virus type 7 (HHV-7) and human herpes virus type 8 (HHV - 8) or Kaposi sarcoma-associated herpes virus[3].

Herpesviridae are further divided into three subfamilies based on serologic differences[2]. These include the alpha, beta and gamma herpes viruses. Where the alpha-herpes viruses are neurotropic viruses that replicate relatively rapidly and infect a wide range of cells in cell culture, the beta-herpes viruses replicate slowly and are restricted in the type of cells productively infected in cell culture. These infected cells often become enlarged. The gamma-herpes viruses on the other hand are lymphotropic, replicate relatively slowly and are restricted in the type of cells productively infected[3]. Conversion of latent infections to productive infection, due to yet clearly undefined factors and stimuli results in activation of virus replication. Recurrence of disease as a result of periodic or sporadic activation of viral replication some authorities say is an important feature of HSV infection.

It should be noted that it has also been argued that herpes infection cannot accurately be described as a recurrent disease, but as a chronic infection of the sensory ganglia with variable levels of epithelial expression[4]. In addition asymptomatic shedding of herpes viruses may play a significant role in transmission from person to person[5-7]. Herpes simplex infection affects skin, mucosal lining, and nerve tissue. The cutaneous manifestations of herpes simplex virus infections were portrayed 2000 years ago by the Greek historian Herodotus as creeping skin disease[8]. Diseases caused by human herpes viruses tend to be relatively mild and self-limited in immunocompetent persons, although with immunodepression, disease could be severe[3]

Pathology and epidemiology of herpes virus infection do not depend only on viral replication with its associated cytotoxicity but also on the capacity of these viruses to establish latent infections. Latent infection or latency means that the genome of invading virus is stably maintained by the cell with only limited expression of viral genes, no production of progeny virus and no evident virus induced cytotoxicity[3].

Although genital HSV infection did not capture public concern in many industrialized countries until the 1970s and is yet to capture public concern in developing nations like Nigeria, recent epidemiological data prove that the global spread of HSV genital tract infection has

Ccorrespondence: OJINMAH UR [Skin Clinic] Department of Dermatology, University of Nigeria Teaching Hospital Ituku/Ozalla, Enugu state.

continued unabated. Human herpes virus infections are endemic. Transmission of virus usually requires intimate contact between persons and often the source is an asymptomatic shedder of infectious virus[5-7].Multiple interactions between HSV-2 and HIV -1, both on epidemiologic and clinical levels [9,10] have further emphasized the need to closely study this infection so as to raise strategies for prevention and control.

Studies from developed countries show that HSV - 2 is a prevalent infection in all sexually active populations. [11-13] In the developing countries the rising incidence of HSV 2 infection [genital herpes] is supported by a study in South Africa which identified HSV 2 in 35.9% of genital ulcer disease [GUD] in 560 patients studied [14] thus overtaking syphilis and chancroid as most frequent causes of GUD.[15-17]

Identification of HIV as the aetiological agent causing AIDS allowed the relationship between inflammatory and ulcerative STDS and the transmission and acquisition of HIV to be studied. The increased risk of HIV acquisition created by a pre-existing genital ulcer disease was shown to be between 2.2 and 11.3 for the adjusted odds ratio from six studies.[18] Since HSV is a major cause of GUD it is likely that this transmission synergy is an important factor in the spread of HIV[19]. In addition HIV infection and the resultant loss of immune function are associated with an increased frequency and duration of HSV-2 reactivation[19].

SOCIAL AND ECONOMIC CONSEQUENCES

Females are more vulnerable than males to sexually transmitted infections including genital herpes (HSV-2).[20] Women, in particular, suffer from painful social consequences of untreated sexually transmitted infection [STI].The social stigma and personal damage due to infertility and pregnancy wastage result in divorce or commercial sex work. Furthermore, significant conflicts arise between couple and families when infertility results and in some cultures, the husband may request the return of her bride price[21].

The economic consequences of STI are also immense. In the United States, the estimated direct costs of hospital admissions from pelvic inflammatory disease alone are $900m a year. In some African countries up to 45% of gynaecological admissions are due to STI. In developing countries many more cases are managed on an out patient basis, and informal alternative health sector, for which no routine statistics are available. It has been estimated that 5 percent of the total discounted healthy life lost in sub-Saharan Africa is due to STI, excluding HIV[21].

Information about the costs of diagnosing and treating STI in the developing world is scarce. However, many people with STI seek care from private providers, where they may pay one-quarter to one-third of their monthly earnings for drugs. Also, STI reduce the productivity of men and women in the prime of their lives[21].

MATERIALS AND METHOD

Patient selection

One hundred and eighty female patients/clients attending skin clinic and antenatal care clinic from May to October 2009 at University of Nigeria Teaching Hospital [UNTH] and Enugu State University Teaching Hospital [ESUTH]; who gave informed consent (written) were selected for the study.

