



A Survey for Human Papilloma Virus Infection among Women Attending Gynaecology Clinic of Jos University Teaching Hospital Jos, Nigeria

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Abstract

Human papilloma virus (HPV) is among the well known causes of cervical cancer and it represents the third most common malignancy in women, however surveillance data on its incidence rates are still needed in this part of the world. Accordingly, this study was conducted to determine the incidence of human papilloma virus in sexually active women aged between 18 and 65 attending gynaecology clinic of Jos University Teaching Hospital, Nigeria (In 2016) using enzyme linked immunosorbent assay ELISA kits (Cusabio Biotech. USA). A total of 90 blood samples was collected and analyzed according to manufacturer's instructions. Out of the samples 28(33.3%) were found to be positive for Human Papilloma virus infection. The incidence of Human Papilloma virus in relation to age shows that those in the age group 16-25 had the highest rate of occurrence of 45.8%, followed by age 36-45(27.8%). Age groups 26-35, 46-55 and 56-65 years have 25.0% respectively. HPV incidence rate of 33.3% was found among women that are not single while 31.0% occurred among the married women. Women with diabetes had 50% incidence of HPV infection while 29.3% occurred among women without diabetes, equally HPV incidence rate of 37.5% occurred among women who had protected sex while 30.5% occurred among women that had unprotected sex. In conclusion high incidence of HPV was detected among the study population, hence, it is recommended that HPV vaccination should be included in the routine immunization among the gynaecology services for sexually active women and regular pap smear screening should be made mandatory.

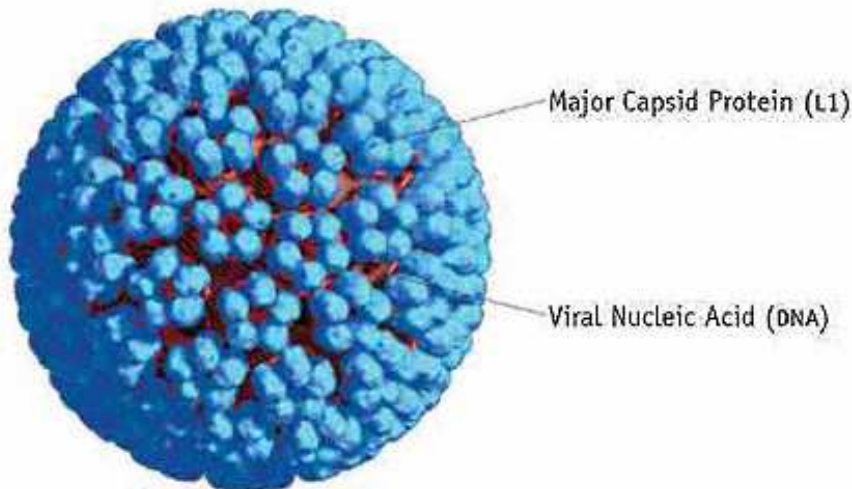
Keywords: Surveillance, Incidence, Infection, Human Papilloma virus, Gynaecology, Women.

INTRODUCTION

Human papilloma virus infection is a highly prevalent sexually transmitted disease and there is evidence of the relationship of Human papilloma virus infection and the development of genital warts, penile intraepithelial neoplasia, invasive penile carcinoma and cervical cancer (Freire *et al.*, 2014). The viral genome of the HPV consists of a single molecule of double-stranded and circular DNA, containing approximately 8000 base pairs and harboring an average of 8 open reading frames ORF (De Sanjose, *et al.*, 2007). In a functional point of view, the HPV genome is divided into three regions. The first is a non-

coding upstream regulatory region (URR) or long control region (LCR) that has regulatory function of the transcription of the E6 and E7 viral genes; The second is an early region (E), consisting of six ORFs: E1, E2, E4, E5, E6, and E7, which encodes no structural proteins involved in viral replication and oncogenesis. The third is a late (L) region that encodes the L1 and L2 structural proteins. The LCR region of the anogenital HPVs ranges in size between 800-900 bp, representing about 10% of the genome, and varies substantially in nucleotide composition between individual HPV types (Bao and Smith 2008).

