Original Research Article

SEROPREVALENCE OF HUMAN PAPILLOMAVIRUS TYPE 16 IMMUNOGLOBULIN G ANTIBODIES (HPV 16-IgG) AMONG WOMEN ATTENDING GENERAL HOSPITAL KAGARKO, KAGARKO LGA, KADUNA STATE

ABSTRACT

A large majority of cervical cancer (more than 95%) is due to the Hhuman Ppapillomavirus (HPV). The HPV type 16 account more than half of cervical cancers.

- ~~The Human apillomavirus (HPV) type 16 account for about half of cervical cancers~~

worldwide. This study investigated the ser-oprevalence of HPV-16 IgG antibodies among women attending General Hospital Kagarko, Kagarko Local government, Kaduna State. Serum samples and questionnaire ~~data~~ were administered to ~~collected from~~ 110 women for collection of data. HPV-16 specific IgG antibodies were detected by the use of an HPV-16 virus-like particle ELISA. The highest HPV-16 IgG sero-positivity was found at the ~~in the studied population~~ age >20-59 years was 24.5% (27/110) at 95% confidence interval. Sero-positivity increased from 9.5% in women having ~~with~~ one lifetime sex partner~~s~~ to 62.5% in women with more than three sex partners (p=0.006). Age at first intercourse, number of lifetime sex partners and having had sex with men who have multiple sex partners were significantly associated with HPV-16 IgG ~~antibobies~~antibodies. Information on HPV sero-epidemiology will be important for designing prevention efforts including vaccine programs.

Keywords: Human Papilloma Virus, Serology, Pprevalence, Immunoglobulin, Antibodies, Women, Nigeria.

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1.0. INTRODUCTION

Human Papillomavirus (HPV) belongs to a large and diverse papillomaviridae family of small, icosahedral, non-enveloped DNA virus, over 200 different subtypes of HPV are known and differentiated on the basis of their genetic sequences. The majority of the types are epitheliotropic infecting mucosa and cutaneous keratinocytes. Mucosal infecting HPV are further subdivided into Low and High risk HPV on the basis of their oncogenic potentials. Low risk HPV types leads to the development of benign neoplasms, such as warts and condyloma acuminatum, whereas high risk HPV types infect the anogenital tract and lead to malignant neoplasms, such as cervical cancer [1, 2]. High risk HPV strains such as HPV 16 and 18 causes about 70% of the cervical cancers, type-16 alone causes more than 50% of cervical cancers and type 18 accounts for more than 10% of cervical cancers. Other high risk HPV genotypes cause more than 20% of cervical cancers globally. Low risk HPV 6 and 11 cause about 90% of genital warts and benign neoplasm, which rarely develop into cancer [3].

According to the International Agency for Research on Cancer, cervical cancer is the fourth most common cancer in women, with an estimated 528,000 new cases and 266,000 deaths worldwide in 2012 [ ]. However, cervical cancer remains a leading cause of cancer related death for women

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in developing countries. In Nigeria cervical cancer ranks as the second cause of female cancer with about 14,089 new cervical cancer cases diagnosed annually [4]. The estimated crude and adjusted HPV prevalence among women with normal cytological findings worldwide were 11.7-12.8% respectively. Sub-saharan African (24.0%), Latin America (16.1%), Eastern Europe (14.2%), Southern Asia (14.0%), Northern America (10.2%) [3]. In Nigeria, no adequate documented studies in relation to the HPV associated cervical cancer. However, the rough estimate of HPV prevalence in Nigeria is high (greater than or equal to 15%) based on research works done in some selected cities across Nigeria [4].

Genital HPV infection is primarily transmitted by genital skin to skin contact, usually but not necessarily during sexual intercourse. HPV infection risk is associated with the number of sex partners that the women or her partners has had over a lifetime and recently. Epidemiologic studies clearly indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity [5]. In addition to sexual activity, age is an important determinant of the risk of HPV infection. Most cervical cancers arise at the squamocolumar junction between the columnar epithelium of the endocervix and the squamous epithelium of the exocervix ~~extocervix~~. At this site, there are continuous metaplastic changes. The greatest risk of HPV infection coincide with the greatest metaplastic activity which occur at puberty and first pregnancy and declines after menopause. However, cervical cancer is more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer [6]. Persistence of infection is more common with the high risk oncogenic HPV types and is an important determinant in development of cervical cancer.

