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Epidemiology of Human Papilloma Virus Genotypes (HPV) in Human Immunodeficiency Virus (HIV) Positive Women from Selected Hospitals in Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author AKO supervised the study. Author OOL carried out the research, performed the statistical analysis, wrote the protocol, managed the analysis, literature draft and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this study is to investigate the epidemiology of Human Papillomavirus genotypes in Human Immunodeficiency Virus (HIV) positive women attending selected Government Hospitals in Ondo state, Nigeria. Questionnaires containing information about age, sex, parity, occupation, socio-demographic characteristics of the women, and exposure to factors which may influence sexually transmitted diseases (STDs) were administered. Information on other signs and symptoms of STD was also obtained. This study was conducted among women ≥20 years attending the HIV or medical outpatient Clinic from selected Hospitals in Ondo State. A total of 185 cervical swab samples collected were transferred in a phosphate buffer transport medium, kept in ice pack and then transferred to the laboratory for analysis. The following analyses were carried out on the samples: DNA extraction, genotyping using polymerase chain reaction (PCR) assay and agarose

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gel electrophoresis, using GP5+/GP6+ primers. Of the 185 cervical swabs samples collected from HIV positive women, 128 (69.2%) had their Human Papilloma Virus (HPV) status as positive, while 57 (30.8%) had their HPV status as negative. The higher prevalence of HPV IgG recorded in this work might be related to varying sexual behaviors, social vices and cultural practice in Ondo State. Prevalence of HPV IgG antibody in HIV women in relation to age of the showed that, age group 31-40 had the highest prevalence of HPV IgG antibody 75 (58.6%). The prevalence might be due to early indulgence in sexual activities and early marriage. The prevalence of HPV antibody in relation to education of the women showed that subjects with no formal education had the highest prevalence of HPV IgG antibody 62 (48.4%). Other parameters like past history of sexually transmitted diseases (STDs), use of oral contraceptives, and age at first sexual experience, occupation, level of education, and marital status also contributed to the high prevalence of HPV. The HR-HPV genotypes detected include: 16, 18, 35, 45, 30, 70, 69, 52, 31, 58, 39, 33, 61, 66 and 74 This study has shown a clear indication of the presence of HPV in HIV positive women, and equally the epidemiological classification of HPV in HIV positive women, and therefore recommends that, there is the need to educate people on the importance of routine HPV screening in order to prevent infection with the disease and development of cervical cancer. Public enlightenment programmes should be organized to educate people on the risk factors associated with HPV, which would also assist women in making responsible choices as regards sexual and other social practices.

Keywords: Prevalence; human immunodeficiency virus; papillomavirus; sexually transmitted diseases; genotypes.

1. INTRODUCTION

Human Papillomavirus (HPV) infection is the most common sexually transmitted disease worldwide, and it particularly affects people living with human immunodeficiency virus (HIV) [1]. While the immune system eliminates over time most HPV infections in immunocompetents individuals, HPV infections tend to persist in immunodeficient ones, such as HIV-positive subjects, probably due to the inability to control the expression and replication of HPV by HIV-compromised immune system [2]. Persistent infections with oncogenic HPV genotypes are causally related to the development of high-grade intraepithelial lesions and invasive carcinoma [3]. For many years, the epidemiological profile of women with cervical cancer was recognized as suggestive of a sexually transmitted process. Agents such as syphilis, gonorrhea etc [4], but in recent times, studies have linked cervical cancer to HPV infection [4]. Other studies likewise have shown that HPV infections priced the development of cervical cancer [5]. Genital warts are common manifestations of genital Human Papillomavirus (HPV) infections [6]. They are commonly associated with HPV6 and HPV11. Human Papillomavirus infection appears to be a necessary factor in the development of almost all cases (90%) of cervical cancer [7].Thirteen high-risk (HR) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are classified as human carcinogens (Group 1/2A) [8]. Large meta-analyses have confirmed HPV16 and HPV18 to cause a majority of cervical cancer in all world regions, including the sub-Saharan Africa, the attributable fraction of HPV16 and HPV 31 in cervical cancer has over the years increased even in Nigeria [9] and slightly higher than HPV45 and HPV35 compared to other regions [10]. However, limited data are available on circulating genotypes in the country, especially among HIV infected women. The objective of this study is to determine the predominant high risk HPV genotypes, in addition to determining the high risk factors associated with the viral infection and suggest possible measures at curbing or reducing the menace caused by HPV infection.

