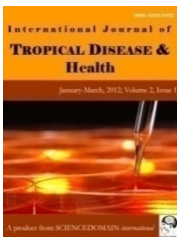

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Seroincidence of Human Herpes Virus 2 Among Ante-natal Clinic Attendees in Benin, Nigeria

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Authors' contributions

This work was carried out in collaboration with all authors. Authors EIK and CKO were involved in the research conceptualization and drawing up of protocol. Authors FEA and KA performed in coordinating cohort follow-up data collection. Authors EOY and VUN performed in specimen analysis. Author EIK produced the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Human Herpes Virus 2 (HSV-2) infection has been shown to be significantly associated with several obstetric complications, especially if the infection occurred during the pregnancy. Advocacy for policy formulation and the design of interventions requires local data on the risk factors for incident HSV-2 infection.

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The aim of this study is to assess HSV-2 sero-incidence among pregnant women in Benin and the effect of socio-demographic factors, HIV status, and HSV-1 status.
Study Location, Design and Duration: Pregnant women attending ante-natal clinic in University of Benin Teaching Hospital and Central Hospital, Benin were prospectively recruited. A cross-sectional study was done and baseline data, obtained. The HSV-2 seronegative participants were followed up till the last clinic appointments [the closest appointment to their expected delivery dates (EDD)]. The study took place between November 2011 and December 2012.
Methods: On recruitment a structured self-administered questionnaire was used to capture their socio-demographic data. Obstetric data was obtained from the patients' case notes. Their HSV-1 and HSV-2 serostatuses were determined using an HSV glycoprotein G-based type-specific ELISA technique. Their HIV statuses were also determined. All those who were seronegative for HSV-2 were retested for HSV-2 six months later and/or on their last clinic appointment before the EDD. Incidence rates were calculated per person-years. Data analysis utilized SPSS version 16 software.
Results: Out of the 674 participants, 315(46.8%) were HSV-2 seropositive while 359 (53.2%) were seronegative. 15.9% (57) of the HSV-2 sero-negative ones were lost to follow-up, giving a response rate of 84.1%. Seroincidence rate was found to be 17.9 per 100 person-years. There was significant association of seroincidence with younger reproductive age groups, unmarried status, and education below secondary level. Relative Risk (RR) and Incidence rate ratio (IRR) of HSV-2 infection among the HIV-infected cohort were 55.15 [95%CI:17.0-179.1] and 72.37 [95% CI:71.11-73.63] respectively. The RR and IRR among the HSV-1-seronegative cohort were 1.43 [95%CI:0.64-3.17] and 11.62 [95%CI:9.60-13.64] respectively.
Conclusion: Seroincidence rate of HSV-2 infection among pregnant women in Benin was found to be 17.9 per 100 person-years. Positive HIV status, young age, unmarried status, and low education level were indicators of increased risk of incident HSV-2 infection.

Keywords: Seroprevalence; seroincidence; HSV-2; HSV-1; HIV; risk factors; incidence rate ratio.

1. INTRODUCTION

Human Herpes Virus 2, the major cause of genital herpes in sub-Saharan Africa, has a worldwide prevalence of 16% among persons of reproductive age group, while the prevalence among females of similar age group in the sub-Saharan Africa region is reported to range between 30% and 80% [1]. Humans are the only known reservoir of the infection [2].

Infection among pregnant women has been associated with several complications. The complications include spontaneous abortion, intrauterine fetal death, preterm labor, low birth weight babies and neonatal herpes [3,4]. These complications are known to be more frequent and severe if the primary infection occurred during the pregnancy, especially, if it occurred in the latter part of the pregnancy [5].

Knowledge of HSV-2 seroincidence, and the associated factors, among pregnant women will give further insight into the burden of the infection and the appropriate interventions.

Among the risk factors of incident HSV-2 infection in pregnancy in this locale, the socio demographic and obstetric factors require assessment. There is a convergence of HIV and

HSV-2 epidemics in sub-Saharan Africa (as shown by the high prevalences of both diseases) [6,7] and both epidemics have been shown to be mutually reinforcing [8,9]. The effect of HIV on incident HSV-2 infection also requires assessment. Furthermore, it is known that the prevalence of HSV-1 antibodies in Nigeria is high [10], but there are conflicting reports on the effect of HSV-1 on incidence of HSV-2 infection [11-13]. It follows, therefore, that the effect of HIV infection on HSV-2 seroincidence and the protective effect of pre-existing HSV-1 antibodies also requires verification [14]. The effect of these factors can be better understood through a longitudinal study. These data should form the basis for formulation of intervention strategies for HSV-2 infection and by extension, other sexually transmitted infections. There's yet no published data on incident HSV-2 and the risk factors in Nigeria.

