RESEARCH PAPER

Low-Grade Cervical Lesion Associated with Rare HPV Genotype 53: Implications for Screening and Risk Stratification

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Abstract

Background: Human papillomavirus (HPV) infection is the principal etiological factor in cervical intraepithelial lesions, with high-risk genotypes such as HPV 16 and 18 being most commonly implicated. However, the clinical significance of less common genotypes, including HPV 53, remains incompletely understood. HPV 53 is classified among probable high-risk types, but its independent role in cervical cytological abnormalities is still under investigation.

Case Presentation: We report the case of a 46-year-old woman who presented for routine cervical screening. Cytological examination of the Pap smear revealed features consistent with low-grade squamous intraepithelial lesion (LSIL), characterized by koilocytic changes, nuclear enlargement, and mild nuclear atypia. Reflex HPV genotyping demonstrated positivity for HPV 53 as the sole detected genotype, while all other high-risk and low-risk HPV types were negative.

Discussion: The detection of HPV 53 as an isolated genotype in association with LSIL is uncommon and raises important considerations regarding its pathogenic potential. Although HPV 53 is not among the most prevalent high-risk types, emerging evidence suggests a possible association with low-grade lesions and, in rare instances, progression. This case highlights the importance of recognizing non-16/18 HPV genotypes in cervical screening programs and underscores the need for careful follow-up and risk stratification according to current guidelines.

Conclusion: This case emphasizes the potential clinical relevance of HPV 53 in cervical cytological abnormalities. Isolated HPV 53 infection may be associated with LSIL and warrants appropriate surveillance. Further studies are needed to clarify its oncogenic risk and implications for patient management.

Keywords: Cervical cytology; LSIL; HPV 53; Real- time PCR; Pap smear.

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1. Introduction

Cervical cancer remains one of the most common female-specific malignancies worldwide and continues to represent a major cause of cancer-related morbidity and mortality. Persistent infection with high-risk human papillomavirus (HPV) is the central etiological factor in the development of cervical intraepithelial neoplasia and invasive carcinoma.[1,2] The spectrum of HPV-associated cervical disease ranges from low-grade squamous intraepithelial lesion (LSIL) to high-grade squamous intraepithelial lesion (HSIL), reflecting a continuum of viral-host interaction and progressive epithelial dysregulation.

In routine cervical screening, Papanicolaou (Pap) cytology remains a cornerstone for early detection of premalignant lesions; however, its diagnostic performance may be limited by interobserver variability and morphological overlap between reactive, benign, and HPV-related changes.[3,4] Concurrently, primary HPV testing or co-testing has been widely adopted to improve detection rates, although HPV DNA testing alone may lack specificity for lesion grading and cannot fully stratify the biological behavior of infection.[5,6] This diagnostic gap has prompted ongoing efforts to refine cervical cancer screening strategies through adjunct molecular and biomarker-based approaches.

Recent advances in molecular pathology have highlighted the potential role of additional biomarkers, including HPV genotyping beyond the classical 16/18 types, as well as host-based molecular alterations such as microRNAs and DNA methylation signatures.[1,2,5] These emerging tools aim to improve risk stratification, particularly in ambiguous cytological categories such as atypical squamous cells of undetermined significance (ASCUS) and LSIL, where management strategies remain variable and sometimes controversial.[4,7] Furthermore, studies have demonstrated that a subset of lesions initially categorized as LSIL may harbor or progress to high-grade disease, underscoring the importance of accurate triage and follow-up strategies.[8] From a clinical perspective, LSIL associated with non-16/18 high-risk or probable high-risk HPV genotypes remains an area of evolving understanding.[5,7] Although many of these infections are transient, certain genotypes may contribute to persistent infection and potential progression, yet are not fully represented in current vaccine coverage or standard risk algorithms. This highlights the importance of comprehensive genotyping approaches and careful clinicopathological correlation. Additionally, current ASCCP risk-based management guidelines emphasize the importance of correlating cytological findings with HPV genotyping results to ensure appropriate clinical follow-up and risk stratification.[3] In this context, we present a case of LSIL detected on liquid-based cervical cytology, in which isolated HPV genotype 53 was identified through a stepwise molecular diagnostic approach. This case highlights the diagnostic difficulty arising from cytomorphological mimics, the limitations inherent in restricted HPV screening assays, and underscores the importance of extended genotyping in improving diagnostic precision and enhancing clinical risk stratification. The clinical significance of single Human papillomavirus 53 infection remains poorly understood, especially in the absence of co-infection with other high-risk genotypes, highlighting the importance of reporting such cases to elucidate its independent pathogenic role. [9]

