BMC Infectious Diseases

https://doi.org/10.1186/s12879-025-12276-5

Article in Press

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Received: 19 March 2025

Accepted: 28 November 2025

Published online: 03 December 2025

Cite this article as: Salhi I., Alzahayqa M., Qrei W. *et al.* Genotypic spectrum and prevalence of HPV associated with cervical cancer in Palestine. *BMC Infect Dis* (2025). https://doi.org/10.1186/s12879-025-12276-5

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Genotypic Spectrum and Prevalence of HPV Associated with Cervical Cancer in Palestine

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Abstract

Background: Cervical cancer remains a significant global public health issue, primarily driven by persistent infection with Human Papillomavirus (HPV). Understanding HPV prevalence, genotype distribution, and epidemiological patterns is essential for effective cervical cancer prevention and management.

Methods: This retrospective study analysed 48 paraffin-embedded cervical tissue samples from Palestinian women diagnosed with poorly differentiated squamous cell carcinoma between 2014 and 2024. DNA extraction was successful for 40 cases. HPV detection was conducted via nested PCR targeting the HPV L1 gene, followed by genotype identification using Sanger sequencing.

Results: HPV was detected in 82.5% of samples, predominantly consisting of high-risk HPV types (77.5%), with HPV16 (32.5%) and HPV18 (10%) being the most prevalent. Women aged 50–59 years exhibited the highest HPV positivity rate. The geographical distribution showed distinct regional variations, particularly clusters of HPV16 infections in specific Palestinian regions.

Conclusions: The high prevalence of HPV, particularly high-risk genotypes such as HPV16 and HPV18, underscores the urgent need for nationwide HPV vaccination and standardized cervical cancer screening programs in Palestine. These findings provide critical epidemiological insights necessary to inform and optimize local public health strategies.

Keywords: Cervical cancer, Human Papillomavirus (HPV), HPV genotypes, Epidemiology, HPV vaccination, Sustainable public health, Prevention, Palestine.

Introduction:

Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide, particularly in developing regions where preventive measures and screening programs are limited [1]. Human papillomavirus (HPV) infection is universally recognized as the primary causative agent of cervical cancer, responsible for approximately 99% of cervical cancer cases globally [2]. HPV comprises a diverse group of DNA viruses with more than 200 genotypes, of which at least 13 are categorized as high-risk due to their oncogenic potential, particularly HPV types 16 and 18, which are responsible for nearly 70% of cervical cancer cases [3].

Despite extensive global research on HPV prevalence and genotype distribution, significant gaps persist in the epidemiological data from the Middle East, specifically Palestine. A recent study in the West Bank, Palestine, based on cervical swab samples collected from women attending gynecology clinics, reported an HPV prevalence of 14.5% [4]. Existing studies highlight variability in HPV genotype distribution due to regional, social, cultural, and healthcare infrastructure differences [5]. Understanding the specific prevalence and genotypic spectrum of HPV in Palestinian women is crucial, not only for accurate epidemiological profiling but also to inform targeted vaccination, screening strategies, and health policies appropriate to the region.

Previous research from neighboring countries indicates substantial heterogeneity in HPV prevalence and genotype distribution, underscoring the importance of localized data [6]. Palestinian women face unique healthcare access challenges due to socio-political factors, further complicating effective cervical cancer prevention and management. Thus, comprehensive local research can critically inform public health interventions, healthcare resource allocation, and contribute significantly to reducing the burden of cervical cancer in Palestine.

This study aims to bridge existing knowledge gaps by determining the prevalence and characterizing the genotypic distribution of HPV in cervical cancer tissues from Palestinian women. These insights will provide valuable epidemiological evidence essential for guiding public health initiatives, optimizing HPV vaccination strategies, and enhancing cervical cancer screening programs tailored to the Palestinian context.

Methodology

Study Design

This retrospective study was conducted using paraffin-embedded tissue (PET) blocks from female patients diagnosed with poorly differentiated squamous cell carcinoma of the cervix.

Sample Collection and Preparation

Forty-eight cases of poorly differentiated squamous cell carcinoma were identified through Laboratory Information Systems (LIS) from three histopathology laboratories located in the West Bank and East Jerusalem. Cases were selected based on histopathological diagnosis and the availability of PET samples from female patients aged 29 to 74 years, diagnosed between 2014 and 2024. Five unstained sections (10 µm thickness) were prepared from PET blocks for each case, along with one hematoxylin and eosin (H&E)-stained slide for histological assessment. The exact location of the cancerous tissue was precisely marked by an experienced histopathologist on the H&E-stained slide for accurate molecular sampling.

