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Short Communication

Molecular and serological prevalence of high-risk human papillomavirus among asymptomatic men in Georgia

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ABSTRACT

Objectives: Human papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide, associated with a range of anogenital and oropharyngeal cancers in men. Despite this, male populations are often underrepresented in HPV surveillance and prevention programs, particularly in Eastern Europe and the Caucasus. This study aimed to assess the prevalence of high-risk HPV in asymptomatic men in Georgia using both serologic and molecular diagnostics.

Methods: We conducted a cross-sectional study of 372 asymptomatic men aged 25-65 years, recruited from urban outpatient clinics in Tbilisi. Serum samples were analyzed using a commercial enzyme-linked immunosorbent assay kit (DiaPro) to detect immunoglobulin G antibodies specific to HPV16 and HPV18. Simultaneously, HPV DNA was extracted and genotyped using the Anyplex™ II HPV28 Detection Assay (Seegene), which identifies 28 genotypes, including 14 high-risk types.

Results: Only five participants (1.34%; 95% confidence interval: 0.6-3.1%) tested positive for HPV16/18-specific immunoglobulin G antibodies. In contrast, HPV DNA was detected in 165 participants (44.4%). The most common genotypes were HPV31 (15.1%), HPV16 (10.0%), HPV33 (5.4%), and HPV18 (4.8%). All detected genotypes were high-risk. Among seropositive individuals, three had HPV16, one had HPV31, and one had HPV18.

Conclusions: These findings reveal a high prevalence of high-risk HPV genotypes in asymptomatic Georgian men, despite low seropositivity. The results support the need for gender-neutral HPV vaccination and underscore the limitations of serological testing in male populations.

Introduction

Human papillomavirus (HPV) remains one of the most ubiquitous sexually transmitted infections globally, with a high lifetime acquisition rate among sexually active individuals [1]. While the burden of HPV-related disease has traditionally been framed in the context of cervical cancer in women [2], a growing body of literature has drawn attention to its significant implications for men [3]. High-risk HPV types, particularly HPV16 and HPV18, are now well-established as causal agents in a range of male malignancies, including anal, penile, and oropharyngeal cancers [4]. Despite this, men continue to be largely underrepresented in HPV surveillance efforts. One contributing factor is the asymptomatic nature of most HPV infections in men, which often resolve without clinical manifestation [5]. As a result, men may unknowingly act as carriers, facilitating ongoing transmission to sexual partners. Moreover, the absence of an established screening infrastructure for pre-cancerous lesions in men, similar to cervical cytology in women, has contributed to a persistent gender imbalance in prevention strategies. Recent meta-analytical estimates suggest that approximately 31% of men globally are infected with genital HPV, with over one-fifth carrying high-risk oncogenic types. HPV16 consistently emerges as the most prevalent genotype, followed by other carcinogenic strains such as HPV18 and HPV31 [6]. However, detecting prior HPV exposure in men presents methodological challenges. Unlike women, men exhibit lower seroconversion rates following infection [7], making serological assays such as enzyme-linked immunosorbent assay (ELISA) less reliable for estimating cumulative HPV burden. Consequently, seronegative status in men does not necessarily indicate an absence of infection or immunity. This challenge

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is particularly pertinent in regions where HPV epidemiology remains poorly characterized. In the Caucasus, and specifically in Georgia, little is known about the prevalence and genotype distribution of HPV among men. This knowledge gap is concerning given the implications for both cancer prevention and public health policy. Understanding the extent of asymptomatic HPV carriage in male populations is essential for developing effective, inclusive vaccination and screening programs. To address this, the present study evaluates HPV infection among asymptomatic Georgian men aged 25 to 65 years using two distinct yet complementary methods. First, serologic testing via ELISA was used to detect immunoglobulin (Ig) G antibodies specific to HPV16 and HPV18, offering insights into immune response and prior exposure. Second, molecular genotyping was performed to assess the current prevalence of high-risk HPV types based on viral DNA detection. This dual approach provides a more comprehensive understanding of HPV epidemiology in men, informing strategies for gender-inclusive prevention in settings where data have previously been scarce.

