Molecular characterisation of genital human papillomavirus among women in Southwestern, Nigeria

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Abstract

Background

Persistent infections with high-risk genital Human papillomavirus (HPV) especially types 16 and 18, are associated with cervical cancer. However, distribution of HPV types varies greatly across geographical regions and the available vaccines target only few types. This study was designed to determine the HPV types circulating in Southwestern Nigeria, thereby providing necessary information for effective control of the virus.

Methods

Endocervical swab samples were collected from a total of 295 consenting women attending routine cervical cancer screening, STI clinics and community-based outreach programme. Viral DNA was extracted from the samples and the consensus region of the HPV DNA was amplified by PCR using GP-E6/E7 primers. Type-specific nested multiplex PCR and Sanger sequencing were used to genotype the HPV isolates.

Results

In this study, 51 (17.3%) individuals were positive for HPV DNA using consensus primers that target the E6/E7 genes but only 48 (16.3%) were genotyped. A total of 15 HPV types (HPV-6, 16, 18, 31, 33, 35, 42, 43, 44, 52, 58, 66, 74, 81, 86) were detected, with HPV-31 being the most predominant (32.8%), followed by HPV-35 (17.2%) and HPV-16 (15.5%). Two rare HPV types; 74 and 86 were also detected. The HPV-74 isolate had three nucleotide (CCT) insertions at E7 gene that translated into amino acid proline. Highest nucleotide substitutions (n = 32) were found in HPV-44 genotype. Among positive individuals, 20.8% had dual infections and 86.2% had High-risk HPV types.

Conclusions

Multiple Human papillomavirus types co-circulated in the study. Most of the circulating Human papillomavirus are high-risk type with type 31 being the most predominant. Although the implication of HPV-74 with proline insertion detected for the first time is unknown, it may
have effect on the transformation potential of the virus. Polyvalent HPV vaccine will be more effective for the infection control in Nigeria.

Introduction
Genital Human papillomavirus (HPV) infection is the most frequent sexually transmitted infection globally and most sexually active individuals will be infected with HPV at some periods in their lives [1, 2]. Young women are more vulnerable to the virus and often become infected by multiple HPV types most especially the high-risk types. Infection by these types of genital HPV is recognised as a causal and essential factor for cervical cancer [3]. Human papillomavirus infection is a major health challenge in developing countries where 80% of cervical cancer occurs. In Nigeria, cervical cancer is the second most common cancer and the 2nd cause of female cancer deaths with estimated 14,550 diagnosed cases and 9659 death annually [4].

Human papillomaviruses are small, non-enveloped, epitheliotrophic viruses with approximately 8 Kbp circular double-stranded DNA, and belongs to the *Papillomaviridae* family [5]. The genome encodes 6 early proteins (E1, E2, E4, E5, E6 and E7) that are responsible for virus replication, and 2 late proteins (L1 and L2) which are the major and minor viral capsid proteins respectively [6]. About 200 HPV genotypes have been identified based on the sequence of their L1 genes [2, 7]. They can be categorized into cutaneous or mucosal types based upon their tissue tropism [8]. The E6 and E7 proteins are the major oncoproteins which are involved in the transformation and immortalisation of host cells. The E6 and E7 proteins bind and inactivate the two major tumour suppressor proteins; p53 and pRb respectively [9]. The sequences of HPV E6 and E7 regions have been used in the classification of some HPV variants [10–12].

Approximately 40 HPV types infect the female genital tract and are further classified as high-risk HPV (HR-HPV) and low-risk HPV (LR-HPV) types according to their oncogenic potential [13]. Persistent infection with high-risk HPV especially types 16 and 18 are associated with 70% of cervical cancers [14]. Two HPV vaccines (Cervarix and Gardasil) are licensed for use in Nigeria [15] and these target two (HPV-16 and 18) and four (HPV-6, 11, 16 and 18) HPV types respectively [16]. The Nigerian National Cervical Cancer Control Policy in 2010 authorized the vaccination of girls aged 9–15 years with Cervarix, but only few privileged individual have been able to use it due to the low knowledge of HPV infection and vaccines, and high cost of the vaccination [12]. The third HPV vaccine (Gardasil-9) is a nonavalent vaccine that was licensed by FDA in December 2014. It protects against HPV-6, 11, 16, 18, 31, 33, 45, 52, and 58 but it is yet to be licensed in Nigeria [16].

