HIGH-GRADE CERVICAL AND ANAL INTRAEPITHELIAL NEOPLASIA IN REPRODUCTIVE-AGE WOMEN WITH HIGH-RISK HPV: A PROSPECTIVE STUDY USING HIGH-RESOLUTION ANOSCOPY

***NINO MUSERIDZE**, MD, PhD;[2] **MADONA JUGHELI**, MD, PhD;[1] **ANA CHOKHONELIDZE**;[2] **BELA JUGHELI**, MD;[1] **NANI TATISHVILI**[2]*

[1] *Caraps Medline Clinic, Tbilisi, Georgia*

[2] *Georgian-German Reproduction Center, Tbilisi, Georgia*

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ABSTRACT

Background: Persistent infection with high-risk human papillomavirus (HR-HPV) is the necessary cause of the vast majority of cervical cancers and the driver of cervical intraepithelial neoplasia (CIN).[1] Fourteen genotypes are considered oncogenic, with HPV16 and HPV18 accounting for the largest share of the global cervical cancer burden.[2] While the cervix has been the focus of screening and prevention for decades, the anal canal – lined by a vulnerable transformation zone analogous to the cervix – can also harbor HR-HPV, develop anal intraepithelial neoplasia (AIN), and progress to squamous cell carcinoma.[3] Anal cancer incidence has risen in many settings, and women constitute a growing proportion of cases.[4]Despite these parallels, routine gynecologic care rarely includes anal assessment.[5] The natural history of anal HPV infection in immunocompetent, HIV-negative women with cervical disease remains poorly characterized, and evidence regarding concurrent high-grade disease at both sites is limited.[6] A more precise estimate of the prevalence of AIN 2/3 among women with CIN 2/3, coupled with HPV genotype concordance data, could inform whether targeted anal evaluation should be integrated into follow-up.[7] Addressing this knowledge gap is particularly important in regions where cervical screening is established but anal screening is not standard of care.[8]

Objectives

This prospective study sought to (1) determine the prevalence of histologically confirmed AIN 2/3 in reproductive-age, immunocompetent women with biopsy-proven CIN 2/3 and HR-HPV infection; (2) evaluate HPV genotype concordance between cervical and anal specimens; and (3) explore associations between cervical disease grade and the presence of high-grade anal lesions. A secondary objective was to describe operational feasibility and patient acceptability of incorporating anal cytology and high-resolution anoscopy (HRA) into routine evaluation pathways after a diagnosis of high-grade cervical disease.[5]

Methods

Design and setting: We conducted a prospective cohort study at two tertiary centers in Tbilisi, Georgia. Eligible participants were 21-49 years old, premenopausal, sexually active, HR-HPV positive, and had histologically confirmed CIN 2/3.[1] Exclusions were pregnancy, known immunodeficiency, including HIV, prior anal cancer or surgery, inflammatory bowel disease, or inability to consent. Ethics committees at both centers approved the protocol, and all participants provided written informed consent.[9]

Procedures: Baseline interviews captured demographics, parity, smoking, sexual history, contraceptive use, and prior cervical treatments. Cervical and anal swabs were collected for HR-HPV genotyping targeting 14 oncogenic types via multiplex PCR with type-specific primers.[2] Anal cytology used a moistened Dacron swab inserted 3-5 cm and processed as liquid-based cytology. Experienced clinicians performed HRA after application of acetic acid and Lugol's iodine; all acetowhite, punctate, or iodine-negative areas underwent directed biopsy. Two independent pathologists, blinded to clinical data, graded lesions as AIN 1, 2, or 3.[6,7]

Outcomes and analysis: The primary outcome was the prevalence of AIN 2/3. Secondary outcomes included cervical-anal HPV genotype concordance and the association between CIN grade and AIN 2/3. We calculated descriptive statistics, 95% confidence intervals for prevalence, and used chi-square or Fisher's exact tests where appropriate, with $p < 0.05$ deemed statistically significant.[10]

