

Prevalence of Cervical Human Pappillomavirus Infection in Awka, Nigeria

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Abstract

Prevalence of cervical Human Papillomavirus infection and type distribution vary with different environments. Knowledge of this will help in some cervical cancer interventions such as vaccine administration. This study aimed at determining the prevalence of cervical HPV infection among women attending a tertiary hospital in Awka, Nigeria. To evaluate cervical HPV Infections, cervical samples were collected over a period of 1 year from 405 consenting women. Multiplex PCR and cytology were used in the study. Semi-structured questionnaire was used to obtain the demographic characteristics of the participants. Statistical analysis was done using IBM SPSS statistics version 21. The findings showed that of the 405 women, 387 (94.4%) had normal cytology, and 18 (4.4%) had Low-grade Squamous Intraepithelial Lesion (LSIL). There was no High-grade Squamous Intraepithelial Lesion (HSIL). HPV prevalence of (79) 19.5% was obtained overall in the 405 women, (75 of 387) 19.4% with normal cytology, (4 of 18) 22.2% with LSIL. Age specific prevalence peaked at age group 30 - 39 and a second peak at 60 - 69. HPV types obtained were HPV 16 31 (7.7%), HPV 18 24 (5.9%), HPV 35 3 (0.7%), HPV 33 9 (2.2%), HPV 68 3 (0.7%) and multiple infections (9) 2.2%. HPV 16 was the only type found in LSIL. Regular HPV typing and screening of our women for HPV infection and Pap's smear can go a long way in the reduction of cervical cancer.

Keywords

Prevalence, HPV Infection, Cervical Cancer, Cytology, Intraepithelial Lesion

1. Introduction

HPV is a sexually transmitted infection and all sexually active women are at the risk of HPV acquisition. Up to 50% of sexually active women will be infected with HPV in their lifetime [1] and it is estimated that, by the age of 50, at least 80 percent of women will have acquired sexually transmitted HPV [1]. Most women are infected shortly after their first sexual intercourse [2]. Although all age groups can be affected, there appears to be a higher prevalence in the young women [3] [4] [5] [6].

HPV 16 has been found to be the most prevalent in most parts of the world [5] [7], though with variations in some places. In Kenya [8], Burkina-Faso [9] and Zimbabwe [10], HPV 52 was more prevalent; in Irun Nigeria [11], and Abuja Nigeria [12], HPV 35 was most prevalent; while in Senegal [13] and Nigeria [14], HPV 16/58 and HPV 16/35 respectively were more prevalent. Hibbits *et al.* (2006) [15] reported high prevalence of HPV 16 and 35.

The prevalence of HPV ranged from 1.5% in Spain [16] to 38.8% in Kenya [8]. In Nigeria, different figures have been documented in the different zones of the country. In Ibadan, South-West Nigeria, cervical HPV was identified in 26.3% of sexually active women above 15 years [14] and 14.7% among 1282 women in Irun [12]. In Okene, North-Central Nigeria, a prevalence of 21.6% among 231 women were documented [17]. In Abuja, the Federal Capital, the prevalence was 37% among 275 women studied [12].

The available HPV vaccines so far can only take care of 9 HPV types. Regional variation in HPV serotypes may influence the effectiveness of the vaccines in the different regions. There is paucity of data on the prevalence of HPV and serotypes in this region. The present study is designed to study the prevalence of and factors associated with cervical HPV infection.

2. Method

The study was carried out at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka in Awka South Local Government Area, Anambra State. Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) is a tertiary hospital serving as a referral centre for Hospitals in Awka North and South Local Government Areas and beyond. The study population was women attending the different clinics at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka. Prospective participants were approached and given information concerning the study and consenting women were recruited for the study. Since human subjects were used, ethical approval was obtained from the hospital ethics committee.

Four hundred and five consenting women were recruited into the study. The sample size was derived using the formula:

$$N = Z^2 pq / d^2 [18],$$

where Z = standard deviation at 1.96 (which corresponds to 95% confidence interval).

P = HPV prevalence at Ibadan = 26.3% [14].

$q = 1 - P$.

d = degree of accuracy/precision expected = 0.05.

Substituting for the above formula:

$$N = 1.96 \times 1.96 \times 0.263 \times 0.737 / (0.05)^2 = 286.577,$$

Addition of 20% attrition, sample size = 345.

Women who had undergone hysterectomy, are physically and mentally unable to undergo an interview and pelvic examination, were pregnant and were menstruating at the time of this study were excluded. Socio-demographic details of the subjects were obtained using previously pretested semi-structured and researcher administered questionnaire. The smoking habits, reproductive history, sexual habit of the women and their partners, previous exposure to STDs, and life time use of contraceptives were also obtained.