Inclusion criteria

1. Age 21 years and above. [22]
2. Unknown HIV-1 Status
3. Unknown HSV-2 Status
4. Female gender
5. Informed consent obtained (written)

3.2.3. Exclusion criteria

1. Age less than 21 years [22]
2. Known HIV-1 Status
3. Known HSV-2 Status
4. Non-female gender
5. Refusal to give consent
6. Critically ill patients

MATERIALS

In this study, female patients that attended skin clinics and clients attending antenatal clinics at UNTH and ESUTTH from May 2009 to October 2009 were informed on the scope and need for this research. With their consent, a standard questionnaire was self administered, with the help of a trained female assistant. The questionnaire was subsequently returned to the researcher by the assistant. The questionnaire had sections on demography, sexual history, past and present features of sexually transmissible disease and section on investigation results.

After administering the questionnaire, they were given a pretest HIV counseling and consenting patients had blood and endocervical swab samples collected from them.

SAMPLE COLLECTION

Blood Samples

Were collected from ante-cubital vein of subjects after cleansing the overlying skin with methylated spirit swab while wearing sterile latex hand gloves. The evacuation tube (vacutainer) collection system was used in blood collection to minimize recurrent puncturing of patients' vein, risk of needle stick injury and blood contact. All samples collected were clearly labeled with subjects' name, hospital number, date and time of collection. 3ml of blood was dispensed into a plain sterile container for HSV 2 studies, HIV screening and VDRL test for

syphilis. Another 3ml was drawn into sterile bottle containing potassium ethylenediamine tetra acetic acid (EDTA) anticoagulant. Blood was thoroughly mixed with EDTA by shaking gently.
This sample was for CD4 T-lymphocyte count.

ENDOCERVICAL SWAB (ECS) SAMPLE
Samples were collected in the periods the subjects were not menstruating. ECS were collected using disposable sterile bivalve (Cusco's) speculum, swab stick and sterile latex hand gloves. With the subject undressed and on a couch, the entire skin (including perineal and perianal skin) were inspected for vesicles, crusted and uncrusted ulcers, and hypo/hyper pigmented macules. The lymph node regions of neck, axillae and groin were also palpated.

Subjects were then placed in Lithotomy position and the introitus cleansed with hibitane lotion. The labia were parted with two fingers and sterile disposable Cusco's speculum was passed.

The cervix was then inspected for vesicles, ulcers, erythema and/or discharge. A sterile swab was passed through the speculum with care so as not to touch vaginal walls. This swab was then introduced into the os of the cervix and, rotated and moved from side to side for 10 - 30 seconds then carefully withdrawn. The speculum was removed and subject's introitus cleaned with dry gauze.

SAMPLE PROCESSING AND STORAGE
(a) The sample for HSV 2 studies, HIV screening and syphilis test was allowed to clot and retract for 30 minutes. It was then centrifuged at 3.000 revolutions per minute (RPM) for five minutes and the clear serum was separated. The test for syphilis and HIV screening and confirmatory where positive were carried out immediately. The remnant of the sample was stored at 4°C and analyzed in batches of seven days for HSV 2 IgM and IgG.

(b) Other blood sample for CD4 T-Lymphocyte count did not require extra processing and were also analyzed immediately after collection.

(c) The endocervical swab was transferred immediately after collection onto a glass slide where a thin and even smear was made by rolling the swab under gentle downward pressure on the glass slide. The smear was air-dried and heat fixed by holding the slide, film upwards, over a flame until just too hot to be borne on the back of hand [23]. The slide was then placed in a rack to cool and stained subsequently.

METHODS
1. HSV 2 IgM and IgG ENZYME IMMUNOASSAY (BIOCHECK)
Principle of Test
Purified HSV 2 antigen is coated on the surface of micro wells. Diluted patient serum is added to wells, and the HVS 2 IgM or IgG-specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of HSV 2 IgM or IgG-specific antibody in sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls .[24,25]

Procedures
First, 1:40 dilution of test samples, negative control, positive control and calibrator was produced by adding 5ul of each to 200ul of diluent respectively and properly mixed. Then 100ul of diluent (used as blank), diluted positive control, negative control, calibrator and sera were placed in the wells. Blank, controls and calibrator were in wells 1-4 while samples were placed subsequently with labeling (the blank was used to set the microwell reader to zero position). The microwell holder was gently tapped to remove air bubbles from the liquids. This was then incubated for 30 minutes at 37°C in a water bath (Heinz Kleef).

At the end of incubation, all liquid were removed from the wells. The micro titer wells rinsed and flicked four times with diluted wash buffer and then once with distilled water. 100ul of enzyme conjugate was dispensed into each well. This was mixed gently for 10-30 seconds and incubated at 37°C for 30 minutes. Enzyme conjugate was removed from all wells. The micro titer wells were rinsed and flicked four times with diluted wash buffer and then once with distilled water. Then 100ul of TMB reagent was dispensed into each well and mixed gently for 10 seconds. This was incubated at 37°C for 15 minutes.

Then 100ul of stop solution (1N HCL) was used to stop the reactions. The stop solution was mixed gently for 30 seconds within which time all the blue colour in the wells changed to yellow. The holder was again tapped gently to remove all air bubbles. The optical densities (O.D.) of the wells were read at 450nM wavelength with Syntron MSR - 1000 microwell reader. The optical densities were read within 15 minutes of completing the procedure and samples were studied singly (i.e. no duplicates). The HSV 2 IgM or IgG indexes of samples were calculated by dividing the optical density of the sample with that of calibrator. The sample index was then compared with interpretation values to classify it positive, equivocal or negative. [24,25.]

