

# MOLECULAR VARIANTS OF HUMAN PAPILLOMAVIRUS AMONG INDIVIDUALS ATTENDING HEALTHCARE CHECKUP IN EVERIGHT DIAGNOSTIC AND LABORATORY SERVICES OWERRI, IMO STATE, NIGERIA.

## ABSTRACT

**Background:** Human papillomaviruses (HPV) are small, non-enveloped, epitheliotropic, double-stranded DNA viruses that infect mucosal and cutaneous epithelia in a wide variety of higher vertebrates in a species-specific manner and induce cellular proliferation. Papilloma viruses are highly epitheliotropic, with a highly host-specific affinity and humans are the only host of HPV. HPV Array Test can detect up to 33 genotypes; which according to the research result of the WHO International Agency for Research on Cancer (IARC), are classified as Low Risk types (6, 11, 42, 43, 44 & 81) which can cause the skin mucosa wart-like lesions; HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 & 68 are classified as high-risk types; HPV 26, 53, 66, 73 & 82 genotypes are classified as middle-risk types. Many countries in West Africa, such as Nigeria have sparing data on the molecular variants of HPV infection and genotype distribution, especially among individuals in the South Eastern part of Nigeria.

**Aim:** This study was designed to determine and classify the molecular variants of HPV among individuals attending healthcare checkup in the Molecular Science and Genetic Studies Department of Everight Diagnostics, Owerri.

**Methodology:** HPV Array test relies on PCR amplification and “Flow-through” hybridization technology. Genomic DNA of the human papilloma viral isolates from tissue samples were extracted using MN Research Bacterial DNA MiniPrep™ Kit. A pool of results was collated from 163 individuals whose urethra and endo-cervical swab samples had been collected and processed accordingly.

**Results:** The demographic distribution of the study population show 66.25% and 33.74% for female and male respectively while the mean age was  $38.34 \pm 13.63$ . Out of the 163 individuals recruited, 50 (30.66%) were HPV positive with the highest prevalence of 22.08 % in females while males recorded 8.58%. High-risk HPV had the highest prevalence of 34 (68%), while 8 (16%) and 8 (16%) were recorded for low and medium risks respectively. The most common genotypes were HPV 16 (8%), HPV 35 (8%), HPV 39 (8), and HPV 51 (8%). High-risk dual infection was recorded in 7 (13%) while 5 (10%) had multiple high-risk HPV infections. HPV 11 (6%) was the highest occurring low-risk HPV infection while HPV 6 and 11 3(6%) were the most occurring dual low-risk HPV genotypes. No cases of multiple low-risk HPV infections were recorded in this study.

**Conclusion:** The research findings shows HPV-16, 35, 39 and 51 as the predominant genotypes amongst the screened individuals. These are high-risk-human papilloma viral genotypes that predispose individuals to cervical and/or urethral cancers, with HPV-16 standing at the top of the hierarchy, followed by HPV-33 and HPV-31. Although HPV genotype is important in determining appropriate triage strategies, other factors should also be considered such as cytology results and fully validated biomarkers such as p16, p16/ki-67 dual-stain, methylation classifiers, viral load and type-specific sequence variants before cancer status is established.

**Keywords:** Human papillomavirus, Genotypes, Variants, Proliferation, Prevalence

## INTRODUCTION

Human papillomaviruses (HPVs) constitute a group of more than 100 different genotypes associated with benign and malignant neoplasms of the skin and mucous membranes [1]. Approximately 40 different HPV genotypes have been detected in the anogenital mucosa [2]. On the basis of their epidemiological association with the development of cervical carcinoma, a group of so-called high-risk HPV genotypes has been defined. These include HPV genotype 16 (HPV-16), HPV-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68 [3]. Other genotypes, such as HPV-6, -11, -42, -43, and -44, are classified as low-risk types [4].

