Prevalence and Determinants of High-Risk Human Papillomavirus Infection among Women Living in Urban and Rural Communities of Edo State, Nigeria

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Abstract: *Introduction:* Oncogenic human papillomaviruses are predominantly sexually-transmitted pathogens of which several high-risk types are associated with nearly all cases of cervical cancer world especially occurring in women in Sub-Saharan Africa where preventive measures against cervical cancer has been very poor. There are little or very few studies in our setting that have evaluated the various high risk HPV subtypes using polymerase chain reaction, with HPV DNA extraction, amplification and Flow-through hybridization.

This study therefore, aimed to determine the pattern and correlates of high risk human papilloma virus infections in Edo State using pooled data from urban and rural Sub regions of the state as knowledge about the distribution of the HPV types circulating in the communities in different regions of the world would be useful in devising the optimum preventive strategy for high risk HPV infection.

Materials and Methods: This was an analytical cross sectional study of consecutive 290 participants (involving 145 urban and 145 rural participants) in Edo state Nigeria. Enrolled participants who consented to the study were administered structured interviewer questionnaire. Cervical swab sample collection using the female cervical cell collection kit for HPV DNA testing was done to determine the presence of high risk HPV infection. The residual cell suspensions from the female cervical cell collection kit was frozen at -20°C until the desired sample size was achieved. The frozen samples were transported to molecular diagnostics laboratory Nigeria limited, Lagos State Nigeria in iced cold packs for analysis. HPV DNA was detected using the hybribio 21 HPV geno array test kit which uses Polymerase Chain Reaction (PCR), amplification and flow through hybridization.

Results: A total of 290 participants (n=145 urban women and n=145 rural women) were recruited across both settings. The mean age of the participants was 41.0±11.0 and 37.0 ± 13.0 for urban and rural participants respectively. The overall prevalence for high risk HPV infection in this study was 23(7.9%) with 11(7.6%) in urban and 12(8.3%) in rural communities (p-value = 0.82). The viral serotypes identified in this study within the urban and rural sub regions of Edo state were types 16,18,31,35,45,51,52 and 58. HPV subtypes 16 and 18 contributed the highest prevalence with 45.5% in the urban setting versus 33.3% in the rural setting, with an overall prevalence of 39.1%. Co-infection of 45 and 58 was highest among urban participants (18.2%) while co-infection of 45 and 52 was highest in rural participants (33.3%). Urban women had variable coinfection (35, 45; 45, 52; 45, 58) whereas in rural women, co-infection occurred with Type 45, 52. Sexual activities irrespective of the number of partners and higher parities were statistically associated with a positive high risk HPV status in both urban and rural participants (p-value 0.041), (p-value 0.001) respectively. There was no statistically significant relationship among circumcision, HIV infection, condom use and HPV positivity in both settings.

Conclusion: The prevalence of high risk HPV infection is comparable among women living in urban and rural communities of Edo state Nigeria, however, variations exist in the pattern of occurrence of high risk human papillomavirus subtypes within these sub regions of the State. Strengthening reproductive and sexual education in both males and females with focus on HPV vaccination, delaying sexual activities and reduction in number of childbirth are strategies which would prevent high risk HPV infection and cervical cancer in these communities.

Keywords: High risk human papillomavirus, cervical cancer, HPV prevalence, Edo State Nigeria.

1. INTRODUCTION

Human Papilloma Viruses (HPV) are DNA viruses, of which there are several types, associated with benign and malignant conditions of the cervix, penis, vulva, vagina, anus and oropharynx [1].

Oncogenic human papillomaviruses are predominantly sexually-transmitted pathogens and several high risk types are associated with nearly all cases of cervical cancer worldwide. Cervical cancer poses a major public health threat to women living in low and medium resourced countries such as in Sub-Saharan Africa and Southeast Asia [1-4]. In particular, the developing world bears a disproportionately high burden of cervical cancer accounting for over 80% of the 500,000 new cases of cervical cancer diagnosed every year and about 250,000 death worldwide [3, 4]. The impact and threat of cervical cancer to the lives of women worldwide is indisputable.

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There are proven risk factors related to the development of cervical cancer. These include infection with high-risk Human Papillomavirus, early sexual debut, high parity, multiple sexual partners and co-infection with Human Immunodeficiency Virus (HIV). Chlamydia trachomatis, herpes simplex virus type 2, immunosuppressant, and certain dietary deficiencies are also known to be associated with the risk [5, 6].

The human papillomaviruses are prime etiologic factor in the development of cervical cancer[5]. In fact, most of the behavioral and sexual risk factors for cervical cancer become statistically insignificant as independent variables after adjusting for HPV infection[6]. Most of the genital infections with HR-HPV type(s) are asymptomatic and heal without treatment. The risk of cervical cancer increases as the HR-HPV infection persists. Over 70% of cervical cancers are attributed to HPV types 16 and 18 and approximately 20% to other high risk subtypes [7-8].