However, there are variations in the distribution of HPV types circulating among Nigerian women in different part of the country which have raised concerns about the effectiveness of the available vaccines in the region. Thus, this study was designed to determine the circulating HPV types among women in Southwestern Nigeria, thereby providing information towards effective prevention and control of HPV infection.

Materials and methods
Sample collection and processing
The sample size for this study was determined by using a statistical formula [17], and the expected prevalence used for the calculation was based on the prevalence reported by Thomas *et al.* [18]. Endocervical samples were collected from a total of 295 sexually active women,
between ages 23 and 77 years. These include women presenting for routine cervical cancer 
screening (Pap smear), sexually transmitted infections (STIs) clinic attendees and women 
enrolled during community based outreach programmes. The participants were enrolled 
between March, 2014 and November, 2015 from Molete community in Ibadan and two health 
facilities [University College Hospital (UCH), Ibadan and Baptist Medical Centre (BMC), 
Saki] all located in Oyo State, Southwestern Nigeria. Women with or without cytological 
abnormalities or symptoms of STIs were included. On the other hand, women who have 
undergone hysterectomy, pregnant, or menstruating at the time of sample collection were 
excluded. Only women who gave informed consent were enrolled for the study. 

Socio-demographic, clinical and sexual history were also obtained from each participant 
using structured questionnaire. Two swab samples were collected from the endocervix of each 
female participant by inserting Cusco’s speculum into the vagina in order to expose the cervix. 
Excess mucus was removed from the cervix and surrounding mucosa using cleaning swab. 
The collection swab was inserted into the endocervix and turned clockwise for 10–15 seconds 
to ensure adequate sampling. The swabs were removed gently and placed in pre-labelled 
screw-capped tubes containing 0.5mL of viral transport medium. The samples were carried on 
ice to the laboratory in the Department of Virology, University College Hospital, Ibadan 
where they were stored at -80˚C until analysed. This study was approved by the University of 
Ibadan/University College Hospital Institutional Review Committee (UI/UCH IRC) with 
research approval number UI/EC/12/0387. The result of this study was analysed using IBM 
SPSS statistic version 21 software. Chi square statistics was used to estimate the degree of cor-
relation between variables with \( p \) values of <0.05 considered as statistically significant.

**DNA extraction and PCR**

Genomic DNA was extracted from each of the samples using commercially available DNA 
extraction kit (Jena Bioscience, Jena, Germany) according to the manufacturer’s instructions. 
The consensus region of the HPV DNA was amplified by PCR using primers targeting the E6/E7 
gene region [one forward primer (GP-E6-3F) and two back primers (GP-E7-5B and GP-E7-6B)] 
as previously described by Sotlar et al. [19]. The cycling conditions for PCRs with GP-E6/E7 con-
sensus primers were preceded by an initial denaturation step at 95˚C for 2 min, followed by 40 
amplification cycles of 95˚C for 30 s, 47˚C for 1 min, and 65˚C for 2 min. The last cycle was fol-
lowed by a final elongation step at 72˚C for 10 min. Type-specific primer pairs for HPV-16, 18, 
31, 33, 35 and 6/11, were used in a nested multiplex PCR (NMPCR) in two cocktails (16,18,35 
and 31,33,6/11) to genotype the HPV isolates [19]. The NMPCRs were performed under the fol-
lowing conditions: the first cycle was preceded by a 2 min denaturation step at 95˚C, followed by 
35 cycles of 95˚C for 30 s, 56˚C for 1 min, and 72˚C for 1 min and the last cycle was followed by a 
final elongation step at 72˚C for 10 min. All PCRs were performed in a final volume of 25μL reaction 
mix containing 5μL of the extracted DNA, 5μL of a premix of PCR buffer, dNTPs, Magnesium 
chloride and Taq Polymerase enzyme in optimized concentration (Jena Bioscience, 
Germany), and 10 pmol of each primer. Five microliters of the PCR product was used as template 
for the nested PCRs. The amplified HPV DNA was detected by electrophoresis on 2% agarose gel 
and visualised using Bio-Rad Gel Doc™ XR+System. The size of the PCR products that were gen-
erated with GP-E6/E7 consensus primers was 630bp while the length of products amplified with 
type-specific primer pairs ranged from 263bp to 457bp as shown in Figs 1–3.