Materials and methods

Study population and design

This cross-sectional study was conducted between October 1 and November 15, 2024, in Tbilisi, Georgia. A total of 372 asymptomatic men, aged between 25 and 65 years, were recruited during routine health check-ups from outpatient departments of urban healthcare centers. This setting was chosen because it serves a diverse patient population across various socioeconomic and occupational groups, making it a practical and accessible recruitment site. The study was approved by the Bioethics International Committee of the Petre Shotadze Tbilisi Medical Academy (IRB #20240902/2), and oral informed consent was obtained from all participants before enrollment. Inclusion criteria consisted of male sex, age between 25 and 65 years, absence of clinical signs or history of HPV-related disease, and no prior HPV vaccination. Participants were excluded only if they declined consent or were not present for a preventive care visit. The selected age range reflects the reproductive and sexually active male population in Georgia. According to the National Statistics Office of Georgia (Geostat) [8], this demographic represents a substantial proportion of the adult male population and is considered a critical group for assessing HPV transmission dynamics. Approximately 15% of eligible men declined participation, primarily due to time constraints or lack of interest. While this introduces a possible source of selection bias, the refusal rate was modest, and no evident demographic patterns were observed among non-responders. The sexual behavior or lifestyle data were not collected at recruitment to preserve participant anonymity and maintain focus on serological and molecular outcomes. Additionally, age-stratified analysis was not performed due to the limited sample size in individual age brackets, which would have undermined statistical power. The recruitment site— the urban outpatient department—is widely accessed by diverse socioeconomic and occupational groups, increasing the generalizability of findings. Moreover, due to the absence of systematic male HPV surveillance or vaccination programs in Georgia, this cohort offers a timely and epidemiologically valid estimate of natural HPV exposure in the country's male population.

Sample collection and serological testing

Peripheral blood was collected by trained phlebotomists using standard venipuncture procedures. The serum was separated by centrifugation and stored at –20°C until analysis. Detection of HPV-specific immunoglobulin G (IgG) antibodies targeting HPV16 and HPV18 was performed using a commercial ELISA kit (DiaPro, Diagnostic Bioprobes S.r.l., Italy). This assay specifically quantifies IgG antibodies directed against L1 viral capsid proteins of HPV16 and HPV18. Optical density (OD) values were recorded at 450 nm using a microplate reader. To determine seropositivity, the OD of each sample was divided by the corresponding cut-off value provided by the manufacturer, yielding a sample/cut-off (S/CO) ratio. Samples with a ratio ≥1.0 were classified as positive, while values <1.0 were interpreted as negative, in accordance with the kit's validation criteria.

Table 1
Distribution of enzyme-linked immunosorbent assay-based human papillomavirus 16/18 immunoglobulin G results.

Result	N (%)
Positive	5 (1.3%)
Negative	367 (98.7%)
Total	**372 (100%)**

HPV genotyping

DNA was extracted from the serum samples using the QIAamp DNA Blood Kit (Qiagen) following the manufacturer's protocol. HPV DNA detection and genotyping were carried out using the Anyplex™ II HPV28 Detection Assay (Seegene), a multiplex real-time PCR method that can simultaneously detect 28 HPV genotypes, including 14 high-risk types (e.g., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Amplification was performed using the CFX96 Real-Time PCR System (Bio-Rad) with thermal cycling conditions according to the manufacturer's instructions. The assay utilizes DPO™ (Dual-Priming Oligonucleotide) and TOCE™ (Tagging Oligonucleotide Cleavage and Extension) technologies for accurate discrimination of multiple HPV genotypes. Internal controls were used to monitor sample adequacy and PCR inhibition. Positive detection of any high-risk HPV genotype was recorded for each sample. Genotyping results were interpreted using Seegene Viewer software.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26.0. Descriptive statistics were calculated for all relevant variables, including means, medians, standard deviations, and interquartile ranges. HPV seroprevalence was expressed as proportions with corresponding 95% confidence intervals (CI) using the Clopper-Pearson method. The distribution of OD values and S/CO ratios was visualized using histograms and boxplots. Frequencies of HPV genotypes were presented as percentages of the total study population. Associations between serologic results and genotype detection were explored using cross-tabulations.