Vaccines against HPV have been developed and provide effective protection against some HPV types. It has been said to prevent the establishment of infection of the high risk HPV types despite contact with the genital tract. However the HPV vaccines available are very expensive and beyond the reach of most people in low resource countries, such that few governmental agencies and even fewer women in developing countries can afford the vaccines.

Primary prevention of high risk HPV infections with these vaccines could decrease the incidence of cervical and other HPV related cancers in both sexes [9, 10]. As the implementation of these vaccines unfolds in the developing world, public health planners within the sub regions of African countries must begin to identify the subpopulations with the highest burden of this disease, so that they can prioritize the allocation of the limited vaccines.

Few studies on the pattern of high risk HPV infection are yet available all over the world with even fewer in west African subregion [11, 12]. Most of these studies are concentrated in urban areas with very little emanating from rural communities [13,14,15]. Existing urban data elsewhere may not be generalizable to other areas even within the same country, where cultural practices, sanitation, health behaviours, and sexual pattern may be far different.

This study sought to determine the patterns of occurrence of high-risk HPV infection in urban and rural communities of Edo state, using pooled data from two cross sectional studies in rural and urban regions of the state.

2. STUDY JUSTIFICATION

The HPV has been recognized as a major aetiological agent in cervical cancer [9-10]. Cervical cancer is a preventable disease of public health importance.

The burden of cervical cancer, difficulties with secondary prevention and deployment of screening and treatment frameworks on a nationwide basis in resource poor countries suggest that HPV vaccination remains a viable strategy in prevention of this disease [11-14].

HPV vaccination of adolescent in Africa may be on the horizon, however the distribution and acceptability still remains very poor. The delivery costs of the vaccine remains very high for most of the people living in various regions of the world thereby prohibiting widespread implementation particularly in low-resource countries [15-17]. Donation or subsidization of the vaccine by Non-Governmental Organizations may provide a limited supply in Sub-Saharan Africa in the near future such that countries will then need to reallocate their stretched public health resources to incorporate HPV vaccination into existing programs. To maximize this, researchers must first generate reliable country and population-specific epidemiological data for HPV, demonstrating the subpopulations of greatest need.

There are little or very few studies in our setting that have evaluated the various high-risk HPV subtypes using polymerase chain reaction based method. The reported trend also has been inconsistent for generalization in other geographical regions were such studies have not been done.

This study therefore, aimed to determine the pattern of high risk human papilloma virus infections in Edo State using pooled data from urban and rural Sub regions as knowledge about the distribution of the HPV types circulating in the communities in different regions would be useful in devising the optimum strategy for cervical cancer preventive programs.

3. RESEARCH QUESTION

1. What is the pattern of distribution of oncogenic HPV infection among women living in the urban and rural communities of Edo state Nigeria?

2. What are the factors associated with the difference in prevalence of oncogenic human papillomavirus infections between these geographical areas?

3.1. HYPOTHESIS

3.1.1. Null Hypothesis

There are no differences in the pattern and correlates of infections with high risk HPV between the urban and rural communities of Edo state Nigeria.

3.1.2. Alternative Hypothesis

There are differences in the pattern and correlates of infection with high risk HPV between the urban and rural communities of Edo state Nigeria.

3.2. Aims and Objectives of the Study

The general aim of this study was to determine the pattern of high risk HPV infection among women living in urban and rural communities of Edo state Nigeria.

3.2.1. Specific Objectives

1. To determine prevalence of HR-HPV infection among subsets of samples of women from rural and urban communities of Edo state.

2. To determine the various high-risk HPV subtypes common within the two sub regions.

3. To compare and correlate the effect of risk factors on the pattern of occurrence of HR-HPV infection in both settings.

4. MATERIALS AND METHODS

4.1. Study Area

The study was conducted in Edo State. The State lies between longitude 5 degrees East and 6.45 degrees east, and latitudes 6.1 degrees North and 7.30 degrees north. It has a total land area of 19,281.93 square kilometers. The State is bounded by Delta State to the South, Kogi State to the North, Ondo State to the West and the River Niger along the Eastern border [7, 6].

Edo State is an inland State in Southern Nigeria, one of the six (6) States in South-South Nigeria. Edo State is divided into three senatorial districts namely Edo North, Edo Central and Edo South. There are eighteen local government areas in the State with six in Edo North, five in Edo Central and seven in the Edo South. The State administrative capital, Benin City is in Edo South Senatorial District [18].

The study was carried out across two Senatorial Districts of the State with urban and rural communities selected from the south and central senatorial districts of the state. The population criteria by the United Nation's Economic Commission for AfricA [19], was strictly followed in delineating the urban from the rural communities in this study.

4.2. Study Design

This was an analytical cross-sectional study.