**DNA sequencing and phylogenetic analysis**

Samples that were not typeable by PCR were sequenced and further analysed for genotype 
identification. The PCR products were purified with a commercially available PCR purification
Fig 1. Agarose gel electrophoresis Image of HPV DNA genotyping with HPV-16 (457bp) and 18 (322bp) primers. Numbers are samples ID; L is a mid-range ladder (Jena Bioscience); NC is the negative control while PC is the positive control. The sizes of the PCR products generated are 457bp for HPV-16 and 322bp for HPV-18.

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Fig 2. Agarose gel electrophoresis Image of HPV DNA genotyping with HPV-31 E6/E7 primers. Numbers are samples ID; L is a mid-range ladder while NC is the negative control. The size of the PCR products generated for HPV-31 is 263bp.

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kit (Jena Bioscience, Germany) according to the manufacturer’s instructions. The sequencing was performed commercially (INQABA BIOTEC, South Africa) on ABI Prism 3130 genetic analyzer (Applied Bio Systems) using the same consensus primers used for the PCR. However, only one of the reverse primers (either GP-E7-5B or GP-E7-6B) was used together with the forward primer (GP-E6-3F) to sequence the DNA samples.

Reference sequences of 179 HPV types available on the International Human Papillomavirus Reference Centre site (https://www.hpvcenter.se/human_reference_clones/) were downloaded and aligned with sequences of the sequenced isolates for phylogenetic analysis. Multiple sequence alignment of the isolates and the reference sequences was carried out using the MUSCLE algorithm on MEGA 6.0.6 software [20]. Evolutionary analyses to estimate divergence between sequences were also conducted on MEGA 6.0.6. The Phylogenetic analysis was inferred using Maximum Likelihood method to generate trees [21]. Multiple alignment and phylogenetic analysis of the isolates’ sequences with existing HPV sequences from other parts of the world were also performed. Nucleotide sequences of 19 isolates were deposited in Genbank and assigned the accession numbers KX545348-KX545366.

Results

HPV detection and genotyping

The socio-demographic characteristics of the study participants have been previously described [22]. Out of the 295 samples analysed, 51 (17.3%) were positive for HPV DNA using...
consensus primers that target the E6/E7 genes but only 48 (16.3%) were genotyped either by type-specific PCR or by sequencing. Human papillomavirus DNAs in 37 samples were genotyped by type-specific primer pairs and identified as HPV-16, 18, 31, 33 and 35. The agarose gel electrophoresis images of the HPV type-specific amplification are shown in Figs 1–3. Overall, 15 HPV types were detected at various frequencies in 58 infections (Table 1). Some individuals (20.8%) were infected with two HPV types (dual infections) although there were significantly more single infections (79.2%, \( p = 0.001 \)). The six most commonly detected types were HPV-31, 16, 35, 18, 33 and 35. The HPV isolates included 8 high risk types (HPV-16, 18, 31, 33, 35, 52, 58, 66) and 5 low risk types (HPV-6, 42, 43, 44, 81). However, two rare HPV types (HPV-74 and 86) with unclassified risk were also identified. High-risk HPV types were detected more frequently (86.2%) among HPV positive individuals than low-risk types (8.6%).

Nine HPV types (HPV-31, 35, 16, 18, 33, 58, 74, 86 and 66) were detected among 26 apparently healthy individuals. However, 11 HPV types (31, 35, 16, 18, 52, 6, 42, 43, 44 and 81) were detected among 22 participants with clinical symptoms of STI (Table 2). There was no significant difference in the HPV types that infected the two groups (\( p = 0.518 \)). High-risk HPV types (31, 35, 16 and 18) were found among both groups of participants. All the detected low risk types were found among symptomatic individuals (\( p = 0.007 \)).

