Original Article

Patterns of Expression of Human Papilloma Virus Gene Products In Cases of Nasopharyngeal Carcinoma: Findings From a University Teaching Hospital in Northern Nigeria

***Yates SM,[1] Tanko ZL,[2] Dogara A,[3] Omisakin OO,[4] Ayuba GI,[1] Iliyasu Y[1]**

[1]*Department of Anatomic Pathology and Forensic Medicine, Barau Dikko Teaching Hospital, Kaduna Affiliated to Kaduna State University, Kaduna, Nigeria.*[2]*Department of Medical Microbiology and Parasitology, Barau Dikko Teaching Hospital, Kaduna Affiliated to Kaduna State University, Kaduna, Nigeria.* [3]*Department of Chemical Pathology and Immunology, Barau Dikko Teaching Hospital, Kaduna Affiliated to Kaduna State University, Kaduna, Nigeria.* [4]*Maxillofacial Division, Department of Surgery, Barau Dikko Teaching Hospital, Kaduna Affiliated to Kaduna State University, Kaduna, Nigeria.*

Article History
Submitted: 11/02/2026; Accepted: 12/02/2026: Published: 27/02/2026

***Correspondence:** Yates SM
Email: simplysannom@yahoo.com

ABSTRACT

Human Papilloma Virus (HPV) is a well-recognized oncogenic virus in several epithelial malignancies of the head and neck; however, its role in the aetio-pathogenesis of Nasopharyngeal carcinoma (NPC) remains controversial and appears to vary by geographic region and histologic subtype. Data on HPV involvement in NPC are limited in Nigeria. This study was therefore undertaken to determine the presence of HPV DNA in NPC tissues from patients seen at Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. A retrospective analysis of 44 histologically confirmed NPC cases diagnosed between 1992 and 2013 was carried out. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were retrieved and DNA was extracted using a commercial FFPE DNA extraction kit. DNA quality was assessed by Polymerase Chain Reaction (PCR) amplification of the human β-globin gene, while HPV DNA detection was performed using GP5+/GP6+ consensus primers targeting the L1 region of the HPV genome, with appropriate positive and negative controls included. All samples showed adequate DNA quality with successful β-globin amplification; however, none of the 44 NPC cases demonstrated detectable HPV DNA by PCR. These findings suggest that HPV may not play a significant role in the pathogenesis of NPC in this environment and support the predominance of non-HPV-related aetiologic factors, such as Epstein–Barr virus, in NPC within this population. Further studies using larger sample sizes and complementary detection methods are recommended.

Keywords: HPV, Nasopharyngeal Carcinoma, PCR

INTRODUCTION

Human Papilloma Virus (HPV) is a small, non-enveloped, epitheliotropic, double-stranded DNA virus that infects mucosal and cutaneous epithelia and induces cellular proliferation. Over 100 types have been identified and designated "high-risk" or "low-risk" depending on their potential for carcinogenicity, notably in the genital tract and the head and neck regions.[1] Recognized high-risk types include HPV types-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82; and low-risk types include HPV types- 6, 11, 40, 42, 43, 44, 54, 61, 72, 81, and 89.[1,2]

All HPV genomes contain approximately eight open-reading frames (ORFs) divided into the early region encoding proteins E1–E7 (necessary for viral replication); the late region encoding the structural proteins L1 and L2 (required for virion assembly); and the largely non-coding long control region (LCR), necessary for the replication and transcription of viral DNA.[3] The early proteins E5, E6 and E7 have been identified as key proteins in the

Article Access
Website: www.wjmbs.org
10.5281/zenodo.19146980

How to cite this article
Yates SM, Tanko ZL, Dogara A, Omisakin OO, Ayuba GI, Iliyasu Y. Patterns of Expression of Human Papilloma Virus Gene Products in Cases of Nasopharyngeal Carcinoma: Findings From a University Teaching Hospital in Northern Nigeria. West J Med & Biomed Sci. 2026;7(1):120-126. DOI:10.5281/zenodo.19146980.