DNA Extraction

DNA extraction involved initial deparaffinization of the PET sections. This process included two consecutive treatments with xylene (10 minutes each) to dissolve paraffin wax, followed by rehydration through graded ethanol washes (100%, 95%, and 70% ethanol) [8]. DNA extraction was subsequently performed using the QIAamp DNA FFPE Tissue Kit (Qiagen,

Catalog No. 69504), specifically designed and optimized for formalin-fixed, paraffin-embedded tissues. The extraction protocol included lysis buffer treatment to disrupt cell structures, followed by silica-based column purification according to the manufacturer's instructions. DNA was eluted in a low-salt buffer and stored at -20°C for subsequent analyses. DNA extraction was successful for 40 out of 48 cases, with eight failures due to inadequate DNA quality or quantity [9]. DNA quantity and purity was verified by spectrophotometry.

HPV Detection

HPV DNA detection was performed using a nested PCR protocol targeting the HPV L1 gene region. PCR amplification was carried out using HPV-specific primers: MY09/MY11 (outer primers) and GP5+/GP6+ (inner primers). PCR reactions were conducted in a total volume of 20 µL, containing 10 µL Q5 High-Fidelity X2 PCR Master Mix (New England Biolabs, Catalogue: M0492L), 5 µL of extracted DNA, 0.5 µM of each primer, and nuclease-free water [10].

Nested PCR was performed on an Applied Biosystems Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific). The outer PCR (MY09/MY11) involved an initial denaturation at 98°C for 1 minute, followed by 35 cycles of 98°C for 20 seconds, annealing at 55°C for 25 seconds, and extension at 72°C for 20 seconds, concluding with a final extension at 72°C for 2 minutes [11].

The inner PCR (GP5+/GP6+) utilized an initial denaturation at 98°C for 1 minute, followed by 35 cycles consisting of denaturation at 98°C for 15 seconds, a complex annealing step involving sequential temperatures at 48°C for 4 seconds, 38°C for 30 seconds, 42°C for 5 seconds, and 66°C for 5 seconds, and an extension at 72°C for 20 seconds. The reaction concluded with a final extension at 72°C for 2 minutes [12]. The house keeping gene beta actin was used to ensure the presence DNA in HPV negative samples.

PCR products were analysed via electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light using the UVITEC gel documentation system. Negative (no-template) and positive controls (known HPV-positive samples) were included to ensure assay reliability.

HPV Genotyping

Positive PCR products underwent purification using the EPPIC Fast ultra-rapid enzymatic purification kit (A&A Biotechnology). Sanger sequencing was performed using the ProDye™ Terminator Sequencing System Kit (Promega), with reactions purified using the Wizard® MagneSil® Sequencing Reaction Clean-Up System (Promega). Sequencing was conducted on a Spectrum Compact Analyzer (Promega), and data were analysed with Proview Software (Promega). HPV genotypes were determined by aligning sequences against HPV reference genomes in the NCBI GenBank database.

Ethical Approval and Consent to Participate

Ethical approval was obtained from the Institutional Review Board (IRB) at An-Najah National University (Ref: MAS. OCT. 2023/6). The research followed all relevant ethical guidelines and regulations and comes in compliance with Helsinki declaration.

Participants were informed about the research objectives and provided consent either through direct telephone communication or via the histopathology laboratories holding their archived samples. Each participant was individually informed of their test results. All participant information and samples were securely managed and anonymized, with access limited exclusively to authorized research personnel

Statistical Analysis

Descriptive statistics (counts and percentages) were used to evaluate HPV prevalence and genotype distribution. No inferential statistical tests were applied, given the small sample size and exploratory design of the study.

Results

HPV Prevalence and Genotype Distribution

As shown in table 1, of the 40 successfully analysed tissue samples, 33 (82.5%) were positive for HPV, while 7 (17.5%) tested negative. These HPV-negative cases are most likely attributable to DNA degradation in FFPE samples rather than true HPV-negative cervical cancers. Therefore, type-specific prevalence was calculated excluding negatives.