Human papillomavirus (HPV) is the most common sexually transmitted infection and at least 50% of sexually active people will get HPV at some time in their lives [5]. Akarolo-Anthony *et al.* [6] reported more than 100 HPV genotypes have been identified based on the sequence of their L1 genes. HPV is classified into high-risk, probable high-risk, and low-risk types, based on HPV-type-specific odds ratios and HPV prevalence among groups of women with cervical cancer and their controls. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered high-risk HPV (hrHPV) [7]. In addition to already established types, the International Agency for Cancer Research (IARC) recently classified HPV39, 59, 51, and 56 as carcinogenic while HPV68, 26, 30, 34, 53, 66, 67, 69, 70, 73, 82 and 85 were classified as possibly carcinogenic, but this classification has been criticized for lack of supporting epidemiological data. The classification of HPV types according to their oncogenic potential is an ongoing process and is dependent on the availability of data from different parts of the world [8].

With an incidence estimated at about 530,000 new cases and 275,000 deaths worldwide each year [9]. Cervical cancer is the leading cancer in women in sub-Saharan Africa and it remains a

serious public health issue. While HPV 16 and HPV 18 genotypes are involved in approximately 70% of cancers of the cervix in the world [8], the distribution of the other genotypes follows geographic variation. This difference in the distribution of genotypes was found in women who were living in different regions of the same country [10].

## **MATERIALS AND METHODS**

### **Study Area**

This cross-sectional analysis was carried out at the Molecular Science and Genetic Studies of Everight Diagnostic and Laboratory Services Limited located in Owerri, Imo State, South Eastern Nigeria.

### **Study Population and Design**

Data was collated from a total of 163 individuals comprising of 108 females and 55 males with an age range of 19-75. The participants poll span from January 2021 to November 2022. Only individuals who gave informed consent were enrolled in the study. The demographic characteristics was obtained from the laboratory information management system.

### **Inclusion and Exclusion Criteria**

Women with or without cytological abnormalities or symptoms of STIs were included. Conversely, women who have undergone hysterectomy, were pregnant, or menstruating at the time of sample collection were excluded.

### **Sample collection**

Endocervical and urethral swab samples were collected from a total of 163 sexually active women and men respectively. These included men and women presenting for routine cervical

cancer screening (Pap smear), sexually transmitted infections (STI) and routine healthcare checkups. Samples were collected by inserting Cusco's speculum into the vagina in order to expose the cervix. The collection swab was inserted into the endocervix and turned clockwise for 10–15 seconds to ensure adequate sampling. Urethral swabs were used to collect male samples. The swabs were removed gently and placed in pre-labeled screw-capped tubes containing 0.5mL of viral transport medium.

### **Principle of Extraction:**

Genomic DNA of the human papilloma viral isolates from tissue samples was extracted using a MN Research Bacterial DNA MiniPrep™ Kit (Zymo Research, Irvine, CA) following manufacturer's instructions. With the NucleoSpin Tissue method genomic DNA was prepared from swab samples of subjects. Lysis was achieved by incubation of the sample material in a proteinase K/SDS solution. Appropriate conditions for DNA binding to the silica membrane in the NucleoSpin Tissue Columns were achieved by the addition of chaotropic salts and ethanol to the lysate. The binding process is reversible and specific to nucleic Acids. Contaminations were removed by subsequent washing with two different buffers. Pure genomic DNA was finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

### **PCR Amplification:**

The human papilloma viral genotypes were amplified using purified genomic DNA as a template, Oligonucleotide primers which was sourced and synthesized to amplify the intact region of the viral genotypes. The PCR mixture consist of 10x reaction buffer with MgCl<sub>2</sub> (1.5Mm), 2ul each of dNTP mix 2.5Mm), 2ul each of forward and reverse primers (10picomoles/ul each primer), 0.3ul of Taq DNA Polymerase (5 U/ul) and 5ul of template DNA

in a total of 20ul all contained in a thin-walled (0.2ml) PCR tube. This was then placed in a Rotor Gene-Q thermocycler (Thermo Fisher Scientific) set to the following pre-optimized conditions:

Initial pre-incubation at 50oC for 5mins.

