



## Prevalence of Human Papilloma Virus among women of child bearing Age in Yola Adamawa State, Nigeria

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### Abstract

Epidemiological, molecular and clinical evidences have shown that cervical cancer is caused by the human papilloma-virus (HPV), especially genotype 16, 18 and 45. This study aims to survey the prevalence of high-risk types of HPV 16, 18/45 among women of child-bearing age. Three hundred (300) women aged 18 years and above were randomly selected from the community and patients attending Specialist Hospital Yola (S.H.Y) and Federal Medical Center (F.M.C) Yola, Adamawa State. Relevant sexual and socio-demographic information was obtained from each subject using a questionnaire. High Vaginal swab samples were collected and analyzed using APTIMA Assay to identify the high-risk HPV genotype 16, 18 and 45. Out of the 300 samples analyzed, 56 (18.7%) were positive, 238 (79.3%) were negative for HPV and 6 (2%) are invalid. Out of the positive samples obtained, 29 (51%) were HPV 16 while 27 (48.2%) were HPV 18/45. The study also showed that the age grade of 24-41 years had the highest prevalence of 25.6%. No positive sample was found among age grades of 18-23. The result shows that HPV prevalence was significantly associated with the number of sexual partners ( $P=0.009$ ).

**Keywords:** Cervical-cancer, Human papilloma virus, High vaginal swab, high risk, sexually transmitted, APTIMA assay.

### INTRODUCTION

Human Papilloma virus (HPV) infection is caused by a human Papilloma virus, a DNA virus from the Papovoviridae family. HPV is a common virus that affects both females and males. Most sexually active men and women will probably acquire a genital HPV infection at some point in their lives (Cohen, 2005). Most types of HPV are harmless, do not cause any symptoms and are self-limiting (CDC, 2003). HPV infection on the genitalia is the most common viral sexually transmitted disease, and it has been estimated that at least 50% of sexually active adults have had a genital HPV infection. Cohort studies indicate that genital HPV infection with oncogenic types is mostly transient and that only a small proportion of those infected become carriers and then develop cervical intraepithelial neoplasia (Hildshein *et al.*, 1994). There are fourteen HPV genotypes that are considered pathogenic or high-risk for cervical cancer disease. Multiple studies have linked genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 to disease progression (Monsonogo *et al.*, 2003). Women with an infection with one of these types have an increased risk for developing severe dysplasia or cervical

carcinoma. HPV infections are very common and most women will clear HPV infections within 6-12 months. The presence of HPV nucleic acid does not mean that cervical dysplasia or cervical cancer is present. However, an effective approach for detecting cervical disease is to target those oncogenic elements of HPV that foster persistent viral infection and cellular transformation (Monsonogo *et al.*, 2003). Studies have shown that different types of high-risk HPV confer different levels of risk for developing severe dysplasia or cervical carcinoma. Globally, HPV types 16, 18 and 45 are associated with approximately 80% of all invasive cervical cancers. These three types are found in 75% of squamous cell carcinomas, with type 16 alone found in over 60% of all squamous cell carcinomas. In adenocarcinomas, HPV types-16, 18 and 45 are found in 80-94% of cases, with types 18 and 45 composing almost half of these infections. The presence of HPV type 18 in an early stage of cervical cancer has been associated with a poor prognosis. HPV type 18 and 45 are under-reported in precancerous lesions, which may be caused by the presence of occult lesion of the cervical canal inaccessible to colposcopy examination (Safaein *et al.*, 2009).



In women infected with HPV 16 and or 18, the cumulative risk of developing cervical diseases is 10- fold higher than other risk types (Walboomer *et al.*, 1999).

Incidence rate of cervical cancer in Nigeria is 25/100,000 while the reported prevalence rate for HPV in the general population is 26.3 %. The incidence of HPV in women with cervical cancer is reported to be 24.8 %. In most, cancer of the cervix, especially in developing countries, present at advanced stages when curative measures are unlikely to be successful (Thomas *et al.*, 2004).

Proper determination and typing of high-risk HPV will help know the prevalence of HPV and create awareness, early detection, and encourage women to prevent the spread of cervical cancer. Therefore, it becomes imperative to determine the high-risk type of human Papilloma-virus among women of child bearing age in the state.

