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Full Length Research Article

HUMAN PAPILLOMAVIRUS 16 SPECIFIC IMMUNOGLOBULIN G ANTIBODIES AND ITS CORRELATES AMONG WOMEN ATTENDEES OF SELECTED HOSPITALS IN SOUTHERN KADUNA, KADUNA STATE, NIGERIA

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ABSTRACT
Human papillomavirus type 16 (HPV-16) is one of the high-risk viruses that cause cervical cancers. Persistent infection with the virus leads to development of precancerous lesions of the cervix in infected women, which without medical intervention can progress to invasive cervical cancer. This study assessed HPV16-specific immunoglobulin G (IgG) antibodies in the serum of women attendees of General Outpatient Department (GOPD) at General Hospital Kafanchan and Kagarko in Kaduna State. This was a cross-sectional study. Structured, self-administered questionnaire was used to collect information and blood samples were also collected for testing. This was in addition to information collected from the laboratory analyzed. Five milliliters (5mls) of blood were aseptically collected from each of the 200 women, who had no history of HPV vaccination at the time of the study for the determination of HPV16-specific IgG antibodies using enzyme-linked immunosorbent assay method. The sero-positivity for HPV 16-specific IgG antibodies among the women was 24.5%. The sero-positivity among women who had their first sexual intercourse at age 13-19 years was 37.6%, and it was significantly different from those who had their sexual debut at ≥20 years (14.8 %) (p=0.001). Sero-positivity also increased from 9.2 % in women with one lifetime sexual partner to 60.0% in those with multiple sexual partners(p=0.001). The finding showed that the women in this study have been exposed to the HPV-16 virus. Further study with a larger population of women in this locality to determine the level of susceptibility or immunity to HPV-16 is strongly advocated, among others.

Keywords: Enzyme-linked immunosorbent assay, Human papillomavirus, Immunoglobulin G, Sero-positivity.

INTRODUCTION

Human papillomavirus (HPV) is recognized as the major cause of skin or mucous membrane infections, leading to genital warts and cervical cancer (Schiffman *et al.,* 2007, Bray *et al.,* 2018). It is a double stranded DNA virus, 55nm with a genome (8kb) in a nucleoshistone core. It belongs to the papilloviridae family that contains more than 130 genotypes (Ma *et al.,* 2013).

The major route of transmission of HPV infection is sexual intercourse (Frazer, 2010). Risk factors such as smoking, prolonged oral contraception consumption, age of sexual debut, multiple sexual partners, co-infections, and multiparty, immune-related diseases have been reported as facilitator of carcinogenesis (Auwal *et al*., 2014). Low risk HPV is associated with benign neoplasms, whereas high risk HPV strains such as HPV-16 and HPV-18 causes approximately 70% of the cervical cancers; type-16 alone accounts for 50% of cervical cancers whereas type 18 is about 10% (Khan *et al.,* 2012).

Cervical cancer is the fourth most common cancer in women, with an estimated 528,000 new cases and 266,000 deaths worldwide (Torre *et al.,* 2012). In Nigeria, cervical cancers is the second cause of cancer in females, with about 14, 089 new cases diagnosed annually (Sung *et al.,*2021)). Since Human papilloma virus is not cultured routinely in the laboratory, immunologic techniques such as ELISAs that detect type-specific antibodies against HPV can be used in population as a measure of exposure to HPV and also serve as a tool to measure or study the immune status/ usually, after vaccination or natural infection, the presence of HPV16 IgG antibodies represents past exposure to HPV (Mohammed *et al.,* 2015).

Even though a minimum level of antibodies required for protection has not been defined for human, systemic level of HPV-specific IgG are readily detectable more frequently in patients with persistent HPV infection, hence HPV- antibodies based test also serves as a diagnostic tool (Thomas *et al.,* 2004). This study determined the level of exposure to HPV (anti-HPV-16 IgG antibodies) among women attendees of GOPD and Family Planning Unit of General Hospital Kafanchan and Kagarko in Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

General Hospital Kafanchan and the General Hospital Kagarko in Southern Kaduna, Kaduna State, Nigeria are the two biggest hospitals serving the people of Southern Senatorial zone of Kaduna state. They are secondary care facilities that provides ranges of services such as general outpatient consultation,

laboratory services, family planning Study Design
This was a cross-sectional study conducted between July and August 2016.

