



PREVALENCE OF HUMAN PAPILLOMA VIRUS INFECTION FROM CERVIX OF WOMEN ATTENDING SELECTED HOSPITALS IN ABUJA, NIGERIA

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ABSTRACT

Introduction: Cervical Cancer is the second most common cancer caused by the persistent infection with Human Papilloma Virus (HPV). A high rate of infection has been recorded worldwide among which Nigeria is inclusive.

Aim: This study was aimed at detecting the prevalence of HPV infection among women and identifying socio demographic and risk factors associated with infection in the study area.

Methods: The study was a hospital based, where cervical swab samples were randomly collected from women seen at the general outpatient department (G.O.P.D) and the gynaecology department of selected hospitals within the Federal Capital Territory.

Results: A total of five hundred and one (501) consenting women were tested using Enzyme Linked Immunosorbent Assay (ELISA) after obtaining signed consent and demographic data from each participant using questionnaires. The Chi Square test was used to determine the relationship of risk factors with the rate of infection statistically using IBM SPSS version 23 software package. The prevalence of HPV infection in this study among participants with mean age 35.90 ± 8.40 was 10.98%. Women who participated were within the age of 15 to 64 years. Demographic data and risk factors such as Age group ($\chi^2 = 9.508$, $P = 0.050$), Educational Status ($\chi^2 = 55.909$, $P = 0.000$), Marital Status ($\chi^2 = 15.390$, $P = 0.000$), HIV Status ($\chi^2 = 11.871$, $P = 0.001$) and Number of sexual Partners ($\chi^2 = 6.252$, $P = 0.012$) were found to have significant association with HPV infection statistically.

Conclusion: The prevalence of the HPV in this study exposes the level of the burden of HPV infection in the study area which justifies the need to increase the level of surveillance on females at risk of infection in Nigeria.

Keywords: Cancer, Human Papillomavirus (HPV), sexually transmitted infections, Infectious disease, Prevalence.



INTRODUCTION

Cervical cancer is the second most common malignancy in women and is a major cause of cancer related death among women worldwide. [1] Studies have shown that about 500,000 new cases are diagnosed every year with approximately 85% of deaths occurring in developing countries of the world. [2] In Nigeria, the incidence of cervical cancer is 14,550 per 100,000 and the mortality rate is 9,659 per 100,000. [3] Approximately more than 200 types of HPV have been identified. The classification of these types and species origin depends on the degree of homology between the viral genomes detected using DNA hybridization. About 40 HPV types infect the genital mucosal and are categorized according to their carcinogenic potential. [4] The virus is transmitted mostly through sex and it is not easily detected at the early stage of infection. Clinical manifestations of HPV infection include genital warts, recurrent respiratory papillomatosis, Cervical Intraepithelial Neoplasia (CIN), and cancers, including cervical, anal, vaginal, vulva, penile, head and neck cancer. [5] Epidemiological studies have shown that human papillomavirus (HPV) is the main cause of cervical cancer and precancerous lesions. The strains involved in cancers are also known as High-risk HPV (HR-HPV) and they include: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 -66 and -68. [6] The prevalence of HPV infection is been reported to be between 10-20% in Sub-Saharan Africa and it is dependent on the age of the patient and the presence of cytological abnormalities. In some populations, cross-sectional studies revealed that 20% - 40% of sexually active young women have detectable HPV infection and that prevalence decreases with age. [4]

Previous studies on Human papilloma virus in among Nigerian women have been conducted but limited to prevalence. [7, 8, 9, 10, 11, 12, 13] To the best of knowledge, scanty information and insufficient data is available on the prevalence of HPV among women leaving in Abuja, which makes it difficult for medical practitioners to recommend effective therapeutics to women at risk. The above-mentioned challenges require urgent attention to reduce the mortality rate of cancer caused by HPV. This will enable the government implement health policy interventions to control cervical cancer and other cancers associated with HPV among population under study.

The use of drugs for the treatment of cancer related issues has failed. One option that appears to be promising is the prophylactic measure (use of vaccine) towards effective prevention and control of cancers in Nigeria. This study intends to determine the prevalence of Human Papilloma Virus (HPV) in circulation within the Federal Capital Territory Abuja, Nigeria and also identify demographic and risk factors associated with the spread of the disease.