Results

Serological detection of human papillomavirus 16/18 immunoglobulin G antibodies

Of the 372 asymptomatic men included in the study, only five individuals (1.34%; 95% CI: 0.6-3.1%) tested positive for IgG antibodies specific to HPV16 or HPV18 using the DiaPro ELISA kit. The mean OD across all samples was 0.110 (±0.107), with a range spanning from 0.019 to 1.796. The calculated mean sample-to-cut-off (S/CO) ratio was 0.335 (±0.350). Only five samples yielded a ratio ≥1.0, meeting the positivity threshold per assay specifications (Table 1).

Human papillomavirus genotype distribution by molecular detection

HPV DNA was successfully amplified and genotyped in 165 of the 372 participants, yielding a positivity rate of 44.35%. Among those with a detectable genotype, HPV31 was the most frequently identified, present in 56 men (15.05%), followed by HPV16 (9.94%), HPV33 (5.38%), and HPV18 (4.84%). Less common high-risk types included

Table 2
High-risk HPV genotype distribution among participants.

Genotype	N (%)
HPV 31	56 (15.05%)
HPV 16	37 (9.94%)
HPV 33	20 (5.38%)
HPV 18	18 (4.84%)
HPV 59	12 (3.23%)
HPV 52	8 (2.15%)
HPV 51	7 (1.88%)
HPV 39	6 (1.61%)
HPV 45	1 (0.27%)
Total	165 (44.35%)

HPV, human papillomavirus.

HPV59, HPV52, HPV51, HPV39, and HPV45 (Table 2). 207 participants (55.65%) were classified as "Not Genotyped" due to either the absence of detectable HPV DNA or low viral load below assay thresholds. Among the five participants who were seropositive by ELISA, molecular genotyping identified HPV16 in 3 cases, HPV31 in 1 case, and HPV18 in 1 case.

Discussion

This study presents the sero-molecular epidemiological investigation of high-risk HPV in asymptomatic men aged 25-65 years in Georgia. Our findings reveal a striking discrepancy between serologic and molecular detection methods: while only 1.34% of participants tested positive for HPV16/18-specific IgG antibodies using ELISA, molecular genotyping identified HPV DNA in 44.35% of the cohort. These findings reflect a substantially lower seroprevalence than the 20-30% range commonly reported in global studies of high-risk HPV antibodies among men [6]. This discrepancy highlights the known biological limitations of serological assays in male populations. Specifically, the low seroconversion rate following natural infection and the typically transient antibody response in men may contribute to the underestimation of cumulative exposure [7,9]. This disparity underscores a key diagnostic limitation: antibody-based serologic testing may severely underestimate HPV exposure in men. Several biological and technical factors likely contribute to this discrepancy. Prior research [7] has shown that men are less likely than women to seroconvert following natural HPV infection, partly due to the keratinized nature of male genital epithelium and the frequently transient or subclinical course of infection. Moreover, the DiaPro ELISA kit used in our study targets only HPV16 and HPV18-specific IgG antibodies, which limits its capacity to detect exposure to other high-risk types. This partial serotype coverage, in combination with potentially short-lived immune responses, may explain the low seroprevalence observed despite high rates of DNA positivity. Among the five participants who tested seropositive, molecular results identified HPV16 in three cases, HPV31 in one, and HPV18 in one—demonstrating only partial concordance. This limited overlap reinforces the lower sensitivity of serologic detection [10] and highlights the importance of DNA-based diagnostics [11] for more accurate HPV surveillance in male populations. Furthermore, the prevalence of high-risk HPV DNA in 44.35% of participants is noteworthy and exceeds global estimates for asymptomatic male populations, where pooled prevalence is typically reported at around 31% [6]. The predominance of HPV31 (15.05%) and HPV16 (9.94%) among genotyped samples mirrors findings from other international studies [12,13], where HPV16 remains the most identified oncogenic type in both men and women. Interestingly, HPV31 exceeded HPV16 in our cohort, a pattern that may reflect regional variation in circulating genotypes or demographic factors specific to the Georgian male population. Other high-risk genotypes—including HPV33, HPV18, and HPV59—were also detected at non-negligible rates, reinforcing the diversity of HPV types men may harbor and transmit.