Table 1. Distribution of identified HPV genotypes and types of infection among individuals tested.

| HPV Risk Type | No. positive (%) | HPV Infection type | No. positive (%) |
|---------------|------------------|--------------------|------------------|
| **High-Risk (HR)** | | | |
| HPV 16 | 9 (15.5) | HPV 16 | 6 (12.5) |
| HPV 18 | 7 (12.1) | HPV 18 | 3 (6.3) |
| HPV 31 | 19 (32.8) | HPV 31 | 13 (27.1) |
| HPV 33 | 2 (3.4) | HPV 33 | 2 (4.2) |
| HPV 35 | 10 (17.2) | HPV 35 | 6 (12.5) |
| HPV 52 | 1 (1.7) | HPV 52 | 1 (2.1) |
| HPV 58 | 1 (1.7) | HPV 58 | 1 (2.1) |
| HPV 66 | 1 (1.7) | HPV 66 | 1 (2.1) |
| **Subtotal** | **50 (86.2)** | HPV 6 | **1 (2.1)** |
| **Low-Risk (LR)** | | | |
| HPV 6 | 1 (1.7) | HPV 44 | 1 (2.1) |
| HPV 42 | 1 (1.7) | HPV 86 | 2 (4.2) |
| HPV 43 | 1 (1.7) | Subtotal | 38 (79.2) |
| HPV 44 | 1 (1.7) | | |
| HPV 81 | 1 (1.7) | Dual | | |
| **Subtotal** | **5 (8.6)** | 16 & 31 | 2 (4.2) |
| **Unclassified Risk (UR)** | | | |
| HPV 74 | 1 (1.7) | 18 & 31 | 2 (4.2) |
| HPV 86 | 2 (3.4) | 18 & 42 | 1 (2.1) |
| **Subtotal** | **3 (5.2)** | 18 & 74 | 1 (2.1) |
| **Total Infections** | **58 (100)** | 31 & 35 | 2 (4.2) |
| | | 35 & 81 | 1 (2.1) |
| HR & HR | 7 (2.4) | Subtotal | 10 (20.8) |
| HR & LR | 2 (0.7) | | |
| HR & UR | 1 (0.3) | HPV Positive | 48 (16.3) |
| HR only | 33 (11.2) | HPV Negative | 247 (83.7) |
| LR only | 3 (1.0) | Untypeable | 3 (1.0) |
| UR only | 2 (0.7) | Total | 295 (100) |
HPV sequencing analysis

Fourteen HPV types (HPV-6, 16, 18, 31, 33, 35, 42, 43, 44, 52, 58, 66, 74, 81 and 86) were identified by sequence analysis from 19 sequenced samples. Each HPV type has unique nucleotide and amino acid differences (Tables 3 and 4).

The overall mean distance within the isolates was 0.659 ± 0.035. HPV 44 sequence had the highest nucleotide variations both at the E6 and E7 region (n = 32). The HPV-74 sequence had an insertion of a triplet codon (CCT) at the E7 region between A512 and G513 position of the reference sequence (Fig 4) that translated to insertion of amino acid Proline (P) between L28 and D29 position. The phylogenetic trees of sequences of the study isolates with their reference sequences are shown (Figs 5–8).

Discussion

This study is a cross-sectional study of Human papillomavirus in Nigeria. Fifteen HPV types (HPV-6, 16, 18, 31, 33, 35, 42, 43, 44, 52, 58, 66, 74, 81 and 86) were detected in this study indicating co-circulation of multiple HPV types in Southwest Nigeria. The prevalence of high-risk HPV (HR-HPV) type was significantly higher than the low-risk type. The high prevalence of high-risk type in this study is a cause for concern because persistent infections with these types have been recognised as a necessary cause for cervical cancer. Similar results of higher prevalence of HR-HPV have been previously reported in several studies in Nigeria [18, 23–29]. Related findings have also been documented in some other African countries [30–33].