MATERIALS AND METHODS

Study Population

The study involved 501 women randomly selected who were attending the Obstetrics and Gynaecology Clinic of the six hospitals selected. The procedures followed were in accordance with the ethical standards of the health management board committee on human experimentation and with the Helsinki Declaration of 1975. Also, the ICMJE requirement on privacy and informed consent was adhered to and to that effect, a structured questionnaire was administered to the

women, after an informed consent duly signed had been obtained from each participant. Anonymity and details given by each participant was kept confidential. Ethical clearance was also obtained from the ethical committee of health management board of the Federal Capital Territory Administration.

Inclusion criteria:

- i. Women who are attendees of the selected hospitals and they are within the age range of 15 to 65 years.
- ii. Women who have given their consent.
- iii. Women who are physically and mentally stable.

Exclusion criteria:

- i. Women who are not attendees of the selected hospitals and they are below 15 years or above 65 years.
- ii. Women who did not give their consent.
- iii. Physically or mentally unstable women.

Determination of Sample Size

The sample size for this study was determined using the equation below

$$n = \frac{t^2 \times p(1-p)}{m^2} \quad [14]$$

Where n = sample size

t = confidence interval at 95% (standard value of 1.96)

p = prevalence rate [14]

m = marginal error at 5% (standard value of 0.05)

To calculate n using the 12.4% prevalence obtained by [12]

See details below:

$$12.4\% = 0.124$$

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

$$n = \frac{1.96^2 \times 0.124(1-0.124)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.124(0.876)}{0.0025}$$

$$n = \frac{0.4172899584}{0.0025}$$

$$n = 166.91598$$
$$n = 167$$

Since larger sample size give minimal errors statistically, [15] 167 was multiplied by 3 to give 501.

Data Collection using Questionnaire

A structured questionnaire was administered to individuals who gave their consent. English and other local dialects were used where necessary to aid in acquiring needed socio demographic information of the participants.

Sample Collection

Cervical smear was collected from February 2018 to June 2018 by a Gynecologist after visual inspection. Sample collection was performed as described, [16] in which each of the participants was placed in a dorsal

position, with her legs flexed at the hip and knee abducted. The labia were parted with gloved thumb and index fingers. A Cusco's bivalve speculum which is not lubricated was passed and fixed to visualize the cervix, under a bright light source. The detachable end of the cervical brush was then inserted into the cervix and rotated through 360° movements, either in a clockwise or counter clockwise direction, to scrape the entire squamocolumnar junction of the transformation zone.^[11] All samples were collected from all hospitals involved using this method.

Sample Preparation

The cervical smear collected was immediately placed in 20mls of Liquid Prep collection vial and stored at freezing temperature before being transported to DNA laboratory in Kaduna for further analysis as described by.^[11]

HPV detection using Enzyme Linked Immunosorbent Assay (ELISA)

The HPV antibodies were detected in cervical swabs of women using HPV ELISA kit by MyBioSource.Inc (Cat No. MBS9358118). All reagents and samples were brought to room temperature naturally for 30min before starting assay procedures. A low speed centrifugation for one or two seconds was done to concentrate the positive and negative controls to the bottom of the vials. Positive control wells, negative control wells and sample wells were set, then 50µl of positive control was added to each positive

control well and 50µl negative control was added to each negative control well. 50µl of each sample was added to each sample wells. The kit was designed to be used with undiluted samples. Then 100µl of HRP-conjugate reagent was added to positive control wells, negative control wells and sample wells and then covered with an adhesive strip and incubate for 60 minutes at 37°C. The plate was then washed using the wash solution four times. 50µl Chromogen solution A and 50µl chromogen solution B was added to each well successively. It was gently mixed and then incubated for 15 minutes at 37°C. Fifty microlitre of stop solution was added to each well. The change in colour was observed in the wells which changed from blue to yellow. Results was been read at an Optical Density (O.D.) of 450 nm using an ELISA reader within 15 minutes after adding Stop Solution.^[17]

Statistical Analysis

Chi square analysis was performed to determine the relationship between the rates of HPV infection and socio-economic factors using IBM SPSS version 23 statistical software package.