According to the International Agency for Research on Cancer (IARC) classification, HPV genotypes were categorized as follows; High-risk HPV types included HPV16 (32.5%), HPV18 (10%), HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59 and HPV68 (2.5% each), low-risk HPV types included HPV83 (2.5%) and unclassified HPV types included HPV26, HPV38 (each 2.5%).

Table 1: Distribution of HPV Positive and Negative samples.

HPV Result	Count	Percentage
Negative	7	17.50%
Positive	33	82.50%
Grand Total	**40**	**100.00%**

HPV16 was the most frequently detected high-risk genotype, present in 13 samples (32.5%), indicating a significant role in cervical cancer pathology in the studied population. HPV18, known globally for its association with aggressive cervical cancer types, was found in 4 samples (10%). Other high-risk genotypes were less common, occurring sporadically with single or double detections. The low-risk HPV83 and unclassified HPV26 and HPV38 genotypes were each detected in single instances, reflecting the diverse genotypic profile of HPV infections within the population, as shown in table 2.

Table 2: Summary of HPV Genotypes Categorized by Risk Level.

HPV Risk Classification	Genotypes	Count	Percentage
High-risk	HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68	31	77.50%
Low risk	HPV83	1	2.50%
Unclassified	HPV26, HPV38	2	5.00%
Negative	-	7	17.50%
Total		**40**	**100%**

The notable dominance (77.5%) of high-risk HPV genotypes underscores their significant epidemiological relevance, stressing their impact on cervical cancer prevalence and the potential implications for public health strategies in Palestine.

Age Distribution of HPV Genotypes

The age distribution analysis revealed variations in HPV genotype prevalence across different age groups. The highest HPV positivity was found in women aged 50 to 59 years, with 13 cases (32.5%), However, due to the small sample sizes, these age-related observations should be interpreted as descriptive only and not attributed to specific etiological patterns**.** HPV16 was most prevalent within this age group, accounting for 5 cases, indicating its persistent infection risk and oncogenic potential. In contrast, HPV18 was predominantly detected among women aged 40–49 years (3 cases).

Other detected HPV types showed variable prevalence across age categories, with isolated occurrences typically observed in the 30–39 and

40–49-year-old groups. Negative results were widely distributed across several age groups.

Geographic Distribution of HPV Genotypes

The geographic analysis showed variation in HPV genotype distribution across different Palestinian cities. Due to the small sample size, regional prevalence comparisons should be interpreted with caution. Given the limited number of cases per region, these findings are exploratory only and likely influenced by random variation. They should not be used as a basis for region-specific public health policy. HPV16 was detected in several cities, including Ramallah and Al-Bireh, Tulkarm, and Nablus, while HPV18 appeared in Hebron, Bethlehem, Tulkarm, and Nablus. Other less common HPV genotypes were sporadically detected in various cities. Negative HPV results were sporadically detected. Negative HPV results were also observed across different regions.

Discussion

HPV Prevalence and Genotype Distribution

The prevalence of HPV infection among cervical cancer cases in this study (82.5%) aligns closely with global findings, where HPV DNA is typically detected in approximately 90% of cervical cancer cases worldwide [13, 14]. The high prevalence of high-risk HPV types (77.5%), especially HPV16 and HPV18, mirrors global patterns and supports previous literature that emphasizes their central role in cervical carcinogenesis [3, 15]. HPV16 was identified as the most common genotype in our study population (32.5%). The combined prevalence of HPV16 and HPV18 (42.5%) is lower than the ~70% reported worldwide, which may reflect regional genotype

diversity, the relatively small cohort size, and possible partial DNA degradation in FFPE samples. [16].

The detection of other high-risk HPV genotypes (e.g., HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68) is also consistent with global epidemiological studies, emphasizing the diversity of oncogenic HPV types associated with cervical cancer [17, 18]. However, the presence of low-risk HPV83 and unclassified types (HPV26, HPV38) underscores the diversity and complexity of HPV infection dynamics. While rare cervical cancers may occasionally be linked to such types, inclusion of these in routine screening would not be efficient due to their very low prevalence [19].

Age Distribution of HPV Genotypes

Our study showed peak HPV positivity among women aged 50–59 years (32.5%). These findings are presented descriptively only and should be interpreted with caution due to the limited sample size.