4.3. Study Population

Eligible women who consented and met the inclusion criteria were recruited for the study.

4.4. Inclusion Criteria

This study included women who were 18yrs or more, drawn from the rural and urban communities of Edo state. No subject was recruited twice.

4.5. Exclusion Criteria

1. History of hysterectomy with removal of the cervix;

2. History of anogenital, breast, oral, esophagus, lung, bladder, liver, or cervical cancer.

3. Women who have undergone cervical cone biopsy.

4. Those with intact hymen (virgins).

5. Mentally challenged women.

6. Women with vaginal bleeding.

7. Pregnant women.

4.6. Sample Size

The sample size was calculated using the formula for cross sectional study based on the study done recently at Ile-Ife which gave a prevalence for high risk oncogenic HPV infection as 21.6% [20].

Assuming there are equal number of cases in both population (Rural and Urban) under study ($n_{1=}n_{2=}n_1$) in the two sub samples, the statistical formula[79] for n^1= 2Z2pq/d2 where;

Z = standard normal deviation which is 1.96,

P = proportion of clients positive for HR HPV = 21.6% = 0.216.

q = proportion of persons negative for HR HPV = 1-0.216 = 0.784,

d = Assuming the observed difference of 10% at a level of significance of 5%,

Therefore, n^1 = 2×1.962×0.216 x 0.7840.12=130.11.

Approximate 130 samples per population.

Assuming the non-response rate (NR) of 10%, with 90% response rate,

Sample per population = 130.110.9 =144.56.

Therefore minimum sample size per study group was 145.

Therefore 290 women were recruited for the study.

4.7. Sampling Techniques

In this study, 290 subjects were recruited, with 145 women recruited per study population (urban and rural dwellers). The sampling method employed was multistage sampling technique.

4.8. Data Collection

4.8.1. Structured Questionnaire

Questionnaires were administered to collect relevant information after informed consent. This included detailed information on sociodemographic, sexual and reproductive history. Each interview was conducted prior to cervical screening and specimen collection. A local interpreter was made available for those who did not understand the information contained in the questionnaire.

Enrolled women where administered a structured questionnaire consisting of;

Section A: Biodata and socio-demographic characteristic.

Section B: Sexual and reproductive history.

Section C: Social History.

Section D: Results of screening test (13 high risk HPV subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), and collection of cervical sample from recruited subjects.

4.9. Clinical Examination and Specimen Collection

Clinical examinations and specimen collection where performed using standardized techniques. Each of the participants was placed in a dorsal position after informed consent under the assistance of a nurse, with the legs flexed at the hip and knees, and abducted. The labia were parted with gloved thumb and index finger. An unlubricated sterile, plastic disposable Cusco's speculum, was gently passed and fixed to visualize the cervix under a bright light source. Excessive mucous or extra secretions were removed using dry sterile cotton swabs. Cervical samples were collected using the female sample collection kit designed for collection and temporary storage of cervical specimen. This kit was comprised of sample vial, collection brush (a cytobrush cervical cell sampler) and 3.5mls of preservation solution.

The cytobrush cervical cell sampler was introduced into the endocervical canal and scraped by a 360-degree rotatory movement (3-5 rotatory movements). The brush was then gently drawn out of the holder and placed in the specimen vial which was a 5-ml vial, containing 3.5mls of preservation solution (serving as specimen transport medium). The lid of the specimen vial was screwed tightly and properly labelled.

4.9.1. HPV DNA Testing

The residual cell suspensions from the female cervical cell collection kit was frozen at -20°C until the desired number of samples where achieved. The frozen samples where then transported to Molecular Diagnostics laboratory Nigeria Limited, Lagos State Nigeria in iced cold packs for analysis using the hybribio 21 HPV Geno array test kit which uses PCR amplification and flow through hybridization technology to characterize the HPV genotypes. This processes involved included DNA extraction, PCR amplification, Flow-through hybridization and Result interpretation.

4.9.1.1. DNA Extraction

Aliquots of cervical samples were repeatedly centrifuged at 14,000 rounds per minutes for 3 times each lasting 5 mins.

After each centrifuge, supernatant was discarded and buffer solutions added respectively to the remaining suspension.

DNA was extracted by the lysis of cells, isolation, precipitation and purification. 1 ml of sample was then pipetted for PCR amplification.

4.9.1.2. PCR Amplification

All PCR reagents were spun for 5 minutes. PCR master mix solution was prepared by mixing appropriate quantities of PCR-mix solution and DNA Taw polymerase for each reaction tube.

One microliter of DNA template was added to each PCR tube. The solution was centrifuged for a few seconds and subsequently placed in a thermal cycler for DNA amplification

4.9.1.3. Flow-through Hybridization

The PCR products were denatured at 95˚C for 5mins. The HybriMem HPV-21 DNA microarray membrane marked with 21 HPV genotype probes was put in place. The PCR products and the pre-warmed hybridization solution were mixed together and then added into sample wells. It was thereafter incubated for 20 mins and blocking solution added. The membrane was washed with hybridization solution and enzyme conjugate added to display the result.