Importantly, all HPV genotypes detected in this study belong to the high-risk category, highlighting the oncogenic potential of HPV infections even in asymptomatic individuals. These findings underscore the role of men as silent reservoirs of HPV [14] and reinforce the case for gender-neutral vaccination strategies. Although the quadrivalent and nonavalent HPV vaccines currently in use offer protection against most genotypes identified here (including HPV16, 18, 31, 33, 52, and 58), vaccine uptake in Georgia remains limited and primarily targeted toward adolescent girls. Expanding vaccination efforts to include boys and young men could meaningfully reduce HPV transmission and associated cancer risk over time.

The study has several strengths, including the combined use of serologic and molecular diagnostics, a well-defined urban cohort, and laboratory methods aligned with international standards. However, certain limitations must be acknowledged. First, while the selection of an urban outpatient clinic allowed for efficient recruitment and reflected healthcare access points for a diverse segment of the adult male population, we recognize that this setting may not fully capture rural or socioeconomically marginalized populations. We recruited participants consecutively from men attending routine check-ups, regardless of the specific reason for the visit, provided they met eligibility criteria. Inclusion was limited to those who were within the 25-65 age range, asymptomatic for HPV-related conditions, and reported no history of HPV vaccination. No additional exclusion criteria such as smoking status or sexual behavior were applied. Participation was voluntary, and approximately 15% of eligible men declined to participate, primarily due to time constraints or lack of interest. While this non-response rate is modest, it introduces a possible source of selection bias. Further studies could improve representativeness by including both urban and rural cohorts and collecting more detailed demographic and behavioral data. Stratified analysis by age groups was not performed in this study due to limited statistical power within each age category. Nonetheless, age-stratified patterns remain a relevant consideration for future expanded studies. Second, the lack of data on participants' sexual behavior, smoking status, or HIV status precludes analysis of risk factors for HPV acquisition. Third, the ELISA assay used was restricted to two genotypes, and the sensitivity threshold for antibody detection may vary across populations. Despite these limitations, this study contributes valuable baseline data on HPV burden in Georgian men and offers a foundation for future public health planning. It supports calls for the inclusion of men in HPV vaccination campaigns, the adoption of more sensitive molecular diagnostics in epidemiological surveillance, and the design of longitudinal studies to assess the natural history of HPV infection in male populations.

Conclusion

This study provides the sero-molecular assessment of high-risk HPV infection among asymptomatic men in Georgia. While seroprevalence of HPV16/18-specific IgG antibodies was low (1.34%), molecular genotyping revealed a substantial proportion of participants (44.35%) carrying high-risk HPV DNA—most notably HPV31 and HPV16. These findings highlight the limitations of serologic testing in men and underscore the importance of DNA-based diagnostics for accurate HPV surveillance.

The predominance of oncogenic HPV types in an asymptomatic male population underscores the role of men as silent carriers and transmitters, reinforcing the need for gender-inclusive HPV prevention strategies. Expanding HPV vaccination to include boys and integrating molecular screening into national public health programs may significantly reduce the burden of HPV-associated cancers in Georgia. Further population-based and longitudinal studies are warranted to guide evidence-informed policymaking and vaccination rollout.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethics approval

Ethical clearance was secured from the Bioethics International Committee of the Petre Shotadze Tbilisi Medical Academy (IRB #20240902/2, Tbilisi, Georgia). All procedures adhered to the Helsinki Declaration of 1975, revised in 2013, with participants receiving comprehensive study information and providing oral informed consent before inclusion.

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Author contributions

All authors equally contributed to this article's preparation.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Clinical trial number

Not applicable.

Human ethics and consent to participate

All procedures adhered to the Helsinki Declaration of 1975, revised in 2013, with participants receiving comprehensive study information and providing oral informed consent before inclusion.

Declaration of generative AI and AI-assisted technologies

During the preparation of this paper, the authors used ChatGPT (https://chat.openai.com/) for stylistic purposes, checking grammar and spelling. After using this tool the authors reviewed and edited the content, they take full responsibility for the content of the publication.

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