2. MATERIALS AND METHODS

2.1 Study Population

This study was conducted among women ≥20 years attending the HIV or medical outpatient Clinic from selected Hospitals in Ondo State. The Hospitals are State Specialist Hospitals in Akure, Ikare, Ondo Okitipupa as well as Mother and Child Hospital, Ondo. Women who had earlier been screened, and had records of being HIV positive were clerked. The study protocol was explained to each woman.

2.2 Data Collection

2.2.1 Enrollment of patients and administration of questionnaires

Enrolled women had their HIV status confirmed using the Determine test kits and then they answered a questionnaire in English. This questionnaire gathered socio demographic characteristics of the women and exposure to factors which may influence sexually transmitted infections. Information on other factors such as signs and symptoms of sexually transmitted diseases (STD) was also obtained.

2.2.2 Sample collection

Samples were collected according to the method described by Micalessi et al. [11] between October, 2017 to April, 2018 in Gynecology unit of the sample collection centres. Gynecological examination was carried out by Nurses with speculum insertion, prior to collection of endocervical and high vaginal swabs. Cervical samples were collected using a cervix brush (Cervex-brush®, Rovers®, Oss, The Netherlands).

2.3 Cytology

Cervical cytology was assessed with conventional Papanicolaou (Pap) smears. Histology of the biopsy specimens were processed and read by a qualified Histopathologist. Cervical cells were collected into an ethanol-based preservative (Surepath TM, Tripath Imaging, Burlington, NC, USA) using the Cervex-Brush ® or Cervex-Brush ® Combi (Rovers Medical Devices BV, KV Oss, The Netherlands), stored at 4°C, and transported to the laboratory for Deoxyribonucleic acid (DNA) and Polymerase chain reaction (PCR) analysis.

2.4 Detection of Genes

2.4.1 Extraction of DNA

A mixture of 500µl nucleic lysis solution and 120µl of 0.5 M Ethylene diamine tetra acetic acid (EDTA) solution (pH 8.0) was added into a centrifuge tube and then kept in ice. About 20µl of sample was added to a micro centrifuge tube. Thereafter, 600µl of EDTA/Nucleic acid solution was added to the tube. It was mixed gently and 17.5µl of 20mg/ml Proteinase K was added. This was then incubated overnight at 55°C with gentle shaking. Also, 3µl of Rnase solution was added to the nuclear lysate and mixed by inverting the tubes 2-5 times. This was then incubated for 15-30 mins at 37°C. The sample was then allowed to cool to 25°C for 5 mins. Similarly, 200µl of protein precipitation solution was added to the sample, and vortexed vigorously at high speed for 20 seconds. The sample was then left on ice for 5 mins. The sample was centrifuged for 4minutes at 13,000-16000 rev/min. The precipitate protein formed a tight white pellet. The supernatant containing the DNA was carefully removed (leaving the protein pellet behind) and transferred to a clean 1.5 ml microcentrifuge tubes containing 600µl of isopropanol. The solution was gently mixed by inversion until the white thread- like strands of DNA formed a visible mass. The supernatant was centrifuged and carefully decanted. Also, 600µl of 70% ethanol was added and the tube was gently inverted to wash the DNA. This was then centrifuged for 1minute at 25°C. The ethanol was carefully aspirated using a sequencing pipette tip. The tube was inverted on a clean absorbent paper, and the pellets air dried for 10-15 mins. Finally, 100µl of DNA rehydration solution was added, and DNA was rehydrated by incubating at 65^{o}C for 1hr. The solution was mixed by gently tapping the tube. The DNA was the stored at 2-8°C [12].

2.4.2 Quantification of DNA extracted

The extracted DNA was quantified using NanoDrop (ND 1000) Spectrophotometer (Thermo Scientific, USA) and the stability of the DNA was checked on 1% agarose gel. The optical density ratio used was between 1.8-2.0 and stored [12].