## MATERIALS AND METHODS

### Study Area

The research was carried in Yola, Adamawa State capital. Adamawa is a state in North Eastern Nigeria. It lays between 80°N and 11N and longitude 11.50 and 13.50 E. It was formed in 1991 from part of Gongola State with four administrative divisions namely: Adamawa, Ganye, Mubi and Numan. It is one of the thirty-six (36) States which constitute the Federal Republic of Nigeria. The State shares border with Gombe State to the North, and Borno State to the North East, while to the West it is bordered with Taraba State as well as the Republic of Cameroon to the East (adamawastate.com).

### Ethical consideration

Ethical approval was obtained from the State Ministry of Health Yola Adamawa with reference number S/MOH/1331/1 and Federal Medical Center Yola, Adamawa State, reference number FMCY ISUB/96NOL.11XX and Specialist Hospital Yola Reference number ADS/SHY/SUB/77/VOL.1. Informed consent approval was signed by the participating population before collecting samples from them.

### Demographical Data of Participant

A questionnaire was given to obtain demographical data of the individual. The questionnaires were filled with individuals' data such as age, marital status, and occupation.

### Study population

The study targeted women of child-bearing ages of 18 years and above within the communities and those attending the two major Hospitals (F.M.C and S.H.Y) for various medical assistance.

### Sample size determination

The sample size was determined using the

formula

$$N = \frac{z^2 p (1-p)}{d^2}$$

Where; N= the sample size, P=Expected prevalence or proportion. Z= Statistic for a level of confidence which is 1.96 at 95 %. d=Precision. For this study, p=25% based on previous prevalence by (Naing *et al.*, 2006), 300 was obtained as the sample size.

### Specimen Collection and Processing

With a sterile swab stick, high vaginal swab samples were obtained from the participants and the swab sticks were returned aseptically to the vials before processing. The samples are then separately transferred into a specimen container containing specimen transport media (S.T.M.) that lyses the cells before the analysis (APTIMA, 2012).

### Assay Procedure

Four hundred microliter 400µl of each sample was pipetted into a container, 100µl of captured reagent was added, A barcode scanner was used to scan each sample before loading into the APTIMA HPV 16 18/45 Assay Machine, it was incubated for 35 minutes at 62 °C, after 35 minutes the temperature was reduced to 25 °C for hybridization to occur, the supernatant was aspirated. 1.0 ml of detergent was added, vortexed, centrifuged and the supernatant aspirated. The temperature was raised to 62 °C and 75 µl of amplification reagent was added and 200 µl of oil was also added mixed and incubated for 10 minutes then the temperature was reduced to 42 °C for 5 minutes, 25 µl of enzyme reagent was added allowed to stand for 60 minutes then the temperature was raised to 62 °C, 100 µl of probe reagent was added vortexed and incubated for 20 minutes to detect the amplicon, 25 µl selection reagent was added vortexed and incubated for 10 minutes to differentiate between the hybridized and unhybridized probes, light emitted from the labeled DNA hybrids is measured as photon signals called Relative Light Units (R..LU) in a luminometer. The samples were also analyzed using positive and negative controls of genotype 16, 18/45 the positive ones are again analyzed using the same controls to rule out false positive. Final assay results were interpreted based on the analyte signal-to-cutoff (S/CO) ratio described in the APTIMA HPV 16, 18/45 Genotype Assay manual (APTIMA).

## RESULTS

Overall prevalence of HPV in the study area was 18.7 % (n=56), 79.3 % (n=238) were negative. Out of the positive samples obtained 51.7 % (n=29) were HPV 16 while 48.2 % (n=27) were HPV 18 and 45 as shown below (Fig. 1).

The age grade 34-41 years had the highest prevalence rate of 25.6 % followed by age grade 42 years above with a prevalence rate of 19.8 % and 0 % rate was recorded amongst age grade 18-25 years (Fig. 2). The results of this study have shown that there is a significant association between numbers of sexual partners and

prevalence of HPV when analyzed statistically using Chi-Square at 95 % confidence level (Table 1). However, HPV prevalence and occupation has been shown not to any association with one another when tested statistically using Chi-Square at 95 % confidence interval (Table 2).