Results

Fifty-three women (median age 34 years, range 21-49) were enrolled. All were HIV-negative and immunocompetent. High-grade anal lesions (AIN 2/3) were histologically confirmed in 44 of 53 participants (83.5%).[3] Rates were comparably high in the CIN 2 subgroup (81.8%) and CIN 3 subgroup (83.9%), with no statistically significant difference between groups. HPV genotype concordance between cervical and anal sites was 91%, primarily driven by HPV16, which was predominant across both compartments; HPV18 was the second most frequent.[2,4] Multiple concurrent HR-HPV infections were present in 28% of participants. A significant association was observed between cervical disease grade and the presence of AIN 2/3 ($p < 0.01$). [6,7] Operationally, HRA with targeted biopsy was feasible in the outpatient setting with high patient acceptance; the majority completed both cytology and HRA in a single visit. There were no serious adverse events, and post-biopsy discomfort was self-limited.[5,9]

Interpretation

The high prevalence of AIN 2/3 in this cohort of immunocompetent, reproductive-age women with CIN 2/3, together with strong HPV genotype concordance, supports the hypothesis that the cervix and anal canal often share an everyday viral exposure and disease trajectory.[2,3] Mechanisms may include autoinoculation during sexual or hygienic practices, a field effect across anogenital epithelium, and shared behavioral risk factors such as smoking.[4,5] These findings align with emerging literature indicating substantial anal disease in women with high-grade cervical lesions and suggest that anal evaluation could be a valuable adjunct to gynecologic follow-up, particularly when HR-HPV persists.[6,7]From a public health perspective, early detection and treatment of anal HSIL may avert progression to invasive carcinoma, potentially offering favorable cost-effectiveness compared with diagnosing cancer at a later stage.[10] Integration of anal cytology and referral HRA into post-CIN pathways could be implemented pragmatically in tertiary centers and extended through capacity building.

Conclusion

Among immunocompetent reproductive-age women with CIN 2/3, high-grade anal lesions are common, and cervical-anal HPV genotype concordance is strong.[3,4] These data reinforce the biologic interconnectedness of anogenital HPV infection and support incorporating targeted anal evaluation into follow-up protocols for women with high-grade cervical disease.[6,7,10]

Keywords

human papillomavirus; cervical intraepithelial neoplasia; anal intraepithelial neoplasia; high-resolution anoscopy; HPV genotyping; reproductive-age women; genotype concordance; screening integration

Supplementary Notes to the Extended Abstract

Supplementary note 1. This investigation was designed to be pragmatic and clinically applicable, mirroring workflows that can be adopted in tertiary gynecology and colorectal clinics. HPV16 predominance is biologically plausible given its higher persistence and carcinogenic potential compared with other oncogenic types. Together, these considerations argue for a risk-adapted model in which women with CIN 2/3 receive structured anal assessment as part of comprehensive care.[2]

Supplementary note 2. We deliberately restricted enrollment to immunocompetent, HIV-negative women to isolate the effect of cervical disease status on anal pathology without immune suppression as a confounder. Genotype concordance across cervical and anal sites suggests either simultaneous acquisition or recurrent autoinoculation within the anogenital tract. Health systems can achieve impact by integrating anal cytology and referral HRA into existing colposcopy clinics, thereby leveraging shared infrastructure.[1]

Supplementary note 3. The analytic plan emphasized clarity and reproducibility, using simple proportions, confidence intervals, and well-established categorical tests for associations. The transformation zone architecture at the anal canal may create a permissive microenvironment similar to the cervical transformation zone. Education for patients and clinicians should clarify that HPV is a multicentric anogenital infection, not confined to the cervix.[10]

Supplementary note 4. Instruments and consumables were selected to match resource-constrained settings, increasing the likelihood of scale-up if results proved clinically meaningful. Smoking acts as a cofactor in HPV persistence, likely mediated by local immunomodulation and epithelial changes that impair viral clearance. Vaccination and screening are complementary; prophylactic vaccination reduces HPV acquisition while screening detects treatable precancer.[9]

Supplementary note 5. Operational definitions were prespecified to minimize misclassification and to enable consistent interpretation by pathologists and clinicians. High acceptance of HRA in this cohort reflects careful counseling, clear explanation of benefits, and attention to comfort during examination. Policy development should be iterative, starting with tertiary referral pathways and expanding as capacity and evidence grow.[6]