2.4.3 Genotyping using GP+5/GP+6 primers (Polymerase chain reaction)

Genotyping was done using Polymerase Chain Reaction (PCR). The extended DNA was used with a master mix of 20mM Tris HCL, 22 Mm KCl, 22 Mm NH_4CL, 1.8 Mm $MgCL_{2,}$ 5% Glycerol, 0.05% Tween^{R} 20, 0.06% IGEPAL^{R}CA-630, 0.02 Mm dNTPs, 25 units/ml one Taq^{R} (Biolabs^{R} inc) and 10µM of each primer (GP+5/GP+6 Primers) to make up a total reaction volume of 20µl for each sample. The primers used were purchased from Inqaba Biotech, South Africa. The PCR cycling conditions adopted by the Thermal Cycler (Thermo Electron Corp., Milford, USA) were as follows: denaturation at 94°C (35 cycles, 1.5mins

each), annealing at 55°C (35 cycles, 1.5 minutes each) and strand elongation at 72°C (1.5 minutes each) [12].

2.4.4 Agarose gel electrophoresis

The PCR products were detected b y an agarose gel electrophoresis (Labnet International Inc, USA). Agarose gel elctrophoresis w as run at 80v for 1,5h using 1.5% gel matrix p repared in 1x TAE buffer. Following electrophor esis, the gels was stained in an ethidium bromidfe bath (3µg/ml) for 5-10 mins, and destained in tap water for 30 mins or until the ba ckground was clear. Gels were visualized un der UV light illumination (BIORAD Gel Doc^{TM}EZ imager, USA) and photographs were taken [12].

2.5 Analysis of Data

Data obtained were presented as means ± standard error (SE). The sig nificance of difference between treatment grou ps was tested using one-way analysis of varia nce (ANOVA) with the aid of Statistical Pack age for Social Sciences (SPSS., IL) version 23 at P≤ 0.05.

3. RESULTS

The overall prevalence of Human papillomavirus (HPV) among Human immunodeficiency virus (HIV) positive women in the stud y population is presented in Fig. 1. Of the 18 5 HIV positive women screened, 128 (69.2%) te sted to Human papilloma virus (HPV) while 57 (30.8%)

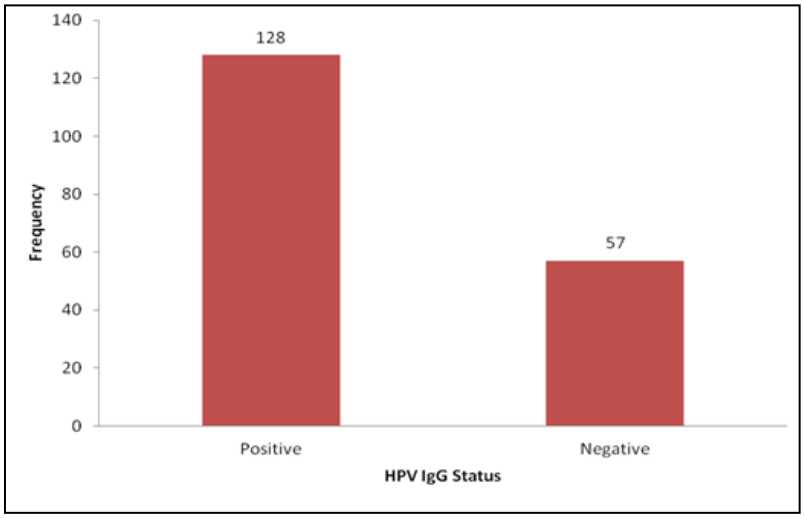

Fig. 1. Overall prevalence of HPV IgG antibody against the frequency of HIV positive women (n=185)