For the collection of cervical smears, each woman was placed in the dorsal position in a consulting clinic where privacy was assured and in the presence of a chaperone. The cervix was exposed using sterile disposable speculum. An Ayre spatula was then passed into the cervix and rotated through 360 degrees. This was immediately smeared on a glass slide and fixed immediately in 95% alcohol before transferring it to the cytology laboratory. A cytobrush was subsequently inserted into the cervical canal and rotated through 360 degrees. The cytobrush was removed, broken and transferred into a collection bottle containing Phosphate Buffered Saline (PBS) and stored at -20°C until it was transferred to the laboratory for HPV DNA test.

2.1. HPV DNA Isolation and Typing

The cervical samples were pre-treated and Viral DNA was extracted from GITC lysates using High Pure Viral DNA kit (Roche, UK) following the manufacturer's instructions. The High Pure Kit uses Spin Column method of DNA extraction. This was carried out according to manufacturer's instruction.

Typing of HPV DNA was done using Multiplex PCR. Four sets of multiplex PCR was carried out using Eppendorf Mastercycler Nexus Gradient (Eppendorf, Germany). The primers were designed based on E1, E6 - E7 and L1 regions of the HPV genome.

The Primer sets were: GP168 (for HPV types 11, 16, 18 and any other type); MY313 (for HPV types 31, 33 and others), OLIS35 (for HPV35 and others), and CpG mix were used for the HPV detection. All primers were synthesized by Invitrogen UK (Life Technology, UK). For all reactions, the Platinum Multiplex PCR Master Mix (Life Technology, UK) was used.

Viral DNA samples were thawed. A worksheet was created. Reactions were set up in 96 well PCR plate (thin walled) and sealed after all additions and placed in a PCR machine (Eppendorf master cycler). The thermal profile was set up and PCR was allowed to run. The plate was brought out and electrophoresis was performed on all the specimens. The gel was carefully brought out and viewed

under the UV light.

The images were captured using Genome-mini Gel documentation system (VWR, UK) and transferred to computer where they were stored for interpretation of the results. Amplicons were read using the known sizes and DNA ladder.

2.2. Cervical Cytology (Papanicolaou Test)

In the cytology laboratory, the slide fixed smears were stained and examined microscopically for the dysplastic cells as characterized by anaplasia, hyperchromatism and large nucleus. The staining was done following the Papanicolaou conventional method and a cytopathologist read all the slides and reported them following the Bethesda classification.

3. Results

3.1. Demographic Characteristics of the Participants

The mean age of the 405 participants was 39.97 ± 9.25 years. They were predominantly of the Igbo ethnic group 388 (95.6%) and Christians 407 (99.3%). The socio-demographic characteristics of the respondents are shown in **Table 1**.

3.2. Prevalence of HPV Infection

Out of the 405 subjects, HPV DNA was detected in 79 giving a prevalence of 19.5%. The commonest HPV serotype identified was HPV 16 which was seen in 7.7% (31/405) followed by HPV 18 accounting for 5.9% (24/405). HPV 33 occurred in 2.2%, HPV 35 occurred in 0.7% while HPV 68 occurred in 0.7%. Infection with multiple serotypes of HPV was present in 2.2% of the women. The prevalence was highest among those aged 30 - 35 years while the lowest prevalence was among ages 50 - 59. **Figure 1** shows the graphic representation of the age specific prevalence of HPV in these women showing a double peak at ages 30

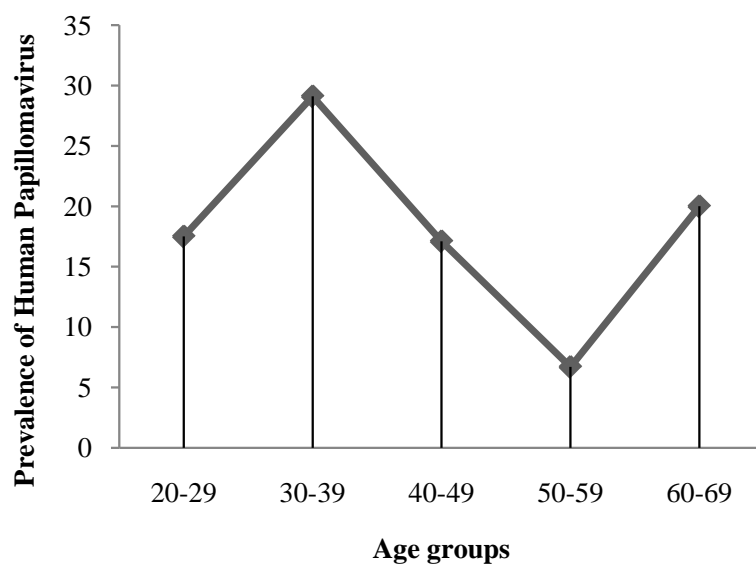


Figure 1. The age specific prevalence of HPV infection among the participants.