Interpretation
Sample index less than 0.90 is negative, sample index of

0.91 , 0.99 is equivocal while sample index greater than or equal to 1 is positive.

2. CD4 T-LYMPHOCYTE COUNT
(Partec Flow Cytometer)
The whole blood samples with EDTA as anticoagulant were analyzed within six hours of collection.

Procedure
20ul of whole blood sample was dispensed into a partec test tube, Then 20ul of CD4 mAb PE (MEM-241, PE-Conjugated monoclonal antibody to human CD4) was added. This was mixed gently and incubated for 15 minutes at room temperature, protected from light.

800ul of no lyse buffer was added and the mixture was shaked gently. The mixture was then placed in Partec cyflow chamber for flow cytometric analysis. The machine gives its result (in cells/ul) automatically on completing the analysis.[26]

3. HIV -1/2 SCREENING TEST
(Global HIV Rapid Test Strip)
Principle
Global HIV-1/2 rapid test strip which uses whole blood, serum or plasma is a qualitative membrane - based immunoassay for the detection of antibodies to HIV-1/2. The membrane is pre-coated with recombinant HIV antigens. During testing, the specimen reacts with HIV antigen coated particles in the test strip. The mixture then migrates towards the membrane chromatographically by capillary action and reacts with recombinant HIV antigen on the membrane in the test region. If the specimen contains antibodies to HIV-1 and/or HIV-2, a coloured line will appear in the test region indicating a positive result. However if the specimen does not contain HIV-1 or HIV-2 antibodies, the coloured line will not appear in the test line indicating a negative result. A coloured line will always appear in the control region as a procedural/quality control.

Procedure
(a) The test strip was removed from the foil pouch and fixed on to sticky test card provided.
(b) 2.5ul of serum was dropped on the specimen pad on the strip using the disposable droppers provided.
(c) 2 drops of the buffer solution was added and the test allowed to stand for 15 minutes and the result read thus:

Positive:
Result showed two distinct coloured lines, one in the control region (C) and another in the test region (T).

Negative: Result produced only one line in the control region (C) and no line in the test region.

Invalid: Result produced only one line in the test region (T) and none in the control region or no line in control and test regions.
The positive samples were then confirmed by western blot method.[27]

4. TEST FOR SYPHILIS (ONE STEP TEST KIT)
All specimen were tested immediately after collection and processing.

Principle
Test is based on the principle of double antigen sandwich immunoassay for determination of syphilis antibodies in serum/plasma. Recombinant antibodies *TpN15, TpN17 and TpN47* are coated on solid membrane. There are two coated lines in the result window. One is the test line (T), coated with recombinant antigens, the other is control line (C) coated with polyclonal antibodies. When the absorbent end of the test strip is immersed into the sample, the sample is absorbed into the device by capillary action; mixes with the antigen-dye conjugate and flows across the pre-coated membrane. When the syphilis antibody is present at appropriate titer value, they bind to the antigen-dye conjugate and are captured by recombinant antigens immobilized in the test region of the device and forms Ag-Ab-Ag-Au (antigen-antibody-antigen-gold) precipitates. This produces a coloured test band and indicates a positive result.[28,29]

Procedure
The test strip was removed from the foil pouch. The strip was then immersed into the specimen with the arrow on it pointing towards the specimen. Strip was removed from specimen after 10 seconds and laid flat on a clean glass surface. Results were read after 15 minutes.

Interpretation
Rose pink bands visible in both the control (C) region and test (T) region indicates positive result. Rose pink band visible in the control region alone indicates a negative result while rose pink band visible in test region alone or total absence of rose pink band over test and control regions declare the result invalid.

5. GRAM STAINING
Principle
Gram staining is an important procedure in routine bacteriology laboratory. It was described by Gram in 1884 and divides bacteria into two - Gram positive and Gram negative. First smear is stained with rosaniline dyes such as crystal violet, methyl violet, or gentian violet. Then smear is treated with iodine, decolourized with acetone, and counter stained with basic fuschin dye like neutral red. Those bacteria that resist decolourization and appear blue or violet are termed Gram-positive, while those that are decolourized and pick up the counter stain appear reddish and are termed Gram-negative [30].

Procedure

The already made slide (with smear) after cooling was flooded with crystal violet, allowed to stand for one minute and then rinsed with water. It was then covered with lugol's iodine solution for two minutes and then rinsed with water. The slide was left to dry partially and then decolourized with acetone until no more colour came off. It was then rinsed thoroughly with water. Next it was flooded with neutral red (as counter stain) for 30 seconds and rinsed in water again. It was allowed to dry.[31]

RESULT

Slides were examined with a microscope under oil immersion. Gonococci were seen as Gram-negative kidney or coffee bean-shaped diplococcic which is 0.6 to 0.8um in size.[31]

ETHICAL CONSIDERATION

Approval for the study was obtained from the research and ethical committee of University of Nigerian Teaching Hospital Ituku/Ozalla, Enugu State.

DATA ANALYSIS

Analysis of results was done using statistical package for social sciences [SPSS] soft ware (Version 13.0) for descriptive and inferential statistics. Confidence interval was 95% while p value was 0.05.