There were significantly more single infections (79.2%) in this study than dual infections (20.8%) which corroborates the findings of past studies [34, 30]. Nonetheless, infections with multiple high-risk HPV types may pose greater risk of developing cervical cancer. Multiple HPV infections could affect HPV testing especially when assay used is not able to detect other types present in multiple infections and this could lead to HPV type-specific prevalence being under reported. Moreover, it would be difficult to achieve effective immunisation against HPV infection where the available vaccines are only able to protect against some HPV types leaving others circulating in the population.

Table 2. Distribution of HPV types by health status of individuals tested.

| HPV Type | No. of Infection | % of Infection | HPV Type* | No. of Infection | % of Infection |
|----------|-----------------|----------------|-----------|-----------------|----------------|
| HPV-31   | 10              | 32.3           | HPV-31    | 9               | 33.3           |
| HPV-35   | 8               | 25.8           | HPV-16    | 4               | 14.8           |
| HPV-16   | 5               | 16.1           | HPV-18    | 5               | 18.5           |
| HPV-18   | 2               | 6.5            | HPV-35    | 2               | 7.4            |
| HPV-33   | 2               | 6.5            | HPV-52    | 1               | 3.7            |
| HPV-58   | 1               | 3.2            | HPV-6     | 1               | 3.7            |
| HPV-66   | 1               | 3.2            | HPV-42    | 1               | 3.7            |
| HPV-74   | 1               | 3.2            | HPV-43    | 1               | 3.7            |
| HPV-86   | 1               | 3.2            | HPV-44    | 1               | 3.7            |
|          |                 |                | HPV-81    | 1               | 3.7            |
|          |                 |                | HPV-86    | 1               | 3.7            |
| **Total**| **31**          | **53.4**       | **27**    | **46.6**        |

STI; Sexually transmitted infection

*Low-risk HPV types are highlighted

bTotal number of infections (some individuals were infected with multiple types)

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There were significantly more single infections (79.2%) in this study than dual infections (20.8%) which corroborates the findings of past studies [34, 30]. Nonetheless, infections with multiple high-risk HPV types may pose greater risk of developing cervical cancer. Multiple HPV infections could affect HPV testing especially when assay used is not able to detect other types present in multiple infections and this could lead to HPV type-specific prevalence being under reported. Moreover, it would be difficult to achieve effective immunisation against HPV infection where the available vaccines are only able to protect against some HPV types leaving others circulating in the population.
The predominant HPV type in this study is HPV-31, followed by HPV-35, 16, 18, 33 and 86. HPV-31 has been reported to be very common in Europe and Latin America [35]. The reason for its predominance in this study is not fully understood. Howbeit, the analysis of the travel history of the participants showed that 63.2% of individuals infected with this HPV type have at one point of their life travelled outside Nigeria, although the exact travel locations were not captured. The only sequenced HPV-31 also clustered with sequence from Portugal.