4.9.1.4. Result Interpretation

Solution membranes were dried on absorbent paper. A positive result was indicated by a clearly visible indigo dot. The HPV genotype result was determined according to the position of specific probes on the HybriMem HPV-21 membrane. Multiple dots indicated multiple infections.

Actual HPV types were determined by comparison of the position of the dots to known reference points

4.9.2. Data Analysis

Data was analyzed using SPSS 20 (Statistical Program for Social Science Version 20) statistical package. The mean and standard deviations where calculated for quantitative variables, while charts, graphs and tables were used to depict qualitative variables. The Chi-square test was used to compare the differences between proportions. Correlates were assessed using bivariate analysis and logistic regression. All statistical analysis was at 5% level of significance with p≤0.05 (95% confidence level).

4.9.2.1. Ethical Consideration

Approval for the study was obtained from the Ethical committee of the Irrua Specialist Teaching Hospital. Ethical considerations in this study was based on the general ethical principles as applicable to human subjects. These are respect for persons, beneficence, non-maleficence and justice.

5. RESULTS

The study included 145 urban and 145 rural women giving a total of 290 participants.

Table 1: Socio-Demographic Characteristics of Respondents

Variables	Urban N=145(%)	Rural N=145(%)	Total N=290(%)
Age group			
18 – 24	4 (2.8)	23(15.9)	27(9.3)
25 – 34	40 (27.6)	54(37.2)	94(32.4)
35 - 44	51 (35.2.)	29(20.0)	80(27.6)
45 – 54	26(17.9.)	15 (10.3)	41(14.1%)
55 & above	24 (16.6)	24 (16.6)	48(16.6)
Mean(SD) Age	40.841(11.02)	37.193(13.01)	39.02(12.17)
Education status			
No Education	2 (1.4)	11(7.6)	13(4.5)
I^0 Education	47 (32.4)	33(22.7)	80(27.6)
2^0 Education	28 (19.3)	52(35.9)	80(27.6)
3^0 Education	68(46.9)	49 (33.8)	117(40.3)
Marital status			
Single	8 (5.5)	39(26.9)	47(16.2)
Married	115 (79.3)	80(55.1)	195(67.2)
Divorced	6(4.1)	0(0.0)	6(2.1)
Seperated	4(2.8)	12 (8.3)	16(5.5)
Widowed	12 (8.3)	14 (9.7)	26(9.0)
Marital setting			
Single	13(9.0)	35(24.1)	48(16.6)
Monogamy	96(66.2)	78(53.8)	174(60.0)
Polygamy	36(24.8)	32(22.1)	68(23.4)

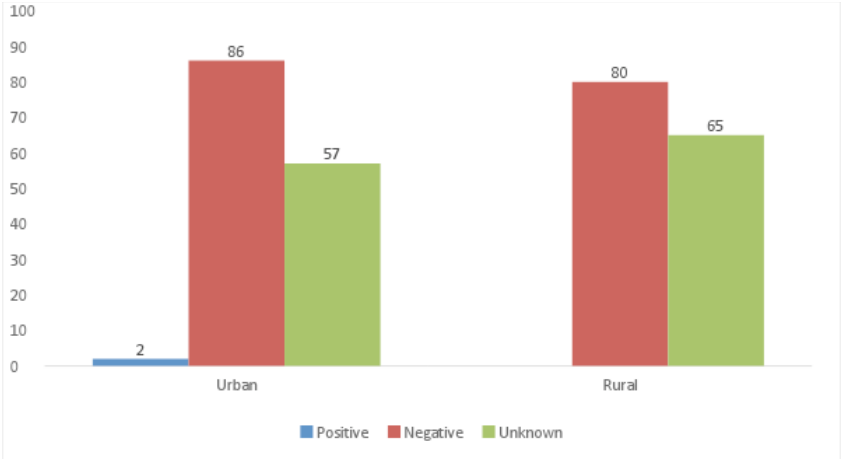

Figure 1: Self-reported HIV Status.

The age was categorized into five age groups which include 18-24years, 25-34years, 35-44years, 45 to 54years and ≥55years. The mean age for women from urban and rural communities where (41.0±11.0) and (37.0±13.0) respectively, with most of the urban women belonging to the age group 35-44years (35.2%) while most of the rural women belonging to the age group 25-34years (37.2%). The overall mean age of participants was 39.0±12.0.