2. Case Presentation

2.2 Patient Information

A 46-year-old woman presented to the gynecology clinic with complaints of persistent vaginal discharge associated with intermittent vaginal spotting. There was no significant prior gynecological or oncological history. A combined cervical screening approach, including Pap test and HPV DNA co-testing, was requested.

2.3 Cytotechniqal Assay

Cervical samples were obtained from both the endocervix and ectocervix using a broom-type collection device. The specimen was transferred into a liquid-based cytology medium (*LBC, BD SurePath*). The container included the sampling device along with a prepared slide. The sample was processed using the SurePath liquid-based cytology system and stained with Papanicolaou (Pap) stain following standard laboratory protocols.

2.4 Adequacy Description

The slides demonstrated thin, evenly distributed smears with good cellularity and clear representation of the transformation zone.

3.1 Cytomorphological Findings

Microscopic examination revealed a predominance of mature squamous epithelial cells exhibiting mild to moderate cytological atypia. These changes were characterized by noticeable nuclear enlargement ranging from mild to moderate degree, accompanied by a slight increase in the nuclear-to-cytoplasmic ratio. Additionally, characteristic koilocytic changes were identified, including well-defined perinuclear clearing and irregular nuclear contours, consistent with cytopathic effects of human papillomavirus (HPV) infection. The chromatin pattern was mildly hyperchromatic but evenly distributed, without significant irregularity or coarse clumping. Importantly, no cytological features suggestive of high-grade squamous intraepithelial lesion (HSIL), such as marked nuclear pleomorphism, coarse chromatin, or abnormal mitotic activity, were observed. A notable diagnostic challenge in this case was the partial morphological overlap with glycogenated squamous cells, which can exhibit perinuclear clearing mimicking koilocytosis. As demonstrated in **Figure 2,** some cells displayed abundant cytoplasm with perinuclear halos but retained relatively uniform, small, and bland nuclei, favoring a glycogenated/reactive appearance. In contrast, **Figure 1** showed cells with more evident nuclear enlargement, irregular nuclear contours, and hyperchromasia, supporting HPV-related cytopathic effect. This distinction was critical, as true koilocytic atypia requires the presence of both perinuclear

clearing and nuclear abnormalities, whereas glycogenated cells typically lack significant nuclear atypia. The integration of cytomorphological features with HPV DNA testing was therefore essential in establishing the diagnosis and avoiding potential over- or under-interpretation.

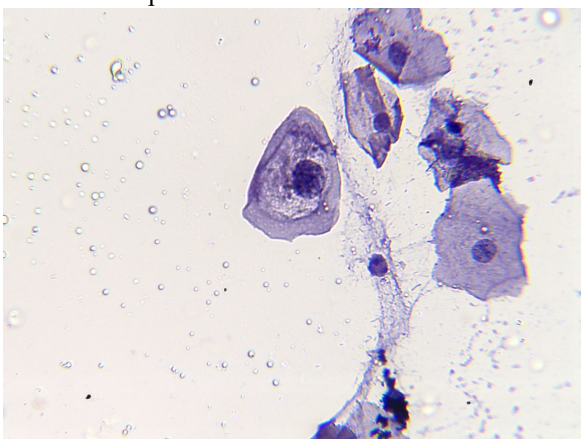

Figure1: Pap smear (LBC, SurePath; Pap stain) showing squamous epithelial cells with features consistent with HPV-related cytopathic effect, including nuclear enlargement, mild hyperchromasia, and distinct perinuclear clearing (koilocytosis), supporting a diagnosis of low-grade squamous intraepithelial lesion (LSIL).