RESULTS

The results of this study indicated that among the five hundred and one (501) women who participated, fifty-five (55) tested positive for the presence of Human Papilloma Virus (HPV) infection making the prevalence rate of HPV infection to be 10.98% (Table 1).

Table 1: Prevalence of Human Papilloma Virus (HPV) among the Women Sampled

HPV Status	Frequency	Percentage
HPV +VE	55	10.98
HPV -VE	446	89.02
Total	501	100

Looking at the socio demographic factors, majority of participant were within the age range of 15-64 and the average mean age was 35.90 ± 8.40 . The prevalence rate was high among women within the age group 35-44 years at 4.8%. About 87% of the participants were married and the HPV prevalence (7.8%) was also higher within this population. About 50.3% of the participants were civil servant and most of the participant (69.9%) had tertiary education and HPV prevalence was higher among this same population (Table 2).

Table 2: Distribution of Demographic Factors among Women infected with Human Papilloma Virus

S/N		Total (N = 501) Number (%)	HPV +VE Number (%)	HPV -VE Number (%)	Mean \pm SD	P-value
1	Age group				35.90\pm8.40	0.050\dagger
	15-24	21(4.00)	1 (0.20)	20(3.80)		
	25-34	204(40.70)	18 (3.60)	186(37.10)		
	35-44	192(38.30)	24 (4.80)	168(33.50)		
	45-54	73(15.00)	8 (1.60)	65(13.4)		
	55-64	11(2.00)	4 (0.80)	7(1.2)		
2	Marital Status					0.000\dagger
	Single	45(9.00)	8 (1.60)	37(7.40)		
	Married	436(87.00)	40 (7.80)	396(79.20)		
	Divorced/widowed	20(4.00)	7 (1.40)	13(2.60)		
3	Occupation					0.427*
	Civil servant	252(50.30)	28 (2.60)	224(47.7)		
	Student	21(4.20)	1 (0.20)	20(4.00)		
	House wife	67(13.40)	5 (1.00)	62(12.40)		
	Business	153(30.5)	21 (4.20)	132(26.3)		
	Farming	8(1.60)	0 (0)	8(1.60)		
5	Educational Status					0.000\dagger
	Primary	59(11.8)	5 (1.00)	54(10.8)		
	Secondary	92(18.3)	10 (2.00)	82(16.3)		
	Tertiary	350(69.9)	40 (7.90)	310(62)		

\dagger significant difference exists, * No significant difference.

The result as shown in Table 3 indicates different risk factors associated with persistence of HPV infection. There was a significant relationship ($P = 0.05$) between incidence of HPV and HIV

status. This indicates that patients with HIV are more likely to have HPV compared to HIV negative patient (23.4 – 9.0%), $\chi^2 (1, N = 495) = 11.87, P = 0.001$. Also, there was a significant relationship between incidence of HPV and number of sexual partner. Patients with multiple sex partners are more likely to have HPV compared to single sex partners (27.3 – 10.2%), $\chi^2 (1, N = 501) = 6.25, P = 0.012$.

Table 3: Prevalence of HPV Infection among Women in relation to Risk Factors

S/N	Factors	Total Number (%)	HPV +VE Number (%)	HPV –VE Number (%)	P- value	Odd ratio
1	Use of Contraceptives					
	Yes	49 (9.80)	3 (0.60)	43(9.20)	0.304*	0.537
No	452 (90.2)	52 (10.40)	400(81.80)			
2	History of sexually transmitted Infection					
	Yes	200(39.90)	26 (5.20)	174(34.7)	0.238*	1.402
No	301(60.10)	29 (5.80)	272(54.3)			
3	Do you smoke					
	Yes	5(1.00)	1 (0.20)	4(0.8)	0.399*	2.513
No	496(99.00)	54 (10.78)	442(89.78)			
4	HIV status					
	Positive	64(13.00)	15 (3.00)	49(10.00)	0.001†	3.077
Negative	431(87.00)	39 (7.8)	392(79.20)			
5	Number of sexual Partner					
	Single	479(95.60)	49 (9.80)	430 (85.8)	0.012†	0.304
	Multiple	22(4.40)	6 (1.20)	16(3.20)		
6	No of children					
	0 – 2	282 (56.30)	31 (6.20)	251(50.1)	0.466*	
	3 – 5	193(38.50)	23 (4.60)	170(33.90)		
	6 and above	26(5.20)	1 (0.20)	25(5.00)		
7	Complication History					
	Miscarriage	143(28.50)	18 (3.60)	125(24.9)	0.635*	
	Stillbirth	19(3.80)	2 (0.40)	17(3.40)		
	Both	31(6.20)	5 (1.00)	26(5.20)		
	None	308(61.50)	30 (6.00)	278(55.5)		
8	Do you Take alcohol					
	No	371(74)	34 (6.80)	337(67.2)	0.078*	

