PREVALENCE, RISK FACTORS OF HUMAN PAPILLOMAVIRUS INFECTION AND PAPANICOLAOU SMEAR PATTERN AMONG WOMEN ATTENDING A TERTIARY HEALTH FACILITY IN SOUTH-WEST NIGERIA

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ABSTRACT

Background: Cervical cancer amongst Nigerian women has been on the increase in the past decade, and is regarded as the second highest cause of cancer deaths among Nigerian women.

Objective: This study was aimed at determining the prevalence, risk factors of HPV infection, and Papanicolaou smear pattern amongst a cohort of women attending the Gynaecology clinic of a tertiary health facility in Ido-Ekiti, South west Nigeria.

Methodology: This was a cross-sectional study involving the screening of women between the ages of 15-64 years for cervical intraepithelial neoplasia using Papanicolaou smear staining technique and serological diagnosis using IgG enzyme linked immunosorbent assay kits. Respondents were selected through convenience sampling of subjects, while interviewer-administered questionnaire and clinical report form were also used to collect data, and data was analyzed using SPSS version 17.

Result: Of the 200 blood samples examined for Human papillomavirus infection, 135 (67.5%) were sero-positive while 65 (32.5%) were sero-negative. For cervical cytology using Papanicolaou smear, 14 (7%) were positive (had presence of cervical abnormality) while 186 (93%) were negative (had no cervical abnormality). Result showed a direct relationship between seropositivity, development of cervical intraepithelial neoplasia and Human papillomavirus infection. The risk factors for the development of HPV infection included age, type of marriage, parity, history of genital infection and tobacco usage. Non circumcision of male partner was also found to be a risk factor.

Conclusion: The presence of abnormal cervical cytology and high level of serological positivity clearly showed why there is need for a holistic approach to the screening, vaccination methodologies and early detection of HPV infection in the country.

Keywords: HPV infection, Cervical cytology, Women, Nigeria

INTRODUCTION

Human papillomavirus is a small non enveloped icosahedral DNA virus that replicates in the nucleus of squamous epithelial cells.[1] Genital human papillomavirus is the most common sexually transmitted infection. More than 100 HPV types have been identified, over 40 of which can infect the genital areas of men and women, including the skin of the penis, vulva, anus, the linings of the vagina, cervix, and rectum. HPV types are classified by their association with cancer. Non-oncogenic or low-risk HPV types (HPV 6 and 11) which causes benign or low grade abnormalities of the cervical cells, anogenital warts and recurrent respiratory papillomatosis (RRP) and oncogenic or high-risk HPV types (HPV 16 and 18) which together causes about 70% of cervical cancers.[2]

Cervical cancer ranks as the second most frequent cancer among women in Nigeria, and the second most frequent cancer among women between 15 and 44 years of age. About 23.7% of women in the general population are estimated to harbor cervical HPV infection at a given time.[3] The prevalence of HPV genotypes in cervical cytological samples varies greatly in different geographical regions and shows a strong correlation with cervical cancer incidence. The HPV type 16, although with different prevalence rates, is the most common viral type being present in 12.3%, 18.4%, 21.4% and 25.5% of HPV-positive cytological normal women from Sub-Saharan Africa (Nigeria), Asia, South America, and Europe, respectively.[4]

Human papillomavirus is widely spread in Nigeria. The overall prevalence of HPV in Ibadan, Nigeria arm of the International Agency for Research on Cancer (IARC) study was 26.3%; and 24.8% in women without cervical lesions.[5] The high risk HPV was predominant (19.7%) and was mostly types 16, 31, 35, 58. Low risk HPV were found in 6.6% and mixed infections with more than one HPV type occurred in 33.5% of HPV positive cases. HPV 16 appeared to a play a smaller role in cervical cancer in Nigeria than in Europe.[4] In Okene, north central Nigeria a similar incidence of 21.6% with high risk HPV prevalence of 16.6% and 3.5% having mixed infections was gotten.[6] The main risk factors contributory to HPV in Nigeria included being unmarried, illiteracy, being positive for anti-Herpes Simplex virus antibodies; also tobacco use multiple sex partners of women and their spouses extramarital affairs.[5]