| S/ N | Name      | HPV Type | E6 Size     | *E6 Nucleotide Substitutions          | E6 Amino acid change |
|------|-----------|----------|-------------|--------------------------------------|---------------------|
| 1    | NGIb106-52 | HPV-52   | 102-548nt   | T404C; C506G                          | No mutation         |
| 2    | NGIb128-31 | HPV-31   | 108-557nt   | C285T; A320T; G404A; A407G; C520T     | H60Y; A138V         |
| 3    | NGIb168-42 | HPV-42   | 114-566nt   | G284A; T310G; T311G                    | F66W                |
| 4    | NGSk203-58 | HPV-58   | 110-559nt   | C187T; G245C; C307T; T322C; C367A; A398G | V46L; D86E; N97D    |
| 5    | NGSk241-86 | HPV-86   | 1-447nt     | T63A; G71A; T372G; A449C              | D21E; S24N          |
| 6    | NGSk246-81 | HPV-81   | 102-566nt   | C269G; C391T                          | N56K; T97I          |
| 7    | NGSk256-18 | HPV-18   | 105-581nt   | T251C; G266A; C287G; G374A; T485C; C491A; A548G; C549A | N129K               |
| 8    | NGSk260-35 | HPV-35   | 110-559nt   | C131A; A295T                          | No mutation         |
| 9    | NGSk266-6  | HPV-6    | 102-554nt   | A221T; C251G; A323C; A365T; C392T; G473A; C479T | S9A; N26H; S39T; Y55F; L70V; N81D; Y82F; V87L; N94K; K95Q; L101F; D122E; D131N |
| 10   | NGSk270-44 | HPV-44   | 105-557nt   | T129G; A180C; C183T; G215C; A218G; G220C; T221C; A268T; C269T; A275T; C312G; A345G; A349T; G363T; A380G; T386G; A387C; C396T; C405T; G407C; C416T; C470G; C482T; A494G; G495A; T524C; A548C; A556G | S12T; N50D; L121V |
| 11   | NGSk271-74 | HPV-74   | 1-453nt     | G35C; A129T; A148G; C331T; T361G | No mutation         |
| 12   | NGSk274-18 | HPV-18   | 105-581nt   | C287G; A476G; T485C; C549A            | No mutation         |
| 13   | NGSk275-16 | HPV-16   | 83-559nt    | T109C; G132T; C143G; G145T; T286A; A289G; C335T; A403G | R171; Q21D; H85Y |
| 14   | NGSk277-86 | HPV-86   | 1-447nt     | T63A; G71A                           | D21E; S24N          |
| 15   | NGSk278-16 | HPV-16   | 83-559nt    | C88G; T109C; G132T; C143G; G145T; T286A; A289G; C335T; A403G | S3P                 |
| 16   | NGSk280-16 | HPV-16   | 83-559nt    | T109C; G132T; C143G; G145T; T286A; A289G; C335T; A403G | S3P                 |
| 17   | NGSk282-43 | HPV-43   | 102-569nt   | A105T; T218G; A256G; T380A; C513T; A543C | T2S; K52R; S148R    |
| 18   | NGSk291-16 | HPV-16   | 83-559nt    | T109C; G132T; C143G; G145T; T286A; A289G; C335T; A403G | R171; Q21D; H85Y   |
| 19   | NGSk294-66 | HPV-66   | 102-569nt   | T108C; C234T                          | R171; Q21D; H85Y   |

*a nucleotide substitutions in bolds translated into different amino acid respectively

*b the two highlighted nucleotide substitutions translated into only one amino acid change

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Therefore, it is not impossible that the infection was acquired through their sexual interactions with infected individuals outside of Nigeria. The prevalence of HPV-31 in this study (4.4%) seemed comparable with 4.0% and 3.8% as previously reported [36, 37]. Similar prevalent HPV types were reported among women in a global study but with HPV-16 predominating [34, 35]. Epidemiological studies among sub-Saharan African women also showed that HPV-16, 18, 45, 35, 33 and 52 are the most commonly detected types [38]. However, some African

| S/N | Name       | HPV Type | E7 Size | E7 Nucleotide* Substitutions | E7 Amino acid change |
|-----|------------|----------|---------|------------------------------|----------------------|
| 1   | NGIb106-52 | HPV-52   | 553-626nt (74bp) | No substitution | No mutation |
| 2   | NGIb128-31 | HPV-31   | 560-657nt (98bp) | G580A; C646T | No mutation |
| 3   | NGIb168-42 | HPV-42   | 542-638nt (97bp) | C607T; A628G; G634A | No mutation |
| 4   | NGSk203-58 | HPV-58   | 574-657nt (84bp) | No substitution | No mutation |
| 5   | NGSk241-86 | HPV-86   | 423-478nt (56bp) | A449C | K9N |
| 6   | NGSk246-81 | HPV-81   | 542-628nt (87bp) | C616T; G625A; C626T | No mutation |
| 7   | NGSk256-18 | HPV-18   | 590-683nt (94bp) | C593T | H2Y |
| 8   | NGSk260-35 | HPV-35   | 562-644nt (83bp) | G595T | V12F |
| 9   | NGSk266-6  | HPV-6    | 530-632nt (103bp) | G617C; T618C; A619T | V30P |
| 10  | NGSk270-44 | HPV-44   | 533-619nt (87bp) | A548C; A556G; C572G; A580T | T6P; Q14E; E16D |
| 11  | NGSk271-74 | HPV-74   | 429-526nt (98bp) | Insertion of CCT | Insertion of P at L28P29D |
| 12  | NGSk274-18 | HPV-18   | 590-620nt (31bp) | T612C | L8S |
| 13  | NGSk275-16 | HPV-16   | 562-646nt (85bp) | A646G | No mutation |
| 14  | NGSk277-86 | HPV-86   | 423-507nt (85bp) | A646G; T648C | N29D |
| 15  | NGSk278-16 | HPV-16   | 562-648nt (87bp) | A646G | No mutation |
| 16  | NGSk280-16 | HPV-16   | 562-646nt (85bp) | A646G | No mutation |
| 17  | NGSk282-43 | HPV-43   | 530-614nt (85bp) | A543C | K5T |
| 18  | NGSk291-16 | HPV-16   | 562-646nt (85bp) | A646G | No mutation |
| 19  | NGSk294-66 | HPV-66   | 572-659nt (88bp) | A586T; G628T; G658A | No mutation |