mechanisms of HPV carcinogenesis. The best known property of the E6 proteins of high-risk HPVs is the inability to bind and degrade the tumour-suppressor protein p53 through the recruitment of the protein ligase, E6-associated protein (E6-AP) resulting in the inhibition of the transcriptional activity of p53 and the abrogation of p53-induced apoptosis.[3] In addition, E6 induces the expression and activity of telomerase responsible for cell immortalization[4] E7 protein also induces apoptosis through the destabilization of p105Rb, a negative regulator of the cell cycle. Another important aspect of E7 biology is its ability to destabilize centrosomes, which cause mitotic defects and genome instability[3,5] E5 protein enhances the immortalization potential of E6 and E7 while also directly inhibiting apoptosis that is induced by ultraviolet (UV) light, Fas-ligand and tumour necrosis factor related apoptosis-inducing ligand.[6]

Well recognized high-risk HPV types, including HPV16, 18 and 31, have been linked to Head and Neck Squamous Cell Carcinoma (HNSCC)[7]. Given this known oncogenic role of HPV in epithelial cancers of head & neck, there has been growing interest in its role in the aetio-pathogenesis of Nasopharyngeal Carcinoma (NPC). Recently, several studies have examined HPV DNA, expression of E6/E7 and expression of p16 (a surrogate marker for HPV oncogenic activity). A systematic review and meta-analysis by Zhao et al found an overall global prevalence of HPV-positive NPC of about 18% among 6,314 NPC patients from 46 studies. These showed regional variation, with higher incidence in the non-endemic North America, and lower incidence in the endemic region of Asia.[8] Another meta-analysis on viral markers in NPC reported HPV DNA prevalence of 34.4% in WHO type I NPC, versus 18.4% in WHO types II/III.9 Although HPV DNA is detectable in a subset of NPCs, consistent and strong evidence of active viral gene product expression (E6/E7 transcripts or proteins) is relatively scarce in NPC compared to that seen in cervical cancer or oropharyngeal carcinoma, and prevalence varies by region, histologic subtype and detection methods.

A more consistent aetiological relationship is seen between NPC and Epstein-Barr Virus (EBV) especially the non-keratinising carcinoma (NKC) histologic subtype in endemic areas (Southern China, Southeast Asia).[10,11] Curiously, HPV appears to modulate EBV biology in NPC as patients with HPV-EBV co-infection seem to be younger and have higher rates of radiation and chemotherapy responses as well as long-term survival with excellent prognosis.[9,12]. The prevailing opinion is increasingly that such patients should be referred to individual therapeutic regimens that are adapted to their HPV-status, which differs from therapeutic strategies for HPV-negative patients. These HPV-positive NPC subsets have been observed to occur more frequently in Caucasians of European ancestry, but they are still poorly understood.[12,13] Furthermore, little is known about the prevalence of oncogenic HPV in NPC and its clinical significance in our environment. We were therefore particularly interested in examining HPV expression in NPC tissues due to its therapeutic and prognostic relevance and because none of the previous Nigerian studies on HPV had examined this.

MATERIALS AND METHODS

The study included 44 cases of histologically diagnosed NPC seen at the Department of Pathology, ABUTH Shika-Zaria from January 1, 1992 to December 31, 2013. Formalin-fixed, paraffin-embedded tissues (FFPE) of all previously diagnosed NPCs within the study period were retrieved and examined with the exclusion of missing, badly damaged, or FFPE with insufficient tissue.

Deparaffinization: About 5-8 freshly cut sections were made from the PETB, each with a thickness of up to 10 μm and a surface area of up to 250 mm^2. The tumoural tissues were retrieved by dewaxing the thin tissue sections in 1mL of xylene and vortexed for 10 seconds. Tubes were then centrifuged at 14,000 rpm for 2 minutes and the supernatant (xylene) was removed carefully by pipetting. To the pellets at the bottom of the tube, 1ml of 96% ethanol was added and vortexed vigorously for 10 seconds before centrifugation at 14,000rpm for 2 minutes. The supernatant (ethanol) was removed by pipetting and the tubes were opened and incubated at room temperature until the ethanol evaporated.