RESULTS

The mean age of females involved in this study was 39.17 +/- 12.8, **[Table 1].** The anti body prevalence of HSV 2 among the 180 females studied in Enugu, Enugu state was found to be 77.80% [n=137], **[Fig 1].** Breakdown of the anti body prevalence showed that IgM alone yielded 5 positive samples. IgG gave 54 positive samples while IgM and IgG combination produced 78 positive samples.

Table 1: Overall Age of Study Population

AGE RANGE [years]	FREQUENCY	PERCENT
21-30	60	34.10
31-40	37	21.02
41-50	42	23.86
51-60	26	14.77
61-70	11	6.25
TOTAL	**176**	**100**

Mean age = 39.17 +/- 12.8

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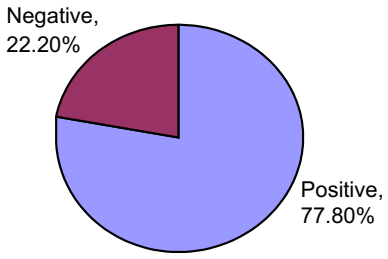


Fig. 1: Herpes Simplex Virus Type 2 Screening Result

[Table 2]. Those that are serologically positive that already had a diagnosis of herpes simplex virus type 2 based on history and / or examination was 14.6% [n=20],

Table 2: Breakdown of Hsv-2 Antibody Prevalence

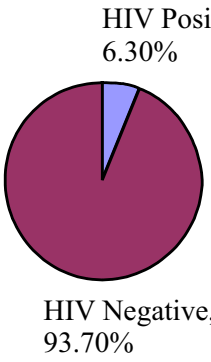
HSV-2 ANTIBODY	FREQUENCY	PERCENT
IgM	5	2.84
IgG	54	30.68
IgM and IgG	78	44.32
Equivocal	3	1.70
Negative	36	20.46
TOTAL	**176**	**100**

Table 3: Relationship Between Hsv-2 Seropositivity And Clinical Diagnosis

CLINICAL DIAGNOSIS	HSV 2 POSITIVE	PERCENTAGE
YES	20	14.6
NO	117	85.4
TOTAL	**137**	**100**

Table 4: Ulcers, Hsv-2 Antibody-Type, Syphilis, And Gonorrhoea Tests

CLINICAL FEATURES	HSV-2 ANTIBODY-TYPE	SYPHILIS TEST [VDRL]	GRAM STAIN FOR GONORRHOEA
Vesicles, ulcers and scabs	IgM	Non-reactive	Gram negative diplococci Absent
Ulcers and scabs	IgM	Non-reactive	Gram negative diplococci Absent
Vesicles, ulcers and scabs	IgM and IgG	Non-reactive	Gram negative diplococci Absent
Vesicles, ulcers and scabs	IgM	Non-reactive	Gram negative diplococci Absent
Vesicles, ulcers and scabs	IgM	Non-reactive	Gram negative diplococci Absent
Vesicles, ulcers and scabs	IgM	Non-reactive	Gram negative diplococci Absent
Vesicles, ulcers and scabs	IgM and IgG	Non-reactive	Gram negative diplococci Absent



All those infected were on WHO stage 1[32].
Fig. 2: HIV PREVALENCE

[Table 3].
Physical examination revealed that out of the 20 patients, 2 [10%] had vesicles, ulcers and scabs over the pubis; 5 [25%] had similar lesions over the buttocks; 6 [30%] had hyper/hypopigmented macules over the buttocks, and 7 [35%] had similar lesions over sacral skin/natal cleft. 2 patients [10%] had inguinal lymphadenopathy. Out of the 7 patients that had ulcers, investigations were used to rule out Syphilis and/or Gonorrhoea as possible cause [s], **[Table 4]**.

The prevalence of HIV in the population studied is 11 [6.30%] while seronegative samples were 165 [93.70%]. All the cases were confirmed positive to HIV 1. The prevalence of HIV and HSV 2 co-infection was 5.11%. This was based on the fact that nine [5.11%] of eleven confirmed HIV positive samples in this study were also HSV 2 positive while two [1.14%] had HIV alone. Out of eleven HIV positive samples, six were serologically positive for HSV 2 also, one for syphilis [VDRL] and HSV2, and two were smear positive for gonorrhoea and also serologically positive for HSV2 [HIV + HSV-2 = 6 samples, HIV + HSV-2 + SYPHILIS = 1 samples, HIV + HSV-2 + GONORRHOEA = 2 samples and HIV alone =

2 samples].
From the foregoing, multiple sexually transmitted infections in HIV yielded 9 samples [81.82% as prevalence]. Based on the fact that only one sample in this study was positive for syphilis, the prevalence = 0.57%.

In the comparison of CD4 count in HIV infection alone and CD4 count in HIV infection with other sexually transmitted infections. Normal CD4 count value among Nigerians = 514-1207 cells / u L [33]
Those with HIV infection alone were two in number and had values of 1140, and 1000 cells/ u L which places them on U.S Centres for disease control [CDC] class A1[34]. The CD4 count values of these patients are as good as those of healthy Nigerians. The average CD4 count for this sub-population is 1070 cells/u L. The sub-population with HIV and multiple sexually transmitted infections had the following results: 779, 601, 369, 622, 912, 720, 744, 392, and 673 cells/u L. The classification in this group show that those with values of 369, and 392 are on CDC class A2 [worsening prognosis] while others are on class A1[34]. The average CD4 count in this sub-population is 641.78cells/u L.