THREE-DIMENSIONAL MODEL OF HUMAN PAPILLOMAVIRUS



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 The PRN Notebook Online at WWW.prn.org
 Three-dimensional model of HPV Created by Louis E. Henderson, PhD,
 Frederick Cancer Research Center.

Figure 1: Structure of Human papilloma virus.

Cervical cancer represents the third most common malignancy in women, and the seventh overall, with an estimate 529,000 new cases and that more than 80% of the sexually active women acquire genital human papilloma virus by 50 years of age (Karthigeyan, 2012).

Documentation of the prevalence of human papilloma virus types in cervical cancer in different regions of India has been used for a prevention program combining both screening and vaccination (Deodhar *et al.*, 2012). Nearly 120 types of Human papilloma virus are known to occur and are categorized into three broad categories based on the potentiality of causing cancer; High risk type – Human papilloma virus (HPV) 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82; Intermediate type 26, 53, 66 and the Low risk type 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108 (Shikha *et al.*; 2012). Human papilloma virus (HPV) types 16 and 18 infections are responsible for about 70% of all cases of cervical cancer worldwide. Human papilloma virus vaccines against type 16 and 18 are now available and have potential to reduce the incidence of

cervical and other anogenital cancers (Shikha *et al.*; 2012). In the year 2012 and 2013, a clinical based survey was conducted in Tiruchirapalli (Tamilnadu, India) among women who attended Obstetrics and Gynaecology clinic which reported incidence rate of 5.6% to 9.3% (Vu and Bui, 2012; Vu *et al.*; 2013). In developing countries like Nigeria and other African countries, information about cervical cancer and other related cancer including cancer of vagina, anus and vulva which causes serious threat to health are limited (Aminu *et al.*, 2014). Therefore, the present work was aimed at determining the incidence of Human papilloma virus among women attending gynaecology clinic of the Jos university teaching hospital between January - June 2016.

MATERIALS AND METHODS

Study Area

This is a hospital based study that was carried out in gynaecology clinic of the Jos University Teaching Hospital (JUTH) Nigeria. This institution is located in central part of Jos.

JUTH is one of the three teaching hospitals situated in North-Central zone of Nigeria. It serves as a referral Centre for Plateau State and neighboring states such as Bauchi, Benue, Kogi, Nasarawa, Adamawa, Taraba and part of Kaduna. Jos is the capital of Plateau state which has over 30 different ethnic groups. The 2006 provisional census puts the population of plateau state at 3,178,712 with 1,585,679 females. Plateau

state lies between latitude 7 and 11 North and longitude 7⁰ and 25⁰ east. The capital city of Jos is a pear-shaped upland which stretches for approximately 140 km from north to south and 80km from east to west, covering an area of about 8,600 km². This region has a height of 1200m above sea level (www.plateau.gov.ng/page/at.a.g glance, 2016)