The link between genital HPV infections and cervical cancer was first demonstrated in early 1980s by Harold Zur Hausen, a German virologist. ~~Since then, the link has become well established~~ [7]. HPV being a necessary cause of cervical cancer but it is not a sufficient cause. Other cofactors are necessary for the progression from cervical HPV infection to cancer. Tobacco smoking, high parity, long time hormonal contraceptive use and co-infection with HPV have been identified as established co-factors. Co-infection with Chlamydia trachomatis and H~~h~~erpes S~~s~~implex V~~v~~irus type 2, immunosuppression and certain dietary deficiencies are other probable cofactors [8].

The primary immune responses to HPV infection is cell-mediated; therefore, conditions that impair cell mediated immunity such as renal transplantation or human immunodeficiency virus

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(HIV) and /AIDS) disease increases the risk of acquisition and progression of HPV. Most HPV-induced cervical cell changes are transient and about 90% regress within 1 to 2 years as the immune system eliminates the virus. The tendency toward regression of HPV correlates inversely with the severity of cervical disease. Only a small proportion of mild and moderate cervical disease develops into invasive cancer, but the risk of progression from severe cervical cellular abnormality to invasive carcinoma is at least 12% [9].

The HPV replication cycle begins with the entry of the virus into the cells of basal layer of the epithelium. Mild abrasion or microtrauma of the epidermis facilitatemicrotrauma of the epidermis facilitates the entry of the virus into the basal layer. HPV 16, like many other viruses attach to the host cell via cell surface heparan sulfate. A secondary receptor or stabilizing protoglycans may also be involved in HPV DNA replicates as the basal cells differentiate and progress to the surface of the epithelium. In the basal layers, viral replication is considered to be non-productive and the virus establishes itself as a low-copy-number episome by using the host DNA replication machinery to synthesize its DNA on average once per cell cycle. In the differentiated keratinocytes of the suprabasal layers of the epithelium, virus switches to a rolling cycle mode of DNA replication, amplifies its DNA to high copy numbers, synthesizes capsid proteins, and causes viral assembly to occur [10]. Since HPVs encode only 8 to 10 proteins, they must employ host cell factors to regulate viral transcription and replication. HPV replication begins with host cell factors which interact with the LCR region of the HPV genome and begin transcription of the viral E6 and E7 genes. The E6 and E7 gene products deregulate the host cell growth cycle by binding and inactivating tumor suppressor proteins, cell cyclin-dependent kinases.

The function of the E6 and E7 genes during productive HPV infection is to subvert the cell growth regulatory pathways and modify the cellular environment in order to facilitate viral replication in a cell that is terminally differentiated and has exited cycle. Cell growth is regulated largely by two cellular proteins, the tumor suppressor protein; p53 and the Retinoblastoma gene product (pRB). The HPV E6 gene product binds to p53 and targets it for rapid degradation via a cellular ubiquitin ligase. The degradation has the same effect as an inactivating mutation. As a consequence, the normal activities of p53 which govern G1 arrest, apoptosis and DNA repair are abrogated. The HPV E7 gene product binds to the hypophosphorylated form of the RB family of

proteins. This binding disrupts the complex between pRB and the cellular transcription factor E2F-1, resulting in the liberation of E2F-1, which allows the transcription of genes whose products are required for the cell to enter the (S) phase of the cell cycle. The E7 gene product can also associate with other mitotically interactive cellular proteins such as cyclin E, the outcome is stimulation of cellular DNA synthesis and cell proliferation [11].

