SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: 10.36346/sarjpm.2024.v05i04.004

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Molecular Characterization of Human Papilloma Virus among Women Attending Selected Hospitals in Sokoto, Sokoto State, Nigeria

Gambo, S[1*], Manga S. B[2], Baki, A. S[2], Muhammad, U-K[2], Habibu, A[3]

[1]Department of Microbiology, Federal University Gusau, Gusau, 860001, Nigeria
[2]Department of Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria
[3]Department of Biological Sciences, Zamfara State University, Talata-Mafara, Zamfara State

***Corresponding Author:** Gambo, S
Department of Microbiology, Federal University Gusau, Gusau, 860001, Nigeria

Article History: | Received: 28.03.2024 | Accepted: 08.05.2024 | Published: 24.07.2024 |

Abstract: Human Papilloma Virus (HPV) infection is the primary cause of virtually all cervical cancers. Cervical cancer despite being largely preventable is still the leading cause of gynaecological cancer related death among females in developing countries. This study was carried out to molecularly characterized Human Papilloma Virus among women attending selected hospitals in Sokoto, Sokoto State, Nigeria. This study involves the analysis of high vaginal swab obtained from 208 women. The high vaginal swab samples were analysed using PCR, DNA sequencing and phylogenetic analysis of the identified genotypes. Only two Human papilloma virus genotypes (HPV type 18 and 58) were detected in this study with Human papilloma virus type 58 as the most prevalent. This study identified only two Human papilloma virus genotypes (HPV types 18 and 58) in Sokoto, Nigeria which can serve as basis for establishing vaccine in the study area.

Keywords: Human Papilloma virus, Molecular Characterization, women and Sokoto.

INTRODUCTION

Human Papilloma Virus (HPV) is a naked double-stranded DNA virus, belonging to the family Papillomaviridae (Sheba *et al.*, 2019). More than 200 Human papillomavirus genotypes have been identified based on the sequence of their L1 genes and approximately half of them infect the genital tract of both sexes. Many types of HPV have been found in cervical cancers, while others are found rarely or not at all in large series of cancers, which gives rise to the nomenclature of 'high-' and 'low-risk' HPVs (Omotayo *et al.*, 2020). In all HPV related cancers, HPV DNA integration into the host cell genome sets the stage for malignancy (Juckett and Hartman-Adams, 2010).

Human papillomavirus testing is more sensitive than cytology for the detection of high grade cervical intraepithelial neoplasia (CIN), although it has been shown to be less specific, especially in younger women (Kitchener *et al.*, 2019). HPV DNA testing can be used in a variety of clinical scenarios that include a primary screening test, particularly in women older than 30 years; as an adjunctive test to cytology. It can also be used to triage women who have an equivocal cytologic finding, or for follow up post-treatment (Mohammed *et al.*, 2015). Most of the burden of Human Papillomavirus associated with malignant and benign disease occurs in developing countries without effective screening programmes and poor access to medical services. In northern Nigeria, cervical cancer is common and accounts for 70.5% of all genital tract malignancies (Mohammed *et al.*, 2015).

In addition, there is no national screening program in Nigeria. In few centres where screening exists it is opportunistic, poorly organised and expensive. This further worsened by the low level of awareness and utilisation of the limited screening resources in Nigeria (Gharoro and Ikeanyi, 2006). Several reasons for low rates of human papillomavirus screening have been observed including low education, demographic, social and psychological factors (Bruni *et al.*, 2019).

In most part of northern Nigeria, the incidence of Human papillomavirus is unknown (Mohammed *et al.*, 2015). Causes for concern are predominantly poor knowledge of Human papillomavirus infection and vaccination thus reflecting the poor knowledge, acceptance, and promotion of Human papillomavirus vaccination by health care professionals across the state. Significant proportion of cervical cancers among Nigerian women would be prevented if a strategy such as Human papillomavirus DNA based screening is implemented. In addition, there is speculation that variation in the distribution of Human papillomavirus types circulating among women in Sokoto and different part of the country which have raised concerns about the effectiveness of the available vaccines in the region (Omotayo *et al.*, 2020). Thus, this study is designed to determine the circulating Human papillomavirus genotypes among women in Sokoto, thereby providing baseline information towards effective prevention and control of Human papillomavirus infection in the state.

MATERIALS AND METHODS

Study Design and Population

This study is Hospital based cross-sectional study. Study subjects were attendees of Usmanu Danfodiyo University Teaching Hospital (UDUTH). Patients of different ages who attended the hospitals during the period of the study were included after written consent is given (see appendix). However, patients menstruating or bleeding per vaginum at the time of specimen collection and those who are not willing to participate in the study were excluded.

Sample Size Determination

The sample size was calculated using formula of calculating prevalence.

$$N = \frac{PQ}{(E \div 1.96)2}$$

Where n = sample size

p = Maximum known prevalence of the condition

q = 1-p (proportion of persons free of the condition)

E = Allowable error margin (0.05)

1.96 = Standard normal deviation (a constant).