METHODOLOGY

Ido-Ekiti is located in Ido/Osi Local Government Area of Ekiti State, Nigeria. It is situated in the Northern part of the state were the routes from Oyo, Osun and Kwara states respectively converge. Ido-Ekiti is the headquarters of Ido/Osi local council. Ido-Ekiti is bounded to the North by Usi-Ekiti; in the South by Igbole-Ekiti and Ora-Ekiti; in the East by Orin-Ekiti and Ipere-Ekiti and lastly to the West by Ilogbo-Ekiti. As at 2006, Ido-Osi LGA had a total population of 159,114 with vastly educated people. With an annual growth rate of 3.2%, the eight year projected population will be 198, 365. The study site, Federal Teaching Hospital, Ido-Ekiti, is located in Ido-Osi Local Government Area of Ekiti State. It is one of the tertiary hospitals in Nigeria with 280 beds; and a referral centre for the over 2.3 million people in Ekiti State.

Ekiti State is situated entirely within the tropics with the capital located at Ado-Ekiti. It is located between longitudes 40º51' and 50º451' east of the Greenwich meridian and latitudes 70º151' and 80º51' north of the Equator. It lies south of Kwara and Kogi State, East of Osun State and Ondo State lies to the South, with a total land Area of 5887.890sq km. The 2006 population census by the National Population Commission put the population of Ekiti State at 2,384,212 people.

This was a descriptive/cross-sectional study carried out among women attending Gynaecology Clinic of the Federal Teaching Hospital, Ido-Ekiti between the ages of 15-64 years who were willing, and met the inclusion criteria. A structured close ended questionnaire was administered to these patients after an informed consent had been obtained, followed by clinical examination.

The study was conducted between February and June, 2014 after approval was obtained from the Ethical Review Committee of the Federal Teaching Hospital, Ido-Ekiti. Informed consent was obtained from patients and/or parents and guardians. The study was done at no cost to the subjects, and information from the patients and/or parents and guardian were confidential.

Women attending the Obstetrics and Gynaecology Clinic of the Federal Teaching Hospital, Ido-Ekiti, within the ages of 15 and 64 years who were willing, and met the inclusion criteria were recruited consecutively during the period of the study, applying the structured questionnaire after an informed consent.

A structured close ended questionnaire was administered to the subjects and after due consent had been taken; cervical smears were collected by a gynaecologist after visual inspection. The smears collected were immediately fixed to slides before being transferred to the laboratory for processing. The fixed smear were stained by the cytotechnologist using Papanicolaou staining procedure and read by a histopathologist using a light microscope. The slides were reported as normal, inflammatory, abnormal (epithelial lesion) or unsatisfactory. Five ml venous blood was collected aseptically from each study participant into a Vacutainer tube marked with a unique identifier (code number). Separation of serum was done by centrifugation of blood samples at 1,600 revolutions per minute (rpm) for 5 minutes with a bench-top centrifuge. Serum samples was collected into 1.8 ml Cryo tubes and was stored at -20°C until analyzed for HPV antibodies.

The sera of the participants were tested *in vitro* for IgG antibodies to human papillomavirus with a commercial Enzyme linked Immunosorbent assay (ELISA) kit (Diagnostic Bioprobes, Milano, Italy). The serologic test and interpretation of results were done according to instructions of the kit manufacturer. Optical signals generated in the microwells were read at 450 nm with an ELISA plate reader. The ELISA kit manufacturer provided the formula for calculating the cut-off OD450nm (OD of negative control plus 0.250) which we used as threshold for determining the reactive and non-reactive serum samples.

Descriptive statistics such as frequency, percentages was used in the discussion of the results, in order to give a lucid representation of the data analyzed, statistical package for social science version 17.0 for windows, was used to test for the level of significance of the result obtained. Both continuous and discreet variables were generated. The relationship between discrete variable and outcome of interest was tested using Chi-squared test at 5% ($p<0.05$) confidence interval.

RESULTS

Table 1 shows the socio-demographic and reproductive characteristics of the respondents. Less than half, 80(40.0%) of the respondents were in the age group 35-44 years and three-quarter, 150(75.0%), of them had tertiary education. About two-thirds, 133(66.5%), of them had sexual debut at ≤15 years of age. A total of 26(13.0%) respondents had more than one sexual partner. Also as shown in Table 1, more than half, 111(55.5%) of the respondents had ever used oral contraceptive.

