



## PREVALENCE AND RISK FACTORS OF HIGH RISK HUMAN PAPILLOMAVIRUS INFECTIONS AMONG WOMEN ATTENDING GYNAECOLOGY CLINICS IN KANO, NORTHERN NIGERIA

\* Auwal<sup>1</sup>, I. K., <sup>2</sup>Aminu, M., <sup>3</sup>Atanda, A.T., <sup>4</sup>Tukur, J. and <sup>1</sup>Sarkinfada, F.

<sup>1</sup>Department of Medical Microbiology and Parasitology, Faculty of Medicine, Bayero University, Kano Nigeria

<sup>2</sup>Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria Nigeria

<sup>3</sup>Department of Histopathology, Bayero University/Aminu Kano Teaching Hospital, Kano Nigeria

<sup>4</sup>Department of Obstetrics and Gynaecology, Bayero University/Aminu Kano Teaching Hospital, Kano, Nigeria

\*Correspondence author: [auwalkabuga@yahoo.com](mailto:auwalkabuga@yahoo.com)

### ABSTRACT

*Cervical cancer is the most common female cancer in northern Nigeria, yet the pattern of infection with human papillomavirus, the principal aetiologic agent is unknown. This was a preliminary study conducted in two referral hospitals in order to establish base-line data on the prevalence and risk factors for the infection in Kano state, Nigeria. Fifty (50) randomly selected women aged 18 years and above were recruited from gynaecology clinics in Murtala Muhammad Specialist Hospital and Aminu Kano teaching Hospital. Relevant sexual and socio-demographic information were obtained from each subject using a questionnaire. Exfoliated cervical cells were harvested and processed using Polymerase Chain Reaction to identify the DNAs of high-risk HPV types 16 and 18. The prevalence rate of HPV infection was 76% [(38/50) at 95% CI=61.8-86.9] with 60.5% (23/38) having co-infections with both HPV type 16 and 18. Risk factors of the infection include low literacy level; living in rural settlements; low parity; early menarche (<15 years of age); early onset of first sexual intercourse (≤16 years of age) and multiple sexual partners. There was however, no statistically significant association between oral contraceptive usage and acquisition of the infections. Findings of this study suggest a high prevalence of HPV types 16 and 18 among women attending gynaecology clinic in Kano and thus called for more elaborate community based study in order to establish the magnitude of the problem from wider perspective.*

**Keywords:** Human Papillomavirus; Prevalence; Risk Factors, Northern Nigeria

### INTRODUCTION

Human Papillomavirus (HPV) infection is a prominent concern in both research and medical fields. It is the most common sexually transmitted infection affecting sexually active women at some point in their lives (Munoz *et al.*, 2003; Gavillon *et al.*, 2010). Although HPV has been associated with many diseases, its association with cervical cancer has particular significance, being the principal cancer in most developing countries (Munoz *et al.*, 2003).

The prevalence of cervical infection with HPV in women varies greatly in the African continent where some of the highest rates are found. Population-based HPV prevalence surveys show a wide variation in the prevalence of HPV infections among sexually active women aged 15-65years, ranging from 2% in Hanoi (Anh *et al.*, 2003), 14.8% in Bagota, Columbia (Molano *et al.*, 2002) to 26.3% in Ibadan, Nigeria (Thomas *et al.*, 2004) and 66.1% in Burkina Faso (Didelot-Rousseau *et al.*, 2006). In northern Nigeria cervical cancer has a relatively high prevalence (Mohammed *et al.*, 2008). However, the prevalence and risk factors for acquisition of HPV infection have not been documented.

The objective of this study was to determine the prevalence of high risk Human Papillomavirus types 16 and 18 and associated risk factors among women

attending gynaecology clinic in Kano, Northern Nigeria.

### MATERIALS AND METHODS

The study was a hospital based multicentered descriptive cross-sectional study that was conducted in two referral hospitals in Kano, Aminu Kano Teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital (MMSH), Kano. Ethical clearance was obtained from Kano State Hospitals Management Board and Aminu Kano Teaching Hospital ethical review boards. Informed consent was obtained from each woman before enrolling into the study.