This study is aimed at assessing the risk factors for incident HSV-2 infection among pregnant women in Benin, Nigeria.

2. METHODOLOGY

2.1 Study Location, Design and Duration

The participants were pregnant women registered in the Obstetrics and Gynecology departments of University of Benin Teaching Hospital and Central Hospital, the two major referral (tertiary) level hospitals serving the Benin metropolis, other parts of Edo State and neighboring states. Cross-sectional study design was used to obtain baseline data. The seronegative participants were followed up to near Expected Delivery Date (EDD) to assess seroconversion. The study took place between November 2011 and December 2012.

2.2 Sampling and Data Collection

Six hundred and seventy four (674) consenting participants were recruited prospectively and consecutively as they booked in the ante-natal clinic registration centres of the two hospitals. Bbaseline data on their socio demographic, obstetric, HSV and HIV profiles was collected. Baseline data on socio-demographic profiles of the participants was obtained using structured questionnaires; obstetric data was obtained from the patient's records; while the presence of HSV-1, HSV-2 and HIV antibodies was assessed by laboratory analyses of archived blood samples.

An HSV seropositive person was defined as any participant whose serum tested positive for IgG antibodies using the gG-based type-specific ELISA assay. Diagnosis of HIV infection was in line with the serial testing algorithm prescribed in the Nigerian Federal Ministry of Health guidelines which is routinely followed in both tertiary hospitals [15].

The HSV-2 seronegative (HSV-2 susceptible) participants' were followed up to appointment dates about 2 weeks close to their EDDs, and re-tested for HSV-2 IgG and IgM antibodies.

Research assistants (consisting of laboratory technicians attached to the ante-natal clinic laboratories and nurses attached to the ante-natal clinics) were trained on the baseline and follow-up protocols. Adequate contact was maintained with the follow-up participants to encourage compliance with clinic appointments.

2.3 Laboratory Procedures

Blood samples were collected in 5 ml plain vacutainer tubes and allowed to clot and sera separated by centrifugation at room temperature. Storage was in cryovials at -20°C.

2.3.1 HSV-1 and HSV-2 IgG assay procedure

This utilized Enzyme Linked Immunosorbent Assay (ELISA) kit by Dia. Pro. Diagnostic Bioprobes Milano–Italy [16,17]. This is an HSV glycoprotein G-based enzyme-linked immunosorbent assay (ELISA) technique and test result was qualitative.

All specimens and kit reagents were brought to room temperature and gently mixed. The laboratory procedures were performed according to the manufacturer's instructions [18-19].

2.3.2 HSV-2 IgM assay procedure

The kit used was Dia. Pro. Diagnostic Bioprobes Milano – Italy and the assays were performed in accordance with manufacturer's instructions [20].

2.3.3 HIV immunoassay procedure

The HIV statuses of the respondents were previously determined in accordance with national guidelines [15], using Determine ® HIV ½ by Inverness Medical Innovations South Africa; and HIV 1 and 2 STAT PAK Assay kit by CHEMBIO Diagnostic system, INC, New York, USA. Each batch of tests ran with both positive and negative controls and results were qualitative.

2.4 Follow-up Protocol

The 302 of the 359 HSV-2 seronegative participants were successfully followed up to appointment dates that were about 2 weeks to their Expected delivery date (EDD) to assess HSV-2 seroconversion. To this end, a consensus appointment date was selected for each follow-up participant. This appointment date was selected to fit, as well as possible into the usual ante-natal clinic appointment pattern, while being about 2 weeks to the EDD.

On the appointed date, serum was collected for re-screening. Each of the 302 participants had one HSV-2 IgM and IgG assay during the follow-up period.

Seroconversion was defined as the detection of HSV-2 antibodies of either IgG or IgM isotype by HSV gG2-based type-specific ELISA assay more than 30 days after previous HSV-2 seronegative assay result [21].