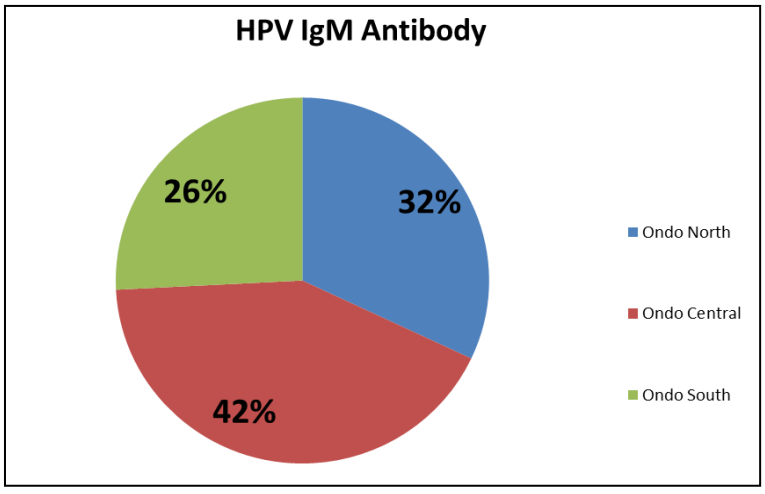

Fig. 2. Senatorial distribution prevalence of HPV IgG antibody in HIV positive women (n=185) in Ondo State

tested negative. Fig. 2 shows the prev alence of HPV antibody in HIV Positive women w ithin the three senatorial districts of Ondo State. The result shows that Ondo Central had the highest prevalence of 42% while Ondo soouth and Ondo North have prevalence rate of 32% and 26% respectively.

The Prevalence of HPV immunoglobin (IgG) antibody in HIV positive women in relation to socio demiographic factors is presented in Table 1. The prevalence in relation to age shows that, age group 31-40 years had the highest prevalence of HPV IgG antibody, 75 (58.6%) and the least in age group 51-60 years had prevalence of 6 (4.7%). The prevalence of HPV antibody in relation to the education of the HIV positive women showed that participants with no formal education had the highest prevalence of HPV IgG antibody 62 (48.4) while others with other training excluding Primary, secondary, post secondary (NCE, OND, HSC) had the least with 9 (7%) respectively. The prevalence of HPV IgG antibody in relation to marital status of HIV women showed that married subjects had the highest prevalence of 126 (98.4%), followed by subjects that were divorced with prevalence rate of 2 (1.6%). The Prevalence of HPV antibody in HIV positive women in relation to their occupation is shows that traders had the highest prevalence of 103 (80.5%) and the least records were in students with 2 (1.6%).

3.1 Prevalence of HIV in Relation to Predisposing Factors

The predisposing factors as responded to in questionnaire are shown in Table 2. None of the 185 respondents smoke. However, 127 (68.6%) subjects had a past history of sexually transmitted infections. Also, the prevalence of HIV among patients in relation to the

Table 1. Prevalence of HPV IgG antibody in HIV positive women by sociodemographic characteristics

Prevalence of HPV IgG antibody in HIV positive women in relation to their age			
Age group (Years)	**Number examined**	**Number positive**	**Percentage (%) positive**
20-30	41	26	20.3
31-40	93	75	58.6
41-50	39	21	16.4
51-60	12	6	4.7
Total	185	128	100
Prevalence of HPV IgG antibody among HIV positive women in relation to education			
Education	**Number examined**	**Number positive**	**Percentage (%) positivity**
No Formal Education	79	62	48.4
Primary	15	11	8.6
Secondary	77	46	35.9
Post-Secondary (NCE, OND, HSC etc)	14	9	7.0
Total	185	128	100.00
Prevalence of HPV IgG antibody in HIV positive women in relation to marital status			
Marital status	**Number examined**	**Number positive**	**Percentage (%) positivity**
Married	176	126	98.4
Divorced	9	2	1.6
Single	0	0.0	0.0
Widowed	0	0.0	0.0
Total	185	128	100
Prevalence of HPV IgG antibody in HIV positive women in relation to occupation			
Marital status	**Number examined**	**Number positive**	**Percentage (%) positivity**
Students	5	2	1.6
Artesians	17	6	4.7
Traders	133	103	80.5
Civil servants	30	17	13.3
Total	185	128	100

Table 2. Prevalence of HPV IgG antibody in HIV positive women in relation to predisposing factors

Variables	Yes		No	
Predisposing factors to HPV	**NY**	**%**	**NN**	**%**
Smoking	0	0	185	100
Alcohol intake	9	4.9	176	95.1
Use of oral contraceptives	40	21.6	145	78.4
Past history of sexually transmitted infection	127	68.6	58	31.4
Relatives who has STD infections	42	27.7	143	77.3
Previous information about cervical cancer	23	12.4	162	87.57
Previous information about HPV	0	0	185	100
Previous screening for HPV	0	0	185	100
Frequent urination	5	2.7	180	97.3
Painful urination	0	0	185	100
Discharge from the vulva	48	25.9	137	74.1
Itching of the vulva	49	26.5	136	73.5
Bleeding of the vagina	0	0	185	100
Bleeding during intercourse	0	0		100