HPV cannot be cultured in the laboratory from clinical specimens. The primary diagnostic tools have been Cytology, Histology, Iimmunologic assay (Serology) and recently Molecular methods to detect HPV DNA sequence i.e PCR. Presently the two methodologies most widely used for generic genital type detection that have equivalent sensitivities and specificities are Hybrid capture version 2(HC2) and PCR with generic primers.

The major types of HPV serology assay is Enzyme-linked Iimmunosorbent Aasssays (ELISA) that detect type-specific antibodies against conformational epitopes on viral like particles (VLPs). As antibodies typically persist, also after clearance of HPV, serology 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. Though, a minimum level of antibodies required for protection has not been defined for human [12]. 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. The primary prevention of HPV infection is through risk reduction practices and administration of HPV vaccines, but- it is unfortunate that Nigeria is yet to implement HPV vaccines as part of her national immunization policy. HPV vaccines are prepared from empty protein shells called virus-like particles (VLP) produced by recombinant technology. They do not contain any live biological product or DNA, so they are non-infectious. Current HPV vaccines are designed to protect against HPV 16 and 18; the quadrivalent vaccine also protectvaccine also protects against low-risk genotypes 6 and 11. One month after the third dose of HPV vaccine, nearly 100% of women aged 15-26 years in trails of either of the vaccines have detectable antibody to each HPV genotype, levels being 10-104 times higher than those in natural infections. Antibody levels achieved after vaccination are inversely related to age (The lower the age, the higher the antibody). Vaccine dose is given three times for effective vaccination that protect last for at least five years (cost $100 per dose). Vaccine protection lasts for at least 5 years [13].

Therefore, the recognition of HPV infection as the necessary cause of cervical neoplasia has created new research fronts in the primary and secondary prevention of cervical cancer. In order to lower the burden of HPV infections, serological survey of HPV infection is important to better elucidate their natural history and to check the potential relevance of HPV vaccines.

The burden of HPV infection is due to its causal role in cervical cancer. Cervical cancer is the second most common cancer in women living in less developed regions with an estimated 530,000 new cases in 2012 (84% of new cases worldwide). In 2012, approximately 270,000 women died from cervical cancer, more than 85% of these deaths occurring in low- and middle-income countries like Nigeria [14]. About 14,089 new cases are diagnosed annually in Nigeria [15]. In northern Nigeria, cervical cancer ranks as the commonest female cancer [16].

Therefore, epidemiological knowledge of the distribution and risk factors of cervical human papillomavirus (HPV) infection in the general population is critical to primary prevention of HPV infection burden. Since there are few studies in HPV serological survey in Kaduna state, Hhence; the need to research on seroprevalence of HPV-16 IgG antibodies among women of childbearing age in Kaduna State.

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2.0. MATERIALS AND METHODS

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Study Area and Population

This research work was a cross-sectional screening of women in General Hospital Kagarko, Kagarko LGA in Southern Kaduna, Kaduna State. The Hospital is located in the Headquarter of Kagarko LGA and is the only General Hospital in the entire Local Government. The facility is fifty bed-space capacities with an average of forty (40) women accessing medical services daily; based on the hospital GOPD register. A total of 110 non-pregnant women within the age range of 15 – 59 years who were able to give informed consent, were recruited for this study.

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Ethical Consideration

An ethical clearance to conduct this research was sought for and obtained from Ministry of Health, Kaduna State. Participants' information was kept confidential. They were not forced to participate; their informed consent was obtained.

Data Collection

Data collection was through administration of questionnaire to all women selected for this study. The questionnaire was structured to contain demographic data, risk factors, sexual & reproductive behavior and immunization status/HPV vaccines.

Sample Collection and Handing

5mls of blood sample were collected aseptically by venipuncture from each subjects into a vacutainer tube (without anticoagulant) and allow clot to form for 30 minutes. The clotted blood sample was centrifuge at 1000g for 10minutes to separate the serum. Serum was carefully removed and aseptically transfer into labeled screw capped cryovials with the aid of sterile pipette. Sera were stored at -20^{o}C in a freezer until assay was ready to be carried out.