Supplementary note 6. Staff training focused on standardized HRA technique and recognition of acetowhite change, mosaicism, punctation, and iodine non-uptake. Liquid-based cytology facilitated consistent sampling and enabled adjunctive HPV testing from the same specimen if needed. Further research should evaluate persistence and clearance of anal HSIL after treatment of cervical disease, including genotype-specific dynamics.[5]

Supplementary note 7. Biopsy targeting followed recognized patterns of HSIL morphology to maximize diagnostic yield while avoiding unnecessary trauma. Directed biopsy under HRA visualization remains the diagnostic cornerstone, with sensitivity superior to random biopsies in focal disease. Cost-effectiveness analyses tailored to regional resources can guide scale-up and inform payer coverage decisions.[7]

Supplementary note 8. Data integrity was protected through double entry and cross-checks, and pathology review was performed independently by two specialists. The lack of serious adverse events supports the safety of outpatient HRA and biopsy when performed by trained clinicians. Equity considerations are paramount; access to HRA should not be limited to urban centers or those with private insurance.[9]

Supplementary note 9. This investigation was designed to be pragmatic and clinically applicable, mirroring workflows that can be adopted in tertiary gynecology and colorectal clinics. Multiple concurrent HR-HPV infections were observed in over a quarter of participants, a pattern that may increase cumulative oncogenic risk. Together, these considerations argue for a risk-adapted model in which women with CIN 2/3 receive structured anal assessment as part of comprehensive care.[4]

Supplementary note 10. We deliberately restricted enrollment to immunocompetent, HIV-negative women to isolate the effect of cervical disease status on anal pathology without immune suppression as a confounder. The strong association between cervical grade and anal HSIL underscores the value of cervical disease severity as a triage signal for anal evaluation. Health systems can achieve impact by integrating anal cytology and referral HRA into existing colposcopy clinics, thereby leveraging shared infrastructure.[3,6]

Supplementary note 11. The analytic plan emphasized clarity and reproducibility, using simple proportions, confidence intervals, and well-established categorical tests for associations. HPV16 predominance is biologically plausible given its higher persistence and carcinogenic potential compared with other oncogenic types. Education for patients and clinicians should clarify that HPV is a multicentric anogenital infection, not confined to the cervix.[5]

Supplementary note 12. Instruments and consumables were selected to match resource-constrained settings, increasing the likelihood of scale-up if results proved clinically meaningful. Genotype concordance across cervical and anal sites suggests either simultaneous acquisition or recurrent autoinoculation within the anogenital tract. Vaccination and screening are complementary; prophylactic vaccination reduces HPV acquisition while screening detects treatable precancer.[2]

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Supplementary note 14. Staff training focused on standardized HRA technique and recognition of acetowhite change, mosaicism, punctation, and iodine non-uptake. Smoking acts as a cofactor in HPV persistence, likely mediated by local immunomodulation and epithelial changes that impair viral clearance. Further research should evaluate persistence and clearance of anal HSIL after treatment of cervical disease, including genotype-specific dynamics.[5]

Supplementary note 15. Biopsy targeting followed recognized patterns of HSIL morphology to maximize diagnostic yield while avoiding unnecessary trauma. High acceptance of HRA in this cohort reflects careful counseling, clear explanation of benefits, and attention to comfort during examination. Cost-effectiveness analyses tailored to regional resources can guide scale-up and inform payer coverage decisions.[6]

Supplementary note 16. Data integrity was protected through double entry and cross-checks, and pathology review was performed independently by two specialists. Liquid-based cytology facilitated consistent sampling and enabled adjunctive HPV testing from the same specimen if needed. Equity considerations are paramount; access to HRA should not be limited to urban centers or those with private insurance.[3,4]

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Supplementary note 18. We deliberately restricted enrollment to immunocompetent, HIV-negative women to isolate the effect of cervical disease status on anal pathology without immune suppression as a confounder. The lack of serious adverse events supports the safety of outpatient HRA and biopsy when performed by trained clinicians. Health systems can achieve impact by integrating anal cytology and referral HRA into existing colposcopy clinics, thereby leveraging shared infrastructure.[9]