Study Population
The study population comprised 200 women aged 13 to 59 years that met the eligibility criteria attending GOPD and Family panning Units, in the two hospital during the period of the study. These included nonpregnant women aged 13-59 years attending GOPD and Family Planning Unit of Kafanchan and Kagarko General Hospitals in Kaduna State and have not received HPV vaccines. Women that have met the inclusion criteria but have received HPV vaccine were excluded from the study.

The 200 women that met the inclusion criteria were recruited at the clinics between July and August 2016 as they were seen at clinics both at Kafanchan and Kagarko General Hospitals. The total number of women recruited in Kafanchan General Hospital was 140 and 60 for Kagarko General hospital. As the women were seen at the clinics, after consultation, they were educated on the study and those that consented and had met the inclusion criteria were then recruited.

Collection and Processing of Blood Samples

Blood samples were collected aseptically by trained medical technician using 5mls syringes and needles and dispensed into sterile plain containers. The blood samples were allowed to clot for 30 minutes and centrifuged at 3,000 revolutions per minute for 20 minutes. The sera were extracted into pre-labeled screw capped cryovials with the aid of sterile pipette. Sera were stored at -20^{o}C until immunoassay were carried out. Anti HPV-16 IgG antibody was determined by ELISA technique as reported by Frazer *et al.* (2010).

Estimation of HPV-16 specific IgG Antibody using Enzyme-linked immunosorbent assay
Enzyme-linked immunosorbent assay was used for detection and quantification of HPV-16 specific IgG antibodies in the serum samples of the women enrolled into the study. The laboratory test was carried out according to manufacturer's instruction: One hundred (100) uL of the standards and serum samples each were added per well, covered with a plate sealer and incubated for 2 h at 37^{0}C. The wash solution of each well was extracted after each wash. One hundred (100) uL of biotin-conjugated antibody was added to each well, incubated for 1 hr at 37^{0}C. Each well was aspirated and washed, repeating the process three times for a total of three washes. The plate was inverted and blotted against clean paper towels. One hundred (100)uL of enzyme-conjugated Avidin was added to each well, covered with a new plate sealer, incubated for 1 h at 37^{0}C protected from light . Fifty (50) uL of stop solution was added to each well. The optical density of each well was determined using a micro plate reader set at 450nm.

Statistical analysis
The collected data was cleaned, entered into Statistical Package for Social Sciences version 21.0 (SPSS version 21.0 inc, Chicago IL, USA) software and analyzed. The results were presented as tables. Chi square test was used to determine the association between categorical variables at 95% Confidence Interval (CI) and p value of < 0.05 was considered statistically significant.

Ethical Approval
Ethical approval was sought and obtained from the Ethical Committee of Kaduna State Ministry of Health and permissions from the Management of Kafanchan and Kagarko General Hospitals. Informed consents were also obtained from the respondents. The respondent had the right to opt out of the study at any stage without any negative consequence. The researchers paid for the cost of the tests conducted. Those with positive results were linked to the appropriate clinics for further evaluation and management.

RESULTS
The result showed that 49/200 (24.5%) of women enrolled into this study had positive HPV 16 specific IgG antibodies status, meaning that they have been exposed to HPV.
Table 1 showed the distribution of human papillomavirus 16-specific IgG antibodies according in relation to the women's age groups. Women of age group 30-39 years had the highest sero-positivity of 28.2%, followed by those less than 20 years of age and 50 to 59 years (25.0 %) each and age group 40 -49 years (22.4%), while for the age group 21-29 years (19.4%). The difference in the sero-positivity by age of women was not statistically significant (p=0.875**).**

Table 1: Human Papillomavirus 16-specific IgG Antibodies and the Ages of the respondents

Age (years)	No. analyzed	No. positive	Percentage	X^2	p-value
<20	20	5	25.0	**1.217**	**0.875**
21-29	36	7	19.4		
30-39	78	22	28.2		
40-49	58	13	22.4		
50-59	8	2	25.0		
Total	200	49	24.5		

From table 2; the highest sero-positivity for HPV–specific IgG antibody was among the widows (36.4 %) followed by the divorcees (31.3%) and the singles (25.4%) while the married recorded lowest (21.6 %). The difference was not statistically significant (p = 0.630). In relation to women that smokes and those on hormonal contraceptives: there was no significant difference between women who smoke (27.3%) and those that do not smoke (24.0 %) (p = 0.685). The women on hormonal contraceptives recorded 21.7% sero-positivity while those who were not on hormonal contraceptives had significant sero-positivity (44.0%) for anti-HPV IgG antibody (p=0.015). Similarly, sero-positivity increased from Nulliparous women (20.0%) to multi-parous (≤5; 25.0 %) and (>5; 28.2 %) women. The increase was not statistically significant (p= 0.539).