Initial denaturation at 95oC for 2mins

45 cycles of denaturation at 94oC for 15 seconds

Cooling at 52oC for 1min.

## RESULTS

The demographic characteristics of the participants are presented in table 1. A total of 163 subjects were enrolled for this study which includes 108 females and 55 males with a total mean age of 38.34±13.63.

**Table 1: Demographic Data of Participants**

Parameter	Female	Male	Total
No of participants (n)	108	55	163
Mean Age	36.58±13.06	41.8±14.04	38.34±13.63

The percentage prevalence of HPV infection among the subjects as shown in table 2 shows that 22.08% of females were infected while 8.58 % of males were infected. The total percentage prevalence of HPV infection as obtained in this study is 30.66 %.

**Table 2: Percentage Prevalence of HPV among the Subjects**

<b>Parameter</b>	<b>Total tested (%)</b>	<b>No Infected (%)</b>
Female	108 (66.25)	36 (22.08)
Male	55 (33.74)	14 (8.58)
<b>Total</b>	<b>163 (100)</b>	<b>50 (30.66)</b>

The identified high-risk HPV genotypes (table 3) show that HPVs (16, 35, 39 & 51) were the most prevalent with a percentage occurrence of 8 % for each of the mentioned genotypes respectively. The high-risk single HPV genotype constituted the highest prevalence of infection at 68 %, this was followed by the high-risk dual genotype with 13 % prevalence while the least prevalence was recorded for multiple high-risk infections with 10%.

**Table 3: Distribution of Identified HPV (High Risk) genotypes and Classifications among**

<b>HPV high Risk (Single)</b>	<b>No. Positive (%)</b>	<b>High Risk (Dual)</b>	<b>No. Positive (%)</b>	<b>High Risk (Multiple)</b>	<b>No. Positive (%)</b>
HPV 16	4 (8)	HPV 16 & 18	1 (2)	HPV 16, 26 & 31	1 (2)
HPV 35	4 (8)	HPV 26 & 53	1 (2)	HPV 16, 53 & 66	1 (2)
HPV 39	4 (8)	HPV 31 & 33	1 (2)	HPV 26, 31 & 53	1 (2)
HPV 51	4 (8)	HPV 35 & 53	1 (2)	HPV 59, 31 & 52	1 (2)
HPV 53	2 (4)	HPV 51 & 53	1 (2)	HPV 59, 52 & 35	1 (2)
HPV 56	2 (4)	HPV 53 & 56	1 (2)		
HPV 59	1 (2)	HPV 68 & 82	1 (2)		
HPV 66	1 (2)				
<b>Subtotal the Subjects.</b>	<b>22 (68)</b>		<b>7 (13)</b>		<b>5 (10)</b>

Low-risk genotypes (table 4) had an 8 % prevalence for both single and dual risks classes respectively. Low-risk multiple HPV classes were not detected in the present study.

**Table 4: Distribution of Identified HPV (Low Risk) genotypes and Classifications among the Subjects.**

<b>HPV Low Risk (Single)</b>	<b>No. Positive (%)</b>	<b>Low Risk (Dual)</b>	<b>No. Positive (%)</b>	<b>low Risk (Multiple)</b>	<b>No. Positive (%)</b>
HPV 6	1 (2)	HPV 6 & 11	3 (6)	-	-
HPV 11	3 (6)	HPV 11 & 42	1 (2)	-	-
<b>Subtotal</b>	<b>4 (8)</b>		<b>4 (8)</b>	-	-

A total of 8 unclassified HPV genotypes (table 5) constituting 16 % prevalence of infection were identified in this study.