Using the maximum reported prevalence of 15.5% (Bruni *et al.*, 2019).

$$N = \frac{0.155 * 0.845}{(0.05/1.96)2}$$

N = 202.

Ethical Approval

Ethical approval for the study was obtained from the ethical committee of Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital Sokoto.

Collection of Demographic and Risk Factors Data

Socio-demographic and risk factors data of the participants including age, marital status, age at start of active sexual activity, age at first pregnancy, number of sexual partners, parity, use of hormonal contraception, use of condom, education and history of smoking were obtained by administration of questionnaire to each participant.

Cervical Sample Collection

A sterile swab stick was used to collect the exfoliated cells from the squamo-columnar junction of the ecto and endo cervix. It was inserted into the cervical canal and rotated five times in a clockwise direction whilst applying light pressure to collect all the necessary cells which adhered to the flat sides of the bristles. This procedure was carried out by Doctors in the department of Obstetrics and Gynaecology of the two study site (Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital Sokoto). The samples were transported in ice boxes to AMR Flemings laboratory, Faculty of Veterinary Medicine Usmanu Danfodiyo University Sokoto for analysis. The cells were suspended in 5 ml phosphate buffered saline and stored at -20ºC in the laboratory for any further analysis (Sheba *et al.*, 2019).

Molecular Detection and Characterization of HPV DNA Extraction

From the cervical specimen obtained in 3.1.7 above, two millilitres (2mls) of the cell suspension was centrifuged at 10,000 rpm for ten minutes (Labnet International, Inc USA). The pooled sediment obtained was re-suspended in 400µl of detergent buffer (10mM Tris-HCl, 50mM KCl, 2.5mM MgCl2, 0.45% Triton X100, 0.45% Tween 20) and 100µg/ml (40µl) proteinase K, mixed and was incubated at 55°C for 2hours. This was followed by the addition of 400µL of phenol chloroform, vortexed and further centrifuged at 10,000 rpm for 20 minutes. The upper layer was carefully pipetted and transferred to a new tube while leaving the middle (proteins and contaminants) and the lower (phenol chloroform) layers in the initial tube which was discarded. About 30µL of 3M Na acetate and 1ml of 100% ethanol was added, vortexed and then kept frozen overnight for DNA to precipitate. The contents was centrifuged at 4°C (ALC high speed refrigerated centrifuge, Italy) for 1 hour before decanting the supernatant (while preserving the pellets) and addition of 200µl of 70% ethanol. Further centrifugation was performed at 14,000 rpm for 10 minutes at room temperature and the whole ethanol was removed through vacuum drying. The precipitated DNA was re-suspended in 30µl of DNAse free water and stored in aliquots at -20°C until ready for PCR (Mohammed e*t al.*, 2015).

HPV Specific PCR

MY09 and MY11 HPV consensus primers were used to detect the virus using PCR. A total of 25µl reaction mixture was prepared in a 0.2ml PCR tube containing the following components; 12.5 of top tag master mix (Qiagen USA), 05µl of each 10mm forward and reverse primers, 6.5µl of free nuclease free water and 5µl of DNA template. The tubes were spun down briefly and transferred into mycycler (Brand USA) PCR machine programmed with the following condition;

initial denaturation at 94^0C for 3minutes followed by 35 cycles of denaturation at 94^0C for 1minute, annealing at 53^0C for 1minute, extension at 72^0C for 1minute and final extension at 72^0C for 10 minutes (Nejo *et al.*, 2019).

Table 1: Primer Sequence for Amplification of HPV Specific Gene

Primers	Sequence	Fragment (Amplicon) size	Reference
MY09 MY11 '	5'CGTCCMARRGGAWACTGATC3' 5'GCMCAGGGWCATAAYAATGG3'	450-bp	Venceslau *et al*., 2014

Detection of PCR Products Using Gel-Electrophoresis

The amplified HPV DNA was detected by electrophoresis on 2% agarose gel and visualised using Bio-Rad Gel Doc™XR+System (Nejo *et al*., 2019).

Optimization and Clean Up

Amplified product obtained using MY09 and MY11were purified using PCR purification kit (Biolab England) following the manufactures instruction (Nejo *et al.*, 2019).

Sequencing

The purified PCR products were sent to Inqaba Biotec, South Africa for single directional Sangers sequencing using the MY11 primer.

RESULTS AND DISCUSSION

Amplicons of the five positive samples from gel electrophoresis were successfully sequenced (MY11) and presented on Table 1. Two Human papilloma virus genotypes (HPV58 and HPV18) were identified after blasting the sequences on National Centre for Biotechnology Information (NCBI) USA website. All the sequences obtained were officially registered and assigned a specific accession number by the Genbank of National Centre for Biotechnology Information (NCBI USA).