2.5 Data Analysis

HSV-2 sero-incidence rate was calculated as the number of seroconversions among HSV-2 seronegative individuals (HSV-2 negative at baseline) divided by the total person-time of observation of population at risk. The person-time was defined as the actual time-at-risk (in months or years) that all the follow-up study participants contributed to the study. It was the calculated sum of the observation times for each participant from baseline screening date to incident infection (seroconversion) dates or to rescreening dates.

The seroconversion time (incident infection time) was assumed to be half the average time between the baseline assay date and the last re-screening date [11,12].

Incidence rate ratios, relative risks and their 95% confidence intervals (95%CI) were calculated and used to analyze association of seroincidence with marital status, level of education, HIV status and HSV-1 status. The effect of age, level of education and parity were subjected to simple linear univariate regression analyses. Fisher's exact test was also used in the analysis of associations between HSV-2 seroincidence and obstetric characteristics and other patients' sociodemographic characteristics. SPSS version 16 software was used in the analyses. P-value of less than 0.05 was adopted as indicative of statistical significance.

3. RESULTS

3.1 Baseline Data of All Participants

The age range of the 674 participants was 18 years to 44 years. Their mean age was 30.6± 5.2 years. Majority (96.6%) of the participants were in the 20-40 age range and none of the participants belonged to the 46-50-year age group.

Most (85.2%) of the participants were married. They were either Christians or Muslims, and the ratio was 4.7:1 respectively.

Most of the members of the baseline study population had good education. Only 6.5% did not achieve complete secondary education Table 1.

Traders/private business operatives were highest in number while teacher's typists/clerks were next in frequency. 13.6% were either housewives or unemployed. The least represented occupation was farming Table 2.

Most (85.3%) of the participants were recruited either in their second or in their third trimesters of pregnancy; while 14.7% were first seen in their first trimesters.

Most (43.5%) of the participants were nulliparae. Twenty-eight percent (28.2%) of them were primiparae; while a total of 27.6% of them were multi-parae. Only two (0.7%) of the participants were grand multiparae Table 3.

3.2 HSV and HIV Statuses of All Participants

46.3% of the participants were HSV-2 sero-positive; while 56.7% were HSV-2 seronegative and were at risk of primary HSV-2 infection.

Prevalence of HSV-1 antibodies among the participants was 96.3%; while the prevalence HIV antibodies were 12.5% Table 4.

Table 1. General characteristics of all participants

Characteristics	Frequency	Percent (%)
Age group (years)		
15-20	7	1.0
21-25	98	14.5
26-30	242	35.9
31-35	225	33.4
36-40	86	12.8
41-45	16	2.4
Religions		
Christianity	556	82.5
Islam	118	17.5
Marital Statuses		
Married	574	85.2
Single	53	7.8
Divorced	22	3.3
Widowed	25	3.7
Levels of education		
Graduate and above	210	31.2
Post-Secondary	292	43.3
Secondary Completed	128	19.0
Secondary uncompleted	14	2.1
Primary completed	27	4.0
Primary uncompleted	3	0.4

Table 2. Occupation of all participants

Occupation	Frequency	Percent
Housewives	51	7.6
Unemployed	41	6.0
Students	65	9.6
Farmers	2	0.3
Traders/Private business operatives	215	31.9
secretaries/Clerks/Typists	100	14.8
Fashion designers/Hair stylists/Tailors	60	8.9
Accountants/Finance institution professionals	20	3.0
Teachers	102	15.1
Nurses	7	1.0
Doctors	8	1.2
Pharmacists	3	0.4
Total	**674**	**100**

Table 3. Obstetric characteristics of all participants

Characteristics Initial gestational ages	Frequency	Percent
1st Trimester	99	14.7
2nd Trimester	268	39.8
3rd Trimester	307	45.5
Parity		
Nullipara	293	43.5
Primipara	190	28.2
Para-2	123	18.2
Para-3	43	6.4
Para-4	20	3.0
Para-5 or more	5	0.7

Table 4. Presence of HSV and HIV antibodies among all the participants

Antibodies	Frequency(N=674)	Percent
HSV-1 antibodies		
Positive	652	96.7
Negative	22	3.3
HSV-2 antibodies		
Positive	312	46.3
Negative	362	56.7
HIV antibodies		
Positive	84	12.5
Negative	590	87.5

3.3 Follow-Up Data

3.3.1 Sociodemographic and obstetric characteristics of follow-up participants

A total of 302 HSV-2 seronegative participants were successfully followed up to a date about 2 weeks to their EDD. This number form the follow-up study population.