**Table 5: Distribution of Identified HPV (Medium Risk) genotypes and Classifications among the Subjects.**

<b>Unclassified HPV Risk</b>	<b>No. Positive (%)</b>
HPV 11, 45, 35, 58, 51 & 73	1 (2)
HPV 16, 6, 59, 56, 53 & 75	1 (2)
HPV 6, 58 & 73	1 (2)
HPV 16, 70, 73 & 51	1 (2)
HPV 6, 45 & 51	1 (2)
HPV 52, 51, 53, 45 39 & 6	1 (2)
HPV 11, 35, 43 & 53	1 (2)
HPV 45, 70 & 18	1 (2)
<b>Subtotal</b>	<b>8 (16)</b>

## **DISCUSSION:**

Human papillomavirus (HPV) is the most common sexually transmitted viral infection worldwide and is associated with occurrence of warts (condylomas) and a variety of cancers in both men and women [11]. The outcome of HPV infection depends on the specific HPV type/s present and can range from asymptomatic infection to severe squamous cell malignancies. Low-risk HPV types (such as types 6 and 11) are associated with anogenital warts and mild dysplasia, while high-risk types (such as 16 and 18) are associated with high-grade dysplasia and cancers of the cervix, vulva, vagina, urethra, penis, anus and oropharynx [12].

This study is a cross-sectional study of Human papillomavirus in Owerri, South East Nigeria. This study to the best of our knowledge is the first of its kind in this city. In this study, the reported age range was between 19 to 70 years with an average of  $38.34 \pm 13.63$  years. This study population is dissimilar to the findings of Kuassi-Kpede et al. [13] who reported age range to be between 17 and 61 with average of  $34.67 \pm 1.2$  years in women in Lome, Togo. Traore et al [14] reported in Bobo-Dioulasso (Burkina Faso) age range between 20 and 56 with an average of  $35.3 \pm 0.6$  years. The 22.08% female prevalence observed in this study is in line with the findings of [14] who reported 25.4 % in females in Bobo-Dioulasso. Data from Benin show overall prevalence of HPV infection of 33.2% in women [15]. The prevalence of HPV positivity of 26.3 % found in Ibadan, Nigeria is in consonant with previous reports of the elevated prevalence of HPV in Sub-Saharan Africa [16]. Numerous reports indicate a higher prevalence of oncogenic HPV types in Sub-Saharan Africa compared to other parts of the world [17,7,18] with an average reported prevalence of 24 % in Sub-Saharan Africa [19]. Few studies are available that have determined the frequency of acquisition and the duration of HPV infection in men. A prevalence of 8.58 % was observed in this study for men. A study by [20] reported a high prevalence with

72.1 % of men testing positive for the virus. This results are in consonant with previous published data showing prevalence ranging from 1.3 to 72.9 % [21-24]. Singh et al. in 2012 [25], demonstrated that in sub-Saharan Africa, the high prevalence of HPV was due to: immune deficiency, poverty, urbanization, poor socio-economic conditions; the precocity of sexual relationships; numerous maternities without strict hygiene rules.

The prevalence of 68% of high-risk HPV obtained in this study is high compared to the worldwide prevalence of HPV infection estimated to be around 11-12% [19]. The prevalence of high-risk HPV in this study is in line with the 23.2% reported in Thiès, in Senegal [14], and the 23% in rural women in Mali [26]. Compared to the low-risk (LR-HVP) type (16%), HPV (HR-HPV) was substantially more prevalent. Concern should be expressed about the high prevalence of high-risk types found in this study, as persistent infections of these kinds have been linked to cervical cancer. Many research in Nigeria has previously reported findings indicating a greater frequency of HR-HPV. [16,6, 27-29]. Related findings have also been documented in some other African countries [15, 32, 14].

This study confirmed the findings of previous studies [33, 15] in that there were substantially more single infections (44%) than dual infections (14%). Multiple HPV infection was 10%. Infections with multiple high-risk HPV types may pose a greater risk of developing cervical cancer. Multiple infections may impact HPV testing, particularly if the assay is unable to identify other kinds present in multiple infections. This may result in underreporting of HPV type-specific prevalence. Furthermore, it would be challenging to provide effective immunization against HPV infection given that the existing vaccines can only provide protection against a subset of the HPV types, leaving the others to circulate in the community.