Urban women attained higher (tertiary) levels of education than the rural women (46.9% vs 33.8%). Rural women had a higher number of women with no level of education, than the urban women (1.4% of urban women vs. 7.6% of rural women). More urban than rural women were married and in monogamous setting (66.2% vs. 55.1%). The rate of polygamy was comparable among urban and rural women (24.8% vs 22.1%). (Table **1**)

Self -reported HIV status was positive in 0.7% of the participants as shown in Figure **1**. 57.2% were negative while 42.1% were unaware of their HIV status

There was no statistically significant difference in the age at coitarche between the urban and the rural women (19.75±2.52 vs 19.15±2.69 p-value 0.051) however the urban women were more likely to engage in protected sexual intercourse than the rural women, with condom use rate of 40.7% for urban women vs. 33.1% for rural women as in Table **2A**.

Table 2A: Reproductive and Sexual-Behavioural Profile of the Participants

Variables	Urban N=145(%)	Rural N=145(%)	Total N=290(%)	P-value
Coitarche 10-14yrs 15-17yrs ≥18yrs	 9(6.2) 14(9.7) 122(84.1)	 19(13.1) 23(15.9) 103(71.0)	 28(9.7) 37(12.8) 225(77.6)	0.051
Mean(SD), age at Coitarche	19.75(2.52)	19.15(2.69)	19.45(2.62)	
Sexual partners (life time) 1-2 3-4 ≥5	 106(73.1) 29(20.0) 10(6.7)	 85(58.6) 37(25.5) 23(15.9)	 191(65.9) 66(22.8) 33(11.4)	0.001
Mean sexual partners(SD)	2.13(1.18)2(	2.71.47)	2.42(1.36)	
Parity 0 1 2-4 ≥5	 15(10.3) 8.0(5.5) 79(54.5) 43(29.7)	 55(37.9) 0.0(0.0) 46(31.7) 44(30.3)	 70(24.1) 8.02.8) 125(43.1) 87(30.0)	
Mean parity(SD)	4.05(2.54)	2.97(2.66)	3.51(2.65)	

Table 2B: Reproductive and Sexual-Behavioural Characteristics of Respondents

Variables	Urban N=145(%)	Rural N=145(%)	Total N=290(%)	P-Value
Ever used a male condom				
Yes	59(40.7)	48(33.1)	107(36.9)	0.181
No	86(59.3)	97(66.9)	183(63.1)	
Genital cutting				
Yes	67(46.2)	57(39.3)	124(42.8)	0.235
No	78(53.8)	88(60.7)	166(57.2)	
Ever had hpv vaccine				
Yes	0(0.0)	16(11.0)	16(5.5)	< 0.0001
No	145(100)	129(89.0)	274(94.5)	
Smoking				
Yes	4(2.8)	8(5.5)	12(4.1)	0.240
No	141(97.2)	137(94.5)	278(95.9)	

Table 3: Prevalence of High Risk HPV In Subset Of Samples From Rural and Urban Communities. Result Of High Risk HPV Test

			Result of HPV-DNA		Total
			Positive	Negative	
Study Area	Urban	Result of High Risk HPV-DNA Test. N (%)	11(7.6)	134(92.4)	145(100.0)
	Rural	Result of High Risk HPV-DNA Test. N (%)	12(8.3)	133(91.7)	145(100.0)
Total		Result of HPV-DNA	23(7.9)	267(92.1)	290(100.0)

X_2 = 0.47 P-value 0.82.

The life time sexual partners were statistically lower among women living in urban communities compared to those living in the rural communities with mean life time sexual partners (2.13 ± 1.18 vs. 2.72±1.47) for urban and rural women respectively and p-value 0.001 as in Table **2A**. Mean parity was significantly higher among the urban than the rural women 4.05±2.54 vs. 2.97±2.66 as in Table **2B**.

Utilization of HPV vaccine in this study was statistically significantly lower among the urban dwellers. Male condom use, genital cutting and number of smokers were comparable across both settings.

The total number of women who were positive to high risk HPV infection was 23 out of the 290 women sampled in both setting. This gave a total prevalence of 7.9%. Eleven (11) women from the urban dwellers and twelve (12) women from the rural dwellers where positive to high risk HPV infection giving a prevalence of 7.6% and 8.3% in urban and rural communities respectively. (8.3% vs 7.6%, p-value = 0.82) as shown in Table **3** below.

A total of 23 of the 290 women sampled in both setting tested positive to 8 high risk HPV serotypes which included types 16,18,31,35,45,51,52 and 58. Eleven (11) of the 145 urban women tested positive to eight (8) high risk HPV serotypes (16,18,31,35,45,51,52 and 58) while 12 of the 145 women sampled in the rural communities were positive to four (4) HPV serotypes including types 16,35,45 and 52.

HPV subtypes 16 and 18 contributed the highest prevalence with 45.5% in the urban setting versus 33.3% in the rural setting, with an overall prevalence of 39.1%.

Co-infection occurred in 39.1% of both urban and rural women who tested positive for high risk HPV infection. Co-infection of 45 and 58 was highest among urban participants (18.2%) while co-infection of 45 and 52 was highest in rural participants (33.3%). Urban women had variable coinfection (31,51; 35,45; 45,52; 45,58) whereas in rural women, co-infection occurred with Type 45,52.