DNA Extraction: EBV DNA was extracted by means of an enzymatic digestion. Genomic DNA extraction was carried out using *QIAamp*® DNA FFPE Tissue kit and performed precisely according to the manufacturer's instruction except for *Proteinase K* digestion which was allowed to stay overnight for complete digestion of the tissues. Pellets were re-suspended in 180 µL ATL buffer and 20 µL of proteinase K was added and allowed to digest overnight at 56^0C. After overnight incubation, the tubes were allowed to cool then incubated at 96^0C for 1hr. Tubes were cooled again and 400 µL of Buffer AL-alcohol mixture (1:1) was added and vortexed vigorously. The entire mixture was carefully transferred to the *QIAamp MinElute* column (in a 2 ml collection tube) and centrifuged at 8000 *rpm* for 1 minute. Changing the collection tubes each time, AW1 and AW2 solutions were used to wash, by centrifuging for 1 minute at 8000 *rpm*. The spin column was dried to remove all traces of alcohol by centrifuging the spin columns at 14,000 *rpm* for 3 minutes. To the dried spin column, 100 µL of TE buffer was added directly to the spin column and allowed to equilibrate for 2 minutes before centrifugation at 8000 rpm for 1 minute. The eluted DNA was stored at -20^0C.

DNA quantification**:** DNA was quantified using NanoDrop 2000C spectrophotometer (Thermos Scientific, USA). Concentration was determined based on absorbance at 260nm. Purity was estimated as ratio of absorbance at 260nm to Absorbance at 280nm (A260:A280).

Polymerase Chain reaction (PCR): PCR for beta Hemoglobin: PCR was run for human Hb-beta subunit to ascertain the quality of the extracted DNA, the viability of the tissue for PCR detection of HPV and as a control gene for human tissues. A primer which targets a 122 bp sequence of the Hb beta sub-unit was used, both forward (5'cttctgacacaactgtgttcactagc 3') and reverse (5'tcaccacaacttcatccacgttcacc 3') primers were obtained from *Inqaba Biotech West Africa*. PCR was carried out in a 25 µL reaction using One T*aq* Quick-load Master Mix (New England Biolab). And amplification was carried out using *Mastercycler Nexus* (Eppendorf, Germany).

Gel Electrophoresis: To confirm amplification of the 120 bp *Hb beta* sub-unit, agarose gel electrophoresis was carried out on 1.2% agarose in TAE buffer according to method suggested by Green and Sambrook.14 Electrophoresis was carried out at 90V for 30min and viewed under UV trans-illuminator. A 100 kb size ladder (*Promega*) was used as the standard size DNA marker and staining was done with Ethidium Bromide.

PCR for HPV using GP5+/GP6+ primers: Only Samples that show positive amplification of the Hb beta sub-unit were selected for the HPV PCR. PCR was carried out with the primer GP5+/GP6+ and using the protocol suggested by Evans *et al*., with the following sequence; GP5+ [5'-TTTGTTACTGTGGTAGATACTAC-3'] and GP6+ [5'-GAAAAATAAACTGTAAATCATATTC-3']. The primer targets the L1 region of the HPV genome and amplifies HPV of all types.[15-17] PCR was carried out in a 25 µL reaction using One T*aq* Quick-load Master Mix (New England Biolab). PCR reactions contain 1.25 U T*aq* polymerase in standard buffer containing 1.8mM $MgCl_2$, 22 mM NH_4Cl, 22 mM KCl, 0.2 µM each of forward and reverse primers and 200 µM dNTPs. To each reaction, 2 µL of extracted DNA of template DNA was added.