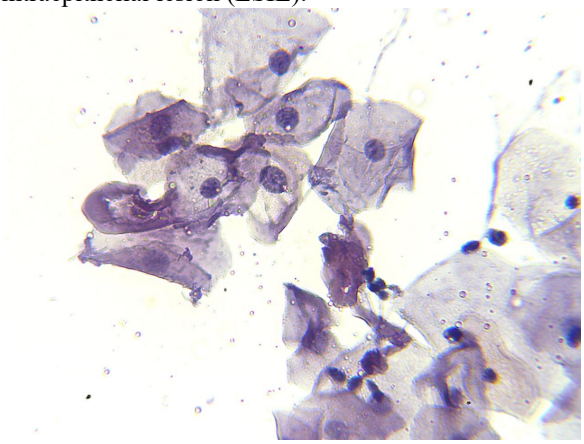

Figure2: Pap smear (LBC, SurePath; Pap stain) demonstrating glycogenated squamous cells with abundant cytoplasm and perinuclear clearing; however, nuclei remain moderate in size , uniform, and lacking significant atypia, highlighting a potential morphological mimic of koilocytosis and emphasizing the importance of nuclear features in differential diagnosis.

4. Molecular Analysis (HPV DNA Testing)

4.1 Initial Screening (Closed System)

Cervical samples were initially processed using a fully automated, closed-system platform (GeneXpert) for primary detection of high-risk HPV. This system was selected due to its high sensitivity, rapid turnaround time, and minimal risk of contamination. However, it is limited to a restricted panel of the most common high-risk HPV genotypes.

4.2 Open-System Workflow and DNA Preparation

To achieve extended genotyping coverage, the sample was further analyzed using an open-system real-time PCR approach. The workflow included multiple sequential steps: Automated nucleic acid extraction to obtain high-quality purified DNA, PCR reaction setup under controlled conditions and Real-time PCR amplification using optimized protocols. This approach allowed broader detection of HPV genotypes beyond those included in closed-system assays.

4.3 Stepwise Genotyping Strategy

The second real-time PCR kit was designed to provide grouped detection of HPV genotypes by categorizing them into three major groups based on their epidemiological and oncogenic profiles. This classification included Group 1, comprising low-risk genotypes (HPV 6, 11, 42, 43, 44, and 81), Group 2, which included the most common high-risk genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), and Group 3, consisting of less common or probable high-risk genotypes (HPV 26, 53, 66, 73, and 82). This grouped detection approach was implemented as an intermediate screening step to narrow down the potential genotype spectrum before proceeding to more specific identification. The analysis demonstrated a positive amplification signal exclusively within Group 3, while no amplification was detected in either Group 1 or Group 2, thereby excluding both low-risk and the most prevalent high-risk HPV types. Based on this result, further characterization was undertaken using a third, highly specific real-time PCR genotyping assay. This confirmatory step was essential to precisely identify the individual HPV genotype present within the positive group. The use of a targeted, high-specificity assay allowed accurate discrimination among the Group 3 genotypes, ultimately enabling definitive identification of the infecting HPV type.

4.4 Molecular Diagnosis

Final analysis confirmed the presence of HPV genotype 53 as the sole detected genotype in the sample. This result was established with high confidence through a structured diagnostic approach integrating closed-system screening and confirmatory open-system real-time PCR genotyping.

4.5 Clinical Management

Following the cytological diagnosis of low-grade squamous intraepithelial lesion (LSIL) and confirmation of isolated HPV genotype 53 infection, the patient was referred for gynecological evaluation. In accordance with current risk-based cervical cancer screening recommendations, colposcopic assessment was advised to further evaluate the cervical epithelium

and exclude the presence of high-grade cervical intraepithelial neoplasia. As no clinical or cytological evidence of high-grade disease was identified, conservative management with close surveillance was recommended. The patient was counseled regarding the generally favorable prognosis of most LSIL lesions, the possibility of spontaneous regression, and the importance of adherence to follow-up. Repeat HPV-based testing with cervical cytology at the recommended interval was planned according to institutional practice and contemporary ASCCP risk-based management guidelines.

5. Ethics Approval and Consent to Participate

This study was conducted in accordance with institutional ethical standards. Ethical approval was obtained from the Ethics Committee of the University Medical Center. Written informed consent was obtained from the patient for the procedure and for publication of anonymized clinical data and images.