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Table 2: Anti-HPV type 16 IgG Antibodies and the respondents' risk factors (n=200)

Variables	No. analyzed	No. positive	Percentage	χ^2	p-value
Marital status					
Married	102	22	21.6	1.733	0.630
Single	71	18	25.4		
Divorced	16	5	31.3		
Widowed	11	4	36.4		
Smoking					
Yes	33	9	27.3	0.164	0.685
No	167	40	24.0		
Hormonal contraceptives					
Yes	175	38	21.7	5.873	0.015*
No	25	11	44		
Parity					
Nulliparous	65	13	20.0	1.237	0.539
Multiparous (≤5)	64	16	25.0		
Multiparous (>5)	71	20	28.2		

Key: * indicates statistical significance

Table 3 showed sero-positivity for HPV 16 –specific IgG antibodies distribution according to sexual and reproductive behaviors. Sero-positivity (37.36%) 32/85 among women who had their first sexual intercourse at age 13-19 years was significantly different from those who had their sexual debut at ≥20 years (14.3 %) (p=0.001**).** Similarly, age at first pregnancy/birth in relation to distribution of HPV 16 IgG antibodies revealed that those within 13-19 years of age had 33.8 % (22/65) sero-positivity while women of age greater and equal to twenty (≥20 years), it was 20.0 %. (p=0.05).
Considering the number of sexual partner(s), women who have one, two, three and more than three (>3) sexual partner(s) had the following sero-positive results: 9.2 %, 29.9 %, 31.0 %, and 60.0 % respectively. While those of the participants spouse/partner's number of sexual partner(s) were: 8.9 %, 21.3 %, 23.4 % and 55.5 % respectively. There was significant difference between number of sexual partners of participants (p=0.006). There was also significant difference between spouse/partner and numbers of sexual partners (p=0.001).

Table 3: Seroprevalence of HPV 16 IgG antibodies and the respondents' sexual and reproduction behavior (n=200)

Characteristics	No. Analyzed	No. positive	Percentage	(χ^2)	p-value
Age at 1st Sex					
13-19	85	32	37.6	13.813	0.001*
≥ 20	115	17	14.8		
Age (first birth or Pregnancy)					
13-19	65	22	33.8	4.547	0.033*
≥ 20	135	27	20.0		
No. of Sexual Partner (s)					
One (1)	76	7	9.2	21.807	0.001*
Two (2)	67	20	29.9		
Three (3)	42	13	31.0		
> 3	15	9	60.0		
Spouse/Partner's number of sexual Partner (s)					
1	56	5	8.9	26.477	0.001*
2	61	13	21.3		
3	47	11	23.4		
> 3	36	20	55.5		

Key: * indicates statistical significance

DISCUSSION

This study determined the HPV 16- specific IgG antibodies among women attendees of GOPD and Family Planning Unit at General Hospitals Kafanchan and Kagarko, in Kaduna State.
About 1ne-fouth of the women were sero-positive for HPV 16-specific IgG antibodies.

Seroprevalence of H.pylori
This is higher than the prevalence of 13.2% reported by Mohammed *et al*. (2015) from Gombe, 15.8% reported by Auwal *et al.* (2014) from Kano, and 22.2% reported by Thomas *et al.* (2004) among women in Ibadan, Nigeria. These lower prevalence rates, in comparison with our work might be due to difference in Assay methodologies. The two researchers Auwal *et al.* (2014), John Hopkins Study Group (1999) employed HPV DNA using a polymerase chain reaction (nPCR) which detects the pathogen (HPV antigens) rather than HPV specific antibodies. ELISA as a screening test for HPV is limited by a high sero-positivity in women with probable prior exposure to HPV 16 without disease manifestation because the immune system might have cleared the HPV-antigen leading to the development of long-lasting memory (due to IgG antibodies).