Mild	80(16.00)	12 (2.40)	68(13.6)
Moderately	50(10.00)	9 (1.80)	41(8.20)
Heavy	0(0)	0 (0)	0(0)

9 Age at first sexual intercourse

>18	105 (21.00)	11(2.20)	94(18.80)		
<18	396(79.00)	44 (8.80)	352(7.20)	0.853*	0.936

† significant difference exists, * No significant difference.

However, there was no significant association ($P > 0.05$) between incidence of HPV and use of contraceptives, history of sexually transmitted infections (STIs), the risk of smoking, number of children, complication history during child birth, alcohol intake and age at first sexual

intercourse. There was also no significant association ($P > 0.05$) between incidence of HPV and level of awareness (Do you know what HPV is, do you know what is cervical cancer is and have you had a cervical screen test done before) as shown in Table 4.

Table 4: Prevalence of HPV Infection among Women in Relation to Level of Awareness

		N = 501				
S/N	Factors	Total Number (%)	HPV +VE Number (%)	HPV -VE Number (%)	P-value	Odd ratio
1	Do you know what HPV is?					
	Yes	255(50.90)	28 (5.60)	227(45.3)		
	No	246 (49.10)	27 (5.40)	219(44.3)		
2	Do you know what Cervical Cancer is?					
	Yes	200(40.00)	26 (5.20)	174(34.8)	0.238	1.402
	No	301(60.00)	29 (5.80)	272(54.2)		
3	Have you had a Cervical Screening Test Done before?					
	Yes	47(9.40)	6 (1.20)	41(8.20)	0.680	1.210
	No	454(90.60)	49(9.80)	405(80.8)		

DISCUSSION

The study showed an overall prevalence rate of 10.98%. The prevalence in this study is lower as compared to studies carried out in Okene Kogi State, Nigeria, which recorded a prevalence rate of 21.6%.^[18] Also studies carried out in Ibadan,^[7] and in Ile Ife,^[1] showed a higher prevalence of 26.3% and 21.6% respectively. A study also from the north central Nigeria reported a higher prevalence of 37%.^[10] A prevalence rate of 48.1% was reported in Gombe the North-eastern part of Nigeria^[19] and a higher prevalence rate of 70% was also recorded in a study in Kano state^[20]. The high level of infection within the North might be due to variation among the study population with varying exposure to different risk factors, diverse culture and geographical location, also owing to the fact that the Northern part of Nigeria is known for polygamous family lifestyle^[19].

Studies on prevalence among HIV infected women in Lagos recorded a rate of 19.6%^[21]. The lower prevalence found in this study was probably due to the fact that it was a hospital-based study and was also not limited to women with cervical lesions or abnormalities, while other studies within Nigeria indicated either community-based studies or targeted towards women with cervical lesions or abnormalities. Previous studies conducted in the Sub-Saharan Africa indicated a generally high prevalence rate with some variation depending on how the target group was selected and also the method of assay used. Using a PCR based assay, HPV prevalence rate were 40% in Mozambique^[22], 31% in Harare, Zimbabwe^[23], 18% in Dakar, Senegal^[24] and 44% in Nairobi, Kenya^[25]. A prevalence rate of 66.1% in Burkina Faso^[26] and 60.7% in Sudan^[27] was also recorded. Using the Hybrid Capture assay, 17% HPV prevalence was reported in rural Uganda^[28]. A higher prevalence rate of 81% has been reported within the Middle East and North African region.^[29]