For negative controls a mixture of all the reagents were used in the PCR mixture preparation with the addition of 1µl of sterile distilled water instead of DNA. For the positive control a preformed HPV from tissue culture was used. The data obtained were statistically analyzed using the Microsoft Excel and presented as simple frequencies in tables and charts.

Ethical approval was obtained from the Ethics and Research Committee of Ahmadu Bello University Teaching Hospital Zaria (ABUTH/HREC/K21/2014).

RESULTS

Real-time PCR detection of HPV done for 44 cases showed no positivity.

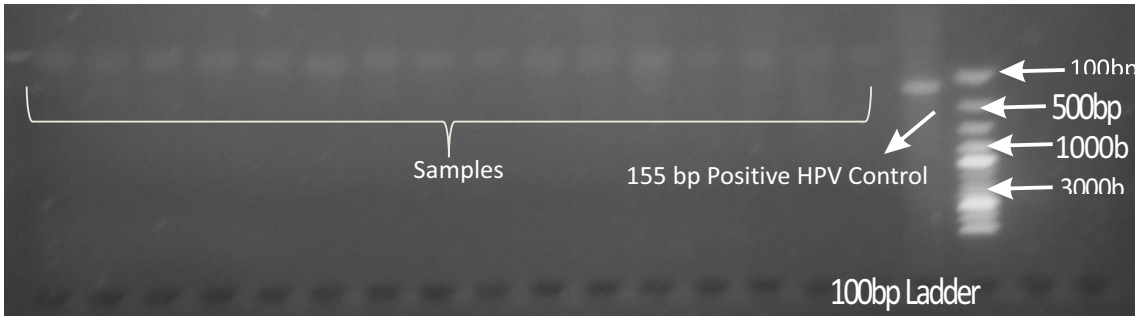

Figure 1: Gel electrophoresis image of PCR detection for HPV. Wells not showing any band are negative. No amplification was observed in the samples. Positive control HPV showed positive band of 155bp Size.

DISCUSSION

The real-time PCR gene detection for HPV done for 44 cases showed no positivity. Markedly contrasting with a previous study of the same population which reported 37.2% positivity for EBV using a similar real-time PCR method.[11] HPV detection in NPC has been carried out by various approaches including consensus PCR (often targeting L1 region with primers such as $GP5^+/GP6^+$ or MY09/MY11), type-specific PCR, in-situ hybridisation (ISH) of HPV DNA, immunohistochemistry for surrogate markers (p16), and more recently E6/E7 mRNA assays. These studies show wide variability in the expression of HPV in NPC perhaps due to the differences in the sensitivity of viral detection methods used and geographical variations.

Identical to the finding in our study, a study in Japan among 43 NPC patients similarly found that no cases were HPV-positive when tested for HPV DNA using ISH.[18] They also found that 14% were EBV-negative with keratinization squamous cell carcinoma (KSCC) and the rest were EBV-positive (86%). A more recent study in the same country among 26 NPC patients (2015-2022) showed 4% prevalence of HPV and 73% prevalence of EBV.[19] These findings would suggest that HPV plays a very limited role for HPV in the development of NPC in non-endemic regions.

However, a slightly higher prevalence is seen in some studies among non-endemic populations. In one study in the UK of 67 NPC cases, HPV DNA was sought by ISH, PCR ($GP5^+/GP6^+$) and p16 IHC and found that 16.4% were HPV DNA positive and 70.1% were EBV-positive.[20] In Accra-Ghana, out of 72 FFPE NPC biopsies, HPV DNA was present in 19.2% of the cases and EBV DNA was present in 25% of the cases.[21] A much higher prevalence is reported from a study in Morocco where 70 NPC biopsies tested for HPV DNA using consensus primers showed about 34% positivity.[22]