DISCUSSION

One hundred and eighty (180) patients and clients attending skin and antenatal care (ANC) clinics in University of Nigeria Teaching Hospital (UNTH), and Enugu State University of Science and Technology Teaching Hospital (ESUTH) in Enugu, Enugu State were recruited with their informed consent (written) into this study. Four of those recruited had incomplete results, leaving a substantive study population of one hundred and seventy-six (176).

HSV 2 ANTIBODY PREVALENCE

The prevalence of herpes simplex virus type 2 (HSV 2) among females in Enugu, Enugu State was found to be 77.8% (Fig. 1). This is quite high and calls for close monitoring. Findings from this study compares fairly with that of Looker et al[35] in a review study in sub-Saharan Africa obtained prevalence of 70% among women and around 55% among men. Corey et al[10] in a review study in Africa found a seroprevalence of > 40% among antenatal care attendees and 60-95% among female sex workers. WHO/UNAIDS/LSHTM report[36] presented seroprevalence of around 50% in sub-Saharan Africa and Caribbean countries. Ramaswamy et al[37] and Cowan et al[38] in London got seroprevalence of 63% and 82.5% respectively. Kjetland et al[39] got rate of 64.5% among rural Zimbabwean women. In Nigeria, Nnoruka[40] reported herpes simplex virus prevalence of 0.2% in South East. Ogunbiyi et al[41] reported same finding in South West. Yahya[42] in North central reported 0.6%. These are very low compared to the finding of this study and could be explained by the fact that they were all clinical studies not supported with serological tests.

BREAKDOWN OF HSV-2 ANTIBODY PREVALENCE

The breakdown of the prevalence in this study into the antibody sub-types gave IgG of 30.68%, IgM of 2.84%, and combined presence of IgG and IgM at 44.32% (Table 2). This might indicate that most people in this study might be in the convalescent phase of illness[43]. The attack might have been mild and asymptomatic. The significantly higher IgG value is similar to the finding of Nizami et al[44] in Adana Turkey who had rates of 63.1% for HSV-2 IgG, and 11.3% for IgM among asymptomatic pregnant women at time of delivery.

HSV 2 POSITIVE SEROLOGY WITH CLINICAL DIAGNOSIS

Out of those who were seropositive for HSV 2 antibodies (77.8%) in this study, only 14.6% had a clinical diagnosis (Table 3). This emphasizes the low sensitivity of clinical diagnosis in this condition. Note that presence of antibody does not translate to active infection. This finding is in tandem with that of Andreoletti et al[45] in France who obtained 10% among HIV and HSV 2 co-infected patients. Siegel et al[46] in San Francisco got 13% for women and 19% for men with HSV 2 antibody. Cowan et al[47] in London obtained a higher figure of 45% for those with symptoms suggestive of genital herpes and 27.4% for those that had had a diagnosis of genital herpes. Ramaswamy et al[37] also in London had clinical diagnosis in 21% of HSV 2 seropositive patients. Opaneye and Bashford[48] in Middlesbrough noted that many of their patients who are seropositive to HSV 2 were

asymptomatic.

ULCERS, HSV-2 ANTIBODY-TYPE, SYPHILIS, AND GONORRHOEA TESTS

The result (Table 4) points at HSV-2 infection as being responsible for all the ulcers found in this study. This is acceptable considering the number of the sub-population with ulcers. Chen et al[14] in a larger study in South Africa found HSV-2 to be the cause of genital ulcer disease in 35.9% of cases.

HIV AND HSV 2 CO-INFECTION

The prevalence of HIV and HSV 2 co-infection was found to be 5.11%. This is surprising since the two infections share similar risk factors and driving force[49, 50, 47, 51,] but this could be explained by the characteristics of the population studied. Higher values were reported by Corey et al[10] who in their review found co-infection rates of 50-90% for Africa, 80% for South Africa and 85% for Peru. Andreoletti et al[45] in France in two cohort studies found rates of 51%, and 66%. Kramer et al[52] in Amsterdam found a lower rate of 15%.

MULTIPLE SEXUALLY TRANSMITTED INFECTIONS [STI] IN HIV

The number with multiple sexually transmitted infections in HIV classified as presence of HIV and HSV 2, (with or without gonorrhoea and or syphilis) was found to be 9 which is the same number as in HIV and HSV 2 co-infection. Sheung et al[53] in their work among female sex workers in Kenya found out that out of 35 that acquired HIV during the study, 16 [46%] had a classical sexually transmitted disease at the time of acquisition. Stamm et al[54] in United States of America found out that infection with HIV was independently associated with a history of syphilis, serologic evidence of syphilis, a history of HSV infection, and antibody to HSV 2.They also found that men with long standing HSV 2 infection have higher HIV antibody prevalence than those with primary HSV 2 manifestation. Sheffield et al [55] in a study among women also in United States of America found that monocytes recruited to genital ulcer disease [Syphilis, HSV 1 or HSV 2] sites express increased levels of CCR5; they concluded that this could account, at least in part, for enhanced HIV-1 transmission in the setting of genital ulcer disease.