In this study, HPV types 16, 35, 39, and 51 were most prevalent, followed by HPVs 53 and 56. In a global survey, women reported having similar prevalent HPV strains, whereas HPV-16 was more common [33, 34]. The most often found HPV types are HPV-16, 18, 45, 35, 33, and 52, according to epidemiological research among women in sub-Saharan Africa [35]. Several HPV kinds are more and less common, according to several research in Nigeria. In Lagos, HPV types 31, 52, 53, and 35 were the most prevalent, even among HIV positive people, while types 18, 16, 52, and 56 were more prevalent among HIV negative people [29]. The type of assay employed, numerous HPV infections, disparities in the research population, and varying exposures of individuals to various risk factors in various geographic regions are among variables that could be to blame for variations in the distribution of HPV types around the world [36]

Regarding the evaluation of changes in HPV type distribution, several cautions are pertinent. Gravitt et al. [36] pointed out that type-specific HPV prevalence may be influenced by the assay utilized as well as by the high rate of multiple HPV infections in some groups. Moreover, research from sub-Saharan Africa has revealed changes in the relative ranking of HPV types that are consistent with chance, and everywhere the prevalence of HPV 16 and 18 increased with the severity of cervical findings [37]

Among 799 cervical cancer biopsies from Africa, the type-specific distribution of HPV revealed that HPV 16 made up 50.2% of samples, HPV 18 made up 14.1%, and HPV 45 made up 7.9% (i.e., a distribution comparable to that reported globally) [37]. The high-risk genotypes of HPV 16 and HPV 18 are included in the vaccinations that are available to prevent cervical cancer. Nonetheless, it was discovered that HPV 16 was the most prevalent genotype worldwide, particularly in Europe [38], the USA [39], and North Africa [40].

## **CONCLUSION**

The research findings show HPV-16, 35, 39 and 51 as the predominant genotypes amongst the screened individuals. These are high-risk human papillomaviral genotypes that predispose individuals to cervical and/or urethral cancers, with HPV-16 standing at the top of the hierarchy, followed by HPV-33 and HPV-31. Although HPV genotype is important in determining appropriate triage strategies, other factors should also be considered such as cytology results and fully validated biomarkers such as p16, p16/ki-67 dual-stain, methylation classifiers, viral load and type-specific sequence variants before cancer status is established.

## **INFORMED CONSENT AND ETHICAL APPROVAL**

All participants of the study were briefed on the nature of the study and informed consent was obtained. They filled out a questionnaire form covering information about their age, and gender. Ethical clearance was obtained by the Institutions Ethical Committee.

## REFERENCES

1. Chan SY, Delius H, Halpern AL, Bernard HU. 1995. Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *J. Virol.* 1995; 69:3074-3083.
2. zur-Hausen H. Papillomavirus infections—a major cause of human cancers. *Biochim. Biophys. Acta.* 1995 1288:55-78
3. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah, KV, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J. Natl. Cancer Inst.* 1995; 87:796-802.
4. Cuzick J, Sasieni P, Singer A. Risk factors for invasive cervix cancer in young women. *Eur. J. Cancer* 1996; 32:836-841.
5. Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virol.* 2010, 401: 70-79. 10.1016/j.virol.2010.02.002.
6. Akarolo-Anthony S.N, Al-Mujtaba M, Famooto A.O. *et al.* HIV associated high-risk HPV infection among Nigerian women. *BMC Infect Dis* 2013; 13:521-53. <https://doi.org/10.1186/1471-2334-13-521>
7. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S, Anh PT, Ferreccio C, Hieu NT, Matos E, et al: Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet.* 2005, 366: 991-998. 10.1016/S0140-6736(05)67069-9.
8. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer.* 2011, 128: 927-935. 10.1002/ijc.25396.
9. Arbyn M, Castellsagué X, de sanjosé S. et al., “Worldwide burden of cervical cancer in 2008,” *Ann. Oncol.* 2011; 22(2), 2675–2686, 2011.
10. Mbaye EHS, Gheit T, Dem A. et al., “Human papillomavirus infection in women in four regions of Senegal,” *J. Med. Viro.* 2014; 86(2), 248–256.