Table 4: Distribution of High Risk HPV Subtypes

			Study Area			Total (N=23)	
		Urban (N=11)	Urban % (n/11)	Rural (N=12)	Rural % (n/12)		%Total (n/23)
Viral Serotype	16	3	27.3	4	33.3	7	30.4
	18	2	18.2	0	0.0	2	8.7
	31,51	1	9.1	0	0.0	1	4.3
	35	1	9.1	4	33.3	5	21.7
	35,45	1	9.1	0	0.0	1	4.3
	45,52	1	9.1	4	33.3	5	21.7
	45,58	2	18.2	0	0.0	2	8.7
Total		11	100.0	12	100.0	23	100.0

Table 5: Correlates of Hr-Hpv Infection, Comparing Urban to Rural Women in Edo State (N=290)

Variables	Prev of HRHPV Urban (%)	Prev OF HRHPV Rural (%)	Total (%)	P-Value
Age Group				
18 – 24	0.0	0.0	0.0	0.010*
25 – 34	5.0	14.8	10.6	
35 – 44	9.8	0.0	6.3	
45 – 54	7.7	0.0	4.9	
55 and above	8.3	16.7	12.5	
Coitarche				
10-14yr	55.6	0.0	17.9	0.08
15-17yr	0.0	0.0	0.0	
≥18yr	4.9	11.7	8.0	
Sexual Partners				
1-2	1.9	10.4	5.5	0.041*
3-4	24.1	10.8	16.7	
≥5	25.0	0.0	6.5	
Parity				
0	0.0	14.5	11.4	<0.0001*
1	0.0	0.0	0.0	
1-4	2.5	8.7	4.8	
≥5	20.9	0.0	10.3	

Type 16 was more common as single high-risk HPV serotype across both setting 27.3% vs. 33.3% in urban and rural women respectively. Serotype 18 was only detected in the urban women in this study (18.2%). Serotype 35 was found more frequently in rural setting (33.3%), while serotype 45 did not occur in isolation, but coexisted with other high-risk HPV subtypes (35,52,58). This is shown in Table **4**.

Age less than 24yrs and delayed coitarche of 15-17years was associated with no prevalence of High risk HPV infection in both setting (p-value 0.010) whereas sexual activity irrespective of the numbers and higher parity were statistically associated with high risk HPV positivity (p-0.041) and (p-0.0001) respectively as in Table **5** above.

Infected women reported a higher mean number of life time sexual partners in urban and rural women compared to those who tested negative (3.4 ± 1.4 vs. 2.0±1.1) and (2.8±1.0 vs 2.7± 1.5) in urban and rural women respectively as in table **6A**. The mean age at coitarche in both setting was lower in high risk HPV positive women than HPV negative women (19±3yrs vs 20±3years) Table **6B**.

Table 6A: Characteristics of HR-HPV+ AND HR-HPV- Women, by Region (n=290)

Variables	HPV Status of Respondents				
	Urban		Rural		P-Value
	Positive	Negative	Positive	Negative	
Age, years	41.8(10.4)	40.8(11.1)	40.0(12.6)	36.9(13.1)	0.710
Age at menarche	14.1(1.0)	14.6(0.8)	14.3(1.0)	14.4(1.0)	0.570
Age at coitarche	17.6(3.6)	20.0(2.4)	19.7(1.8)	19.1(2.8)	0.940
Sexual partners	3.4(1.4)	2.0(1.1)	2.7(1.0)	2.7(1.5)	0.046
Parity	6.3(1.8)	3.9(2.5)	1.3(2.0)	3.1(2.7)	0.0001

Table 6B: Correlates of HR-HPV Infection Comparing Urban to Rural

Variables	Urban		Rural		P-Value
	HPV positive n (%)	HPV negative n(%)	HPV Positive n (%)	HPV Negative n(%)	
HIV					
Positive	0(0.0%)	2(100%)	0(0.0%)	0(0.0%)	0.552
Negative	5(5.8%)	81(94.2%)	4(5.0%)	76(95.0%)	
Unknown	6(10.5%)	51(89.5%)	8(12.3%)	57(87.7%)	
Condom use					
Yes	5(8.5%)	54(91.5%)	8(16.7%)	40(83.3)	0.305
No	6(7.0%)	80(93.0%)	4(4.1%)	93(95.9)	
Genital cutting					
Yes	7(10.4)	60(89.6)	4(7.0)	22(84.6)	0.146
No	4(5.1)	74(94.9)	8(9.1)	111(93.3)	
HPV vaccination					
Yes	0(0.0)	0(0.0)	0(0.0)	16(100%)	<0.0001
No	11(7.6)	134(92.4)	12(9.3)	117(90.7)	

Condom use and female circumcision did not appear to positively correlate with risk for HPV infection in both setting.