The age pattern showed peak of HPV positivity in women within the age range of 35-44 years (4.8%). This is similar to studies in Ibadan which reported a peak of HPV infection (mainly high-risk types) among women younger than 25 years

and a consistently high prevalence among middle aged and older women.^[1] There was a high prevalence recorded among women within the age range 35-44 years and a higher prevalence of HPV infection among younger women decreased with age^[10]. Statistically, there was a significant difference between HPV infection and the different age groups ($P = 0.050$). Although the variation in age prevalence of HPV is well documented and appears to largely reflect differences in sexual behaviour across geographical regions^[30]. The difference between this study and that of the previous studies from Nigeria may be due to the characteristics of the populations and region surveyed. The explanation for the persistent high prevalence of HPV in middle and older aged women, specifically in Nigerian societies where polygamy is generally accepted, is that a fraction of men and women (mainly men) may continue to have multiple sexual partners throughout their life and therefore re-infect themselves and their spouses.^[7] Additionally, as previously suggested, women in developing countries, like Ghana, Nigeria and India, may have decreased ability to clear HPV infections, possibly due to accompanied genital infections or nutritional deficiencies, since the development of an efficient immune response against HPV acquired over age is the generally accepted reason for the decline in HPV prevalence observed in other populations.^[7] The groups of women who remain persistent carriers of HPV by middle age are now considered the high-risk group for cervical cancer.^[31] Prevalence in relation with age group is also similar to a finding^[31] and the report from Costa Rica who found a high prevalence among women within 35-54 years and those from Mexico who reported a high prevalence among 35-44 years in Mexico^[32].

Lack of education had been associated with the high-risk of sexual practices and a poor health seeking attitude^[33] this will cumulate in the increased presence of sexually transmissible infections like HPV. The statistical analysis had a significant difference between the rate of HPV infection in relation to the level of education and this is similar to a report^[19] This study showed a significant association between the HPV infection and HIV status ($P = 0.001$) agreeing with

the result of some studies, this suggests that HIV infection is a known risk factor for HPV infection.^[34] This study is also similar to a study in Jos north central Nigeria,^[35] who recorded a prevalence of 9.0% among HIV positive women. A study in Lagos recorded a prevalence rate of 44.9% among HIV positive women in Lagos Nigeria. Other studies suggest that the probability of HPV infection is increased by about 100% in HIV infected persons.^[37] A report found out that the sexual contact at young age increases the time for HPV infection to progress to precancerous lesions and eventually invasive cancer especially in HIV positive persons^[38]. This suggests that, infection with HIV is an important risk factor for HPV infection and development of HPV associated lesion in the female genital tract and also immune damage caused by HIV increases the risk of developing cervical cancers. A meta-analysis reported a prevalence rate of 56%^[29] and also a report from a study conducted on HIV population in Kenya recorded a prevalence rate of 61%^[39] which suggests that the risk of HPV among people living with HIV is high in African countries.^[29] International study shows a prevalence of 27% among women receiving treatment in an STD clinic in America in a study.^[40] The prevalence was highest among person aged 14-19 years and it was observed that it decreased with increasing age.^[40]

This study indicated no association between HPV infection and the number of birth or parity, but studies stated otherwise^[19]. This study suggested a higher rate of HPV infection among women who had between 0 to 2 numbers of children. Another study however indicated a higher prevalence rate among women who had between three to four numbers of children.^[41] It was also observed in a study that women with high parity (three pregnancies or more) accounted for 83.7% of women with high risk HPV^[1,13] and a study done in Washington D.C, USA also reported same.^[42]

Various reasons have been put forward to explain the rise in positivity among this population such as hormonal changes in pregnancy resulting in reducing immunity, to the exposure of the ectocervix during repeated child birth resulting in easy attachment of the Human Papilloma Virus in

addition to damage of the cervical epithelium during childbirth and easy accessibility of the virus to be incorporated into the cellular matrix of the cervix.^[43] The Cancer research UK also stipulated that the women who have had 7 or more children had double risk of HPV infection. This indicates that the risk of getting infected with the virus doubles with the number of times a woman gives birth because of the occurrence of cervical trauma at the time of delivery. There are tendencies of weakening and rupture of epithelial cells around the cervix thereby enhancing chances of infection when in contact with the virus. It has also been reported among group of low-income earners that the prevalence of HPV increases with the number of pregnancies, although another study reported no association between numbers of pregnancies and HPV infection.^[32]

There is still insufficient data to give final conclusions about the effect of number of births on the risk of HPV infections.