Reagent and Preparation of Assay

The laboratory test was conducted with MyBiosource-MBS9315865 ELISA Kit Human papillomavirus type 16 IgG. Before beginning the test, all samples and reagents were brought to room temperature (22- 25)^{0}C and gently mixed.

Principle of the Assay

The kit uses a double- antigen sandwish enzyme-linked immunosorbent assay (ELISA) to analyze the existence or not of Human PV-type 16-IgG in samples. Add samples the wells pre-coated with one HPV-16 IgG antigen, at same time add HRP-conjugated HPV IgG antigen to bind the analyte followed by incubation and washing procedures to remove unbounded substance. Finally, HRP (Horseradish peroxidase) are added, incubated for detection, and a blue color is developed. Reaction is stopped and color turns to yellow when stopping solution (acidic) is added. The existence or not of HPV-16-IgG in the samples is then determined by comparing the O.D of the samples to the CUT OFF [17].

Test Procedure

The serum samples in the freezer were thawed and the samples were diluted in HB-PBS in ratio 1/10, 1/31.6, and 1/100. Fifty (50) microliter of serially diluted serum was added into each well

of the plate. The plates were washed by adding 150 microliter of PBS-T per well in a microplate washer and the washing step was repeated for a total of five times. Fifty (50) microliter of anti-human IgG-HRP diluted in 1/1000 in HS-PBS was added to each well. The plates were washed by adding 150 microliter of PBS-T per well. Washing step was repeated for a total of five washes. Fifty (50) microliter of peroxidase substrate was added to each well of the plates. Each plate was incubated at room temperature for 30 minutes and air bubbles were avoided. Before reading the plates, they were carefully dispersed in order to obtain a uniform colour development. Plates were read in a microplate reader at 415nm. Controls were prepared along and read at the same time and same wavelength [17].

Interpretation of Result

A standard curve was obtained by plotting a graph of absorbance of standard against concentration of standard given. The concentration of IgG in each sample was estimated from the graph using their various absorbance and results interpreted.

CONCENTRATION(IU)	INTERPRETATION
≤ 15	Negative
15-20	Equivocal
≥20	Positive

A negative result indicates no immunity to HPV 16 IgG. (Mybiosource, 2016)

Data Analysis

Data obtained from the questionnaires and results observed from the laboratory analysis were analysed using the statistical Package for Social Sciences (SPSS version 21.0) software. Percentage prevalence rate were calculated and Chi-square test was used to determine association between variable and infection at 95% Confidence Interval (CI) and P-values of 0.05 or less was considered as statistically significant.

3.0. RESULT

Table 1: Shows overall distribution of HPV 16 IgG antibodies among subjects. The result revealed that out of 110 women screened for HPV I6 immunoglobulin G antibodies, 27(24.5%) were seropositive while 83(75.5%) were seronegative.

Table 1: Overall result of HPV 16 IgG antibodies

Total Examined	HPV 16 IgG Status	
	No positive (%)	No Negative (%)
110	27 (24.5)	83 (75.5)

Table 2: Shows the distribution of HPV I6 IgG antibodies according to different age group of the evaluated women. The highest prevalence of 27.9% was obtained in 30-39yrs age group. This is followed by 40-49yrs age group with 21.9%, while the least prevalence rate of 25.0% was found in women within age group 50-59years.

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Table 2: Overall result of HPV 16 IgG antibodies according to age group

Age (years)	No Examined	No positive (%)	No Negative (%)	χ^2	p-value
< 20	11	3 (27.3)	8 (72.7)	**2.509**	**0.643**
21-29	20	4 (20.0)	16 (80.0)		
30-39	43	12 (27.9)	31 (72.1)		
40-49	32	7 (21.9)	25 (78.1)		
50-59	4	1 (25.0)	3 (75.0)		
TOTAL	**110**	**27 (24.5)**	**83 (75.5)**		

Key: **No**=Number, **(%)** =Percentage, χ^2=Chi-square, Significant association exist at ($p \leq 0.05$) at 95% CI

TABLE 3: Indicates the distribution of HPV 16 IgG based on marital status. The highest seroprevalence of 33.3% was obtained in divorced/widow women, followed by single women with 25.6% while the least prevalence of- 21. 4 % ~~1.8%~~ was obtained among married women. Seronegative results were obtained in d~~D~~ivorced with 66.6%, married with 78.6% and single with 74.4%.

