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Cytomorphological Reflections of HPV Positivity in Concurrent HPV Testing and Smear Specimens

Eş Zamanlı HPV Testi ve Smear Örneklerinde HPV Pozitifliğinin Sitomorfolojik Yansımaları

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Abstract

Background: Cervical cancer is one of the most common malignancies affecting women worldwide, accounting for approximately 12% of all cancers in women. High-risk human papillomavirus (hrHPV) is the principal etiological agent. Although cytology-based screening remains a cornerstone of early detection, molecular HPV testing enhances sensitivity, particularly for the detection of high-grade lesions and asymptomatic infections. The aim of this study was to investigate cytomorphological features associated with hrHPV positivity in liquid-based papanicolaou smear samples and to evaluate the distribution of hrHPV subtypes according to age groups within a single regional population.

Materials and Methods: This retrospective study included 1,500 patients who underwent simultaneous hrHPV DNA testing and liquid-based cytology at the Department of Pathology, Dicle University Faculty of Medicine between 2015 and 2016. hrHPV testing was performed using a fully automated Roche Cobas X4800 system. Cytological evaluations were conducted in accordance with the 2014 Bethesda System. Associations between cytomorphological findings and hrHPV subtypes were analyzed statistically using SPSS v18.0, with p-values < 0.05 considered significant.

Results: hrHPV positivity was detected in 141 (9.4%) of the 1,500 cases. Of these, HPV 16 was identified in 26 cases (18%), HPV 18 in 14 cases (10%), and other hrHPV subtypes in 101 cases (72%). No HPV 18 positivity was observed among the ASC-US, LSIL, and HSIL groups. Cytomorphological features, such as perinuclear halo, multinucleation, and parakeratosis, were frequently detected in hrHPV-positive cases. Notably, 138 of 1,418 cases with normal cytology exhibited hrHPV positivity (9.7%), indicating latent or subclinical infection.

Conclusions: The combination of molecular hrHPV testing and cytomorphological evaluation enhanced the diagnostic yield of cervical cancer screening. Recognizing the specific cytological features associated with hrHPV can assist in risk stratification and guide clinical management, particularly in cases lacking access to molecular testing. Larger multicenter studies are warranted to validate these associations further.

Keywords: High-risk HPV, cervical cytology, Bethesda system, cervical cancer screening, HPV subtypes

Öz

Amaç: Serviks kanseri, dünya genelinde kadınları etkileyen en yaygın maligniteler arasında yer almakta olup, tüm kadın kanserlerinin yaklaşık %12'sini oluşturmaktadır. Yüksek riskli insan papillomavirüsü (hrHPV) bu hastalığın başlıca etiyolojik etkenidir. Sitolojiye dayalı tarama yöntemleri erken tanıda temel bir rol oynamaya devam etmekle birlikte, moleküler HPV testleri, özellikle yüksek dereceli lezyonların ve asemptomatik enfeksiyonların saptanmasında duyarlılığı artırmaktadır. Bu çalışmanın amacı, likit bazlı papanicolaou smear örneklerinde hrHPV pozitifliği ile ilişkili sitomorfolojik özellikleri incelemek ve tek bir bölgesel popülasyon içinde yaş gruplarına göre hrHPV alt tiplerinin dağılımını değerlendirmektir.

Materyal ve metod: Bu retrospektif çalışmaya, 2015-2016 yılları arasında Dicle Üniversitesi Tıp Fakültesi Patoloji Anabilim Dalı'nda eş zamanlı hrHPV DNA testi ve sıvı bazlı sitoloji yapılan 1.500 hasta dahil edildi. hrHPV testi tam otomatik Roche Cobas X4800 sistemi ile yapıldı. Sitolojik değerlendirme 2014 Bethesda sistemine göre gerçekleştirildi. Sitomorfolojik bulgular ile hrHPV alt tipleri arasındaki ilişkiler SPSS v18.0 programında, p<0,05 anlamlılık düzeyi ile analiz edildi.

Bulgular: Bin beş yüz olgunun 141'inde (%9,4) hrHPV pozitifliği saptandı. Bu olguların 26'sında (%18) HPV 16, 14'ünde (%10) HPV 18 ve 101'inde (%72) diğer hrHPV alt tipleri tespit edildi. ASC-US, LSIL ve HSIL gruplarında HPV 18 pozitifliği gözlenmedi. Perinükleer halo, multinükleasyon ve parakeratoz gibi sitomorfolojik bulgular hrHPV pozitif olgularda sık görüldü. Normal sitolojiye sahip 1.418 olgunun 138'inde (%9,7) hrHPV pozitifliği saptandı ve bu durum latent/subklinik enfeksiyona işaret etti.