* Nucleotide substitutions in bolds translated into different amino acid respectively

b Insertion of CCT (highlighted) translated to Proline (P)

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Fig 4. Nucleotide sequence alignment of HPV-74 isolate with reference sequence and sequence from France, showing insertion of CCT. The top row specifies the positions where variations were observed in the sequences; positions without variations were marked with an asterisk (’’) while no asterisk indicated a variation position. The letters indicated their nucleotide base sequences. REF; Reference sequence.

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studies revealed variations in the prevalent HPV types that were also different from the types detected in this study [34, 39, 33].

Several studies in Nigeria have shown disparities in the prevalent HPV types. In Lagos, HPV type 31 also predominated albeit among HIV positive individuals, followed by types 52, 53 and 35 while among HIV negative group, type 18, 16, 52 and 56 were the commonest [27]. The most prevalent types found among women in Port Harcourt [40] and Ibadan [18] agrees with the result of this study except for HPV-18 and 16 dominating respectively. Some factors

Fig 5. Phylogenetic tree of sequences of study isolates with HPV reference sequences. All the nineteen study isolates clustered with only fourteen HPV reference sequences (REF). Study isolates are indicated by shaded triangles. *REF; Reference Sequences.  

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that could be responsible for variations in the distribution of HPV types across the world
include: the type of assay used, multiple HPV infections, differences in the study population,
and varying exposures of individuals to different risk factors in different geographical regions
[41].

High-risk HPVs found both among apparently healthy individuals and symptomatic partic-
ipants further buttress the fact that most infections with HPV do not manifest clinically. Rout-
tine HPV testing among general population would therefore be important for diagnosis.
Further studies on the rare types (HPV-74 and 86) detected in this study would be essential to
access their distribution and determine their association with cervical carcinoma. All the low-
risk HPV types detected were found among symptomatic individuals, although, only one indi-
vidual out of those with genital wart had low-risk HPV (type 81). This could imply that HPV
types other than low-risk types may also be associated with genital warts. Hence, regular HPV
testing and not the mere presence of a wart will be required to detect HPV types in an infected
individual.

The phylogenetic analysis of the 19 sequenced isolates showed that the E6 and E7 sequences
of HPV types are conserved within the types but varies between types. The overall mean dis-
tance between the isolates being 0.659 showed a high level of diversity among the isolates in
the E6/E7 region. To the best of our knowledge; this seems to be the first study that describes
the sequences of HPV types by E6/E7 genes among Nigerian women.

The analysis of HPV E6 and E7 sequences has been used to determine the variants of some
HPV types [10–12]. Although the specific effects of HPV variants on cervical carcinogenesis is
still poorly understood, several reports suggested that variants of some high-risk HPVs espe-
cially types 16 and 18 may be more strongly associated with persistence and progression of
lesions to cervical cancer [10–12]. Four of the sequenced isolates were HPV-16 and are identi-
fied as variants of African lineage (Af) as a result of 3 missense mutations (R17I, Q21D and
H85Y) found in their E6, as previously described [42–45]. The G132T and A403G substitu-
tions also observed in these isolates identified them as Af-2a sub-lineage group as described
[10, 45–47] or lineage B [48, 49]. Literatures have shown that African lineages are the most
prevalent in African regions [42, 50, 51] and are more highly oncogenic than the European
variants [52–54]. The Q21D alteration has been shown to increase the affinity of E6 with p53
by 180% [55] while R17I could lead to decreased affinity with p53 and its degradation [56, 57].