COMPARISM OF CD4 COUNT IN HIV ALONE TO CD4 COUNT IN HIV WITH MULTIPLE SEXUALLY TRANSMITTED INFECTIONS

Comparison of CD 4 T-lymphocyte count in HIV infection alone and in multiple sexually transmitted infections showed a higher average value for those with HIV alone compared to those with concomitant multiple sexually transmitted infections. It was also noticed that some of those with HIV and multiple sexually transmitted infection have already dropped to CDC class A2[34] with worsening prognosis while all those with only HIV infection were all on CDC class A1[34].

Thus one can assume that multiple sexually transmitted infections presents a higher burden to the immune system of the patient than HIV infection alone. This finding compares fairly with Abimiku et al[56] who in their work in Nigeria among pregnant HIV-positive women found out that co-infection with other sexually transmitted diseases was one of the reasons for low CD4 + T-lymphocyte count in the population. Sheth et al[57] in Canada found that among other things there is reduction in proliferative response in CD4 + T-cell count in people with HIV and HSV 2 co-infection. Habib et al[58] in Zaria, Nigeria found similar mean CD4+ T-Lymphocyte count [562.9 +/- 99.2 cells/ u L] between patients with HIV and those with HIV complicated by other sexually transmitted diseases. This is at variance with the finding in this study and might indicate the need for coordinated study involving the North and East of Nigeria. Onyemelukwe et al[59] in Zaria, Nigeria found mean CD4 T-Lymphocyte count among HIV patients [240 cells/ u L] to be significantly lower than value in controls [600 cells/ u L].

PREVALENCE OF HIV AND SYPHILIS

The prevalence of HIV in this population of women attending skin and ANC clinics was 6.3% (Fig. 2). This is in tandem with prevalence of 6.6% for Enugu State in the 2005 National HIV seroprevalence sentinel survey[60, 61]. Kjetland et al[39] found prevalence rate of 29.3% among rural Zimbabwean women. Zhang et al[62] in Beijing, China got HIV-1 prevalence of 2.1% among men that have sex with men. Chen et al[63] also in China got rate of 1.2% among sexually transmitted disease clinic attendees in Guangxi.

The prevalence of syphilis in this study was 0.57%. This indicates increasing prevalence as it is much higher than 0.13% which Ozumba et al[64] found about a decade ago among pregnant women in Enugu. This could be explained by changes in the people's lifestyle brought on by progressive urbanization. Note that prevalence of syphilis in this study is in tandem with less than 1% for most states as recorded in 2005 National HIV seroprevalence sentinel survey[60] which is a more recent study.

CONCLUSION

Herpes simplex virus type-2 (HSV 2) antibody prevalence among females in Enugu, Enugu State was found to be high and among them, the proportion with clinical manifestation was low. The CD4 T-lymphocyte count of patients with only HIV infection was higher than that of those with HIV and co-infection of other sexually transmitted infections.