This study indicated that there was also a significant association between the HPV infection rate and the number of sexual partners ($P = 0.012$). This is similar to a study in Brazil^[44] and in Nigeria^[19] which showed a significant association with HPV positivity in relation to number of sexual partners ($P = 0.001$). Also, a similar research work done in Guinea also demonstrated a significant difference between number of sexual partners and HPV infection.^[45]

The influence of the sexual orientation of women as a contributory factor to the occurrence of HPV is evident in this study as majority of the women with more than one lifetime partner and those with spouses with multiple sexual partners are associated with higher risk of acquiring oncogenic strains of HPV. This role of multiple sexual partners in acquiring HPV infection was also observed in a study done in Columbia^[46]. The presence of multiple sexual partners increases the risk of acquisition of sexually transmitted infections. Looking at previous work done in comparison with this study, one can come to a conclusion that since HPV is sexually transmitted, having more than one partner is a risk

factor and could increase the rate of infection in location.

Jensen *et al* had established a link between heavy smoking and persistence of oncogenic strains of HPV. [47, 48, 49] These had been attributed to the nicotinic inhibition of the phagocytic property of cervical macrophages resulting in the persistence of the virus. Contrary to the above, this study did not show any association between smoking and the occurrence of HPV. Cultural inhibitions in the area under study frowned at smoking among women, which may have contributed to the low number of smokers in the study. Nevertheless, smoking was found not to be associated with HPV prevalence. There was no significant association between the rate of HPV infection and oral contraceptive use. A possible explanation is that the use of oral contraceptive is characterised by low intensity exposure and will often need longer exposure for more than 5 years to become a risk factor. [50]

CONCLUSION AND RECOMMENDATIONS

The prevalence of HPV infection in the studied environment was significant (10.98%) among participants with mean age 35.90 ± 8.40 . Demographic data such as age group, educational status, marital status, and risk factors such as HIV status and number of sexual partners were found to have significant association with HPV infection statistically. The findings observed in this study has exposed the level of the burden of HPV infection in the study area. There is therefore the need to increase the level of surveillance on females at risk of cervical cancer in this environment. More so, Government agencies should promote a more inclusive HPV vaccine in their national immunization scheme, which has a wider coverage as against the one currently available in the country. This should be administered to girls at the appropriate age if HPV transmission is to be reduced. There is also need for sexual behaviour education and awareness in order to reduce the impact of the risks factors identified in this study. Population based study need to be done for further characterization of HPV genotypes in our environment.

Acknowledgement

The authors wish to acknowledge all the staff of the Gyneacology and Cytology units of Abaji General Hospital, Asokoro district Hospital, Bwari General Hospital, Kuje General Hospital, Kwali General Hospital and Nyanya General Hospital for their dedication towards working with us all through the stage of specimen collection. Also we would like to appreciate the staff of DNA Labs Kaduna for opening their laboratory to conduct this study. Finally, the authors acknowledged Dr. Umobong for her inputs during this research work.

Competing Interest: All authors have declared that no competing interest exist among them.

Authors' Contribution

Author A designed the study, wrote the protocol and the first draft of the manuscript, Author C performed the statistical analysis, Author B managed the analysis of the study and Author D managed the literature searches while author E reviewed the protocol and literature. All Authors read and approved the final manuscript.

Declaration of Originality

We hereby declare that the present article has not been submitted before but has been independently written. All passages including those from the internet which were used directly and in modified form especially those sources using text, graphs, charts or pictures are indicated as such. We realized that an infringement of these principles would amount to attempt of deceit leading to proceedings against myself.

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