Table 3: Seroprevalence of HPV 16 IgG antibodies according to marital status:

Marital Status	No Examined	No positive (%)	No Negative (%)	χ^2	p-value
Married	56	12 (21.4)	44 (78.6)	**0.944**	**0.815**
Single	39	10 (25.6)	29 (74.4)		
Divorced	9	3 (33.3)	6 (66.7)		
Widow	6	2 (33.3)	4 (66.7)		
TOTAL	**110**	**27 (24.5)**	**83 (75.5)**		

Key: No=Number, **(%)** =Percentage, χ^2=Chi-square, Significant association exist at ($p \leq 0.05$) at 95% CI

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TABLE 4: Shows distribution of HPV 16 IgG antibodies based on residential settings. Positive results among those that lived in Urban, Semi-urban and Rural settings were 27.8%, 25.0% and 23.3% respectively. Women that live in urban area have the highest seropositive result of 27.8%.

Table 4: Seroprevalence of HPV 16 IgG antibodies according to residential setting.

Residence	No Examined	No positive (%)	No Negative (%)	χ^2	p-value
Urban	18	5 (27.8)	13 (72.2)	**0.153**	**0.926**
Semi-Urban	32	8 (25.0)	24 (75.0)		
Rural	60	14 (23.3)	46 (76.7)		
TOTAL	**110**	**27 (24.5)**	**83 (75.5)**		

Key: **No**=Number, **(%)** =Percentage, χ^2=Chi-square, Significant association exist at ($p \leq 0.05$) at 95% CI

TABLE 5: Reveals seroprevalence based on risk factors. 5 (27.8%) out of 18 respondents that said YES to smoking were seropositive, while 92 respondents that said NO, 22 (23.9%) were positive and 70(76.1%) were negative. On uses of hormonal contraceptives; 96 said YES with

21(21.9%) seropositive rate while 14 that answered NO have 6(42.9%) seropositive and 8(57.1%) seronegative

Seroprevalence of HPV according to number of children (parity) characteristics show that women without children have 19.4% seropositive rate, women with less and equal (≤5) five number of children have 25.7% positive rate while women with more than five number of children have 28.2% positive and 71.8% negative rate respectively.

Table 5: Seroprevalence of HPV 16 IgG antibodies according to risk factors.

Characteristics	**No Examined**	**No positive (%)**	**No Negative (%)**	**χ^2**	**p-value**
Smoking					
Yes	18	5 (27.8)	13(72.2)	**0.121**	**0.728**
No	92	22(23.9)	70(76.1)		
Hormonal Contraceptives					
Yes	96	21(21.9)	75(78.1)	**2.904**	**0.088**
No	14	6(42.9)	8(57.1)		
Parity					
None	36	7(19.4)	29(80.6)	**0.814**	**0.666**
≤5	35	9(25.7)	26(74.3)		
>5	39	11(28.2)	28(71.8)		
TOTAL	**110**	**27 (24.5)**	**83 (75.5)**		

Key: **No**=Number, **(%)** =Percentage, **χ^2**=Chi-square, Significant association exist at ($p \leq 0.05$) at 95% CI

TABLE 6: This table shows seroprevalence of HPV 16 IgG antibodies according to sexual and reproductive behaviors. The respondents who had their first sexual intercourse at age 13-19yrs are 47 with 38.3% highest seropositive rate, while on other hand those who had their sexual

debut at age greater and equal to twenty(≥20) have 14.3% positive and 85.7% negative results respectively.

Age at first birth/pregnancy in relation to HPV 16 IgG antibodies reveals that those within 13-19yrs of age have 33.3% positive and 66.7% negative results while respondents of age greater and equal to twenty(≥20) have 20.3% seropositive results.