11. Giuliano AR, Tortolero-Luna G, Ferrer E, Burchell AN, de Sanjose S, Kjaer SK, Munoz N, Schiffman M, Bosch FX. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. *Vacc.* 2008; 26(10), 17–28.
12. Müller EE, Rebe K, Chirwa TF. *et al.* The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa. *BMC Infect Dis.* 2016. 16: 440-452.
13. Kuassi-Kpede A.P, Dolou E, Zohoncon T.M. *et al.* Molecular characterization of high-risk human papillomavirus (HR-HPV) in women in Lomé, Togo. *BMC Infect Dis.* 2021; 21: 278-285.
14. Traore IMA, Zohoncon MT, Ndo O, Djigma WF, Obiri-Yeboah D, Compaore T, Guigma S, Yonli A, Traore, G, Ouedraogo P. Simpore J. Oncogenic human papillomavirus infection and genotype characterization among women in Orodara, Western Burkina Faso. *Pak J Biol Sci.* 2016; 19:306-314.
15. Piras F, Piga M, De Montis A. *et al.* Prevalence of human papillomavirus infection in women in Benin, West Africa. *Virol J.* 2011; 8: 515-523.
16. Thomas J, Herrero R, Omigbodun A. *et al.* Prevalence of papillomavirus infection in women in Ibadan, Nigeria: a population-based study. *Br J Cancer.* 2004; 90, 638–645.
17. Castellsagué X, Menéndez C, Loscertales M.P. *et al.* Human papillomavirus genotypes in rural Mozambique. *The Lancet.* 2001; 358(9291): 1429–1430
18. Mayaud P, Weiss HA, Lacey CJN, Gill DK, Mabey DCW. Genital human papillomavirus genotypes in northwestern Tanzania. *J.Clin. Microbio.* 2003; 41(9): 4451–4453.
19. Formana D, de Martel C, Lacey CJ, *et al.* Global burden of human papillomavirus and related diseases. *Vacc.* 2012; 30(5): 12–23.
20. Marcos FP, Pires D, Forjaz R, Sato S, Cotrim I, Stiepcich M, Scarpellini B, Truzzi JC. Genital prevalence of HPV types and co-infection in men. *Int Braz J Urol.* 2014 Jan-Feb;40(1):67-71.
21. Giuliano AR, Anic G, Nyitray AG. Epidemiology and pathology of HPV disease in males. *Gynecol Oncol.* 2010; 117: 15 -19.
22. Dunne EF, Markowitz LE. Genital human papillomavirus infection. *Clin Infect Dis.* 2006; 43: 624-629.