Generally, viral detection by PCR has been found to be affected by several factors including the type and duration of fixation, prolonged archiving of PETB, and the RNA/DNA extraction methods.[23-25] The type of fixative used routinely for specimens in our laboratory is 10% neutral formal saline and specimens are kept for a duration of 24-48 hours, long enough for adequate fixation. Formalin-fixed archival samples were thought to be poor materials for molecular biological applications, however Masuda et al analyzed the modification of formalin fixation and found that de-modification (by proteinase K and temperature elevation) enabled extraction of RNA from fixed samples to the same amount as from fresh samples or at least removed more than half of the modification.[25]

The oldest specimen in our study was archived for a period of about 21 years at the time of the study. In a comprehensive study using a large number of decades-old samples of over 20 years, similar to ours, Iwamoto et al concluded that the age of the test samples was not as great a problem as was the source of procurement, thus extracted DNA can be used for all types of assays that require PCR amplification, such as restriction fragment length polymorphism, single-strand conformation polymorphism, and direct sequencing. They demonstrated the feasibility of using decades-old archival tissues for use in large-scale molecular studies.[26] Considering the adequate fixation of tissues in or study and the proven

feasibility of PCR in archived FFPE tissues, DNA degradation due to poor sample quality is an improbable explanation for the lack of HPV expression in NPC in this study but rather due to other biological and technical reasons.

Firstly, studies have shown that the L1 gene consensus primers ($GP5^+/GP6^+$), used in this study, may be undetected at low viral loads even with PCR amplification.[27] The viral load in non-cervical sites, including the nasopharynx, have also been shown to be much lower or distribution more heterogeneously, so consensus L1 primers may be sub-optimal.[28] Similarly, sampling bias due to tumour heterogeneity and contamination with non-tumour tissue as well as co-infection with EBV may complicate HPV detection resulting in widely varied prevalence coupled with differing detection methods.[8]

In addition, a major event in high-risk HPV-related carcinogenesis is integration of the viral genome into the host genome which involves the disruption of the E1/E2 genes (that regulate E6/E7) and may also truncate or delete other regions including the late genes L1/L2.[29,30] A recent study found that L1 expression in oesophageal and other cancers declines with advancement of the disease, associated with progressive viral integration and a simultaneous upregulation of p16.[31] HPV testing that primarily targets L1, as this study does, may be less reliable than that targeting E6/E7 oncogenes, as L1 expression can be lost while E6/E7 expression is almost always present.[27,29,30] Even without deletion, the late genes (including L1) may be silenced epigenetically (via methylation of viral DNA, histone modifications) or by host regulatory mechanisms once integration occurs and the virus moves from a replicative to transforming mode. The disruption of E2, changes in upstream regulatory region (URR) methylation, and reduced expression of viral late transcripts are observed.[32] Furthermore, EBV is the dominant etiologic virus in the aetio-pathogenesis of NPCs, therefore, HPV may play a secondary or incidental role and may be present in a latent or integrated form which may reduce expression and explain the lack of L1 gene expression and detection.

Finally, a comparative study of HPV18 in NPC and cervical cancer noted that the expression levels of E6/E7 differed between the two sites (higher E6 in cervical, higher E7 in NPC). It also noted variation in the L1 region sequences and LCR region in NPC compared with cervical cancer, suggesting adaptation of virus at different sites[33]. These site-specific differences in viral pathogenesis in NPC may further suggest a lower L1 expression and detection.

CONCLUSION

The observed lack of expression of HPV in NPC in this study is likely multifactorial reflecting a combination of technical and biological realities. For studies in NPC, it is advisable to adopt a more comprehensive HPV testing strategy including E6/E7 detection, integration assessment, and good quality samples, to avoid underestimating HPV's potential role. In NPC research, given the dominance of EBV, it may be helpful to test for both viruses and evaluate viral load, integration status, and surrogate markers (p16, E6/E7) rather than relying solely on one method.

Recommendation

The interpretation of negative PCR results should be done cautiously, as they may reflect technical limitation or viral genome disruption rather than a true absence of HPV.