4. Discussion

Cervical cancer remains one of the most significant malignancies affecting women globally, with strong molecular epidemiological evidence confirming persistent high-risk human papillomavirus (HPV) infection as the primary etiological driver of disease progression.[12] Cervical cytological abnormalities, particularly low-grade squamous intraepithelial lesion (LSIL), represent an early manifestation of HPV-induced epithelial disruption, although their biological behavior remains heterogeneous and often unpredictable. When comparing the diagnostic role of Papanicolaou (Pap) smears in cervical cytology, previous studies have highlighted their variable accuracy and the clinical significance of identifying low-grade squamous intraepithelial lesion (LSIL) cells within atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) categories, which may influence diagnostic interpretation and patient management. [9]

In the present case, LSIL was identified on liquid-based cervical cytology and was associated with isolated HPV genotype 53 detected through a stepwise molecular diagnostic approach. This finding is of particular interest, as HPV 53 is not among the most commonly recognized high-risk genotypes and its oncogenic potential remains controversial. Available epidemiological data suggest that HPV types such as 53 and CP8304 demonstrate a low prevalence ratio in high-grade lesions and carcinomas compared to low-grade lesions or normal specimens, supporting a likely low carcinogenic risk profile. [14] However, the occasional detection of HPV 53 in dysplastic cervical lesions highlights the need for cautious interpretation, particularly when it is the sole identified genotype, as demonstrated in this case.

From a cytological perspective, LSIL is typically characterized by koilocytic changes, nuclear enlargement, and mild dyskaryosis, but its distinction from benign mimics such as glycogenated squamous cells may be challenging. In the present case, a diagnostic overlap was observed between true koilocytic atypia and glycogen-rich squamous epithelium, emphasizing a key limitation of morphology-based diagnosis alone. This reinforces the importance of correlating cytological findings with molecular HPV testing to improve diagnostic confidence and reduce interpretative variability.

When compared with published literature, LSIL lesions are frequently associated with transient HPV infections and often regress spontaneously; however, a subset may persist or progress, particularly when associated with high-risk HPV types.[10,11] Furthermore, emerging evidence suggests that molecular alterations such as DNA methylation changes in genes like p16(INK4a), as well as miRNA expression profiles, may provide additional prognostic stratification in LSIL and CIN1 lesions, helping to identify cases with higher risk of progression.[10,11] In contrast, the current case demonstrates an isolated HPV 53 infection without evidence of high-grade cytological features, suggesting a potentially lower immediate risk profile.

Interestingly, HPV genotype distribution studies have shown variability across populations, with low-risk and high-risk HPV types coexisting in different disease spectra, and certain genotypes such as HPV 6 and 11 being highly prevalent in benign lesions, while high-risk types predominate in more severe disease.[13]The detection of HPV 53 in this case further expands the spectrum of HPV types encountered in routine cervical screening and highlights the importance of extended genotyping beyond the classical high-risk panel.

Overall, the present case underscores both similarities and differences with existing literature. The similarities lie in the association between LSIL and HPV infection, the cytomorphological features consistent with HPV effect, and the necessity of HPV testing for accurate classification. The differences include the unusual isolation of HPV 53 as the sole detected genotype and its uncertain oncogenic potential compared with well-established high-risk HPV types. This highlights a diagnostic and clinical gray zone where extended molecular testing and careful follow-up are essential for optimal patient management.

5. Conclusion

This case highlights a low-grade squamous intraepithelial lesion (LSIL) associated with isolated HPV genotype 53 infection, a relatively uncommon finding in routine cervical screening. The case

underscores the importance of integrating cytomorphological assessment with molecular HPV testing to ensure accurate diagnosis, particularly when morphological overlap exists with benign entities such as glycogenated squamous cells. The presence of koilocytic changes together with confirmatory HPV genotyping was essential in establishing a definitive diagnosis and avoiding potential misinterpretation on cytology alone. This report further supports the clinical relevance of non-16/18 HPV genotypes, including HPV 53, in cervical epithelial abnormalities and emphasizes the need for continued surveillance and further studies to clarify its oncogenic potential and clinical behavior. The lesion caused by HPV53 infection progressed slowly. The pathogenicity of a single HPV53 infection was low.

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