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Detection of Human Papillomavirus 16-Specific Immunoglobulin-G Antibodies among Women Attending General Hospital Kagarko, Kaduna State, Nigeria

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ABSTRACT

Background: Human papillomavirus type 16 (HPV-16) is one of the high-risk viruses that cause cervical cancers. The virus significantly accounts for cervical cancers in the world today. Persistent infection with HPV-16 leads to development of precancerous lesions of the cervix in infected women, which without medical intervention can progress to invasive cervical cancer.

Aim: This study aimed at determining HPV16-specific Immunoglobulin G (IgG) antibodies in the serum of consecutive consenting women attending General Out Patient Department (GOPD) at General Hospital Kagarko in Kaduna State.

Methods: This study was a cross-sectional, laboratory-based study. Self-administered structured questionnaire in addition to laboratory based information were used to collect data for analysis. Five (5) milliliters (mls) of blood was aseptically collected from 110 women, who had no history of HPV vaccination at the time of investigation, for the determination of HPV 16-specific IgG antibodies using enzyme-linked immunosorbent assay (ELISA) method.

Results: We reported 24.5% sero-positivity for HPV 16-specific IgG antibodies among the women. Sero-positivity increased from 9.5% in women with one lifetime sexual partner to 62.5% in those with multiple sexual partners (p= 0.006). Sero-positivity among women who had their first sexual intercourse at age 13-19yrs (38.3 %) is significantly different from those who had their sexual debut at ≥20 years (14.3 %) [P=0.004].

Conclusion: This 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 for possible intervention with HPV vaccine.

Key words: Enzyme-linked immunosorbent assay, Human papillomavirus, Immunoglobulin G, Sero-positivity

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INTRODUCTION

In the recent decades, human papillomavirus is recognized as the major cause of skin or mucous membrane infections, leading to gental warts and cervical cancer [1, 2]. Human papillomavirus (HPV) is 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.

The major route of transmission of human papillomavirus (HPV) infection is sexual intercourse (Ma et al. Infection with HPV leads to increased risk of developing cervical cancer [3, 4]. Risk factors such as smoking, prolonged oral contraception consumption, age of sexual

debut, multiple sexual partner, co-infections, and multiparty, immune-related diseases have been reported as facilitator of carcinogenesis [5]. Low risk HPV is associate 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% [6].

Cervical cancer is the fourth most common cancer in women, with an estimated 528,000 new cases and 266,000 deaths worldwide [7]. In Nigeria cervical cancer ranks the second cause of cancer in females, with about 14,089 new cervical cancer cases diagnosed annually [8]. 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 [9].

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 tool10. This study was conducted to determine the level of exposure to HPV (anti-HPV-16 IgG antibodies) among women GOPD and Family Planning Unit, General Hospital Kagarko, in Kagarko LGA of Kaduna State, Nigeria to contribute to the field of knowledge in cervical cancer.

MATERIALS AND METHODS

Study Area and Study Population

The study population comprises 110 women aged (13-59 years) attending GOPD and Family Planning Unit, the General Hospital Kagarko in Kagarko Local Government Area of Kaduna State. It is one of the biggest hospitals in Southern Senatorial Zone Kaduna, Kaduna State Nigeria.

Study Design

This was a cross-sectional, laboratory-based study. Structured, self-administered questionnaire and laboratory-based proforma were used to collect data for analysis.

Ethical Approval /Consent

After obtaining ethical approval from the Kaduna State Ministry of Health (KSMOH)'s ethical committee and permission from the management of General Hospital Kagarko; women who gave written consent were recruited and enrolled into the study. Those who had no history HPV vaccination or refused consent were excluded from the study but were not denied access the hospital services.

Inclusion Criteria and Exclusion

Inclusion Criteria

Non pregnant women age 13-59 years attending GOPD and Family Planning Unit

Women who gave informed consent

Exclusion

Confirmed pregnant women

Women who refused consent

Women above 60 years

Collection and Processing of Blood Sample

Blood samples were collected aseptically by venipuncture using 5mls syringes and needles into sterile plain container. 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 -20oC until immunoassay were carried out. Anti HPV-16 IgG antibody was determined by ELISA technique as reported by Frazer.

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 Antibody 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 hours at 370C. 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 370C. 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 hr at 370C 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.