#### Sample size

A sample of 300 women aged 18 years and above among patients attending gynaecology clinics at Aminu Kano Teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital (MMSH) was determined using the equation described by Sar Mukaddam and Garad (2006) based on a previously established prevalence of HPV infections in Ibadan, Southern Nigeria.

#### Questionnaire Administration

A guided questionnaire was administered to each of the subjects to obtain relevant socio-demographic, sexual and reproductive characteristic profiles before collecting cervical scrapings.

Pregnant, post-hysterectomy, post-cervical conisation, physically or mentally challenged women and women bleeding per vaginum were excluded (Thomas *et al.*, 2004).

### **Sample Collection and Processing**

Sterile wooden Ayres' spatula was used to exfoliate cells from the ecto- and endocervix of the 300 eligible subjects. All the 300 samples were screened for cervical cancer using conventional cytology. A sample of 50 women (comprising 18 with abnormal cytology and 32 with normal cytology among the screened women) selected by simple random sampling were further screened for HPV types 16 and 18 DNA using the cervical cells. Exfoliated cells from each of the 50 subjects were collected and immersed in 5mls of phosphate buffered saline (a transport medium) in universal rubber container, stored at 4°C and transported in ice packs to the Biotechnology and Research Center of Ahmadu Bello University, Zaria (ABU) for Polymerase Chain Reaction (PCR) detection of viral DNA (Baseman and Koustky, 2005).

### **Detection of Viral DNA using Polymerase Chain Reaction (PCR)**

DNA extraction was carried out using the ZR genomic DNA™-tissue mini prep kit (Zymoresearch cooperation, South Africa). HPV types 16 and 18 DNA amplification was carried out using individual Primer sets (Inqaba biotechnical industries Pty Ltd, South Africa). The reaction was performed according to the PCR master mix manufacturer's protocol (Fermentas life science, South Africa).

### **Statistical Analysis**

Statistical analysis was performed using SPSS version-10, Minitab version-15 and Epi info Version-3.5.1 statistical packages. Chi-square test and Odds ratio were used at 0.05 significance level and at 95% confidence intervals to test for statistical association between HPV positivity and the risk factors for HPV infection.

### **RESULTS**

The age distribution of the women as depicted in Table 1, shows that 30 (60%) were in the age range of 21 to 39 years while those 50 years and older constituted only (8%) of the studied women. Table 1 also shows that 39 (78%) of the women attained menarche at ≤ 15 years of age, 26 (52%) had their first sexual experience at ≤ 16 years of age with 1 out of every 3 either being in a polygamous setting or had multiple sexual partners in the last 12 months. Multiparity was a feature of most (64%: 32/50) of the women with only 4 (8%) having used oral contraceptives pills before.

Of the total 300 women screened for cervical cancer, 282 (94%) had normal cytology while 18 (6%) had some form of abnormal cytology results (Table 2).

Up to 38 (76%) of the 50 women (95% CI=61.8-86.9) were positive for HPV DNA while the remaining 12 (24%) were negative. Fifteen (39.5%) of those

infected had single infection [6 (15.8%) with HPV 16; 9 (23.7%) with HPV 18)] while the remaining 23 (60.5%) were co-infections (Table 3). The trend of the infection rate (Table 3) shows 100% (9/9 cases) infection rate in women ≤20 years of age and in women between 40 and 49 years (7/7); 65% (11/17) in women aged between 21 and 29; 62% (8/13) in women aged between 30 and 39 years and 75% (3/4) in women > 50 years of age. The age group 30 to 39 years however, showed the highest co-infection rate of 75% (6/8 cases) and those 21 to 29 years had the lowest rate of 36.4% (4/11).

The highest risk factor for infection with HPV was the level of education of the subjects (Table 4). Women who had no formal education were four times more likely to have HPV infection than those who had formal education (OR=1:4.5). The risk of HPV infection in women living in the rural settlements was three-fold more than in women living in urban areas (OR=3.4:1). Women at low parity were also more than three times likely to have HPV infection than their multiparous counterparts (OR=1:3.3). Menarche before the age of 15 years, age at first sexual intercourse ≤16 years and multiple sexual partners were each also associated with about two-fold increased risk of being infected (OR=1:2.2; 1:1.7; 1:1.8). There was however, no association between oral contraceptive usage and risk of the infection among the studied group (OR=1:0.9).