Most of the general characteristics of the follow-up cohort were essentially similar to those of the baseline study population: the mean age of the follow-up study population relative to baseline population was respectively slightly reduced (29.9 vs 30.6); proportion of age-above-40 is lower (10.2 vs 15.2); the ratio of Christians to Muslims was, respectively, slightly increased (5:1 vs 4.7:1); the proportion of the married relatively and respectively increased (92.1 vs 85.2); the proportion of the nulliparae increased (85.2 vs 83.5); and the proportion of the participants recruited in their first trimester also slightly increased (19.5 vs 14.7). All these differences were statistically insignificant. However, the follow-up participants were significantly more likely to be HIV-infected than the baseline study population (p=.02) Tables 1, 5 and 6.

Similarly, the characteristics of those who were lost to follow-up were essentially similar to those who were re-screened. However, the proportion of the HIV-infected among the 57 participants who were lost to follow-up was, respectively, less than that of the follow-up study population (5.3% vs 7.3); although the difference in proportion was not significant (p=.58).

3.3.2 HSV-2 seroincidence

16 (5.3%) of the 302 participants seroconverted during the follow-up period. The 59 participants who were recruited in the first trimester were generally rescreened about 6.5 months (0.54 year) later; the 110 participants recruited in their second trimester were rescreened about 4.5 months (0.38 year) later; while the 133 participants recruited in their third trimesters were generally rescreened about 2 months (0.17year) later.

Total Person-Time contributed by all the participants was 1071.24 person-months or 89.27 person-years. The HSV-2 incidence rate was therefore 17.9 per 100 person-years (95% CI: 0.30 – 35.5 P-Y) Table 5.

Table 5. Obstetric characteristics of follow-up participants and HSV-2 seroincidence

Characteristic	Total no tested (%) (N = 302)	No of seroconverters (%) (N=16)	P-value
Gestational ages at recruitment			
First trimester	59(19.5)	7 (11.9)	**P = .001**
Second trimester	110(36.4)	9 (8.2)	
Third trimester	133(44.0)	0 (0.0)	
Parity			
Nulliparae	149(49.3)	10 (6.7)	**P=0.06**
Primiparae	82 (27.2)	2 (2.4)	
Para-2	53 (17.5)	1 (1.9)	
Para-3 or more	18 (6.0)	3 (25.0)	
Para-4	2 (0.7)	0 (0.0)	
Grand-multiparae	4 (1.3)	0 (0.0)	

All the seroconversions took place among those who were recruited either in the first or in the second trimesters of their pregnancy. The greatest proportion of seroconversions took place among para-3 participants but there was no significant association between parity and seroincidence (p=.06).

All, but one, of the seroconversions occurred among participants aged 26 to 30 years. There was significant association between age and HSV-2 seroincidence, p=.001.

There was no statistically significant association between HSV-2 seroincidence and religion p = 1.00.

A higher proportion of the unmarried participants seroconverted than the married ones. The association between marital status and HSV-2 seroincidence was statistically significant. (p =.01) Table 6.

All participants who did not complete primary education seroconverted, and level of education was a significant risk factor for seroconversion.

With HIV-uninfected as the control cohort, the relative risk of seroconversion among the HIV-infected was very much higher than 1.0 RR=55.2 (95%CI:17.0-179.1). The incidence rate of HSV-2 infection among the HIV-infected was 248 cases/100 P-Y and was 72.4 times more than the 3.4 cases/100 P-Y found among the HIV-uninfected.

The association between pre-existing HSV-1 infection and incident HSV-2 infection was not significant. p=0.15. The 95% CI of the relative risk of incident HSV-2 infection among HSV-1 –uninfected participants includes 1.0, which indicates no RR Table 7.