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Supplementary note 20. Instruments and consumables were selected to match resource-constrained settings, increasing the likelihood of scale-up if results proved clinically meaningful. The strong association between cervical grade and anal HSIL underscores the value of cervical disease severity as a triage signal for anal evaluation. Vaccination and screening are complementary; prophylactic vaccination reduces HPV acquisition while screening detects treatable precancer.[1,2]

Supplementary note 21. Operational definitions were prespecified to minimize misclassification and to enable consistent interpretation by pathologists and clinicians. HPV16 predominance is biologically plausible given its higher persistence and carcinogenic potential compared with other oncogenic types. Policy development should be iterative, starting with tertiary referral pathways and expanding as capacity and evidence grow.[6,10]

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Supplementary note 23. Biopsy targeting followed recognized patterns of HSIL morphology to maximize diagnostic yield while avoiding unnecessary trauma. The transformation zone architecture at the anal canal may create a permissive microenvironment similar to the cervical transformation zone. Cost-effectiveness analyses tailored to regional resources can guide scale-up and inform payer coverage decisions.[3,7]

Supplementary note 24. Data integrity was protected through double entry and cross-checks, and pathology review was performed independently by two specialists. Smoking acts as a cofactor in HPV persistence, likely mediated by local immunomodulation and epithelial changes that impair viral clearance. Equity considerations are paramount; access to HRA should not be limited to urban centers or those with private insurance.[10]

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Glossary of Terms (for reader clarity)

High-risk HPV (HR-HPV): Oncogenic HPV genotypes associated with CIN and anogenital cancers; in this study, genotyping targeted 14 high-risk types, including HPV16 and HPV18.
CIN 2/3: Histologically confirmed high-grade cervical intraepithelial neoplasia representing substantial risk of progression to invasive carcinoma without treatment.
AIN 2/3: High-grade anal intraepithelial neoplasia, the immediate precursor to anal squamous cell carcinoma, diagnosed histologically on directed biopsy.

High-resolution anoscopy (HRA): Magnified examination of the anal canal after application of acetic acid and Lugol's iodine to identify HSIL morphology for targeted biopsy.

Anal cytology: Liquid-based cytologic sampling of the anal canal using a moistened Dacron swab inserted 3-5 cm, analogous to cervical cytology.

Genotype concordance: Detection of the same HR-HPV genotype in paired cervical and anal specimens, suggesting shared exposure or autoinoculation.

Transformation zone: Region of squamous-columnar junction susceptible to HPV-mediated neoplastic change; present at both cervix and anal canal.

Autoinoculation: Self-transfer of virus between anogenital sites via contact during sexual or hygienic practices.

Directed biopsy: Sampling of visually suspicious lesions under HRA guidance to increase diagnostic yield for HSIL.

Liquid-based cytology: A Sample preservation technique enabling improved cellular morphology and ancillary testing compared with conventional smears. Multiplicity of infection: Presence of more than one HR-HPV genotype in a given individual or site at the same time.

Field effect: The Concept that adjacent or related epithelia share carcinogenic exposure and susceptibility, enabling multicentric disease.

Persistence: Failure to clear HPV over time; persistence of HR-HPV is a critical step toward the development of high-grade intraepithelial lesions.

Clearance: Immunologic elimination of HPV; most infections are apparent spontaneously, but persistence is more likely with HR-HPV types and cofactors such as smoking.

HSIL morphology: Colposcopic or HRA patterns including dense acetowhitening, punctation, mosaicism, and iodine negativity that suggest high-grade disease.

Confidence interval: Range of values around an estimate that likely contains the actual population parameter; used to convey the precision of prevalence measures.

Chi-square test: Statistical test assessing association between categorical variables; used here for CIN grade versus AIN 2/3 presence.

Fisher's exact test: Exact test for small samples to evaluate associations between categorical variables when expected counts are low.

Outpatient feasibility: Practicality of delivering HRA and biopsy in clinic without general anesthesia, minimizing resource use and patient burden.

Acceptability: Participant willingness to undergo procedures; in this study, acceptability was high with appropriate counseling and comfort measures.

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