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

ABSTRACT

Comment [Ma1]: Abstract section is too long, the author may minimize the background section and the conclusion in the abstract to meet the journal criteria regarding the length of abstract no more than 300 words, also there are very basic information which is not nessary

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 MiniPrepTM 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±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.

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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.

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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.

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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.

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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.

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Principle of Extraction:

Genomic DNA of the human papilloma viral isolates from tissue samples was extracted using a MN Research Bacterial DNA MiniPrepTM KIt (Zymo Research, Irvine, CA) following manufacturer's instructions. With the NuceloSpin 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 $MgCl_2$ (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.

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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

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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

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

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

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HPV high Risk (Single)	No. Positive (%)	High Risk (Dual)	No. Positive (%)	High Risk (Multiple)	No. Positive (%)
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)				
Subtotal **the Subjects.**	**22 (68)**		**7 (13)**		**5 (10)**

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.

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

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.

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Unclassified HPV Risk	No. Positive (%)
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)

Subtotal	**8 (16)**

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±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±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±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

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The study is in accordance with Kuassi-Kpede et al. (2021) nearly age range and age average

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In the discussion section must compare the study results with recent 5 years or at most the last 10 years, otherwise the author just report the study results with suitable interpretation supported with robust analysis from textbooks with new editions

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].

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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.

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