REFERENCES

1. Papillomaviruses H. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon Fr IARC. 2011; Available from:http://www.mojinbio.com/private/Files/20210819/6376498867801235939889166.pdf(accessed on 22 December 2025).
2. IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100B: A Review of Human Carcinogens: Biological Agents. IARC Monogr Eval Carcinog Risks Hum. International Agency for Research on Cancer Lyon; 2012. Available from: https://monographs.iarc.who.int/wp-content/uploads/2018/08/14-002.pdf (accessed on 22 December 2025).
3. Fehrmann F, Laimins LA. Human papillomaviruses: targeting differentiating

epithelial cells for malignant transformation. Oncogene. 2003;22(33):52017.

4. Oh ST, Kyo S, Laimins LA. Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and GC-rich Sp1 binding sites. J Virol. 2001;75(12):555966.
5. Duensing S, Münger K. Centrosome abnormalities, genomic instability and carcinogenic progression. Biochim Biophys Acta BBA-Rev Cancer. 2001;1471(2):M818.
6. Zhang B, Spandau DF, Roman A. E5 protein of human papillomavirus type 16 protects human foreskin keratinocytes from UV B-irradiation-induced apoptosis. J Virol. 2002;76(1):22031.
7. Pim D, Banks L. Interaction of viral oncoproteins with cellular target molecules: infection with high-risk vs low-risk human papillomaviruses. APMIS. 2010;118(67):47193.
8. Zhao BY, Hirayama S, Goss D, Zhao Y, Faden DL. Human papillomavirus-associated nasopharyngeal carcinoma: A systematic review and meta-analysis. Oral Oncol. 2024;159:107057.
9. Tham T, Machado R, Russo DP, Herman SW, Teegala S, Costantino P. Viral markers in nasopharyngeal carcinoma: A systematic review and meta-analysis on the detection of p16INK4a, human papillomavirus (HPV), and Ebstein-Barr virus (EBV). Am J Otolaryngol. 2021;42(1):102762.
10. Yates SM, Iliyasu Y, Ahmed SA, Liman AA. Immunohistochemical expression of epsteinBarr virus Latent Membrane Protein-1 in nasopharyngeal carcinoma. Ann Trop Pathol. 2017 July 1;8(2):99.
11. Yates SM, Agyigra IA, Lamido-Tanko Z, Ayuba GI, Iliyasu Y. Molecular Detection of Epstein-Barr Virus in Nasopharyngeal Carcinoma. Ann Trop Pathol. 2024 July 6;15(1):238.
12. Dogan S, Hedberg ML, Ferris RL, Rath TJ, Assaad AM, Chiosea SI. Human papillomavirus and EpsteinBarr virus in nasopharyngeal carcinoma in a low-incidence population. Head Neck. 2014;36(4):5116.
13. Huang SH, Jacinto JK, OSullivan B, Su J, Kim J, Ringash J, et al. Clinical presentation and outcome of human papillomavirus-positive nasopharyngeal carcinoma in a North American cohort. Cancer. 2022;128(15):290821.
14. Green MR, Sambrook J. Molecular cloning. Lab Man 4th. 2012;448.
15. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol. 1995;76(4):105762.
16. Snijders PJ, van den Brule AJ, Schrijnemakers HF, Snow G, Meijer CJ, Walboomers JM. The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. J Gen Virol. 1990;71(1):17381.
17. Evans MF, Adamson CS, Simmons-Arnold L, Cooper K. Touchdown General Primer (GP5+/GP6+) PCR and optimized sample DNA concentration support the sensitive detection of human papillomavirus. BMC Clin Pathol. 2005;5(1):10.
18. Saito Y, Ushiku T, Omura G, Yasuhara K, Yoshida M, Takahashi W, et al. Clinical value of the Epstein-Barr virus and p16 status in patients with nasopharyngeal carcinoma: a single-centre study in Japan. ORL. 2017;78(6):33443.
19. Liu L, Hirai N, Kondo S, Moriyama-Kita M, Nakazawa R, Komura S, et al. Recent Trends in Prevalence of HPV Infection in Nasopharyngeal Carcinoma in Japan. Microorganisms [Internet]. 2025;13(11). Available from: https://www.mdpi.com/2076-2607/13/11/2514
20. Robinson M, Suh Y eun, Paleri V, Devlin D, Ayaz B, Pertl L, et al. Oncogenic human papillomavirus-associated nasopharyngeal carcinoma: an observational study of correlation with ethnicity, histological subtype and outcome in a UK population. Infect Agent Cancer. 2013;8(1):30.