Although the effect of missense mutation (N29D) found in isolate NGSk278-16 was not stud-
ied, previous studies [58, 45] have shown that mutation at this site (N29S) alters the affinity
of E7 for pRB and modifies its oncogenic potential.

The two HPV-18 sequenced in this study have 3 silent mutations at their E6 regions as pre-
viously found [59, 60]. Isolate NGSk256-18 belongs to the HPV-18 Af lineage (now B lineage)
based on the presence of A548G in addition to a C549A [46] while isolate NGSk274-18 belongs
to A lineage due to C549A substitution. The HPV-6 E6 nucleotide alterations observed in this
study were previously found among HPV-6a group [61–63] or HPV-6 sublineage B3 [48]. Sub-
stitutions at the E6 region of HPV-31 study isolate have been previously described as muta-
tions that occur in HPV-31 variants lineage C [64–66]. However, the association of these
variants with morbidity and progression to cancer is unknown.

The HPV-44 sequence with the highest nucleotide substitutions had only two of the E6 sub-
stitutions (C183T and C396T) reported by Maver et al. [67]. The isolate clustered mostly with
HPV-55 now considered a subtype of HPV-44 [68, 8]. The E6/E7 sequences of other sequenced types (HPV-35, 42, 52, 58, 66 and 81) have been described by fewer studies [65, 69, 70, 66, 59] but were not linked to any variant type.

HPV-86 and 74 sequenced in this study are very rare. Their prevalence has been reported by only limited studies [71–73]. The two HPV-86 (isolates NGSk241-86 and NGSk277-86) clustered differently on the phylogenetic tree. The HPV-74 sequence had a triplet codon (CCT) insertion that translated to amino acid Proline (P) at the E7 region. Based on information available in the literature, this is the first report of the insertion. Although the implication of the insertion is unknown, it may have effect on the oncogenic potential of the virus.

The presence of HPV-74 and 86 among participants in this study could not be linked to any factor. However, the genome of HPV-74 was first identified from an immunosuppressed woman with persisting low-grade vaginal intraepithelial neoplasia [74], while that of HPV-86 was initially isolated from the cervicovaginal cells of a woman with cervical intraepithelial neoplasia grade 1 [75]. Future studies that will detect HIV infection and cytological abnormalities of all participants will be conducted in addition especially to access the association of these rare types with cervical abnormalities.
In this study, 3 (5.9%) out of the 51 positive samples could not be successfully genotyped. The three samples had unreadable sequences due to mixed peaks shown on their sequenced data which means that it is still possible to have some more HPV types in the samples. Hence, the genotypic result of this study might not be representative of the overall circulating genotypes in Southwest Nigeria. This limitation however can be tackled in future studies by the use of Next Generation Sequencing technology.

In conclusion, the high prevalence of high-risk HPV types detected in this study even in multiple infections reveals the burden of HPV infection in the country. Infections with high-risk types have the tendency of progressing to malignancy; thus an increased surveillance to determine the women at risk of cervical cancer is advocated. Further studies into detecting HPV-74 and 86 (rare types) will be important to determining their association with cancer. Multiple HPV types detected with non-HPV 16 and non-HPV 18 dominating indicated that HPV-16 and 18 might not be the major circulating types in Nigeria and the available vaccines in the country may be less effective in controlling the infection. However, 7 (HPV-6, 16, 18, 31, 33, 52 and 58) out the 15 circulating types, detected in 40 infections (69.0%) are vaccine types in Gardasil-9. It is therefore necessary for policy makers to consider a more protective polyvalent vaccine like Gardasil-9 for effective control of HPV infection, and the vaccine should be made available at a subsidised rate to the target population in Nigeria.

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