In Table 2, of the 200 blood samples examined for Human papillomavirus infection, using serological diagnosis, 135 (67.5%) were seropositive while 65 (32.5%) were seronegative. For cervical cytology using Papanicolaou smear, 14 (7%) were positive (had presence of cervical abnormality) while 186 (93%) were negative (had no cervical abnormality). The difference observed in the prevalence of abnormal cervical cytology and HPV serology was found to be statistically significant ($p<0.0001$)

In Table 3, the prevalence by age showed that the age group 45-54 years had the highest positive cytology while those in the age group 35-44 years had the highest proportion of HPV sero-positive result. For both the cervical cytology and HPV serology, married respondents had the highest proportion of proportion of positive/abnormal result. These differences across the age group ($p<0.0001$) and among the married respondents ($p<0.0001$) were found to be statistically significant.

As shown in Table 4, a higher proportion of the women in the age group 45-64 years compared with the younger women, 15-44 years, had more positive cervical cytology. However, the younger age group (15-44years) had more positive HPV serology. The observed difference was statistically significant for both the cervical cytology ($p<0.0001$) and HPV serology ($p=0.016$)

Similarly, more than three-quarters of those with more than a sexual partner had a higher proportion of positive cervical cytology and HPV serology compared with those with 0-1 sexual partner. The observed difference was also statistically significant for both the cervical cytology ($p<0.0001$) and HPV serology ($p<0.0001$)

DISCUSSION

A high level of HPV seropositivity (67.5%) was recorded in this study and it corresponds with various theories which state that seroprevalence of HPV infection could range from as low as 1% to as high as 80%. Aminu *et al*[7] had a prevalence of 42.9% in their study. Adekunle *et al*.[8], recorded a seroprevalence of 6.6% in women of child bearing age, Wang *et al.*[9] recorded a prevalence of 16% in a cohort of 10000 women, while Castro *et al*.[10] recorded a baseline seropositivity of 43.2% and a follow up prevalence of 50.2%.

Age distribution of the subjects showed that the largest population of subjects were within the ages of 35-44yrs with a frequency of 80 (40%). This could be attributed to the fact that most women who visit the Obstetrics and Gynecology clinic where this research was carried out are middle aged and young women of child bearing age. The highest number, 48(24.0%), of HPV seropositive subjects was found within the age group of 35-44yrs, with a statistical significance ($p<0.0001$) between seropositivity and the age of the subjects. Castro *et al*.[10] indicated a statistical significance ($p=0.001$) between subjects age and HPV seropositivity.

The highest amount of HPV sero-positivity was found amongst married women (62.5%) while single women accounted for the rest (5.0%). This was in contrast to results obtained by Stone *et al*.[11] where no statistical significance was drawn between the marital status of women and HPV sero-positivity in the United States. However, Vaccarella *et al*.[12] in a study to determine the seroprevalence of antibodies against Human papillomavirus (HPV) types 16 and 18 in four continents, alluded to the findings of this study that marital status played a role in an increase in the risk of HPV seropositivity. In this study, the observation was also noticed in the type of marriage of the respondents contributing significantly ($p=0.004$) to an increase in HPV sero-positivity among the subjects. A statistical relationship ($p=0.032$) was also found between the type of marriage of the subjects and development of abnormal cervical cytology.

The age of sexual debut of the subjects did not constitute a significant risk factor to the development abnormal cervical cytology ($p=0.392$) and HPV seropositivity ($p=0.943$). This was rather surprising as majority of studies such as Thomas *et al*.[5] Durowade *et al*.[13] Wang *et al.*[9] all stated an association between the age at sexual debut of their subjects with presence of abnormal

cervical cytology or an increase in HPV seropositivity. This might be due to the subjects' sincerity in answering some of the questions put forward in the questionnaire.