Sonuç: Moleküler hrHPV testi ile sitomorfolojik değerlendirmenin birlikte kullanılması, serviks kanseri taramalarında tanısal verimi artırmaktadır. hrHPV ile ilişkili belirli sitolojik bulguların tanınması, özellikle moleküler testlere erişimi olmayan durumlarda, risk sınıflamasına ve klinik yönetime katkı sağlayabilir. Bu ilişkilerin doğrulanması için daha geniş, çok merkezli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Yüksek riskli HPV, servikal sitoloji, Bethesda sistemi, servikal kanser taraması, HPV subtipleri

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Introduction

Cervical cancer remains a significant global health issue, particularly among women in low- and middle-income countries. It accounts for approximately 12% of all malignancies worldwide (1). Cervical cancer continues to cause substantial morbidity and mortality, despite being largely preventable through screening and vaccination. The high disease burden in developing regions is primarily due to the lack of structured and accessible screening programs, limited public awareness, and inadequate access to early diagnostic interventions (2). Conversely, countries with well-organized screening protocols have seen a marked decrease in cervical cancer incidence and mortality, underscoring the vital role of early detection and prevention.

The papanicolaou (PAP) smear test is the cornerstone of cervical cancer screening and facilitates the microscopic evaluation of exfoliated cervical epithelial cells. Advances in sample processing have led to the development of liquid-based cytology (LBC), which has now become widely adopted in many screening programs. LBC offers several advantages over conventional smear techniques, including improved sample adequacy, enhanced cellular preservation, reduction in obscuring elements, such as blood or mucus, and greater reproducibility of results (3). In this method, cervical cells are suspended in a liquid preservative, allowing the creation of a monolayered smear that provides a clearer and more standardized view for cytological examination.

A fundamental etiological factor in cervical carcinogenesis is Human Papillomavirus (HPV), a double-stranded DNA virus with tropism in squamous epithelial cells. HPV is considered a necessary, although not solely sufficient, cause of cervical cancer. The virus exerts its oncogenic effects primarily through the expression of two viral proteins, E6 and E7, which inactivate the tumor suppressor proteins p53 and Rb, respectively, leading to unchecked cellular proliferation, genomic instability, and malignant transformation (4,5).

Among the more than 200 identified HPV genotypes, a subset is classified as high-risk HPV (hrHPV) owing to its strong association with cervical intraepithelial neoplasia (CIN) and cervical carcinoma. Notably, HPV types 16 and 18 are the most prevalent, and are detected in nearly 70% of all cervical cancer cases worldwide (6). Their high oncogenic potential justifies their inclusion in molecular testing panels and prophylactic vaccination programmes.

With the growing recognition of HPV's pivotal role of HPV in cervical cancer development, molecular HPV DNA testing has been integrated into the primary screening algorithms. Molecular testing is more sensitive than cytology alone for the detection of precancerous lesions and enables the early identification of women at a significant risk for disease progression (6). This is especially relevant in cases where cytological findings are equivocal, such as Atypical Squamous Cells of Undetermined Significance (ASC-US). In these situations, reflex HPV testing assists in risk stratification by identifying women who may safely return to routine screening versus those who require colposcopic evaluation (7).

Cytomorphologically, hrHPV infections are associated with a spectrum of nuclear and cytoplasmic changes that reflect virus-induced cellular dysregulation. These features include nuclear enlargement, hyperchromasia, irregular nuclear membrane contours, binucleation, perinuclear clearing (koilocytosis), and increased mitotic figure (8). Koilocytosis, in particular, is considered a hallmark of productive HPV infection and can serve as an important clue for cytological interpretation.

Combining cytological screening and molecular HPV testing provides complementary diagnostic information. Cytology allows for morphological assessment of epithelial abnormalities, while HPV testing identifies the presence of high-risk viral DNA before overt morphological changes occur. This combined approach enhances the sensitivity and specificity of screening protocols and aids in the accurate classification of cervical lesions, improves clinical management, and reduces overtreatment.

Given the regional differences in HPV genotype distribution and variations in cytomorphological expression, localized studies are important for tailoring screening strategies. The prevalence and cytological manifestations of hrHPV infection may differ based on population demographics, sexual health education, vaccine uptake, and access to health care services.