NN = Number that said NO; NY = Number that said YES

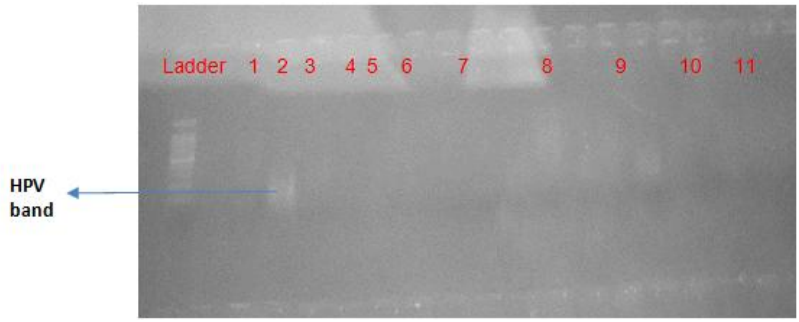

Plate 1. Gel electrophoresis showing samples with HPV

Ladder (100bp); Lane 1 to 11 = samples from HIV positive women tested for HPV

Plate 2. Gel electrophoresis showing samples with HPV

Ladder (100bp; Lane 11 to 28 = samples from HIV positive women tested for HPV

predisposing factors showed that 137 (74.1%) subjects did not have painful urination, while 176 (95.1%) subjects did not take alcohol. Similarly, only 5 (2.7%) subjects have heard about HPV before now. Age at first sex ranged from 15-25 years, thus majority, 139 (75.1%), of the women had first sex at age 15-20 years, while 46 (24.9%) subjects had sex at age 21-25 years. Also, many 164 (88.6%) of the subjects reported that they had multiple sex partners.

Table 3. Percentage DNA of HPV detection using oligonucleotide primers pairs (GP5+/GP6+)

DNA of HPV	GP5+/GP6+	
	Frequency	**Percentage (%)**
Negative	57	30.8
Positive	128	69.2
Total	185	100.0

DNA: Deoxyribonucleic Acid; HPV: Human Papilloma Virus

Table 4. Prevalence of 15 high risk HPV genotypes among HIV positive women in selected hospitals in Ondo State

HPV genotypes	Prevalence	
	Frequency (N)	Percentage (%)
16	18	14.1
18	12	9.4
35	7	5.5
45	9	7.0
30	5	3.9
70	3	2.3
69	1	0.78
52	13	10.2
31	1	0.8
58	8	6.3
39	1	0.8
33	13	10.2
61	22	17.2
66	12	9.4
73	3	2.3
Total	128	100.0

N = number of HIV positive women with HPV genotypes

Table 3 shows the percentage DNA of Human papilloma virus (HPV) detection using oligonucleotide primers pairs. The DNA of HPV positive was detected in 69.2% (128/185) of the total population using GP+-PCR while 30.8% are negative. HR-HPV genotype among HIV women in Ondo State is shown in Table 4. The HR-HPV genotypes detected include: 16, 18, 35, 45, 30, 70, 69, 52, 31, 58, 39, 33, 61, 66 and 74. However, the most frequent high risk Human papilloma virus (HRHPV) genotype found in women in Ondo State was HPV 61 (17.2%) and HPV 70 and 73 with prevalence rate of 2.3%.

Plates 1 and 2 shows the Gel electrophoresis picture of the HIV positive samples showing HPV genotypes band with the Ladder.

4. DISCUSSION

This study reports the overall prevalence of Human papillomavirus IgG antibody among HIV positive women attending selected Hospitals in Ondo State, Nigeria. Human papilloma virus antibody was detected in 128 (69.2%) of the HIV positive women screened. This result is similar to the finding of Nweke and Banjo [13] who reported prevalence of 54.90% HPV antibody among the HIV positive women attending Lagos University Teaching Hospital Lagos, but higher than the report of [1] in their study, an estimate of HPV prevalence at 22.2% in Lome, Togo. Thus, the higher prevalence of HPV IgG been recorded in this work might be related to varying sexual behaviour, social vices and cultural practice in Ondo state. However, the prevalence is higher compared with the results of several authors [14,15]. The increased prevalence of HPVs among HIV positive women may be due to the fact that HPV replication may be more efficient in immune-deficient host which could result in increased detection rate as well as a higher chance of developing persistent HPV infection, also variations among the different study populations with varying exposures to different risk factors based on diverse socio-cultural and geographical differences [16].