Table 6. Sociodemographic risk factors for HSV-2 seroincidence

Factors	Total number re-tested (%)	Number that seroconverted (%)	P-value
age			
15-20	1 (0.3)	0 (0.0)	**P=.001**
21-25	54 (17.9)	0 (0.0)	
26-30	106 (40.1)	15 (14.2)	
31-35	94 (31.5)	1 (1.1)	
36-40	24 (7.9)	0 (0.0)	
41-45	7 (2.3)	0 (0.0)	
Religion			
Christianity	240 (84.1)	14 (5.8)	**P= 1.00**
Islam	46 (15.9)	2 (4.3)	
Marital status			
Married	278 (92.1)	12 (4.3)	**P=.01**
Single	13 (4.3)	2 (15.4)	
Divorced	4 (1.3)	0 (0.0)	
Widowed	7 (2.3)	2 (28.6)	
Level of education			
Graduate and above	100(33.1)	4 (4.0)	**P = 001**
Post-secondary	131(43.4)	9 (6.9)	
Secondary completed	58 (19.2)	0 (0.0)	
Primary completed	10 (3.3)	0 (0.0)	
Primary uncompleted	3 (1.0)	3 (100)	
Occupations			
Housewives	25 (8.2)	2 (8.0)	**P =0.255**
Unemployed	14 (4.6)	3 (21.4)	
Students	31(10.3)	2 (6.5)	
Farmers	1(0.3)	0 (0.0)	
Traders/Privatebusiness operatives	103 (34.1)	4 (3.9)	
Secretaries/Clerks/Typists	46 (15.2)	2 (4.3)	
Fashiondesigners/Hair stylists	26 (8.6)	3 (11.5)	
Accountants/Finance professionals	8 (2.6)	0 (0.0)	
Teachers	42(13.9)	0 (0.0)	
Nurses	2 (0.7)	0 (0.0)	
Doctors	4 (1.3)	0 (0.0)	

Table 7. Association of HIV and HSV-1 statuses with HSV-2 seroincidence

Status	Total number tested N=302	Number of serocon-Ver sions	Person-years at risk	Relative risk (rr)	Incidence rate ratio (irr)(95%ci)	P-value
HIV status						
HIV-infected	22 (7.3)	13 (59.9)	5.24	55.15 [17.0–79.1]	72.37[71.11 -73.63]	.001
HIV-uninfected	280 (92.7)	3 (1.1)	87.51	0.41 [0.25 -0.68]		
HSV-1						
HSV-1-infected	299 (99.0)	15 (5.0)	92.35	.15 [0.03 - 80]		.15
HSV-1-uninfected	3 (1.0)	1 (33.3)	0.53	1.43 [0.64-3.17]	11.62 [9.60 – 13.64]	

4. DISCUSSION

The follow-up study population had essentially similar characteristics to the baseline study population. It could be assumed that the difference is essentially the HSV-2 seronegativity of the follow-up cohort. It follows that findings from the follow-up study could be generalizable on the baseline population from which it was drawn. Furthermore, a random distribution of the multiple determinants of seroincidence could also be assumed. Thus, in the various classifications of the follow-up cohort into study cohort (exemplified by the HIV-infected) and the control cohort (exemplified by the HIV-uninfected), and vice versa, it could be assumed that determinants like husband's/partner's serodiscordance were randomly distributed.

The seroincidence of HSV-2 of 17.9/100 P-Y found in this study is high. To our knowledge, this is the first documentation of HSV-2 seroincidence among pregnant women in Nigeria. There is also no documented data on the incidence among other population groups. So, it is not possible to make comparisons or draw inferences on incidence trends in Nigeria. Previous study reports from other African countries were on specific high risk subpopulations like commercial sex workers and Hotel/Bar workers and not pregnant women. This seroincidence value was less than the 23/100P-Y found among Kenyan commercial sex workers [22] and 28.6/100 P-Y among women working in food/recreational centres [23]. The value of 20.5/100 P-Y found among female attendees of a sexually transmitted clinic in USA is also higher than that of this study [24].

These finding highlights the fact that transmission of HSV-2 is efficient among pregnant women in Benin, Nigeria and it is instructive of an increasing burden of HSV-2-associated complications, which may be obstetric and non-obstetric [3-5]. This result should stimulate research interest in the incidence of obstetric complications among pregnant women in Nigeria, as these complications are known to occur more frequently and more severely when primary HSV-2 infections occur during the pregnancy [1,5].