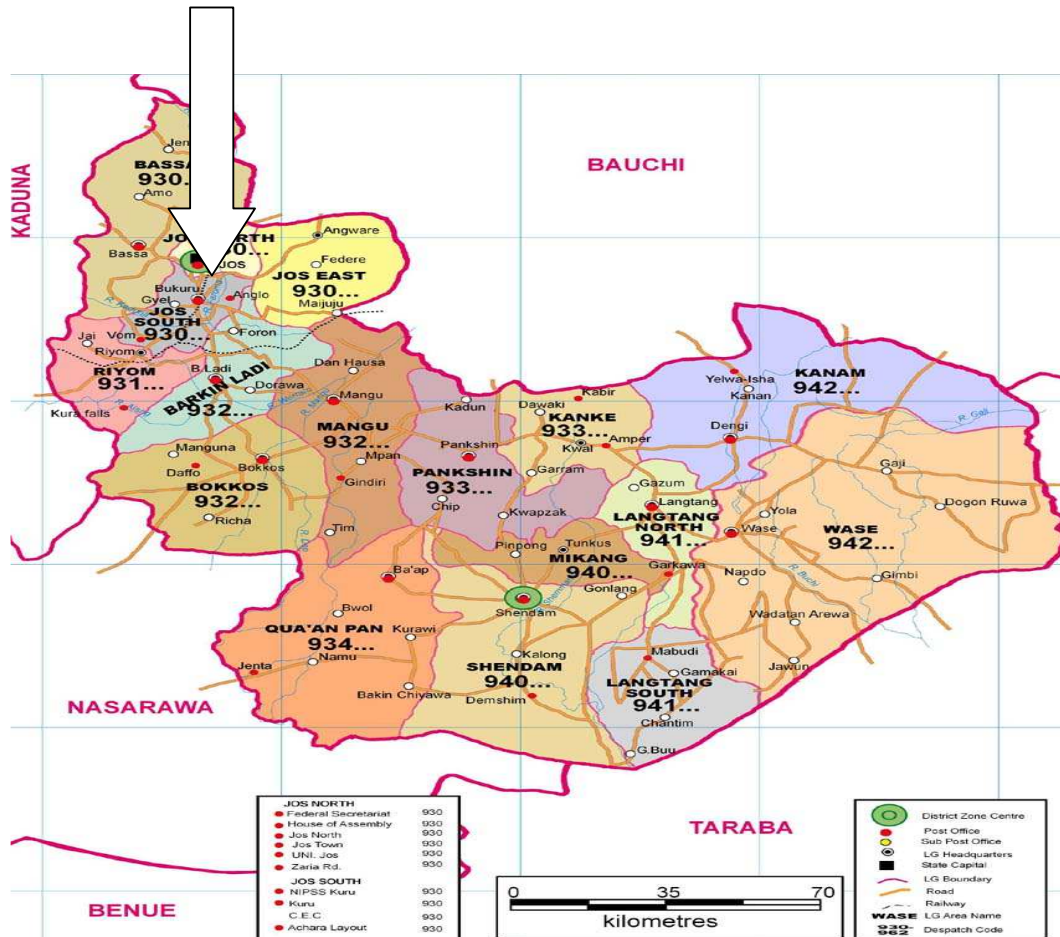


Fig 2: Map of Plateau State.(Plateau zip code map. Assessed 7th December 2016)
Down Arrow: Indicating the location of Jos university Teaching Hospital (JUTH)

Ethical Clearance and Consent of the participants

Ethical approval was sought and obtained from the ethical committee of Jos University Teaching Hospital, Plateau State, Nigeria (Appendix I) and consent of the subjects were individually sought before the commencement of the research (AppendixII)

Study Population

The study population consist of all consenting women attending the gynaecology clinic of the Jos university teaching hospital (JUTH) with symptoms and signs of cervical cancer (Between ages 18 years and 65).

Sample Size Determination

A total of 90 women were randomly selected and enrolled for the study between January – June 2016, from the calculated sample size using the formula:

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

Where; n=sample size $z = 1.96$ (level of confidence 95%) $d = 0.05$ (error margin)

$q = (1-p) = 0.94$ $p =$ Reported prevalence 6.0% (Vem, *et al.*, 2012 in Jos Nigeria)

$$n = \frac{1.96^2 \times 0.06 \times 0.94}{0.05^2}$$

The calculated sample size $n = 86.6$ approximately 87, hence, sample was rounded up to 90 for convenience.

Data collection

Data were collected using a semi-structured interviewer administered questionnaire. It consists of three sections; Section A which includes information on socio-demographic data such as age, marital status. Section B focused on information on the participants' knowledge of the infection. Section C included information on the predisposing factors of the infection such as history of diabetes, smoking and multiple sex partners (Appendix III)

Sample Collection

Five milliliters (5ml) each of the blood was collected from the participants through venipuncture and emptied into sterile tubes, allowed to clot and centrifuged at 1500 rpm for 10 minutes. The serum aliquots were transferred to plain sterile sample bottles, labeled appropriately and stored at -20°C until ready for use.

Detection of Human Papilloma Virus

All the specimens collected were analysed for the presence of Human Papilloma virus using commercially available enzyme immunoassay (ELISA) kit (Cusabio, Biotech, USA). The assay was performed according to the manufacturer's instructions.