Data Analysis

Results from laboratory tests and data from questionnaires were reduced to percentages and presented on tables; statistical analysis was done using the statistical Package for Social Sciences version 21.0 (SPSS version 21.0 Inc., Chicago, IL, USA) software. Percentage prevalence rate were calculated and Chi-square test was used to determine association between the variables at 95% Confidence Interval (CI) and P-values ≤ 0.05 were considered significant.

RESULTS

The HPV 16 –specific Ig G antibodies was detected in 24.5 % of the 110 pregnant women analyzed. The result revealed that 27 (24.5 %) of women enrolled into this study had positive HPV 16 specific Ig G antibodies status while 83 (75.5%) of the women were not exposed.

Table 1 shows result of distribution human papillomavirus 16-specific IgG Antibodies According to Age. Result showed that women of age group 30-39 years had the highest percentage sero-positivity (27.9%) followed by those within the age of < 20 years with (27.3%). Women within the age groups 50-59 years and 40-49 years had 25.0 % and 21.8 % sero-positivity respectively, while for the age group 21-29 years, it was 20.0%. The difference in the sero-positivity by age of women was not statistically significant (p=0.643, χ2 = 3.509).

From table 2 the highest sero-positivity for HPV–specific Ig G antibody was among the divorced women and the widows (33.3%) followed by the singles (25.6%) while the married recorded 21.4 %. The difference is not statistically significant (p-value = 0.815).

In relation to women that smokes and those on hormonal contraceptives: there is no significant different between women who smoke (27.8%) and those that do not smoke (23.9 %) (χ 2=Chi-square P-value = 0.728). The women on hormonal contraceptives recorded 21.9 % sero-positivity while those who were not on hormonal

Table 1: Distribution Human Papillomavirus 16-specific IgG Antibodies According to Age.

Age (years)	No. analyzed	No. Positive	Percentage	Chi-square (χ2)	p-value
< 20	11	3	27.3	2.509	0.643
21-29	20	4	20		
30-39	43	12	27.9		
40-49	32	7	21.8		
50-59	4	1	25		
Total	110	27	24.5		

Key: No=Number, (%) =Percentage, χ 2=Chi-square, Significant Association Exist (p<0.05)

Table 2: Distribution of Anti-HPV type 16 IgG Antibodies According to risk factors.

Variables	No. Analyzed	No. positive	Percentage	Chi-square (χ^2)	p-value
Marital Status					
Married	56	12	21.4	0.944	0.815
Single	39	10	25.6		
Divorced	9	3	33.3		
Widow	6	2	33.3		
Smoking					
Yes	18	5	27.8	0.121	0.728
No	92	22	42.9		
Hormonal Contraceptives					
Yes	96	21	21.9	2.904	0.088
No	14	6	42.9		
Parity					
Nulliparous	36	7	19.4	0.814	0.666
Multiparous (≤5)	35	9	25.7		
Multiparous (>5)	39	11	28.2		
TOTAL	**110**	**27**	**24.5**		

Key: No=Number, (%) =Percentage, χ 2=Chi-square, Significant association exist at (p≤0.05) at 95% CI

Table 3: Seroprevalence of HPV 16 IgG antibodies according to sexual and reproduction behavior.

Characteristics	No. analyzed	No. positive	Percentage	Chi-square (χ2)	p-value
Age at 1st Sex					
13-19	47	18	38.3	8.38	0.004*
≥ 20	63	9	14.3		
Age (first birth or Pregnancy)					
13-19	36	12	33.3	2.231	0.135
≥ 20	74	15	20.3		
No. of Sexual Partner (s)					
One (1)	42	4	9.5	12.307	0.006*
Two (2)	37	11	29.7		
Three (3)	23	7	30.4		
> 3	8	5	62.5		
Spouse/Partner's No. of sexual Partner (s)					
One (1)	31	3	9.7	13.944	0.003*
Two (2)	33	7	21.2		
Three (3)	26	6	23.1		
> 3	20	11	55		
Total	110	27	24.54		

Key: No=Number, (%) =Percentage, χ 2=Chi-square, * = Significant Association Exist

contraceptives had 42.9 % sero-positivity for anti-HPV IgG antibody. Furthermore, sero-positivity increased for women understudy from Nulliparous women (19.4 %) to multi-parous (≤5; 25.7 %) and (>5; 28.2 %) women. The increase is not statistically significant (p-value = 0.666).