### **DISCUSSION**

The socio-demographic characteristics of women in this study show relative non-exposure to western education, early age at first sexual intercourse, multiple sexual partners and high parity. This socio-demographic pattern parallels that found in a similar study from Ghana, another West African country (Domfeh *et al.*, 2008).

The high prevalence of 76% for HPV infection recorded in our study reflects the observation that has been reported in other community based studies (Clifford *et al.*, 2005; De sanjose *et al.*, 2007) that developing countries, particularly within Africa, have the highest prevalence rates for the disease. Similar high prevalence rates were reported from other parts of Africa; 66.1% in (De vuyst *et al.*, 2003) and 40% in rural Mozambique (Castellsauge *et al.*, 2001). In Nigeria, Thomas *et al.*, 2004 documented a 26.3% prevalence rate of HPV infection in a community based study in Ibadan, South-Western Nigeria. The very high prevalence rate from our study sites may reflect the high prevalence of cervical cancer in communities in Kano and its environs (Mohammed *et al.*, 2008). However, a community based survey is required to establish the prevalence of HPV infection among women in the state. The age pattern observed in our study, with bi-modal appearance; peak infection rates in the under twenties and those in the age group 40 to 49 years is also typical of the pattern described for populations, like ours, with high incidence of cervical cancer (Wiredu and Armah, 2006).

**Table 1: Socio-demographic, sexual and reproductive characteristics of the women**

Characteristics	Frequency(%)
<b>Age</b>	
≤20	9(18)
21 – 29	17(34)
30 – 39	13(26)
40 – 49	7(14)
≥50	4(8)
<b>Formal education</b>	
Yes	20(40)
No	30(60)
<b>Place of living</b>	
Urban	40(80)
Rural	10(20)
<b>Age at menarche</b>	
≤15yrs	39(78)
>15	11(22)
<b>Age at 1<sup>st</sup> sexual contact</b>	
≤ 16yrs	26(52)
>16yrs	24(48)
<b>No. of sex partners</b>	
One (1)	33(66)
Two (2)	15(30)
≥3	2(4)
<b>Parity</b>	
(≤1)	18(36)
Multiparous	32(64)
<b>Oral contraceptive use</b>	
Never	46(92)
Ever	4(8)

Mean age of the women=28years,

Median age at first sexual intercourse=16years.

**Table 2: Frequency of Cervical Cytology Findings (Pap smear)**

Cytologic Classification	Frequency(%)
<b>Abnormal</b>	
ASC-US	4(1.3)
LSIL	10(3.3)
HSIL	2(0.7)
SCC	2(0.7)
<b>Normal</b>	282(94)
<b>TOTAL</b>	300(100)

Prevalence of abnormal cervical cytology (Pap smear) was 6% with a 95% confidence interval CI of (3.6-9.3).

**NB: ASC-US:** Abnormal squamous cells of undetermined significance.

**LSIL:** Low grade squamous intraepithelial lesion.

**HSIL:** High grade squamous intraepithelial lesion.

**SCC:** Squamous cell Carcinoma

**Table 3: Single and co-infections in different age groups.**

Age grp (yr)	No tested	Single infection(%)	Co-infection(%)	Overall positive(%)
≤ 20	9	3(33.3)	6(66.7)	9(100)
21 – 29	17	7(63.6)	4(36.4)	11(65)
30 – 39	13	2(25)	6(75)	8(62)
40 – 49	7	2(28.6)	5(71.4)	7(100)
≥ 50	4	1(33.3)	2(66.7)	3(75)
<b>Total</b>	<b>50</b>	<b>15(39.5)</b>	<b>23(60.5)</b>	<b>38(76)</b>