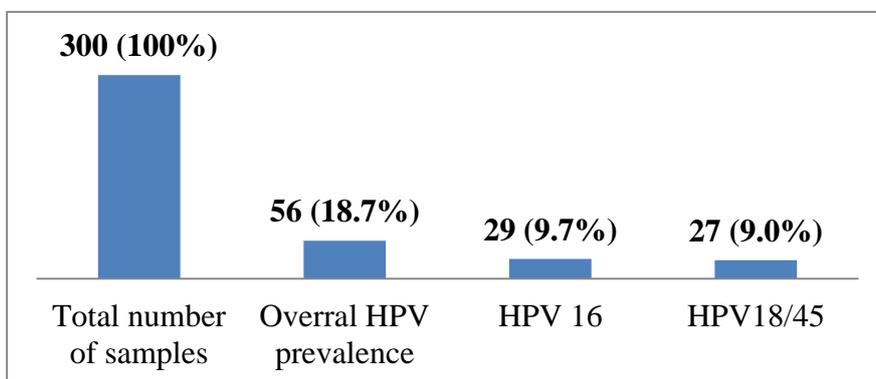


Figure 1: Prevalence of HPV among women of child bearing age in Adamawa State

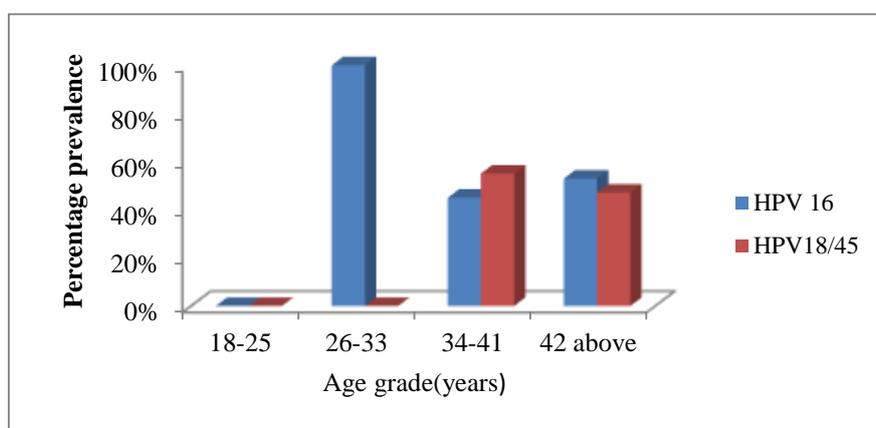


Figure 2. Prevalence of HPV genotype with respect to age

Table 1. Association between HPV prevalence and number of sexual partner

Age years)	Number of Sexual Partner						Total
	One		Two		Three and above		
	Pos	Neg	Pos	Neg	Pos	Neg	
18-25	0 (0.2)	15 (5.6)	0 (2.2)	6 (7.8)	0 (3.3)	0 (12)	31 (31.0)
26 -33	0 (0.1)	4 (3.4)	1 (1.3)	4 (4.8)	1 (2.0)	9 (7.3)	19 (19.0)
34-41	0 (0.5)	9 (14.0)	7 (5.5)	21 (19.5)	13 (8.3)	28 (30.2)	78 (78.0)
42 above	2 (1.1)	26 (31.0)	13 (12.0)	44 (43.0)	18 (18.3)	69 (66.0)	172 (172.0)
<b>Total</b>	<b>2 (2.0)</b>	<b>54 (54.0)</b>	<b>21 (21.0)</b>	<b>75 (75.0)</b>	<b>32 (32.0)</b>	<b>116 (116.0)</b>	<b>300 (300.0)</b>

Pearson chi (15) = 30.9235, Pr = 0.009

Values in the brackets are the expected frequencies.