There was lot of socio-demographic factors that were predictive of high risk HPV status in the HIV positive population in relation to age group. The highest Prevalence of HPV IgG antibody in HIV women in age group 31-40 years is similar study carried out among the general population of Ibadan, by Thomas et al. [17] and In Rwanda by Singh et al. [18] and contrasting to the report of Fadahunsi et al. [19]. The prevalence of HPV in this age group might be due to early indulgence in sexual activities and early marriage as explained by Christine and Holschneider [20]. This may be explained by the time taken for persistence to develop in the 25-34 years age range and the lower incidence of sexual activity in the >51 years age range because 70% of HPV infection disappears in 1 year and 90% in 2 years [21].

In this study, women who do not use contraceptives had the highest prevalence of HPV which is in agreement with the studies of Naucler et al. [22], in which a record of higher prevalence of the infection among women who did not use any form of contraceptives. The higher prevalence in this study might be due to unprotected sex which is the major route of transmitting HPV infection as been reported by Cliffordet al. [23].

The prevalence of HPV IgG in the marital status of the HIV subjects showed that married subjects had the highest prevalence of 126 (98.4%). This finding is in agreement with the findings of several authors [24-25] they observed a higher prevalence among married women. This is in agreeement to the general belief that sexual activities among different partners expose them more to the infection than a single partner. This could be due to the husbands not being faithful

to their wives and getting involved with multiple partners and as a result infecting their wife. However, the result of this study was in contrast to the findings of several authors [26-27] who both reported high prevalence of HPV antibody in single women. The difference in these findings could be due to similar rate of sexual activities among the single and married women and the singles involving themselves in multiple sexual partners.

The DNA of HPV was detected in 69.2% of HIV positive subjects used in this study. The HPV Genotpye code GP5+GP6+ used in this study detected these following 15 HR-H PV genotypes (16, 18, 35, 45, 30, 70, 69, 52, 31, 58, 39, 33, 61 and 74). The prevalence of HR-HPV 16, 18, 33, 35 in this study agrees with the report of Irabor et al. [9] from Calabar, Nigeria. The predominance of HPV types 61 and16 as observed in this study follows the global trend and is in conformity with some Nigerian studies [28].

Also, Fadahunsi et al. [19] reported the predominant HPV types 16, 53, 18 and 52 among pregnant women in Osun state and types 16, 35, 58 and 31as seen in Lagos state [13] which was in contrast to the result documented in this study. This sharply contrasts with a worldwide prevalence rate of HPV 16 and 18; as reported Sanjose et al. [29]. Few studies are available to justify this assertion, geographical differences associated with HPV type distribution had been previously reported from other parts of the world The predominant HPV types were also different from those reported in Abuja, Nigeria This may be attributable to geographical variations in addition to culture and other life styles or partly because most of the studies restricted their search to specific HPV types while this study had the advantage of using DNA sequencing to detect wider variety.

5. CONCLUSION

Human papillomavirus IgG antibody was detected in the study population and has the highest prevalence among the age group 31-40 years. Women may likely develop cervical cancer if the infection persists for years. Other risk factors associated with the virus in this study were occupation, marital status, educational status and the age at first sexual Intercourse.

HPV types that have been found in studies in Nigeria are in variance with high risk HPV types detected across the country. However in this study, the most frequent HR-HPV genotype found in this study was HPV 61. This observation of such variation necessitates the development of a prospective study that will allow the definition of the spectrum, of HPV genotypes.

CONSENT AND ETHICAL APPROVAL

Ethical approval for the study was obtained from the Ondo State Health Research Ethics Committee with reference number NHREC/18/ 08/2016 and protocol number OSHREC/29/ 01/2018/038.Written informed consent was then obtained from every participant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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