Considering the number of sexual partner(s), women who have one, two, three & >3 sexual partner(s) have the following seropositive results: 9.5%, 29.7%, 30.4%, & 62.5% respectively. The seronegative results were 90.5% for one partner, 70.3% for two partners, 69.6% for 3 partners and 37.5% for sexual partners (>3).

When antibodies distribution were evaluated in relation to subjects' spouse/partner's number of sexual partner(s), the following seropositive results were obtained; for subject's spouse/partner with one 9.7%, with two 21.2%, with three 23.1% and with (>3) 55.0%.While their seronegative results were as follow: with one 90.3%, with two 78.8%, with three 76.9% and with (>3) 45.0%.

Table 6: Seroprevalence of HPV 16 IgG antibodies according to sexual and reproduction behavior.

Characteristics	**No Examined**	**No positive (%)**	**No Negative (%)**	**χ^2**	**p-value**
Age at 1st Sex					
13-19	47	18 (38.3)	29 (61.7)	**8.380**	**0.004***
≥ 20	63	9(14.3)	54(85.7)		
Age at 1st Birth/Pregnancy					
13-19	36	12(33.3)	24(66.7)	**2.231**	**0.135**
≥ 20	74	15(20.3)	59(79.7)		
No of Sexual Partner (s)					
One (1)	42	4 (9.5)	38(90.5)	**12.307**	**0.006***
Two (2)	37	11(29.7)	26(70.3)		
Three (3)	23	7(30.4)	16(69.6)		

> 3	8	5(62.5)	3(37.5)		
Spouse/Partner's No. of sexual Partner (s)					
One (1)	31	3 (9.7)	28 (90.3)	**13.944**	**0.003***
Two (2)	33	7 (21.2)	26(78.8)		
Three (3)	26	6(23.1)	20(76.9)		
> 3	20	11(55.0)	9(45.0)		
TOTAL	**110**	**27 (24.5)**	**83 (75.5)**		

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

4.0. DISCUSSION

This study determined the Human Papillomavirus type 16 immunoglobin G antibodies (HPV 16-IgG) among women attending General Hospital Kagarko, Kagarko Local Government Area of Southern Kaduna, Kaduna State. In the studied population, seroprevalence of 24.5% (27/110) HPV-16 IgG antibodies were detected. This seroprevalence rate of 24.5% is in agreement with the study conducted by Vaccerella *et al* and IARC HPV study group [18], which revealed that 24.8% of study population in Nigeria have HPV-16 antibodies. This results also correlates with research conducted in Australia for seroprevalence of HPV type 6, 11, 16 and 18 by Anthony *et al.*, [19] which found 22% seropositivity of HPV-16 among women. John Hopkin Hospital study group (1999) also found similar seroprevalence rate of 24.2% among women in Brazil with invasive cervical carcinoma [ ]1. Thomas *et al.,* [20] who researched on prevalence on Human papillomavirus infection in women in Ibadan, Nigeria, documented HPV-16 antibodies seroprevalence of 22.2%.

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Although, the studies conducted in ~~there are only a few studies from~~ Northern Nigeria, on HPV type 16 IgG, lower prevalence of 15.8% was reported by Auwal *et al.,* [21] from Kano and 13.2% was also reported by Mohammed *et al.,* [22] from Gombe. This lower in prevalence rate observed in the research work of Auwal *et al.,* [21] and Mohammed *et al.,* [22] in comparison

with our work might be due to difference in Assay methodologies. The two ~~researcher~~ employed HPV DNA using a polymenase chain Reachan (nPCR) which detect HPV current antigens but not the antibodies. HPV-16 VLP ELISA as a screening test for HPV infection is limited by a high seroprevalence in women with probable prior exposure to HPV 16 but without disease because the immune system might have clear-of-the HPV-antigen leading to the development of long lasting immunoglobulin G antibodies.