21. Asante DB, Asmah RH, Adjei AA, Kyei F, Simpong DL, Brown CA, et al. Detection of Human Papillomavirus Genotypes and Epstein-Barr Virus in Nasopharyngeal Carcinomas at the Korle-Bu Teaching Hospital, Ghana. Sci World J. 2017;2017(1):2721367.

22. Laantri N, Attaleb M, Kandil M, Naji F, Mouttaki T, Dardari R, et al. Human papillomavirus detection in moroccan patients with nasopharyngeal carcinoma. Infect Agent Cancer. 2011;6(1):3.

23. Ben-Ezra J, Johnson DA, Rossi J, Cook N, Wu A. Effect of fixation on the amplification of nucleic acids from paraffin-embedded material by the polymerase chain reaction. J Histochem Cytochem Off J Histochem Soc. 1991 Mar;39(3):3514.

24. Greer CE, Lund JK, Manos MM. PCR amplification from paraffin-embedded tissues: recommendations on fixatives for long-term storage and prospective studies. PCR Methods Appl. 1991 Aug;1(1):4650.

25. Masuda N, Ohnishi T, Kawamoto S, Monden M, Okubo K. Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples. Nucleic Acids Res. 1999 Nov 15;27(22):443643.

26. Iwamoto KS, Mizuno T, Ito T, Akiyama M, Takeichi N, Mabuchi K, et al. Feasibility of using decades-old archival tissues in molecular oncology/epidemiology. Am J Pathol. 1996 Aug;149(2):399406.

27. Isayeva T, Li Y, Maswahu D, Brandwein-Gensler M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. Head Neck Pathol. 2012;6(Suppl 1):10420.

28. Winder DM, Ball SL, Vaughan K, Hanna N, Woo YL, Fränzer JT, et al. Sensitive HPV detection in oropharyngeal cancers. BMC Cancer. 2009;9(1):440.

29. Xing B, Guo J, Sheng Y, Wu G, Zhao Y. Human papillomavirus-negative cervical cancer: a comprehensive review. Front Oncol. 2021;10:606335.

30. Nkili-Meyong AA, Moussavou-Boundzanga P, Labouba I, Koumakpayi IH, Jeannot E, Descorps-Declère S, et al. Genome-wide profiling of human papillomavirus DNA integration in liquid-based cytology specimens from a Gabonese female population using HPV capture technology. Sci Rep. 2019;9(1):1504.

31. Vythilingam DI, Gautam SD, Santos LD, Xuan W, Rabiei M, Rajendra S. L1 capsid protein expression in human papillomavirus positive Barretts metaplasia-dysplasia-adenocarcinoma lesions. Clin Transl Gastroenterol. 2022;10.14309.

32. Hatano T, Sano D, Takahashi H, Oridate N. Pathogenic role of immune evasion and integration of human papillomavirus in oropharyngeal cancer. Microorganisms. 2021;9(5):891.

33. Santa S, Brown CA, Akakpo PK, Edusei L, Quaye O, Tagoe EA. HPV18 L1 and long control region sequences variation and E6/E7 differential expression in nasopharyngeal and cervical cancers: a comparative study. Infect Agent Cancer. 2023;18(1):78.

Conflict of Interest: None to be declared.