REFERENCE

1. Chang Y, Cesarmen E, Pessin MS, et al.

Identification of herpes virus-like DNA sequences in AIDS-associated Kaposi sarcoma. Science. 1994; 266:1865-1869.
2. Raizman B, Baines J: The diversity and Unity of Herpes viridae. Comp Immunol Microbiol Infec Dis. 1991; 14: 63-70.
3. Pertel PE, Spear PG. Biology of herpes viruses in: King K. Holmes, P. Frederick Sparling, Per-Anders Mardh, et al (eds). Sexually transmitted diseases. Third edition. New York. McGraw-Hill, 1999:269 - 283.
4. Olumide YM. A self-Instructional text book of sexually transmitted infections for Medical Students, Resident Doctors and Medical Practitioners. Longman Nigeria Plc. 2003; 43-45.
5. Gillier AC, Meyers JD, Corey L, et al. Cytomegalovirus infection in homosexual men: Relationship to sexual practices, antibody to human immunodeficiency virus, and cell-mediated immunity. AMJ med. 1987; 82:593 - 601.
6. Bryson Y, Dillon M, Bernstein DI et al: Risk of acquisition of genital herpes simplex virus type 2 in sex partners of persons with genital herpes: A prospective couple study. J. Infect Dis. 1993; 167:942- 946.
7. Miller G, Niederman JC, Andrews LL. prolonged oropharyngeal excretion of Epstein-Barr virus after infectious mononucleosis. N EngI J Med. 1973; 288: 229-232.
8. Marques AR, Straus SE. Herpes simplex virus type 2 an update. Dis Mon 2000; 46: 325-350.
9. Wald A. Link K. Risk of human Immunodeficiency virus infection in herpes simplex virus type 2 seropositive persons: a meta analysis. J Infect Dis 2002; 185:45-52.
10. Corey L, Wald A, Celum CL, et al. The effects of Herpes Simplex Virus 2 on HIV-1 acquisition and transmission: A review of two overlapping epidemics. J Acquir Immune Defic Syndr 2004; 35(5):435-445.
11. Patrick DM, Dawar M, Cook DA, et al. Antenatal seroprevalence of herpes simplex virus type 2 (HSV-2) in Canadian women: HSV-2 seroprevalence increases throughout the reproductive years. Sex Transm Dis 2001; 28:424-428.
12. Fiersgren M. Genital herpes simplex virus infection and incidence of neonatal disease in Sweden. Scand J Infect Dis 1990; 69 (SuppI): 37-41.
13. Nahmias AJ, Lee FK, Beckman-Nahmias S. Seroepidemiological and Sociological patterns of herpes simplex virus infection in the world. Scand J infect Dis 1990; 69 (suppi): 19-36.
14. Chen CY, Ballard RC, Beck-Sague CM, et al. Human Immunodeficiency virus infection and genital ulcer disease in south Africa: the herpetic connection. Sex Transm Dis 2000; 27:21-29.
15. Bogarts J., Ricart CA, Van Dyck E, et al. The etiology of genital ulceration in Rwanda. Sex Transm Dis 1989; 16:123-126.
16. Nsanze H, Fast MV, D'Costa U, et al. Genital ulcers in Kenya, clinical and laboratory study. Br J Vener Dis 1981; 57: 378-381.
17. Mabey DC, Wall RA, Bello CS. Aetiology of genitalulceration in the Gambia. Genitourin Med 1987; 61:312-315.
18. Flemming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 1999:75:3-17.
19. Schacker T, Zeh J, Hu HL, et al. Frequency of symptomatic herpes simplex virus types 2 reactivations among human immunodeficiency virus infected men, J infect Dis 1998; 178: 1616- 1622.
20. STIs and gender; vulnerability of women. Guidelines for the care of sexually transmitted infection in conflict affected settings. Developed by the women's commission for Refugee women and children on behalf of the Reproductive Health Response in conflict consortium 2004: 8-9.
21. Olumide YM. A self-Instructional text book of sexually transmitted infections for medical students, Resident Doctors and Medical Practitioners. Longman Nigeria Plc. 2003; 6.
22. Federal Ministry of Health (Nigeria).2003.National HIV/AIDS and Reproductive Health Survey, 2003. Federal Ministry of Health Abuja Nigeria: 26.
23. Armstrong GL, Schillinger J, Markowitz L, et al, Incidence of herpes simplex virus type 2 infection in the United States. AM J Epidemiol. 2001; 153:912-920.
24 Vestergaard BF, Grauballe PC, Spangaard H. Titration of herpes simplex virus antibodies in human sera by the enzyme-link immunosorbent assay [ELISA]. Acta Pathol. Microbiol.Scand.Sect.1997; B (85): 446-448.
25. Coleman RM, Pereira PD, Bailey D, et al. Determination of herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 1983;18: 287.
26. Cassens U, Gohde W, Kuling G, et al. Simplified volumetric flow cytometry allows feasible and accurate determination of CD4 T-Lymphocytes in immunodeficient patients worldwide. Antiviral Ther. 2004; 9: 395-405.
27 Schable CA and George JR. Serological detection of HIV infection in : R. Madhok, C.D Forbes and B.L Evatt (eds). Blood, Blood products and HIV. Second edition. London. Chapman and Hall Medical, 1994: 162.
28 .George R, Pope V, Fears M, et al. An analysis of the value of some antigen-antibody interactions used as diagnostic indicators in a treponemal western blot test for syphilis. J. Clin Lab Immunol.1998; 50(1): 27-44.
29 .Schachter J. Office and laboratory testing for STDs:

Present and Future. Adolesc Med. 1990; 1(3) : 583-596.
30. Baker FJ, Silverton RE, Pallister CJ. Introduction to medical laboratory technology. Seventh edition. Oxford. Butterworth-Heinemann, 1998 : 354-362.
31. Olumide YM. A Self-instruction text book of sexually transmitted infections for Medical students, Resident doctors and Medical practitioners. Longman Nigeria PLC. 2003 : 80, 102.
32 .www.who.int/hiv/pub/guidelines/HIV staging 150307.pdf. 30/03/09.
33 .Njoku MO, Sirisena ND, Idoko JA, et al. CD4+ T-Lymphocyte counts in patients with Human Immunodeficiency Virus type-1 (HIV) and healthy population in Jos, Nigeria. Nat. Postgrad. Med. J. 2003; 10 : 135-139.
34 Centre for Disease Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep. 1992;18[41(RR-17)]:1-19.
35. Looker KJ, Garnett GP. and Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bull World Health Organ. 2008; 86 (10) : 737-816.
36. Herpes simplex virus type 2. Programmatic and research priorities in developing countries. Report of a WHO/UNAIDS/LSHTM workshop (London 14-16 February 2001); 4.1.
37. Ramaswamy M, Sabin C, McDonald C.et al. Herpes Simplex Virus type 2 (HSV 2) seroprevalence at the time of HIV-1 diagnosis and seroincidence after HIV-1 diagnosis in an ethnically diverse cohort of HIV infected persons. Sex Transm Dis. 2006; 33(2): 96-101.
38. Cowan FM, Humphrey JH, Ntozini R, et al. Maternal herpes simplex virus type 2 infection, syphilis and risk of intrapartum transmission of HIV-1: results of a case control study. AIDS 2008; 22(2): 193-201.
39. Kjetland EF, Gwanzura L, Ndhlovu PD, et al.Herpes simplex virus type 2 prevalence of epidemic proportions in rural Zimbabwean women: association with other sexually transmitted infections. Arch. Gyn. Obstet. 2005; 272(1): 67-73.
40. Nnoruka EN. Skin diseases in South-east Nigeria: A current perspective. Int J Dermatol 2005; 44**:** 29-33.
41 Ogunbiyi AO, Daramola OOM, Alese 00. Prevalence of skin diseases in Ibadan, Nigeria. Int J Dermatol 2004; 43:31-36.
42 Yahya H. Change in pattern of skin disease in Kaduna, North-Central Nigeria. Int. J Dermatol. 2007 Sep; 46 (9): 936-43.
43 Ho DW, Field PR, Irving WL, et al. Detection of Immunoglobulin M antibodies to glycoprotein G-2 by western blot [immunoblot] for diagnosis of initial herpes simplex virus type 2 genital infections. J. Clin. Microbiol. 1993;31(12):3157-3164.
44. Nizani D, Fugen Y, Cuneyt E, et al. Asymptomatic herpes simplex virus type 2 (HSV-2) infection among pregnant women in Turkey. Indian J. Med. Res. 2004;120(8):106-110.
45 Andreoletti L, Piednoir E, Legoff J, et al. High Seroprevalence of Herpes Simplex Virus Type 2 Infection in French Human Immunodeficiency Virus type 1- Infected Out patients. J. Clin Microbiol. 2005; 43(8): 4215-4217.
46 Siegel D, Golden E, Washington AE, et al. Prevalence and Correlates of herpes simplex infections. The population-based AIDS in multiethnic Neighborhoods study. JAMA 1992; 268 (13): 1702-8.
47 Cowan FM, Johnson AM, Ashley R, et al. Antibody to herpes simplex virus type 2 as serological marker of sexual lifestyle in populations. BMJ 1994; 309[11]:1325-1329.
48 Opaneye AA and Bashford J. Seroprevalence of antibodies to herpes simplex virus types 1 and 2 among sexually active female populations in Middlesbrough, England. The Journal of the Royal society for the promotion of health 2002; 122 [2]*:* 108-111.
49 Haddow LJ, Sullivan EA, Taylor J, et al. Herpes simplex virus type 2[HSV-2] infection in women attending an antenatal clinic in the south Pacific Island nation of Vanuatu. On line J Am Sex Transm Dis Assoc. September 13, 2006.
50 Stover CT, Smith DK, Schmid DS et al. Prevalence of and Risk factors for viral Infections among Human Immunodeficiency Virus (HIV) Infected and High-Risk HIV-Uninfected women. J. Infect. Dis 2003; 187: 1388-1396.
51 Celum C, Levine R, Weaver M, et al. Genital herpes and human Immunodeficiency virus: double trouble. Bull World Health organ 2004; 82 (6): 447-453.
52 Kramer MA, Uitenbroek DG, Ujcic-Voortman JK, et al. Ethnic differences in HSV 1 and HSV 2 seroprevalence in Amsterdam, the Netherlands. Euro Surveill, 2008; 13 (24):pii=18904.
53 Sheung A, Rebbapragada A, Shin LYY, et al. Mucosal Nisseria gonorrhoea co-infection during HIV acquisition is associated with enhanced systemic HIV-specific CD8 T-cell responses. AIDS.2008; 22(14): 1729-1737.
54 Stam WE, Handsfield HH, Rompalo AM, et al. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA.1988; 260(10): 1429-33.
55. .Sheffield JS, Wendel GD[Jr], McIntire DD, et al. Effect of genital ulcer disease on HIV-1 co-receptor expression in the female genital tract. J.Infect.Dis. 2007; 196(10): 1509-16.
56. Abimiku A, Valualba-Diebold P, Dadik J, et al. Risk factors associated with low CD4 + Lymphocyte count among HIV-positive pregnant women in Nigeria. Int J. GynecolObstet 2009; 106 (3): 227-231.

57. Sheth PM, Sunderji S, Shin LY, et al. Coinfection with herpes simplex virus type 2 is associated with reduced HIV-specific T cell responses and systemic immune activation. J Infect Dis 2008; 197 (10): 1394 -13401.
58. Habib AG, Keshiro IB, Gebi UI, et al. Clinical presentation of HIV infection in Northern Nigeria and its relationship to CD4+ T- cells count. Nig. Med. Pract. 1998; 35 (½): 3-8.
59. Onyemelukwe GC, Musa BOP. CD4+ and CD8+ lymphocytes and clinical features of HIV seropositive Nigerians on presentation. Afri.J. Med. Med. Sci. 2002; 31(3): 229-233.
60. Federal Ministry of Health (Nigeria). 2005 National HIV seroprevalence survey: process and findings. National AIDS/STDs control programme (NASCP). Federal Ministry of Health Abuja, Nigeria: 7, 9.
61. www.census.gov/ipc/hiv/nigeria08.pdf. 2/01/10.
62 Zhang X, Wang C, Hengwei W, et al. Risk factors of HIV infection and prevalence of co-infections among men who have sex with men in Beijing, China. AIDS.2007; 21(suppl 8): S53-7.
63 Chen XS, Yin YP, Tucker JD, et al. Detection of acute and established HIV infections in sexually transmitted disease clinics in Guangxi, China: implications for screening and prevention of HIV infection. J. Infect Dis.2007; 196(11): 1654-61.
64 .Ozumba UC, Oshi DC, Nwokeji CM, et al. Trends in seroreactivity of syphilis among pregnant Nigerian women. Sex. Transm. Inf. 1999;75:120-123.19