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

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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

Date Accepted for Publication: 21st September, 2012
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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. 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.

Herpesviridae are further divided into three subfamilies based on serologic differences. 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. 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. In addition asymptomatic shedding of herpes viruses may play a significant role in transmission from person to person. 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. Diseases caused by human herpes viruses tend to be relatively mild and self-limited in immunocompetent persons, although with immunodeficiency, disease could be severe.

Pathology and epidemiology of herpes virus infection do not depend only on viral replication with its associated cytopotoxicity 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 cytopotoxicity.

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
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. Multiple interactions between HSV-2 and HIV-1, both on epidemiologic and clinical levels 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. 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 thus overtaking syphilis and chancroid as most frequent causes of GUD. 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. Since HSV is a major cause of GUD it is likely that this transmission synergy is an important factor in the spread of HIV. In addition HIV infection and the resultant loss of immune function are associated with an increased frequency and duration of HSV-2 reactivation.

**SOCIAL AND ECONOMIC CONSEQUENCES**

Females are more vulnerable than males to sexually transmitted infections including genital herpes (HSV-2). 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.

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.

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.

**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.
2. Unknown HIV-1 Status
3. Unknown HSV-2 Status
4. Female gender
5. Informed consent obtained (written)

**Exclusion criteria**

1. Age less than 21 years
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 ESUTH 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. The slide was then placed in a rack to cool and stained subsequently. 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.

**Interpretation**

Sample index less than 0.90 is negative, sample index of...
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 shaken 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.

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.

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

Principle 
The 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.

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.
**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 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.

**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.

**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>
<thead>
<tr>
<th>AGE RANGE [years]</th>
<th>FREQUENCY</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>60</td>
<td>34.10</td>
</tr>
<tr>
<td>31-40</td>
<td>37</td>
<td>21.02</td>
</tr>
<tr>
<td>41-50</td>
<td>42</td>
<td>23.86</td>
</tr>
<tr>
<td>51-60</td>
<td>26</td>
<td>14.77</td>
</tr>
<tr>
<td>61-70</td>
<td>11</td>
<td>6.25</td>
</tr>
<tr>
<td>TOTAL</td>
<td>176</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean age = 39.17 +/- 12.8

**Table 2: Breakdown of Hsv-2 Antibody Prevalence**

<table>
<thead>
<tr>
<th>HSV-2 ANTIBODY</th>
<th>FREQUENCY</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>5</td>
<td>2.84</td>
</tr>
<tr>
<td>IgG</td>
<td>54</td>
<td>30.68</td>
</tr>
<tr>
<td>IgM and IgG</td>
<td>78</td>
<td>44.32</td>
</tr>
<tr>
<td>Equivocal</td>
<td>3</td>
<td>1.70</td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>20.46</td>
</tr>
<tr>
<td>TOTAL</td>
<td>176</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>CLINICAL DIAGNOSIS</th>
<th>HSV 2 POSITIVE</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>20</td>
<td>14.6</td>
</tr>
<tr>
<td>NO</td>
<td>117</td>
<td>85.4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>137</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>VESICLES, ULCERS AND SCABS</th>
<th>HSV-2 ANTIBODY</th>
<th>SYPHILIS TEST [VDRL]</th>
<th>GRAM STAIN FOR GONORRHOEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>Non-reactive</td>
<td>Gram negative diplococci</td>
<td>Absent</td>
</tr>
<tr>
<td>IgM and IgG</td>
<td>Non-reactive</td>
<td>Gram negative diplococci</td>
<td>Absent</td>
</tr>
<tr>
<td>IgM</td>
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<td>Absent</td>
</tr>
<tr>
<td>IgM</td>
<td>Non-reactive</td>
<td>Gram negative diplococci</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**HIV Positive, 6.30%**

**HIV Negative, 93.70%**

All those infected were on WHO stage 1.

**Fig. 1: Herpes Simplex Virus Type 2 Screening Result**

**Fig. 2: HIV PREVALENCE**
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 [8].

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 / uL. Those with HIV infection alone were two in number and had values of 1140, and 1000 cells/ uL which places them on U.S Centres for disease control [CDC] class A1. The CD4 count values of these patients are as good as those of healthy Nigerians. The average CD4 count for this subpopulation is 1070 cells/ uL. 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/uL. 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.

The average CD4 count in this sub-population is 641.78 cells/uL.

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. in a review study in sub-Saharan Africa obtained prevalence of 70% among women and around 55% among men. Corey et al. 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 presented seroprevalence of around 50% in sub-Saharan Africa and Caribbean countries. Ramaswamy et al. and Cowan et al. in London got seroprevalence of 63% and 82.5% respectively. Kjetland et al. got rate of 64.5% among rural Zimbabwean women. In Nigeria, Nnoruka reported herpes simplex virus prevalence of 0.2% in South East. Ogunbiyi et al. reported same finding in South West. Yahya 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. The attack might have been mild and asymptomatic. The significantly higher IgG value is similar to the finding of Nizami et al. 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. in France who obtained 10% among HIV and HSV 2 co-infected patients. Siegel et al. in San Francisco got 13% for women and 19% for men with HSV 2 antibody. Cowan et al. 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. also in London had clinical diagnosis in 21% of HSV 2 seropositive patients. Opaneye and Bashford 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 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 but this could be explained by the characteristics of the population studied. Higher values were reported by Corey et al who in their review found co-infection rates of 50-90% for Africa, 80% for South Africa and 85% for Peru. Andreoletti et al in France in two cohort studies found rates of 51%, and 66%. Kramer et al 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 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 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 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.

COMPARISION 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 with worsening prognosis while all those with only HIV infection were all on CDC class A1.

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 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 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 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 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. Kjetland et al found prevalence rate of 29.3% among rural Zimbabwean women. Zhang et al in Beijing, China got HIV-1 prevalence of 2.1% among men that have sex with men. Chen et al 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 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 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.

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