Globally, it has been reported that HPV persistence and progression risks may increase in older age groups, in part due to waning immune responses and limited screening opportunities [19,20,21]. This information provides useful epidemiological context, but it is not used here to explain the distribution observed in our data. Within our cohort, HPV16 was most frequently detected in women aged 50–59 years (5 cases), whereas HPV18 was more common in women aged 40–49 years (3 cases).

Other high-risk HPV types occurred sporadically across different age groups, while negative HPV results were distributed in multiple categories. These observations remain descriptive and do not suggest age-related causation.

Geographic Distribution of HPV Genotypes

The geographic analysis showed variation in HPV genotype distribution across different Palestinian regions. Due to the small sample size, these findings are exploratory and should be interpreted with caution.

HPV16 was more frequently detected in Ramallah and Al-Bireh (3 cases), Tulkarm (4 cases), and Nablus (3 cases), while HPV18 appeared with single occurrences in Hebron, Bethlehem, Tulkarm, and Nablus. Other high-risk and low-risk HPV genotypes were identified sporadically in various cities. Similar geographic variability in genotype distribution has been described in international reports, where socio-demographic, cultural, and healthcare access differences influence epidemiological patterns [13,22,23]. These references are cited as general background and are not intended to explain our observed variation.

HPV-negative results were identified in several regions, most frequently in Nablus (3 cases), with additional cases in Jenin, Ramallah and Al-Bireh, and Tulkarm. Given the retrospective nature of FFPE samples and potential DNA degradation, these negatives should be interpreted with caution and are unlikely to represent true HPV-negative cancers.

In summary, the observed regional differences in HPV genotypes are descriptive and reflect the limited dataset. They should not be considered evidence for region-specific interventions. Instead, these findings support the broader need for nationwide preventive strategies such as HPV vaccination and standardized cervical cancer screening.

Conclusion

This study provides new data on the prevalence and genotypic spectrum of HPV in cervical cancer tissues from Palestinian women. HPV was detected in most cases, with high-risk genotypes—particularly HPV16 and HPV18—being the most common.

While other oncogenic, low-risk, and unclassified HPV types were also observed, their contribution remains rare and requires further investigation. Our findings add to the limited body of evidence from Palestine but should be interpreted with caution due to the small sample size and retrospective nature of the material.

Overall, the results confirm the relevance of oncogenic HPV types in cervical cancer in Palestine and underscore the importance of

implementing established preventive measures, including HPV vaccination and cervical cancer screening, as effective strategies to reduce disease burden at the national level.

Declarations

Acknowledgements

The authors would like to express their sincere gratitude to the Faculty of Medicine and Allied Health Sciences and the Faculty of Graduate Studies at An-Najah National University (www.najah.edu), the Medicare Labs team, and the collaborating histopathology laboratories for their valuable cooperation and support throughout this study.

Author Contributions

IS conceptualized and designed the study, performed the literature review, analysed the data, and wrote the manuscript. MQ and ZS conceptualized and designed the study, supervised laboratory experiments, critically revised the manuscript, and significantly contributed to the writing and editing process. MZ, WQ, and TQ performed all laboratory experiments and were responsible for data collection. All authors participated in the interpretation of results, provided critical feedback, and approved the final version of the manuscript.

Funding

No external funding was received for this study. This work was supported by An-Najah National University as part of a doctoral degree project.

Availability of Data and Materials

The nucleotide sequence data generated and analyzed during the current study have been deposited in the GenBank database under accession numbers PV455512–PV455544. These sequences correspond to samples patient 1 through patient 33. The data will be publicly available online

via the NCBI GenBank database. For further details, please refer to the following link: (https://www.ncbi.nlm.nih.gov/Genbank/update.html) .

Additional details regarding data availability, including access to the raw data analyzed in this study, can be obtained by contacting the corresponding authors (Mohammad Qadi; m.qadi@najah.edu and Zaidoun Salah; zaidouns@medicare.ps).

Consent for Publication

Informed consent for publication was obtained from each participant after clearly explaining the purpose and scope of the study.

Competing Interests

The authors declare no competing interests.

Conflict of Interest

The authors declare no conflicts of interest concerning the research, authorship, and/or publication of this article.