Worldwide the incidence and prevalence of HPV peaks at younger age soon after the start of sexual activity between the age of 25-35 years but subsequently declines with infections clearing as the infected individual grows older [23]. In this study, the highest peak of HPV 16 seropositivity 27.9% occurred among those who were 30-39 years of age, though when compared with other age group; it is not statistically significant. Anthony *et al.*, [19] observed similar trend among Australian women between the ages of 30-39 years. But the decreased in antibodies distribution with age in line with global observation could not be established in this work, this may be as a result of fewer number of subject within the older age group of 50-59 years in our study population.

The higher positivity rate of 27.3% in very young women age (<20) years among the study population may be attributed to early acquisition of infection as a result of early indulgence in sexual activity and early marriage. Women in the study area often marry as young as age 15years, and it has been reported that age of sexual debut in Nigeria is 9-10 years [24].

The seroprevalence of HPV-16 IgG antibodies according to marital status of the woman was not statistically significant in this study. Single women have 25.5% and married women have 21.4% in this study, indicating a similar seroprevalence of HPV-16, which implies a similar rate of HPV infection. The seroprevalence of HPV-16 was same and highest in Divorced and widows in contrast to other studies, which reported a higher prevalence among married women [25, 26]. Other studies have reported a highest prevalence among single women [27, 28].

This means that all women, regardless of marital status were at similar risk of being infected depending on their various sexual lifestyles.

The prevalence of HPV-16 antibodies among our studied population showed that women from rural area had a lower HPV prevalence (23.3%) than that of the urban population (27.8%). Although, there was no significant difference between the urban and rural women. Quamrun *et al.*, [29] and Baloch *et al.*, [30] from Bangladesh and China respectively reported same in their

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works. But Li *et al,* [31] had a contrary view. Distribution of HPV-16 antibodies among rural and urban may be due to socioeconomic environment, lifestyles and early sexual activity in the respective area.

Smoking and contraceptives variables were observed in this study, no significant association with HPV-16 antibodies distribution among the study population. In contrast to this study, Adegbesan-Omilabu *et al.,* [32], and Rocha-Brischilear *et al.,* [33] all reported that use of oral contraceptive pills, cigarette smoking are significant risk factors of HPV infection. This may be attributed to the relatively few percentage of women that respond to question on smoking history in this work. Quamrun *et al.,* [29] had a similar report to this study, where they established no significant association between oral contraceptive use and HPV-16 antibodies. So also a study conducted by Auwal *et al.,* [21] in Kano, Nigeria, did not find a significant association between use of oral contraceptives and HPV infection. In this study, women who had more than five children had highest prevalence of antibodies to HPV 16, follow by women with less than 5 children and the least HPV-16 antibodies was observed among women with no children. Although, this difference in prevalence did not reach statistical significance. This observation is consistent with a report by Okolo *et al.,* [34]. This increase in prevalence of HPV infection with increasing parity (number of children) has been attributed to increased sexual activity [35].

HPV-16 antibodies prevalence and selected risk factors for cervical cancer. Age at first intercourse / sex 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 and equal twenty ($\geq$20) years.

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 [36, 37]. Acquisition of HPV infection has been shown to be strongly related to sexual behaviour and the prevalence of HPV increases with increasing number of sexual partners and early sexual debut [35]. This is very evident in this study.

Conclusion

In light of the findings in the present study, there is evidence of significant exposure to Human papillamavirus type 16 in the study population. HPV-16 IgG seropositivity was found to be associated with a lifetime number of sexual partners as expected of sexually transmitted infections (STI). Given that HPV infection is a necessary cause of cervical cancer and HPV serotype 16 which is the most implicated as the leading cause of cervical cancer in the world; preventing it is the most effective way to prevent cervical intraepithellal neoplasia and invasive cervical carcinoma. HPV vaccines have been proven to be effective and safe measure to prevent HPV infection. Our results confirm that primary prophylactic HPV vaccination programs should be targeted at pre-adolescents, because HPV seropositivity begins to increase markly in women less than twenty (<20) years of age. This unique population data provided by this study will inform disease transmission models, assessing optimal vaccination strategies as regards ideal age for vaccination.

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