Screening Procedure

All the reagents and the micro titre ELISA plates were brought to room temperature for

30 minutes before use. A micropipette was used to dispense 100 μl of the negative and positive control into well 1 and 2 respectively, while well 3 was left as blank. One hundred microlitre (100 μl) each of the samples was added to the appropriate wells and covered with adhesive strip and incubated for 30 minutes at 37°C . The wells were then washed vigorously with wash buffer and excess buffer was removed after the last washing by slapping the plate on a clean absorbent tissue. Two drops of reagent 1 (blue solution) was added to each well (except blank) and further incubated at room temperature for 5 minutes. The wells were washed thoroughly using wash buffer and two drops of reagent 2 (Red solution) was then added to each well (except blank) and incubated for 5 minutes and then washed. Two drops of chromogen was then added to each well and incubated for 5 minutes. Finally, two drops of stop solution was then added to each well and mixed gently by tapping the side of the plate with index finger taking blank as zero, the optical density of each well was determined within 10 minutes, using a micro-plate reader set at 450nm. However, the results were also read visually for colour changes. Any sample well with absorbance reading of 0.15 and above with yellow coloration as the positive control was regarded as positive while samples wells with absorbance reading of less than 0.15 and appeared colorless to faint yellow was regarded as negative. (Aminu *et al.*, 2014).

RESULTS

Out of a total of 90 samples screened 28 samples were positive for Human Papilloma virus with a prevalence of 31.3%. Table 1 shows the incidence of Human Papilloma virus in relation to age, an incidence of 45.8% was observed among the age group 16 -25 years, this was followed by women aged 35-45 with 27.8%, while, age bracket 26-35, 46-55 and 56-65 years had 25.0% each. The incidence of Human Papilloma virus in relation to marital status was depicted in table 2.

The incidence among the single women was 33.3% while the married had 31.0%. Table 3 shows the incidence of Human Papilloma virus in relation to some associated risk factors. Those with diabetes had incidence rate of 50.0% for Human Papilloma virus infection while 29.3% was

recorded among those without diabetes. Among women that have had protected sex (sex with condom), 37.5% were positive while 30.5% incidence rate was recorded among those that had unprotected sex.

Table 1: Incidence of Human Papilloma Virus in Relation to Age Among Women attending Gynaecology Clinic in JUTH

Age(years)	Number screened	Number positive	Percentage (%)
16 -25	24	11	45.8
26 -35	32	8	25.0
36 -45	18	5	27.8
46 -55	12	3	25.0
56 -65	4	1	25.0
Total	90	28	31.1

Key: JUTH= Jos University Teaching Hospital

Table 2: Incidence of Human Papilloma virus in Relation to Marital status Among Women attending Gynaecology Clinic in JUTH

Marital status	Number screened	Number positive	Percentage (%)
Married	87	27	31.0
Non-married(single)	03	1	33.3
Total	90	28	31.1

Key: JUTH= Jos University Teaching Hospital

Table 3: Incidence of Human Papillomavirus in Relation to Risk factors Among Women attending Gynaecology Clinic in JUTH

Risk factor	Number screened	Number positive	Percentage (%)
Diabetes			
Yes	08	4	50.0
No	82	24	29.3
Unprotected Sex			
Yes	82	25	30.5
No	08	3	37.5

Key: JUTH= Jos University Teaching Hospital

DISCUSSION

The study revealed a total incidence rate of 31.1% for Human Papilloma virus infection among women attending gynaecology clinic at Jos University Teaching Hospital. To my knowledge this is the most recent data on the serological incidence of HPV from the study

area despite the information available on cases of cervical cancer in Jos (Envuladu, *et al.*, 2013).The incidence of HPV reported in this work is in agreement with the work done by Turki *et al.*,(2013) in Saudi Arabia, who reported an overall prevalence of 30.0%.

However, the result was lower compared to that of Sally *et al.*, (2014) at Abuja university teaching hospital (AUTH) and Aminu *et al.*,(2014) at Ahmadu Bello Universty Teaching Hospital Zaria (ABUTH) who reported prevalence rate of 37.0% and 42.7% respectively. However the result was higher compared to the work done by Kornya *et al.*,(2002) where a prevalence of 17.6% was reported among Hungarian female population. The difference in the prevalence may be due to sample size, seasonal variation, difference in the study population and could be due to the sensitivity of the test.