This study aimed to evaluate cytomorphological alterations associated with high-risk HPV infections using liquid-based PAP smear samples, a method that improves sample quality and standardization. Specifically, we aimed to identify the frequency and distribution of characteristic cytological features in hrHPV-positive cases and correlate them with HPV subtypes where possible. Furthermore, we sought to assess the regional prevalence of high-risk HPV genotypes among the women screened at our institution. Understanding the dominant cytological patterns in hrHPV-positive patients will enhance the diagnostic accuracy and may offer clues regarding lesion severity, persistence, or regression potential.

Our findings are expected to contribute to the existing body

of knowledge by providing cytological reference data that are specific to our population. Additionally, the study supports efforts to optimize screening algorithms, particularly in borderline or ambiguous cytology cases, by highlighting the cytomorphological spectrum of hrHPV infections. The integration of cytological and molecular data can ultimately improve patient outcomes by ensuring timely and appropriate clinical follow-up.

Materials and Methods

This retrospective study was conducted at the Department of Pathology, Faculty of Medicine, Dicle University between 2015 and 2016. A total of 1,500 female patients who underwent simultaneous high-risk human papillomavirus (hrHPV) testing and liquid-based PAP smear cytology were included in the study.

High-Risk HPV Testing

Detection of high-risk HPV DNA was performed using a fully automated Roche Cobas X4800 system (Roche Diagnostics, Switzerland). Cervical cell samples were transferred into 11 mm tubes containing 2 mL liquid-based cellular medium. The Cobas system performs DNA extraction, amplification, and detection in an integrated and standardized workflow. Sample identifiers and processing protocols were recorded electronically. The obtained data were analyzed for HPV DNA positivity and genotype distribution, focusing on high-risk subtypes, such as HPV 16, HPV 18, and others.

Cytological Evaluation

LBC samples were collected using a cytobrush and transferred into specialized vials containing preservative medium. The automated processing system ensured monolayered smear preparation on the glass slides. All smears were stained using a conventional PAP staining technique.

Cytological assessments were independently reevaluated by an experienced pathologist using a Nikon Eclipse 50i light microscope (Nikon, Japan). The following cytomorphological features were specifically investigated: peripheral halos, cytoplasmic vacuolization, multinucleation, dyskeratosis, parakeratosis, nuclear hyperchromasia, and a clear cytoplasm. These features have been documented and correlated with HPV status.

Ethical Considerations

The study protocol was reviewed and approved by the Clinical Research Ethics Committee of the Faculty of Medicine at Dicle University (approval number: 01, date: November 27, 2015).

The study was conducted in accordance with the principles of the Declaration of Helsinki and applicable national and international ethical guidelines. All patient data were anonymized to maintain confidentiality and used exclusively for scientific purposes.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). Categorical variables are summarized as frequencies and percentages, while continuous variables are presented as mean ± standard deviation. Comparisons between cytomorphological features and HPV status were performed using the Chi-square test or Fisher's exact test, as appropriate, depending on the expected cell counts. A p value of <0.05 was considered statistically significant.

Results

A total of 1,500 patients were included in this study. The mean age of the patients was 42.6 ± 11.8 years, with the youngest patient aged 16 years and the oldest 102 years. High-risk HPV (hrHPV) positivity was detected in 141 cases, corresponding to a prevalence of 9.4%. Among these, HPV 16 was identified in 26 cases (18%), HPV 18 in 14 cases (10%), and other high-risk HPV subtypes in 101 cases (72%).

Cytological evaluations were performed in accordance with the 2014 Bethesda System guidelines. Based on this classification, 32 cases were diagnosed as Atypical Squamous Cells of Undetermined Significance (ASC-US), 42 cases as low-grade squamous intraepithelial lesions (LSIL), 8 as high-grade squamous intraepithelial lesions (HSIL), and 1,418 cases exhibited normal cytology (Figure 1).

Among the 32 ASC US cases, HPV 16 was detected in 2 cases and other hrHPV subtypes in 3 cases, yielding a total of 5 hrHPV-positive cases. In the 42 LSIL cases, one was positive for HPV 16 and three for other hrHPV subtypes, totaling 4 hrHPV-positive cases. Among the eight HSIL cases, HPV 16 was detected in one case and other hrHPV subtypes in three cases, resulting in four hrHPV-positive cases. Notably, no HPV 18 positivity was observed in any of the ASC-US, LSIL, or HSIL groups.