Table 1. Socio-demographic characteristics of the respondents.

Variable	Frequency	Percentage
Age (N = 405)		
20 - 29	57	14.1
30 - 39	125	31.4
40 - 49	152	37.5
50 - 59	59	14.6
60 - 69	10	2.5
Marital Status (N = 405)		
Married	273	67.4
Single	81	20.0
widowed	51	12.6
Level of Education (N = 405)		
No formal education	17	4.2
Primary level	40	9.9
Secondary level	123	30.4
Tertiary level	225	54.9
Ethnicity (N = 405)		
Igbo	388	95.6
Hausa	4	1.0
Yoruba	4	1.0
Others	9	2.2
Religion (N = 405)		
Christianity	402	99.3
Islam	3	0.7
Age at coiterchy (N = 405)		
≤17 years	76	18.8
>17 years	329	81.2
HIV status (N =364)		
Positive	126	34.6
Negative	238	65.4
Not sure	45	8.9
History of STI (362)		
Yes	177	48.9
No	185	51.1

- 39 and 60 - 69. Eighteen women out of the 405 had abnormal cervical cytology in the form of Low-Grade Squamous Intraepithelial Lesion giving a prevalence of 4.4%. The prevalence of HPV infection in these women with abnormal cervical cytology was 22.2% while it was 19.4% among those with normal cervical cytology. Only HPV 16 was isolated from women with abnormal cytology.

With bivariate analysis, significant factors associated with HPV infection were age less than 40 ($P = 0.008$), being married ($P = 0.001$), early coitarchy ($P < 0.001$), multiple sexual partners ($P = 0.02$), history of previous STI ($P < 0.01$), and cigarette smoking ($P = 0.001$) (**Table 2**). However, when subjected to multiple logistic regression, only young age ($P = 0.01$, OR = 0.42 [0.21, 0.84]), early coitarchy ($P \leq 0.001$, OR = 0.21 [0.1, 0.43]) and multiple sexual partners ($P = 0.01$, OR = 0.4 [0.19, 0.82]) remained significant.

Table 2. Association between HPV infection and select variables.

Variable	HPV positive		Chi Square	P-value
	No	%		
Age				
<40 (184)	46	25.0	6.5	0.010
≥40 (221)	33	14.9		
Marital status				
Single (132)	13	9.8	11.6	0.0006
Married (273)	66	24.2		
Age at coitarchy				
≤17 (76)	30	39.5	23.8	<0.001
>17 (329)	49	14.9		
Type of marriage				
Monogamy	64	21.7	0.53	0.47
Polygamy	8	27.6		
Multiple sex partners				
Yes (193)	46	23.8	5.2	0.02
No (203)	30	14.8		
History of STI				
Yes (177)	52	29.4	18.3	<0.001
No (185)	21	11.4		
On Contraceptive				
Yes (162)	40	24.7	3.2	0.07
No (210)	36	17.1		
Cigarette smoking				
Yes	3	100	12.4	0.0004
No	76	19.1		
HIV status				
Yes (126)	19	15.1	2.7	0.10
No	54	22.2		
Anti-retroviral drug				
Yes (102)	12	11.8	4.6	0.03
No (24)	7	29.2		
Cervical smear				
Normal (387)	75	19.4	0.09	0.77
Abnormal (18)	4	22.2		

4. Discussion

The prevalence of HPV infection obtained in this work (19.4%) among cytologically normal women and 19.5% in the overall women is comparable to other results obtained from different parts of the country. Prevalence of 14.7% was obtained from Irun Nigeria [11], 21.6% in Okene, Nigeria [17], 26.3% among cytologically normal women in Ibadan Nigeria [14], 19.6% in western Nigeria [19] and the highest prevalence was obtained 37% in Abuja Nigeria [12]. Higher prevalence has been recorded in other African countries—38.8% in Kenya [8], 74% among young girls in Tanzania [6] and even lower prevalence (12.5%) in Senegal [13].

The differences may be influenced by the age group studied, environment and variations in assays methods.

Age specific prevalence recorded in this research was highest in age group 30 - 39 (29.1%) and a slight peak in the age group 60 - 69 (20.0%). The two peaks in the middle and old ages were also observed by Thomas *et al.* [14] in Ibadan, Nigeria. In Irun Nigeria, HPV prevalence did not decline with age but with slight peaks in women 15 - 29 and 60 - 69 years old [11] while in Abuja (Nigeria), there was a steady decline of HPV prevalence in older women [12]. In other parts of the world, very high prevalence in the younger age [3] [4] [5] and a steady decline in the older age [8] [20] [21] have been recorded.