23. Wikström A, Hedblad MA, Syrjänen S. Penile intraepithelial neoplasia: histopathological evaluation, HPV typing, clinical presentation and treatment. *J Eur Acad Dermatol Venereol* . 2012; 26: 325-330.
24. Vaccarella S, Plummer M, Franceschi S, Gravitt P, Papenfuss M, Smith D, et al. Clustering of human papillomavirus (HPV) types in the male genital tract: the HPV in men (HIM) study. *J Infect Dis*. 2011; 204: 1500-1504.
25. Singh, G.K, Azuine RE, Siahpush M. Global inequalities in cervical Cancer incidence and mortality are linked to deprivation, low socioeconomic status, and human development. *Int J MCH AIDS*. 2012; 1(1), 1–17
26. Schluterman N.H, Sow S.O, Traore CB, et al. Differences in patterns of high-risk human papillomavirus infection between urban and rural low-resource settings: cross-sectional findings from Mali. *BMC Woms Heal*. 2013; 13(4): 1–9.
27. Dareng EO, Ma B, Famooto AO, Akarolo-Anthony SN, Offiong RA, Olaniyan O, et al. Prevalent high-risk HPV infection and vaginal microbiota in Nigerian women. *Epidemiology & Infect*. 2016; 144 (1): 123–137.
28. Fadahunsi OO, Omoniyi-Esan GO, Banjo AA, Esimai OA, Osiagwu D, Clement F, et al. Prevalence of High Risk oncogenic HPV types in cervical smears of women attending well women clinic in Ile-Ife. *Gynaecology Obstetrics*. 2013; 3(6): 1000185.
29. Nweke IG, Banjo AAF, Abdulkareem FB, Nwadike VU. Prevalence of Human Papilloma virus DNA in HIV positive women in Lagos University Teaching Hospital (LUTH) Lagos, Nigeria. *Brit Microbiol Res J*. 2013; 3(3): 400–413.
30. Adegbesan-Omilabu MA, Okunade KS, Omilabu SA. Oncogenic human papillomavirus infection among women attending the cytology clinic of a tertiary hospital in Lagos, South-West Nigeria. *IJRMS*. 2014; 2(2): 625–630
31. Ezechi OC, Ostergren PO, Nwaokorie FO, Ujah IAO, Odberg PK. The burden, distribution and risk factors for cervical oncogenic Human papillomavirus infection in HIV positive Nigerian women. *Virology J*. 2014; 11:15.
32. Zohoncon TM, Bisseye C, Djigma FW, Yonli AT, Compaore TR, Sagna T, Ouermi D, Ouédraogo CM, Pietra V, Nikiéma JB, Akpona SA, Simpore J. Prevalence of HPV High-Risk Genotypes in Three Cohorts of Women in Ouagadougou (Burkina Faso). *Mediterr J*

*Hematol Infect Dis.* 2013 Sep 2;5(1) :e2013059. doi: 10.4084/MJHID.2013.059. PMID: 24106609; PMCID: PMC3787662.

33. de Sanjosé S, Diaz M, Castellsague X, Clifford G, Bruni L, Muñoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect. Dis.* 2007; 7(7): 453–59. [https://doi.org/10.1016/S1473-3099\(07\)70158-5](https://doi.org/10.1016/S1473-3099(07)70158-5) PMID: 17597569
34. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjosé S. Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1 Million Women with Normal Cytological Findings. *J. Infect. Dis.* 2010; 202(12): 1789–1799. <https://doi.org/10.1086/657321> PMID: 21067372.
35. Denny L, Adewole I, Anorlu R, Dreyer G, Moodley M, Smith T, et al. Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. *IJC.* 2014; 134: 1389–1398
36. Gravitt PE, Kamath AM, Gaffikin L, Chirenje ZM, Womack S, Shah KV. Human papillomavirus genotype prevalence in high-grade squamous intraepithelial lesions and colposcopically normal women from Zimbabwe. *IJC.* 2002; 100: 729–732. <https://doi.org/10.1002/ijc.10538> PMID:12209615
37. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Br J Cancer* 2003; 89: 101–105
38. Tjalma WA, Trinh XB, Rosenlund M, et al. A cross-sectional, multicentre, epidemiological study on human papillomavirus (HPV) type distribution in adult women diagnosed with invasive cervical cancer in Belgium. *Facts Views Vis Obgyn.* 2015; 7(2):101–108.

39. Monsonogo J, Cox JT, Behrens C, et al. Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol. Oncol.* 2015; 137(1):47–54.
40. Guettiti H, Ennaifer E, Attia L, et al. Pre-vaccination prevalence and genotype distribution of human papillomavirus infection among women from urban Tunis: a cross-sectional study. *APJCP.* 2014; 15(21): 9361–9365. doi: 10.7314/apjcp.2014.15.21.9361.

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