Among the 1,418 cases with normal cytology, HPV 16 was detected in 32 cases, HPV 18 in 14 cases, and other high-risk HPV subtypes in 92 cases, yielding a total of 138 hrHPV-positive cases among cytologically normal patients (Table 1).

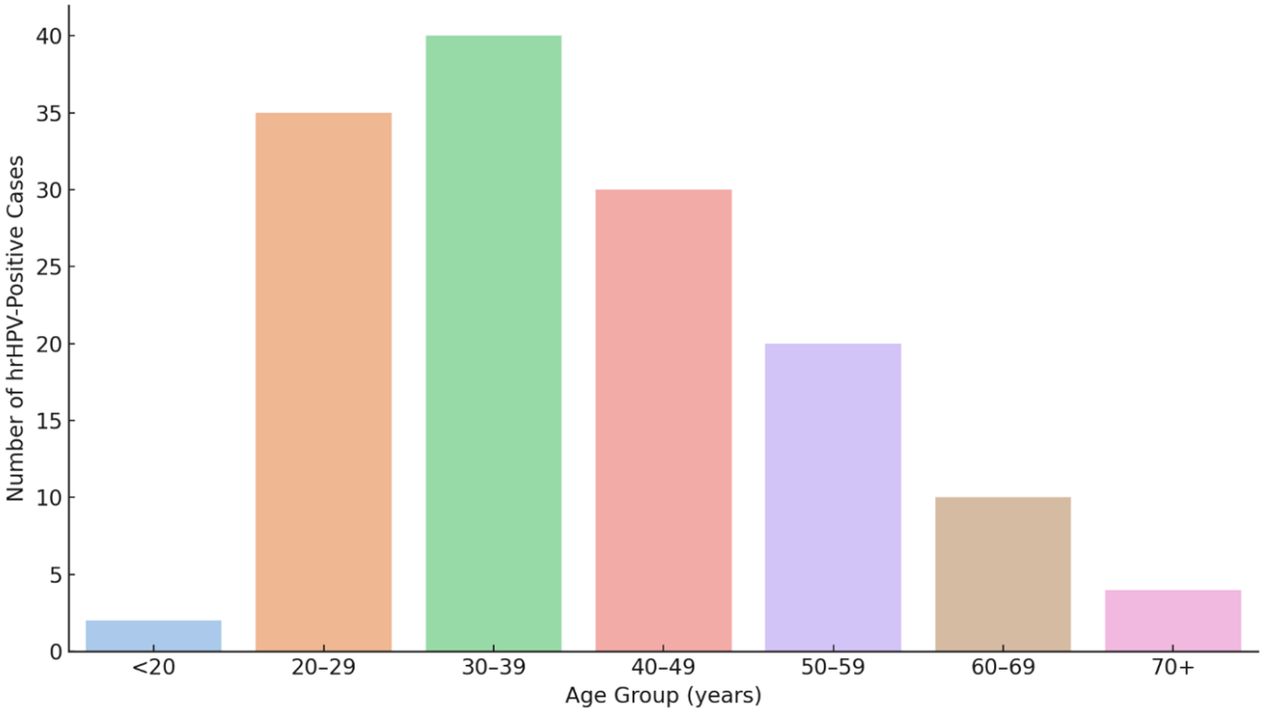

Figure 1. Distribution of hrHPV-positive cases by age group

Table 1. Distribution of HPV subtypes by cytological diagnosis

Cytological diagnosis (n)	HPV 16, n (%)	HPV 18, n (%)	hrHPV other subtypes, n (%)	Total hrHPV+ , n (%)
ASC-US	2 (5.6%)	0 (0%)	3 (3%)	5 (3.4%)
LSIL	1 (2.8%)	0 (0%)	3 (3%)	4 (2.6%)
HSIL	1 (2.8%)	0 (0%)	3 (3%)	4 (2.6%)
Normal cytology	32 (88.8%)	14 (100%)	92 (91%)	138 (91.4%)

HPV: Human papillomavirus, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, hrHPV: High-risk human papillomavirus

Age-stratified analysis demonstrated hrHPV positivity across all age groups, with the highest prevalence observed in individuals aged 20-60 years. Across all age groups, HPV 16 and 18 were detected less frequently than other hrHPV subtypes (Figure 2). The relationship between cytomorphological findings and hrHPV subtypes was further examined based on Bethesda classification. These associations are summarized in Table 2, highlighting the distribution of specific viral subtypes across the ASC-US, LSIL, and HSIL categories.