**Table 4: Risk factors for HPV infections among women in Kano**

Variable	No. Tested	N (%)	OR <sup>b</sup>	95%CI	P-value
<b>Age (yrs)</b>					
<30	26	20(76.9)	1		
≥30	24	18(75)	1.1	(0.3-4.9)	0.9
<b>Educational level</b>					
Yes	30	20(66.7)	1		
No	20	18(90)	4.5	(0.8-34.5)	0.057
<b>Place of living</b>					
Urban	40	29(72.5)	1		
Rural	10	9(90)	3.4	(0.4-80.4)	0.2
<b>Age at menarche (yrs)</b>					
>15	11	7(63.6)	1		
≤5	39	31(79.5)	2.2	(0.4-11.8)	0.2
<b>Age at 1<sup>st</sup> sex. contact</b>					
>16	24	17(70.8)	1		
≤16	26	21(80.8)	1.7	(0.4-7.8)	0.4
<b>No. of sex. Partners</b>					
One (1)	33	24(72.7)	1		
>1	17	14(82.8)	1.8	(0.3-9.9)	0.3
<b>Parity</b>					
≤1	17	15(88.2)	1		
>1	33	23(69.7)	3.3	(0.5-25)	0.2
<b>Oral contraception</b>					
Never	46	35(76.1)	1		
Ever	4	3(75)	0.9	(0.1-26.1)	1.0

<sup>b</sup>Adjusted for age.

The high positivity rate in very young women may be attributed to peak period of recent infection and acquisition from sexual partners. This is reflected in poor usage of contraceptive methods by this age group in our study. As for the high infectivity rate that has been observed in older (peri- and postmenopausal) women Thomas *et al*, 2004 have suggested that changes in sexual behaviour of both the male and female sexual partners may be responsible for this, with male partners continuing to have multiple sexual partners. In addition to this it is our opinion that, this period coincides with periods when polygamous men in our population take younger wives, and the choice of new wives are usually in women around their twenties, who themselves are in their peak periods of infectivity. The men then re-infect their older spouses.

The risk factors for HPV infection identified by our study are similar to those identified for cervical cancer in Northern Nigeria (Adewuyi *et al*, 2008); foremost of these being illiteracy. Dwelling in rural areas compared to urban living was also associated with increased risk of being positive for oncogenic cervical HPV infection. This finding contrasts with data from other authors who have either noted a higher prevalence among urban women than rural women or have noted no difference (Kliucinskis *et al*, 2006; Pinto *et al*, 2011). A possible explanation for this is reflected in our findings that higher percentages of women from polygamous settings were from the rural areas and also have low literacy level. Early menarche, early sexual exposure due to early marriages, and multiple sex partners usually secondary to high rates

of divorce and polygamy among communities in Kano could be associated with about 2-fold risk of HPV acquisition as reported in other studies (Thomas *et al*, 2004; Domfeh *et al*, 2008).

While much attention has been paid to the natural history and epidemiology of genital HPV infection among females little inference has been drawn concerning male genital infection. The high prevalence of female genital infection with oncogenic HPV described in this study would suggest a correspondingly high male genital infection with the virus and low acceptance of barrier contraceptive use among both sexes. Nicolau *et al*, 2005 on the other hand have shown as much as 60% prevalence of detectable oncogenic HPV among male sexual partners of infected women; the prepuce (44% of cases) and distal urethra (30% of cases) being the more common sites from which viral DNA was detected.

From the foregoing it follows that a community based survey on the prevalence and risk factors of HPV infection among women in communities particularly in Kano and Northern Nigeria is needed to establish the magnitude of HPV infections and the associated risk factors at the community level. This could justify the need for establishing a readily accessible cervical cancer screening programme to enable early detection of cervical HPV infection. However, to minimize the risk of HPV infection, there is an immediate need to improve levels of education of the general populace, of women in particular and with special focus on rural areas.

This strategy, though not targeted at changing marriage practices such as polygamy could improve the awareness of both women and men on reproductive health and compliance with intervention strategies such as cervical smear testing and the use of contraceptives. There is also need to establish readily accessible cervical screening program in the country to enable early detection of early abnormalities of the cervical epithelium following HPV infection as means to reducing the enormous cervical cancer burden in this region.

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- In conclusion, this study suggests a relatively high prevalence of high-risk HPV types 16 and 18 infection among women attending gynaecology clinics in Kano. Women at higher risk of HPV infection were those with low level of literacy (OR=1:4.5; P=0.057), attaining menarche  $\leq$ 15 years of age (OR=1:2.2; P=0.2), being  $\leq$ 16 years at first sexual contact (OR=1:7; P=0.4), having multiple sexual partners (OR=1:1.8; P=0.3), low parity (OR=1:3.3; P=0.2) and living in rural settlements (OR=1:3.4; P=0.2).
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