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References

1. World Health, O., *Cervical Cancer.* 2023.

2. World Health, O., *Human papillomavirus (HPV)*. 2017, World Health Organization, Regional Office for Europe.
3. Schiffman, M., et al., *Carcinogenic human papillomavirus infection.* Nature Reviews Disease Primers, 2016. **2**(1): p. 16086.
4. Salhi, I., Z. Salah, and M. Qadi, *Prevalence, genotyping and phylogenetic analysis of HPV infecting Palestinian women.* BMC Infect Dis, 2024. **24**(1): p. 1299.
5. Obeid, D.A., et al., *Human papillomavirus epidemiology in populations with normal or abnormal cervical cytology or cervical cancer in the Middle East and North Africa: A systematic review and meta-analysis.* J Infect Public Health, 2020. **13**(9): p. 1304-1313.
6. Elmi, A.A., et al., *Human Papillomavirus (HPV) Infection: Molecular Epidemiology, Genotyping, Seroprevalence and Associated Risk Factors among Arab Women in Qatar.* PLOS ONE, 2017. **12**(1): p. e0169197.
7. AlWaheidi, S., et al., *Mammographic Screening in the Occupied Palestinian Territory: A Critical Analysis of Its Promotion, Claimed Benefits, and Safety in Palestinian Health Research.* JCO Glob Oncol, 2020. **6**: p. 1772-1790.
8. Snow, A.N., et al., *A simple and cost-effective method of DNA extraction from small formalin-fixed paraffin-embedded tissue for molecular oncologic testing.* BMC Clinical Pathology, 2014. **14**(1): p. 30.
9. Oba, U., et al., *An efficient procedure for the recovery of DNA from formalin-fixed paraffin-embedded tissue sections.* Biology Methods and Protocols, 2022. **7**(1): p. bpac014.
10. Forslund, O., H. Ly, and G. Higgins, *Improved detection of cutaneous human papillomavirus DNA by single tube nested 'hanging droplet' PCR.* Journal of Virological Methods, 2003. **110**(2): p. 129-136.
11. Mant, C., et al., *Polymerase chain reaction protocols for the detection of DNA from mucosal human papillomavirus types -6, -11, -16, -18, -31 and -33.* Journal of Virological Methods, 1997. **66**(2): p. 169-178.
12. Erhart, S.M.M., et al., *Comparative evaluation of the GP5+/6+, MY09/11 and PGMY09/11 primer sets for HPV detection by PCR in oral squamous cell carcinomas.* Experimental and Molecular Pathology, 2016. **100**(1): p. 13-16.
13. Bruni, L., et al., *HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010-2019.* Preventive medicine, 2021. **144**: p. 106399.
14. de Sanjosé, S., et al., *Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis.* Lancet Infect Dis, 2007. **7**(7): p. 453-9.
15. World Health, O., *Human papillomavirus (HPV) and cancer*. 2024, World Health Organization.
16. Bruni, L., et al., *Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis.* Lancet Glob Health, 2016. **4**(7): p. e453-63.

17. Muñoz, N., et al., *Epidemiologic classification of human papillomavirus types associated with cervical cancer.* N Engl J Med, 2003. **348**(6): p. 518-27.
18. Serrano, B., et al., *Human papillomavirus genotype attribution for HPVs 6, 11, 16, 18, 31, 33, 45, 52 and 58 in female anogenital lesions.* European Journal of Cancer, 2015. **51**(13): p. 1732-1741.
19. Bosch, F.X., et al., *Comprehensive control of human papillomavirus infections and related diseases.* Vaccine, 2013. **31 Suppl 5**: p. F1-31.
20. Castle, P.E. and A.R. Giuliano, *Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients--assessing their roles as human papillomavirus cofactors.* J Natl Cancer Inst Monogr, 2003(31): p. 29-34.
21. Rodríguez, A.C., et al., *Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection.* J Natl Cancer Inst, 2010. **102**(5): p. 315-24.
22. Elfgren, K., et al., *Management of women with human papillomavirus persistence: long-term follow-up of a randomized clinical trial.* Am J Obstet Gynecol, 2017. **216**(3): p. 264.e1-264.e7.
23. Schiffman, M., et al., *Human Papillomavirus Testing in the Prevention of Cervical Cancer.* JNCI: Journal of the National Cancer Institute, 2011. **103**(5): p. 368-383.