Table 3 shows sero-positivity for HPV 16 – specific IgG antibodies distribution according to sexual and reproductive behaviors. Sero-positivity among women who had their first sexual intercourse at age 13-19yrs (38.3 %) is significantly different from those who had their sexual debut at ≥20 years (14.3 %) [P=0.004].

Similarly, age at first pregnancy/birth in relation to distribution of HPV 16 IgG antibodies reveals that those within 13-19yrs of age had 33.3 % sero-positivity while women of age greater and equal to twenty (≥20), it was 20.3 %.

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: 9.5 %, 29.7%, 30.4 %, and 62.5 % respectively. While those of participants spouse/partner's number of sexual partner(s) were: 9.7 %, 21.2 %, 23.1 % and 55.0 % respectively. There is significant difference between number of sexual partners of participants (P=0.006). There is also significant difference between spouse/partner and numbers of sexual partners (P=0.003) [10].

DISCUSSION

This study was conducted to determine the HPV 16- specific IgG antibodies among women attending GOPD and Family Planning Unit at General Hospital Kagarko, in Kaduna State. In the studied population, sero-positivity of 24.5% HPV 16-specific IgG antibodies was determined. This is higher than the prevalence of 13.2% reported by Manga et al., [11] from Gombe, 15.8% reported by Auwal et al.,5 from Kano, and 22.2% reported by Thomas et al., [12] 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 employed HPV DNA using a Polymenase 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 also found similar sero-positivity rate of 24.2% among women in Brazil with invasive cervical carcinoma.

In this study, the highest HPV 16 sero-positivity of 27.9 % was found in the age group 30-39 years. Newall et al., [13] observed similar trend among Australian women between the ages of

30-39 years. The higher positivity rate of 27.3% 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. Furthermore, women in the study area often marry at as young as age 15years, and Aminu, et al., [14] in his 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 [15] Menendez et al., [16]. Other studies have reported a highest HPV prevalence among single women [17, 18]. This means that all women, regardless of marital status were at similar risk of being infected depending on their various sexual lifestyles.

Furthermore, in this study smoking and contraceptives was not significantly associated with HPV-16 antibodies distribution among the study population. In contrast to this study, Adegbesan-Omilabu et al., [19] and Rocha-Brischilear et al.,[20] reported that use of oral contraceptive pills, cigarette smoking are significant risk factors of HPV infection. This may be attributed to the relatively few number of women involve in this study. Quamrun et al., [21] had a similar report to this study, where they established no significant association between oral contraceptive use and anti-HPV-16 IgG antibodies. Another study conducted by Auwal et al., in Kano, Nigeria, reported an insignificant association between use of oral contraceptives and HPV infection.

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., [22]. This increase in seropositivity 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 4.

Age at first intercourse was related to HPV-16 antibodies and it was found to be statistically significant (But age at first birth/pregnancy was not statistically significant, though women who had their 1st birth/pregnancy at age 13-19years had higher prevalence of antibodies than those who had theirs at age greater than or equal to twenty (>20) years. Olsen et al., [23] made similar observation and they established that seropositivity against HPV 16 capsid is a better marker of past sexual behaviors than presence of HPV DNA.

The analysis of HPV-16 antibodies in relation to number of sexual partner(s)/spouse's number of lifetime sexual partner(s) showed that Seroprevalence markedly increased with an increasing number of life time sexual partners. A Seroprevalence increased from 9.5% for a woman with one sexual partner to 62.5% for women with more than three life time sexual partners. Statistical significant was well established. 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. Acquisition of HPV infection has been shown to be strongly related to sexual behavior and the prevalence of HPV increases with increasing number of sexual partners and early sexual debut [24, 25].

CONCLUSION

In light of the findings in this study, there is evidence (24.5 %) of significant exposure to human papillomavirus type 16 in the study population. Human Pappillomavirus-16 IgG seropositivity 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 PHV in the Kaduna state.

There is need for public health education/ awareness on the mode of transmission of the virus and risks factors associated with the HPV infection'.

Those who are positive for HPV should go for screening (Pap smear) for possible intervention in order to p cervical cancer.

LIMITATIONS

The study is hospital based and cannot be extrapolated to the general populace

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

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