The HPV vaccination rate in this study was 5.5%. The 16 women vaccinated, where from the rural region of the state. These women also tested negative to high risk HPV infection.

DISCUSSION

The prevalence of high risk HPV infection in this study is 7.9%. The urban women had a prevalence of 7.6%, while the rural women had a relatively higher prevalence of 8.3%. The prevalence reported in this study is comparable with the point prevalence of HPV infection worldwide which is 10.1% [21]. The overall prevalence of 7.9% is at variance with the prevalence which has been reported in some parts of Nigeria including 21.6% in Ille-Ife, [22], 19.7% in Okene central Nigeria [23], 37% in Abuja Nigeria [24] and 76% in kano [25]. A similar study done in Mali [26] which recruited women in rural and urban communities in same state in Mali, had an overall prevalence of 18%, however the prevalence of 12% among the urban women is comparable to that found in this study [26].

The International Agency for Research on Cancer (IARC) on HPV prevalence in various countries Survey described inconsistent trend in HPV infection in Africa. The lower prevalence of HR-HPV seen in this study is not easily explained by the methodology. In this study, a population that is generally very receptive of medical research studies was encountered and were eager to participate, regardless of current or previous medical conditions or experiences. The sampling techniques produced samples that were comparable on many socio-demographic characteristics, providing evidence that the women who decided to participate were similar across the settings. The baseline differences that was apparent-for example, the lower number of 18–24year-old urban women, did not appear to bias the overall prevalence figures within settings, as demonstrated by subgroup prevalence figures.

The higher prevalence in other studies done in Nigeria, may probably be since most of these studies were hospital based studies conducted within one region of the state while this, was a population based study conducted in two regions of the state. Also, some of the few studies on high risk HPV prevalence available in Nigeria did not focus solely on high risk HPV infection as done in this study, but also included other subtypes of HPV infection in the overall studies. Apart from the differences in sexual behavioral practices between the Nigerian and Malian women in these two studies (lower mean age at coitarche, higher number of sexual partners and higher polygamy among the Malian women), the higher risk could be explained by intrinsic biological and cultural characteristics, or to environmental factors associated with this ethnic group; these possibilities were not taken into consideration in this study, but should be analyzed in further studies. The laboratory method of analyzing the samples may also not have contributed to the difference in prevalence recorded in both setting as the specificities and sensitivities of polymerase chain reaction and other laboratory modalities such as hybrid capture 2 assays are comparable. While this study employed Polymerase Chain Reaction (PCR) in analyzing all samples, the study from Mali combined both PCR and Hybrid capture to analyze the samples from urban and rural women recruited in Mali. Previous HPV surveys in Sub-Saharan Africa have generally shown relatively high prevalence with some variations, depending on how women were selected and how HPV was tested for.[81]Using the Hybrid Capture (HC) assay, 17% prevalence of HR-HPV types was found in rural Uganda, while a prevalence of 8.2% was found in women in rural India [27]. Polymerase Chain Reaction (PCR)-based assays showed HPV prevalence of 40% in rural Mozambique, 31% in Harare, Zimbabwe, 18% in Dakar and Pikene, Senegal, and 44% in Nairobi, Kenya [28].

The age-specific point prevalence of cervical high-risk HPV infection in this study was higher in two age groups in both setting. Among the urban women, the prevalence was higher in women aged 35-44 and women ≥55years while in the rural setting, it was highest at age 25-34years and above 55years. This age pattern in both setting are comparable to the pattern of age distribution of human papilloma viral infection as reported in a study from rural Nigeria in which the peak prevalence in young women was accompanied by a second but smaller increase in prevalence among older women [29,30]. This also followed the pattern as reported by a South American study[83] in which the peak prevalence of high risk HPV infection followed a U-shaped curve; with first peak in women under 30years, and second peak in women above 55yrs. In two other sub-Saharan African studies, HPV prevalence showed no significant decline with age, and indeed, in one of these two studies high-risk HPV were more frequently detected in older than younger women [15].

The peak age prevalence in this study in both setting also corresponded to the peak age prevalence of cervical intra-epithelial neoplasia at age 25-35 years and above 40yrs of age worldwide [6]. This further buttress the relationship between high-risk HPV infection and dysplastic changes of the cervix.