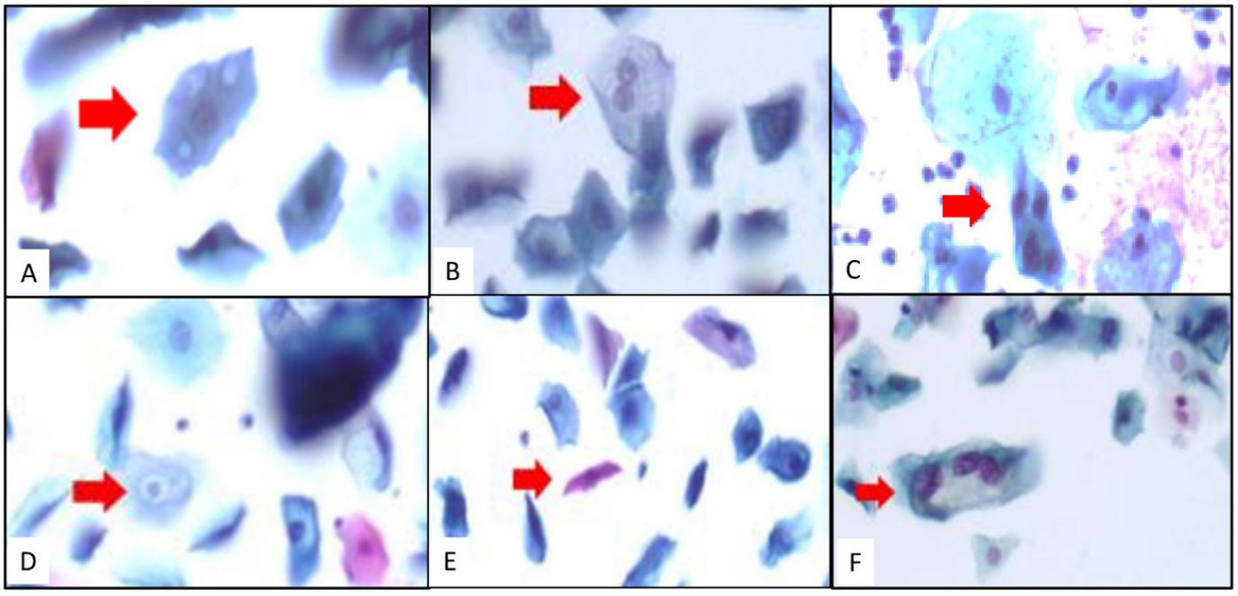

Figure 2. Smear cytomorphological findings. Cytoplasmic cavitation (A), binucleation (B), multinucleation (C), perinuclear halo (D), parakeratosis (E), multinucleation and nuclear hyperchromasia (F) (PAP stain, x200)

Table 2. Cytomorphological findings in HPV status

Cytomorphological feature	**HPV 16, n (%)**	**Other hrHPV, n (%)**	**HPV negative, n (%)**	**p**
ASC-US (n=32)				
Nuclear enlargement	0 (0%)	1 (33.3%)	10 (37.0%)	
Hyperkeratosis	0 (0%)	0 (0%)	5 (18.6%)	0.345
Perinuclear halo	2 (100%)	2 (66.7%)	12 (44.4%)	
LSIL (n=42)				
Koilocytosis	0 (0%)	1 (33.3%)	29 (76.3%)	0.065
Nuclear irregularity	1 (100%)	2 (66.7%)	9 (33.7%)	
HSIL (n=8)				
Increased N/C ratio	1 (100%)	2 (66.7%)	2 (66.7%)	0.123
Marked chromatin increase	0 (0%)	1 (33.3%)	1 (33.3%)	

HPV: Human papillomavirus. ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, hrHPV: High-risk Human Papillomavirus

Discussion

Cervical cancer is a preventable malignancy detected early through organized screening programs, which can significantly reduce disease-related mortality. Supporting this, studies reported an approximately 80% reduction in cervical cancer mortality in countries with well-established screening protocols between 1930 and 2012. One of the primary tools in cervical

cancer screening is the Papanicolaou (PAP) test, first introduced by George Papanicolaou in the 1940s, and it remains the most widely used screening method globally (9). However, the conventional PAP smear has notable limitations, including low sensitivity, suboptimal transfer of cellular material, inflammatory artifacts, cell overlap, and uneven distribution of atypical cells on slides. In response to these challenges, LBC techniques have been developed to improve sampling quality and diagnostic reliability (10).