Pos. Positive samples

Neg. Negative samples

Table 2. Prevalence of HPV in association with occupation

Age-Grade	Students		Civil Servant		Traders		Housewives		Farmers		Total
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	
18-25	0 (0.3)	8 (2.5)	0 (1.6)	5 (6.1)	0 (2.3)	8 (7.4)	0 (0.7)	6 (4.5)	0 (0.9)	4 (4.7)	31 (31.0)
26-33	0 (0.2)	1 (1.5)	0 (0.9)	2 (3.7)	1 (1.4)	3 (4.6)	0 (0.4)	4 (2.8)	1 (0.6)	7 (2.9)	19 (19.0)
34-41	1 (0.8)	6 (6.2)	7 (3.9)	20 (15.3)	8 (5.7)	19 (18.7)	2 (1.8)	6 (11.4)	2 (2.3)	7 (11.7)	78 (78.0)
42-above	2 (1.7)	9 (13.8)	8 (8.6)	32 (33.8)	13 (12.6)	42 (41.3)	5 (4.0)	28 (25.2)	6 (5.2)	27 (25.8)	172 (172.0)
Total	3 (3.0)	24 (24.0)	15 (15.0)	59 (59.0)	22 (22.0)	72 (72.0)	7 (7.0)	44 (44.0)	9 (9.0)	45 (45.0)	300 (300.0)

Pearson chi-square (27)= 40.9799 Pr= 0.041

Values in the brackets are the expected frequencies.

Pos. Positive samples

Neg. Negative samples

## DISCUSSION

The prevalence of HPV positivity of 18.7% found in this study in some part of Adamawa state; Nigeria is consistent with previous reports of the elevated prevalence of HPV in women in Nigeria and Sub-Saharan Africa. Studies carried out in Ibadan Nigeria showed a prevalence of 26.3% in a population base study according to Thomas *et al.*, (2004). Prevalence of 66.10 % was reported in Burkina-Faso (Didelot-Roussen *et al.*, 2006). HPV surveys in sub-Saharan Africa have generally shown relatively high HPV prevalence with some variation, depending on how women were selected, and how HPV was tested. For example, 17 % prevalence of high-risk HPV types was found in rural Uganda (Serwada *et al.*, 1999), while 25 % prevalence was found among HIV-negative women in Harare, Zimbabwe (Womack *et al.*, 2000). Prevalence of 18 % was also recorded in Dakar and Pikene, Senegal (Xi and Toure, 2006). Prevalence of HPV with specific genotype shows that HPV 16 has the highest prevalence with the highest rate amongst age grade 26- 33 years, followed by HPV 18/45 with the highest rate amongst age grade 34-41 years. This finding is in line with some work carried out in some Africa countries, with variations in the relative ranking of HPV types that are compatible with chance and everywhere the predominance of HPV 16 and 18 rises with the increasing severity of cervical findings (Clifford *et al.*, 2003). Also type-specific distribution of HPV among cervical cancer biopsies from Africa showed that HPV 16 accounted for 50.2 % of samples, HPV 18 for 14.1 %, and HPV 45 for 7.9 % i.e., a distribution similar to that found worldwide) [Clifford *et al.*, 2006]. The age pattern of HPV prevalence also differs somewhat from one country to another. The predominant pattern includes an early peak, soon after the start of sexual intercourse (Jacobs *et al.*, 2000, Kiaer *et al.*, 2001), followed by

lower levels of HPV positivity in middle age [Jacobs *et al.*, 2000, Annh *et al.*, 2003, Sanjose *et al.*, 2003]. In three studies from sub-Saharan Africa (Serwada *et al.*, 1999, Castellsague *et al.*, 2001, DeVust *et al.*, 2003), the prevalence of HPV declined with an increased in age. In contrast, a study by Xi *et al.* (Xi and Toure, 2006) indicated that high-risk, but not low-risk HPV types, were more frequently detected in older than younger women.

According to Nweke *et al.*, (2013) older females between the ages of 25-34 years were more likely to be infected with high risk HPVs than those less than 25 years and those 55 years and above. This is similar to what was discovered in this study and among HIV positive Rwandan women in whom prevalence peaked in those aged 25-34 years and declined in those greater than 55 years old (Singh *et al.*, 2009). This may be explained by the time taken for persistence to develop in the 25-34 years age range and the lower incidence of sexual activity in the >55 years age range.

Statistical analysis of HPV prevalence based on the number of sexual partners shows that there is significant association between number of sexual partner and HPV prevalence as majority of the positive subjects are having more number of sexual partners. This is in line with World Health Organization (WHO, 2006) report that risk factors for persistent HPV infections include early age of first sexual intercourse, multiple sexual partners, smoking, and poor immune function. Bosch, *et al.* (2006) also reported that HPV is transmitted by direct skin-to-skin contact with vaginal and anal sex being the most common methods. Occasionally, it can spread from a mother to her baby during pregnancy. It does not spread via common items like toilet seats.



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