Table 2: Identity of Human Papilloma Virus circulating in Sokoto

Sample code	Accession no.	% Similarity	E-Value	Query Cover	HPV Type
SPH-187	OR722814	100	0.0	99	58
SPH-101	OR722815	99.77	0.0	99	58
UTH-14	OR722816	100	5E-157	100	18
SPH-52	OR722817	100	0.0	99	58
SPH-120	OR722818	99.7	0.0	99	58

Key: SPH 187 = Specialist Hospital 187, SPH 101 = Specialist Hospital 101, UTH 14 = Usmanu Danfodiyo University Teaching Hospital, SPH 52 = Specialist Hospital 52, SPH 120 = Specialist Hospital 120

Human papilloma virus type 58 and 18 are the only genotypes identified in this study. They are among the global most common cervical types among women (HPV type 16, 18, 31, 33, 35, 45, 52 and 58). Human papilloma virus type 58 and 18 are all considered to be high risk Human papilloma virus types. However, Human papilloma virus type 58 is more prevalent in this study. This agrees with the finding of Mohammed *et al.*, (2015) and Auwal *et al.*, (2013). There is a variation particularly in predominance between the Human papilloma viruses genotypes found in this study in comparison to most other Nigerian studies. This may be attributable to geographical differences in addition to culture and other life styles. Additionally, the inability of this study in detecting low risk Human papilloma virus genotypes may be due to the fact that, they are generally fewer in cervical specimens and are also more common among males than females. Phylogenetic analysis of HPV 52 and 18 revealed that HPV 52 could be regarded as United State of America cluster and HPV 18 as Middle East cluster.

CONCLUSION

Only two Human papilloma virus types (HPV type 18 and 58) were detected in this study with Human papilloma virus type 58 as the most prevalent. Molecular detection needs to be adopted as national screening technique because it provides information on particular genotypes involve in the infection which is very critical in vaccine production and administration.

REFERENCES

- Auwal, I. K., Aminu, M., Atanda, A. T., Tukur, J., & Sarkinfada, F. (2013). Prevalence and risk factors of high risk human papillomavirus infections among women attending gynaecology clinics in Kano, Northern Nigeria. *Bayero Journal of Pure and Applied Sciences*, *6*(1), 67-71.
- Bruni, L., Albero, G., Serrano, B., Mena, M., Gómez, D., Muñoz, J., Bosch, F. X., & De Sanjosé, S. (2019). ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). *Human Papillomavirus and Related Diseases in Nigeria*. Available at www.hpvcentre.net (Accessed on 22nd September, 2021).
- Farahmand, M., Moghoofei, M., Dorost, A., Abbasi, S., Monavari, S. H., Kiani, S. J., & Tavakoli, A. (2020). Prevalence and genotype distribution of genital human papillomavirus infection in female

sex workers in the world: a systematic review and meta-analysis. *BMC Public Health*, *20*, 1-14.

- Gharoro, E. P., & Ikeanyi, E. N. (2006). An appraisal of the level of awareness and utilization of the Pap smear as a cervical cancer screening test among female health workers in a tertiary health institution. *International Journal of Gynecologic Cancer*, *16*(3).
- Juckett, G., & Hartman-Adams, H. (2010). Human papillomavirus: clinical manifestations and prevention. *American family physician*, *82*(10), 1209-1214.
- Kitchener, H. C., Almonte, M., Thomson, C., Wheeler, P., Sargent, A., Stoykova, B., ... & Peto, J. (2009). HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. *The lancet oncology*, *10*(7), 672-682.
- Manga, M. M., Fowotade, A., Abdullahi, Y. M., El-Nafaty, A. U., Adamu, D. B., Pindiga, H. U., ... & Osoba, A. O. (2015). Epidemiological patterns of cervical human papillomavirus infection among women presenting for cervical cancer screening in North-Eastern Nigeria. *Infectious agents and cancer*, *10*, 1-9.
- Nejo, Y. T., Olaleye, D. O., & Odaibo, G. N. (2018). Prevalence and risk factors for genital human papillomavirus infections among women in Southwest Nigeria. *Archives of basic and applied medicine*, *6*(1), 105.
- Nejo, Y. T., Olaleye, D. O., & Odaibo, G. N. (2019). Molecular characterisation of genital human papillomavirus among women in Southwestern, Nigeria. *Plos one*, *14*(11), e0224748.
- Omotayo, F. F., Kehinde, K. K., Emmanuel, O. A., Kayode, E. O., Mike O., Samuel O. V., Taiwo, O. I., Habeeb, O. A., Semeeh, A. O., & Precious, A. K. (2020). Knowledge of HPV and HPV Vaccine among Senior Secondary School Students in Nigeria: Implications on Cancer Prevention Strategies, the Chance Study. *Population Medicine*, *2*, 31-39.
- Sheba, J. M., Aminu, M., Inabo, H. I., Oguntayo, A. O., & Ahmed, S. A. (2019). Prevalence and Risk Factors of Human Papilloma Virus Infection among Women in a Nigerian State. *EC Gynaecology*, *8*, 814-823.
- Venceslau, E. M., Bezerra, M. M., Lopes, A. C. M., Souza, É. V., Onofre, A. S. C., Melo, C. M. D., ... & Onofre, F. B. D. M. (2014). HPV detection using primers MY09/MY11 and GP5+/GP6+ in patients with cytologic and/or colposcopic changes. *Jornal brasileiro de patologia e medicina laboratorial*, *50*, 280-285.