Age group 16-25 years recorded a higher incidence of 45.8% in this research. This may be due to life style of this age bracket, the women are younger within this age group and sexually active, which might have exposed them in having multiple sex partners and sometimes unprotected sex. Aminu *et al.*,(2014) collaborated this outcome when he reported that, age group 20-23years had a higher prevalence of HPV with 50% compared with other age brackets from a work conducted at Ahmadu Bello University Teaching Hospital..

The incidence according to marital status in this research revealed a higher incidence of 33.3% among the single compared to the married. The sexual life style of the single probably having multiple sex partners might be the reason for the high incidence. Similar study done by Eileen *et al.*,(2007) reported a prevalence of 31.1% in the United State among the single. Katia *et al.*,(2009) in Brazil reported prevalence of 45% among the single. The difference in prevalence recorded among this group in the different countries could be due to their sexual life

style and possibly due to the difference in tradition and culture.

The incidence of human papilloma virus among the diabetic subjects in this study was 50%, however, this rate is almost similar compared with the work done by Albert *et al.*,(2014) who recorded a prevalence of 47.5% in HIV/AIDS patient in Brazil. The similarity could be due to the fact that HIV/AIDS is also a dilapidating infection as diabetes.

Those that had protected sex (use of condom) had higher incidence of 37.5% compared to those that had unprotected sex with incidence of 30.5%. The major factor for this outcome could be the sample size. This report is in contrast to the study conducted by Hai-Rim *et al.*,(2003) in South Korea where they reported a lower prevalence of 18.0% among those that had protected sex compared with those that had unprotected sex. The difference may be due to sample size, test sensitivity, culture, and study population.

CONCLUSION

This study revealed an incidence rate of 31.1% for human Papilloma virus infection among women attending gynaecology clinic in Jos University Teaching Hospital, Nigeria. According to these findings, women can acquire Human Papilloma virus infection (HPV) regardless of the age or marital status of the individual. It is recommended that routine immunization should be introduced in the national immunization schedule in other to reduce the current high prevalence of the infection in Nigeria. Equally awareness programs should constantly be organized in the villages and cities to educate the women on the prevention and management of the infection.

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Appendix I: Ethical clearance Certificate.

**JOS UNIVERSITY TEACHING HOSPITAL
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Cables & Telegram: JUTH
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JOS

Ref: JUTI/DCS/ADM/127/XIX/6323

Date: 19th October, 2015.

Seri Bonmwa Faith,
Department of Microbiology,
Faculty of Natural Sciences,
University of Jos,
Jos-Nigeria.

RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 14th September, 2015 on the research proposal titled:

"Seroprevalence of Human Papillomavirus among Women Attending Gynaecology Department in Jos University Hospital" and your appearance before the Ethical Committee on 2nd October, 2015.


Following recommendation from the Institutional Health Research Ethics Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

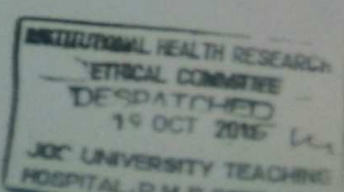
You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the Secretary, Administration Department, please.

On behalf of the Management of this Hospital, I wish you a successful research outing.


Hajia R. Danfillo
For Chairman, MAC



Appendix II : INFORMED CONCENT FORM

I, **Seri Bonmwa Faith (UJ/2011/NS/0534)** a student of University of Jos wish to collect blood sample for B.Sc research work on “ Seroprevalence of human papilloma virus among women attending gynaecology clinic in Jos University Teaching Hospital,Nigeria.”

If you agreed to participate, five milliliter (5ml) blood samples will be collected form you through venipuncture with minimal pains and analyzed for the presence of human papilloma virus.

The result and information obtained may be used in finding ways of providing proper medical care to you and the community at large.

I will assure you that all information about you will be kept confidential and even if the research will be published you will not be identified.

Participation is voluntary and you are free to ask questions if you need more clarifications. Also the research will be conducted at no cost to you.

I..... have read (or have been read to) and understand the informed consent form and agreed to allow medical information and samples to be used in the research.

.....

.....
Signature of Participant

Date

I have read and explained in details to the above participant and have answered questions about this information.

.....

.....
Seri Bonmwa Faith

Date