The viral serotypes identified in this study within the urban and rural sub regions of Edo state were types 16,18,31,35,45,51,52 and 58. Urban women tested positive to eight (8) high risk HPV serotypes 16,18,31,35,45,51,52 and 58 while the rural dwellers were positive to four (4) of the HPV serotypes including types 16,35,45 and 52. The presence of more high risk HPV subtypes among the urban women may be related to higher population mobility including immigration of populations who may be at higher risk of HPV seropositivity of varied types. Inter-ethnic sexual contact and racial mixing which is more common in the urban communities could have also accounted for this difference as has been reported in California USA [31]. HPV subtypes 16 and 18 contributed the highest prevalence with 45.5% in the urban setting and 33.3% in the rural setting, with an overall prevalence of 39.1%. This is worrisome considering the oncogenic potential of these two subtypes of high risk HPV. Urban women had variable coinfection (31, 51; 35, 45; 45, 52; 45, 58) whereas among rural women, co-infection occurred with Type 45, 52. Type 45 did not occur in isolation but coexisted with other HPV types (89% of women who had more than one type). HPV type 16 was the single most common HR-HPV (30.4%), found in this study. This is consistent with previous studies done in Sub-Saharan Africa [13,83,84,85]. It was followed by HPV type 35 which was 21.7%. In Mozambique HPV 35 was found to be slightly higher than HPV16[32].

Importantly in this study was that serotype 45 was not found in isolation in any of the women who tested positive for high risk HPV infection, but coexisted with other subtypes, and was equally distributed among the urban and rural women in this study. This followed a similar pattern as has been reported in South America and other HPV co-infection studies [33,34,35].

It has been established that the transmission of the human papillomavirus is predominantly by sexual contact. Early sexual debut and multiple sexual partner are known risk factors. Other routes have been shown to be of lesser significance [36]. In this study sexual activities irrespective of the number of partners and higher parities were statistically associated with a positive high risk HPV status in both urban and rural participants. The HR-HPV positive women had a lower mean age at first sexual intercourse compared to HR-HPV negative women (17.6 ±3.4years vs. 20.0 ± 2.4years) in the urban setting. This was not the case in rural setting. The infected women in the rural setting had a higher mean age at coitarche. Furthermore, the infected women reported a higher mean number of life time sexual partners in urban setting. The idea that the higher rural prevalence of 8.3% compared to the urban prevalence of 7.9% in this study is driven by that group's riskier sexual behavior-a somewhat lower mean age at first intercourse and slightly higher number of partners, is refuted by the lack of a convincing association between either of those risk factors and HR-HPV status in the rural women. These factors were more convincing among those who were positive within the urban community. It is possible that the sexual and social network construction may inherently be different in the rural and urban setting. Also, what is obvious in the study is that the uptake of HPV vaccine was higher among the rural women. 5.5% of the rural women had received HPV vaccine at the time of this study, while non-had received the vaccine among the urban women. This may have offered some protection to some of these rural women with multiple sexual partners.

It was observed in this study that a higher mean parity correlated positively with high risk HPV infection. This is consistent with a study done in south western Nigeria in which high parity correlated with positive HPV status among the study participants[45] but was contrary to a South American study which could not demonstrate this relationship[82]. Historically, studies in the developing world-but not in developed countries-have found a positive relationship between parity and HPV or cervical cancer. This phenomenon suggests that the measured relationships with parity may be the result of residual confounding due to sexual activity[26]. There is still insufficient data to give final conclusions about the effect of number of births on the risk of HPV infections. For some factors, such as female circumcision and use of condoms, the sample was too homogenous to detect any meaningful association with HR-HPV status.

There was also no meaningful association between HIV infection in this study and high risk HPV status. This probably may be due to the very low HIV infection rate in this study of 0.7%. Studies that have focused solely on high risk HPV infection among HIV positive women have found a higher infection rate [37-39].

This study had some limitations. First, as with all studies using self-reporting of variables, especially variables of a sensitive sexual nature, the quality of our data was contingent on accurate reporting. Secondly, because of the cross sectional design of this study temporal trends of potential correlates of HPV and the risk factors for persistent HPV infections could not be examined. With continued follow up of this population and repeated sampling, it is possible to be able to report the pattern and spectrum of persistent HPV infection in future.

CONCLUSION

This study showed that the prevalence of high risk HPV infection do not differ significantly between the urban and rural sub regions of Edo state Nigeria. However, variations exist in the HPV subtypes such that the urban dwellers are more exposed to varied high risk HPV serotypes than the rural dwellers. HPV vaccination, low parity, age less than 24yrs and delayed sexual debut in this population were protective features of high risk HPV infection. Strengthening reproductive and sexual education in both males and females with focus on HPV vaccination, delaying sexual activities and reduction in number of childbirth are strategies which could prevent high risk HPV infection and cervical cancer in these communities.

RECOMMENDATIONS

It is advocated that HPV vaccines made available to urban and rural dwellers in Edo State taking into cognizance the peculiarities of HPV genotypes present in these communities. Establishment of laboratory facilities for HPV DNA typing which is accessible and affordable to the general public in various regions will improve HPV screening rate in Nigeria

Population based study of this nature needs to be replicated in other Sub-regions in Nigeria for further characterization of HR-HPV status and genotypes in these environments as well follow up studies for viral persistence as these would strengthen the preventive measures for cervcal cancer Nationwide.

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