Sherwani et al. demonstrated that the use of LBC improved the detection rates of LSIL from 10.6% to 18.1% and HSIL from 0.6% to 4.3%. Furthermore, the sensitivity of LBC was reported to be 97.6% compared to 53.7% with conventional smears (11). Consistent with this, our study employed the LBC method to enhance the diagnostic accuracy.

HPV infection is a known etiological factor of cervical carcinogenesis, although not all infections result in clinically significant lesions (12). Although molecular HPV DNA testing is more sensitive than cytology in detecting precancerous lesions (13,14), cytology remains essential for identifying morphological changes that influence clinical decision-making. Our study utilized PCR-based HPV testing and LBC simultaneously to provide a comprehensive diagnostic perspective.

Current U.S. guidelines recommend hrHPV testing for patients aged ≥30 years who present with ASC US cytology (15). In a study by Veijalainen et al., hrHPV positivity was observed in 40.9% of ASC-US cases and 68.6% of LSIL cases (16). In comparison, our study reported hrHPV DNA positivity in 15.6% (5/32) of ASC-US cases and 9.5% (4/42) of LSIL cases. These lower rates may be attributable to the small sample size and regional differences in HPV prevalence.

Wong et al. previously reported that cytomorphological features, such as multinucleation, nuclear hyperchromasia, and atypical changes, were 6 to 12 times more frequent in hrHPV-positive patients than in those with low-risk HPV infections. Specific findings in ASC-US and LSIL cases with hrHPV include koilocytosis (23.3%), perinuclear halos (50%), cytoplasmic vacuolization (16.7%), multinucleation (46.7%), dyskeratosis (18.3%), parakeratosis (35%), nuclear hyperchromasia (43.3%), minimal nuclear hyperchromasia (83.3%), opaque cytoplasm (25%), and atypical metaplastic cells (35%) (17).

In our analysis of hrHPV-positive ASC-US cases, we observed perinuclear halos in 3 cases, cytoplasmic vacuolization in 2 cases, multinucleation in 4 cases, parakeratosis in 4 cases, and minimal nuclear hyperchromasia in 1 case. Notably, dyskeratosis and opaque cytoplasm were not observed in any of the HPV-positive ASC-US cases. Minimal nuclear hyperchromasia was detected only in HPV 16-positive cases, suggesting its potential utility as a cytological marker for HPV 16 in settings in which genotyping is unavailable.

In patients with normal cytology, hrHPV DNA positivity may reflect a latent infection or early stage of the disease. Despite the absence of cytological abnormalities, hrHPV warrants close monitoring due to the risk of progression to high-grade lesions. Our study supports this finding, as a considerable number of hrHPV-positive cases were found in patients with normal cytology, highlighting the importance of integrating molecular HPV testing into screening protocols (18).

This study had some limitations. The relatively small number of ASC-US and LSIL cases likely contributed to the lower observed hrHPV positivity compared with larger studies. Furthermore, as this was a single-center study, the generalizability to a broader population is limited. Future research should incorporate multicenter, large-scale cohorts with longitudinal follow-up to better characterize the cytomorphological and clinical progression of hrHPV infection.

Conclusion

Cervical cytology and molecular HPV testing are essential complementary tools for early detection and prevention of cervical cancer. Our study demonstrated that hrHPV infections may be present even in cytologically normal patients, reinforcing the necessity of HPV DNA testing in screening programs. Additionally, in settings where molecular testing is unavailable, specific cytomorphological featuressuch as multinucleation, perinuclear halos, and parakeratosismay serve as valuable diagnostic indicators of HPV infection. Further multicenter studies with larger sample sizes are needed to better define cytological changes associated with distinct HPV subtypes and refine screening strategies in diverse populations.

Ethical Approval: *This study was approved by the Clinical Research Ethics Committee of the Faculty of Medicine at Dicle University (approval number: 01, date: November 27, 2015).*

Author Contributions:

Concept: B.S., G.T.
Design: B.S., G.T.
Control: B.S., E.A.
Data collection and processing: B.S., E.A., U.F.
Analysis and interpretation: B.S., G.T.
Literature Review: B.S., E.A.
Writer: B.S., U.F.

Conflict of Interest: *The authors have no conflicts of interest to declare.*

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