Age group as a factor in HPV acquisition has remained almost the same in all parts of our country—Nigeria with slight differences. High prevalence of HPV infection in a very young age [11] [12], middle age group [14] and a second peak in the old age has been consistent. This work equally witnessed the same pattern, though none of the participants were less than 20 years (the age at which the highest prevalence occurs). The high prevalence in the middle group may have been because this is a hospital-based research, thereby attracted more women of child bearing age that tend to go to hospital more often. The second peak in the old age may probably be because of acquisition of new sexual partners later in life especially among the widows [14].

In this study, HPV 16 was the most prevalent serotype and this is consistent with findings from other studies [5] [7] [21]. However, the finding differs from others within Nigeria and Africa where serotypes 35 and 52 were more prevalent [8] [9] [11] [12]. This difference in type specific prevalence from place to place according could be influenced by the type of assay used and by the high proportion of multiple HPV infection in certain populations. Okolo *et al.* [22]., argued that even though HPV 35 may have been reported in Nigeria, the risk of developing invasive cervical cancer was more in the individuals infected with HPV 16 and 18 than those with other high-risk types.

HPV is a sexually transmitted infection and this study has demonstrated a relationship between sexual behaviour and HPV infection. Early commencement of sexual activity, history of STI and having multiple sexual partners were significantly related to HPV infection.

Early age at first sexual intercourse may be considered an indicator for early age at first exposure to HPV and other STIs [23] [24]. In this study, HPV infection was found to be higher among the participants that had their first sexual intercourse before the age of 17 and this is consistent with findings from other studies [25] [26] [27].

Contrary to what would be expected, HIV positivity did not significantly affect the prevalence of HPV infection. This may however be because the proportion of the respondents is small and so not powered enough to detect such difference. Again, the use of anti-retroviral drugs may also be modifying the influence of HIV infection on host response to other infective organisms. This may actually explain the finding of a significantly higher prevalence among HIV pos-

itive respondents receiving anti-retroviral drugs in this study. Ezechi *et al.* [19] equally reported higher prevalence of HPV infection among HIV patients that were not on ARD in South Western Nigeria.

5. Conclusion

Prevalence of HPV infection in this study is 19.4% among women with normal cytology, and 22.2% among women with low grade squamous intraepithelial lesion (LSIL). Five different HPV types (16, 18, 33, 35 and 68) were identified using multiplex PCR. Multiple HPV types (2.2%) were equally detected in some samples. The most prevalent HPV type was HPV 16 (7.7%). HPV prevalence was bimodal with the first peak in the age group 30 - 39 and second peak at age group 60 - 69. Some factors including early age at first sexual intercourse, marriage, polygamous marriage, multiple sexual partners, smoking, and age were found to be positively associated with HPV transmission. This study has also shown that the currently available HPV vaccines will cover for the predominant HPV types in this study population.

6. Limitations and Strengths

The strength of this work was our ability to type the HPV DNA, thereby making it possible to identify the prevalent HPV type in Awka. The limitation was that it was a hospital-based research, hence may not represent the true population. The young girls that usually have the highest prevalence of HPV infection [3] [4] [5] were not captured in this work and that may have affected the age prevalence peaks.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix I: Questionnaire

QUESTIONNAIRE 1 (the information given here is confidential. This is to be used in determining the risk factors associated with HPV infection

- 1) Age..... Phone number.....
- 2) Place of residence.....
- 3) Occupation (a) trader (b) civil servant (c) commercial sex worker (d) others (specify)
- 4) Marital Status (a) single (b) married (c) widowed (d) divorced
- 5) Husband's occupation (if married).....
- 6) Level of education (a) no formal education (b) primary (c) secondary (d) tertiary
- 7) Ethnicity (a) Igbo (b) Hausa (c) Yoruba (d) others
- 8) Religion (a) traditionalist (b) Muslim (c) Christianity (d) others
- 9) Denomination (for Christians) (a) Roman Catholic (b) Pentecostal (c) protestants
- 10) Age at first sexual intercourse.....
- 11) Type of marriage (a) monogamous (b) polygamous
- 12) Does your husband have any extramarital relationship (a) yes (b) no (c) not sure
- 13) Number of sexual partners (cumulative) (a) one (b) two (c) more than two (d) numerous
- 14) How many times have you delivered before?
- 15) Do you smoke (a) yes (b) no (c) smoked before
- 16) If you have stopped smoking, when was that (a) <a month (b) <a year (c) >a year
- 17) How often do you smoke (a) very often (b) once in a while
- 18) HIV status (a) positive (b) negative (c) not sure
- 19) Are you on ARD (Anti-Retroviral Drugs) (a) yes (b) no
- 20) What kind of contraceptive do you use (a) condom (b) hormonal contraceptive (c) intra-uterine device
- 21) Do you have any abnormal vaginal discharge (a) yes (b) no (c) not sure
- 22) Do you have any vaginal rash (a) yes (b) no (c) not sure
- 23) Have you been treated of any sexually transmitted infection before (a) yes (b) no (c) not sure