It was necessary to assess the association of incident HSV-2 infection with gestational age, as previous reports indicate that neonatal herpes and some other HSV-2-associated complications are more associated with primary infections occurring in the latter half of the pregnancy, especially in the third trimester. [5] This is due to the possible lack of development of effective (high avidity) antibodies during puerperium, if primary infections occurred late [25]. In this study, none of the participants recruited in the third trimester seroconverted during the follow-up period. Although the person-months of follow-up was short, a deduction of low frequency of primary infection in the third trimester can be made and this may be explained by the fact that, in the Nigerian sociocultural environment, the females and their husbands tend to avoid sexual intercourse during the late pregnancy period. This finding implies that the incidence of the obstetric complications that are related to third trimester primary infections may not be high in this environment.

All seroconversions took place among first and second trimester recruitees. It is not expected that these primary infections could be strongly associated with neonatal herpes because they should have developed protective antibodies by the puerperal period [22]. Nevertheless, there are other obstetric complications that are prenatal. The high seroincidence in first and second trimester recruitees implies that primary prevention strategies are more likely to be effective if pregnant women at risk of primary HSV-2 infection (the HSV-2 seronegative) are detected early. Early booking should therefore be promoted.

Although seroprevalence studies show that there is a consistent increase in prevalence with age [26], seroincidence findings, as in this study, indicate that the infection is acquired at early reproductive age-groups; and that higher seroprevalence values observed with increasing ages is the result of the fact that each infection is lifelong. Moreover, the older age-groups tend to be less susceptible, as shown by reduced proportion of older age-groups in our follow-up cohort. Similar finding was reported in a review of worldwide HSV-2 seroincidence [1]. This observed effect of age on seroincidence suggests that the younger reproductive age groups should be the major targets of infection control interventions.

As also reported in Tanzania, unmarried status, and education below primary school level was associated with increased HSV-2 seroincidence in this study [23]. In the same vein, seroincidence was highest among the unemployed in this study. These findings have implications for individual case assessment and prevention strategies. Indeed, socioeconomic development seems fundamental to addressing sexually transmitted infection control challenges.

The findings in this study also have implications for HIV control. In this study, the 55.2 (CI:17-179.1) estimated relative risk of acquisition of HSV-2 infection between the HIV-infected and HIV-uninfected is very high and indicates a very strong association between the two infections, as has been corroborated by previous reports [27,28].

Our findings indicate that there is no significant association between HSV-1 infection and HSV-2 seroincidence. The relative risk estimate of 0.15 [95%CI:0.03–0.80] suggests reduced risk of HSV-2 transmission among the HSV-1-infected. If the HSV-1-uninfected cohort is made the study cohort, the relative risk estimate of 1.43 [95%CI:0.64–3.17], suggests no consistent association, since the 95% CI includes 1.0. Contrarily, incidence rate ratio (IRR) indicate definitely increased incidence among HSV-1-negative cohort relative to the HSV-1-seropositive cohorts. The significance of our findings is limited by the small number of HSV-1-negative participants which is also due to small sample size. However, some protective effect of HSV-1 antibodies and vulnerability of HSV-1-seronegative persons to incident HSV-2 infections can be inferred, although statistical significance was not achieved. This finding agrees with a report from Italy [29].

5. RECOMMENDATIONS

While the debate on cost-effectiveness of routine HSV-2 screening of pregnant women continues, all pregnant women should be informed of the need to book early, and to be tested for HSV-1, HSV-2 and HIV on booking. Meanwhile the investigations should be prescribed on individual bases until adequate evidence is found for programmatic application of these investigations.

6. LIMITATIONS

HSV-2 IgM antibodies were not assessed at baseline. HSV-2 IgM seropositive ones could produce spurious seroconversions. Seroconversion was used as the only evidence of incidence. Incident infections, especially those of third trimester recruits, that had not evoked detec table antibody response could have been missed. The contributions of these 2 counter cases are expected to be small. Their net effects on sero-incidence could cancel out.

CONSENT

Informed consent was obtained from the participants for effecting and publishing this work.

ETHICAL CONSIDERATION

Certificate of ethical approval was obtained from the Ethical Committee of University of Benin Teaching Hospital for this work.

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COMPETING INTERESTS

The authors declare that no competing interests exist.

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