Subjects that had more than three children had the highest amount of seropositivity while the least was found among nulliparous subjects. This supports the works of Anh *et al*.[14] which found an association between HPV seropositivity and parity, but in contrast with the works of Aminu *et al*.[7] which had no statistical relationship (p=0.316) between this risk factor and sero-positivity. Development of HPV infection was directly related to the subjects' history of genital infection as 13 (92.86%) subjects with abnormal cervical cytology, all had a history of genital infection. This also is in support of the work of Reis *et al*.[15]

Alcohol usage did not constitute a risk factor of statistical significance in this study. With just 31 (15.5%) subjects indicating they used alcohol, there was no statistical difference (p=0.136) between alcohol usage and HPV seropositivity among the subjects. Also, in cervical cytology, alcohol usage did not constitute a risk factor in the development of abnormal cervical cytology. There was no statistical difference (p=0.456) between the consumption of alcohol and development of abnormal cytology. Unlike alcohol usage, tobacco usage constituted a high risk factor for both the development of seropositivity and abnormal cervical cytology. Results showed that only 9 subjects used tobacco and all the 9 subjects were all positive for HPV serology, with a statistically significant association (p<0.0001) existing between tobacco usage and sero-positivity. Results further revealed that 8 out of the 9 subjects that used tobacco were positive for abnormal cervical cytology. There was also a statistically significant relationship (p=0.035) between the subjects tobacco usage and development abnormal cytology. Though this is in contrast with the works of Aminu *et al*.[7] it is fully supported by that of Castro *et al*.[10] Marais *et al*.[16] and Durowade *et al*.[13] Male partner circumcision of subjects constituted a critical risk factor in this study. Serological results indicated that all the 8 (100%) subjects whose male partners were not circumcised were positive for serology.

In this study, one hundred and eleven subjects utilized oral contraceptive, with 97 of them positive for serology and 11 of them positive for abnormal cervical cytology. While there was a statistical difference (p=0.001) between the subject's oral contraceptive use and HPV seropositivity, there was no significant association (p=0.072) between oral contraceptive use and development of abnormal cervical cytology. Various studies have clearly shown that, use of oral

contraceptive contributes to HPV seropositivity. Castro *et al*[10], Marais *et al*[16] Aminu *et al*[7], and Anh *et al*.[14] all alluded to the role played by oral contraceptive use of subjects to acquiring HPV infection.

CONCLUSION

This study provides for the estimates of HPV sero-prevalence and prevalence of abnormal cervical cytology. The high level of seropositivity in this study showed an urgent need for a national policy for screening against HPV. The high HPV sero-prevalence seen among respondents below age 20 years, as well as the increase after age 50 years supports the need of an initiation of vaccination programs at young ages and the established screening practice at older ages across the country. Although sero-prevalence may underestimate cumulative exposure to HPV due to low seroconversion rates, it seems to be a useful molecular epidemiologic tool to assess HPV infection in young women unwilling to undergo gynecological examination, with potential application in monitoring the effect of HPV vaccines. Similarly, the high level of seropositivity, and presence of HPV in all abnormal cervical smears in this study, showed a dire need for a National programme geared towards scaling up vaccination against HPV infection in this country.

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Table 1: Socio-demographic and reproductive characteristics of the respondents

Variables	Frequency (%)	Variables	Frequency (%) N=200
Age (years)		**Age at sexual debut(years)**	
15-24	5(2.5)	≤15	133(66.5)
25-34	50(25.0)	>15	67(33.5)
35-44	80(40.0)		
45-54	47(23.5)	**Parity**	
55-64	18(9.0)	0	45(22.5)
		1-3	116(58.0)
		>3	39(19.5)
Level of Education			
Primary	4(2.0)	**Previous genital infection**	
Secondary	23(11.5)	Yes	126(73.0)
Tertiary	150(75.0)	No	74(37.0)
None	27(13.5)		
Marital status		**Number of sexual partners**	
Single	19(9.5)	0-1	174(87.0)
Married	176(88.0)	>1	26(13.0)
Divorce	5(2.5)		
Alcohol intake		**Male partner circumcision**	
Yes	31(15.5)	Yes	192(96.0)
No	169(84.5)	No	8(4.0)
Tobacco use			
Yes	9(4.5)		
No	191(95.5)		
Type of Marriage**			
Monogamy	57(31.5)		
Polygamy	124(68.5)		
Use of Oral pills			
Yes	111(55.5)		
No	89(44.5)		

**N=181

Table 2: Prevalence of abnormal cervical smears and HPV infection among the respondents