The result of this study agrees with the report of the John Hopkin Hospital study group (1999) who also found similar sero-positivity rate of 24.2% among women in Brazil with invasive cervical carcinoma (Rocha-Brischillori *et al*., 2014).
In this study, the highest HPV 16 sero-positivity of 28.2 % was found in the age group 30-39 years. Anthony *et al*, (2008) observed similar trend among Australian women between the ages of 30-39years. The higher positivity rate of 25.0 % in women age ≤ 20 years in this study population may be attributed to early acquisition of infection as a result of early indulgence in sexual activity and early marriage. This implies that the young age group of women in this study may not have good knowledge on cervical cancer,

preventive measures and may likely not go for screening and utilize the vaccine against the cancer.

In addition, women in the study area often marry at younger age of 15 years. Aminu *et al*. (2014) in their study found out that the age of sexual debut in Nigeria is 9-10 years.
We also found in this study similar sero-positivity for anti-HPV-16 IgG antibodies among the divorced and widows indicating similar rate of HPV infectivity. This agrees with the findings of Schiecht *et al.* (2001), Menendez *et al.* (2010). Other studies have reported a highest HPV prevalence among single women (Adegbesan-Omilabu *et al.,* 2014, Rocha-Brischillori *et al.,* 2014). This means that all women, regardless of marital status were at risk of being infected depending on their various sexual lifestyles.

Furthermore, in this study hormonal contraception and not smoking was significantly associated with HPV-16 infection. In agreement with our findings, Adegbesan-Omilabu *et al*. (2014) reported that use of oral contraceptive pills, are significant risk factors for HPV infection among women attending the cytology clinic of a tertiary hospital in Lagos, South-West Nigeria. Our data however, disagree with report of study conducted by Auwal *et al*. (2014) in Kano, Nigeria. They reported an insignificant association between use of oral contraceptives and HPV infection. Again, our data, strongly disagrees with findings Quamrun *et al.* (2014) who conducted a population-based survey in Bangladesh where they established no significant association between oral contraceptive use and anti-HPV-16 IgG antibodies among women in Bangladesh. This may be attributed to the relatively few numbers of women involved in this study.

It was also noted in this study that women who had more than five children had higher seropositivity for antibodies to HPV 16, compared to those with 5 children and the less. This observation is consistent with a report by Okolo *et al.* (2010). This increase in sero-positivity of antibody to HPV 16 with increasing parity (number of children) has been attributed to increased sexual activity and hence increases likelihood of exposure to HPV (Okolo *et al.,* 2010). Age at first intercourse and pregnancy or birth were related to HPV-16 antibodies and it was found to be statistically significant. Our data disagrees with the findings of Olsen *et al. (*1997) worked on HPV 16 capsids. This may be because HPV 16 capsid is a better marker of past sexual behaviors than presence of HPV DNA and seropositivity to HPV16 capsid is positively associated sexual activity (Olsen *et al., (*1997).

The analysis of HPV-16 antibodies in relation to number of sexual partner(s)/spouse's number of lifetime sexual partner(s) showed that sero-prevalence markedly increased with an increasing number of life time sexual partners. A significant sero-prevalence increased from 9.2% for a woman with one sexual partner to 60.0% for women with more than three life time sexual partners. This pattern has been a consistent finding in epidemiological studies using HPV VLP-based ELISA and would be expected for a sexually transmitted infections agent (Dillne *et al.,* 1996, Viscid *et al.,* 1997). Acquisition of HPV infection has been shown to be strongly associated with sexual behavior and the prevalence of HPV increases with the number of sexual partners and early sexual debut (Mohammed *et al.,* 2015).

Limitations: This work was limited to the determination of HPV type 16 IgG antibodies. Some participants were not willing to disclose information related to their sexual activity. Due to cost, Pap smear for the cervical cancer screening was not done for the sero-positive women.

Conclusion
About one-fourth of the study population showed evidence of exposure to human papillomavirus type 16. Human Pappillomavirus-16 IgG sero-positivity was found to be associated with a lifetime number of sexual partners as expected of sexually transmitted infections (STI). Early sexual debut and multiple sexual partners were at higher risk of infection with human papillomavirus.

Recommendation
Further investigation on larger population; covering the entire state is strongly advocated to provide a more accurate picture of the epidemiology of HPV in the Kaduna state.

There is need for health education by the health
The study is hospital based and cannot be extrapolated to the general populace workers on the mode of transmission of the virus and risks factors associated with the HPV infection'.

Those who tested positive for HPV should go for screening (Papsmear) for possible intervention in order to prevent cervical cancer.

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