Variables	Frequency	Percentages (%)	p value
Papanicolaou smear			
Positive	14	7.0	
Negative	186	93.0	
Total	200	100.0	
			<0.0001
HPV Serological test			
Positive	135	67.5	
Negative	65	32.5	
Total	200	100.0	

Table 3: Cervical cytology and HPV serology by Age and Marital status of the respondents

Variable	Cervical Cytology N=200		HPV Serology (%) N=200		
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	p value
Age (Years)					
15-24	0(0.0)	5(2.5)	0(0.0)	5(2.5)	< 0.0001
25-34	3(1.5)	47(23.5)	30(15.0)	20(10.0)	< 0.0001
35-44	0(0.0)	80(40.0)	48(24.0)	32(16.0)	< 0.0001
45-54	8(4.0)	39(19.5)	40(20.0)	7(3.5)	< 0.0001
55-64	3(1.5)	15(7.5)	17(8.5)	1(0.5)	< 0.0001
Marital Status					
Single	0(0.0)	19(9.5)	10(5.0)	9(4.5)	< 0.0001
Married	14(7.0)	162(81.0)	125(62.5)	51(25.5)	< 0.0001
Divorced	0(0.0)	5(2.5)	0(0.0	5(4.5)	< 0.0001

Table 4: Socio-demographic/reproductive characteristics and cervical cytology/HPV serology

Variables	Cervical cytology		OR	p value	HPV serology		OR	p value
	Pos (%)	Neg (%)	95%CI*		Pos (%)	Neg (%)	95%CI*	
Age(years)								
15-44	3(2.2)	132(97.8)	0.11	<0.0001	78(57.8)	57(42.2)	2.19	0.016
45-64	11(16.9)	54(83.1)	0.02-0.46*		25(38.5)	40(61.5)	1.15-4.20*	
Marital status								
Ever married	14(7.7)	167(92.3)	0.00	0.369	125(69.1)	56(30.9)	2.01	0.146
Never married	0(0.0)	19(100.0)	0.00-0.00*		10(52.6)	9(47.4)	0.70-5.72*	
Type of marriage								
Monogamous	6(4.5)	127(95.5)	0.29	0.032	85(63.9)	48(36.1)	0.29	0.004
Polygamous	8(14.0)	49(86.0)	0.08-0.98*		49(86.0)	8(14.0)	0.12-0.70*	
Age at sex debut(yrs)								
≤15	11(8.3)	122(91.7)	1.92	0.392	90(67.7)	43(32.3)	1.02	0.943
>15	3(4.5)	64(95.5)	0.47-9.04*		45(67.2)	22(32.8)	0.52-2.00*	
Parity								
Nulliparous	2(4.4)	43(95.6)	0.55	0.740	21(46.7)	24(53.3)	0.31	0.001
Parous	12(7.7)	143(92.3)	0.08-2.77*		114(73.6)	41(26.4)	0.15-0.66*	
Past genital infection								
Yes	13(92.9)	113(7.1)	8.40	0.016	115(91.3)	11(8.7)	28.33	<0.0001
No	1(60.7)	73(39.3)	1.11-175.58*		20(27.0)	54(73.0)	11.85-68.95*	
Alcohol intake								
Yes	3(9.7)	28(90.3)	1.54	0.459	25(80.6)	6(19.4)	2.33	0.136
No	11(6.5)	158(93.5)	0.32-6.51*		110(65.1)	59(34.9)	0.81-6.47*	
Tobacco usage								
Yes	8(92.9)	1(7.1)	246.7	<0.0001	9(100.0)	0(0.0)	0.00	<0.0001
No	6(60.8)	185(39.2)	23.73-621.68*		126(66.0)	65(34.0)	0.00-0.00*	
Male circumcision								
Yes	9(4.7)	183(95.3)	0.03	<0.0001	127(66.1)	65(33.9)	0.00	0.056
No	5(62.5)	3(37.5)	0.00-0.17*		8(100.0)	0(0.0)	0.00-1.37*	
Sexual partners								
0-1	5(28.7)	169(71.3)	0.06	<0.0001	110(29.9)	64(70.1)	0.07	<0.0001
>1	9(92.3)	17(7.7)	0.01-0.21*		25(96.2)	1(3.8)	0.00-0.49*	

OR=Odds Ratio; *95% Confidence Interval