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ORIGINAL RESEARCH

Histopathological Features and p16/p62 Expression as Indicators of High-Risk HPV Co-Infection in Anogenital Condyloma Acuminata

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Background: Condyloma acuminata (CA) is primarily caused by low-risk human papillomavirus (LR-HPV); however, high-risk HPV (HR-HPV) co-infection is increasingly reported and cannot be reliably distinguished based on clinical appearance alone. Histopathological assessment and selected immunohistochemical markers may help identify lesions with greater biological risk.

Objective: To evaluate the association between histopathological features, p16 and p62 expression, and HR-HPV co-infection in anogenital CA.

Methods: This cross-sectional study included 81 histopathologically confirmed CA cases. Semi-quantitative scoring was applied to lesion morphology, keratinization patterns, koilocytosis, atypical mitoses, and lymphocytic infiltration. p16 and p62 expression were assessed immunohistochemically. HPV genotyping was performed using real-time PCR. Variables significant in bivariate analysis were entered into multivariate logistic regression.

Results: HR-HPV co-infection was detected in 59.3% of cases, with HPV-16 as the most frequent genotype. Hyperkeratosis (aOR = 4.29; $p = 0.039$) and atypical mitotic activity (aOR = 14.29; $p < 0.001$) were independently associated with HR-HPV co-infection. Parakeratosis and koilocytosis showed inverse associations. p16 and p62 were not independent predictors, although p16 block positivity correlated with high p62 expression ($p < 0.001$).

Conclusion: Certain histopathological features, particularly atypical mitoses and hyperkeratosis, are associated with HR-HPV co-infection in anogenital CA. Routine histopathological evaluation may therefore provide practical clues to identify lesions that warrant closer clinical attention, while p16 and p62 offer complementary biological information.

Plain Language Summary: Condyloma acuminata, also known as genital warts, is usually caused by low-risk types of human papillomavirus (HPV). However, some genital warts also contain high-risk HPV types that are associated with a greater chance of developing serious disease. These high-risk infections cannot always be recognized by visual examination alone, especially in healthcare settings without access to specialized HPV tests. In this study, we examined genital wart tissue under a microscope for features that might indicate a high-risk HPV infection. We focused on visible changes in the skin cells, such as abnormal cell division and thickening of the outer skin layer. We also studied two laboratory markers, p16 and p62, which reflect how cells respond to HPV infection. We found that abnormal cell division and certain keratinization changes were more common in warts with high-risk HPV. In contrast, p16 and p62 alone could not reliably identify high-risk HPV. These results show that routine microscopic examination can still provide useful clues about which genital warts may require closer medical attention. This is particularly valuable in settings where advanced HPV testing is unavailable, helping doctors and pathologists determine which patients may benefit from closer monitoring.

Keywords: condyloma acuminata, human papillomavirus, high-risk HPV, atypical mitoses, p16, p62

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Introduction

Human papillomavirus (HPV) infection remains a major global public health concern, affecting both men and women. It is responsible for a broad spectrum of anogenital diseases, ranging from benign warts to invasive malignancies.[1,2] *Condyloma acuminata (*CA) is among the most common HPV-associated anogenital lesions and is predominantly caused by low-risk HPV (LR-HPV), particularly genotypes 6 and 11, which account for more than 90% of cases.[3–5] Although CA is generally regarded as a benign condition, increasing evidence indicates that co-infection with high-risk HPV (HR-HPV) is more frequently reported.[6]

The presence of HR-HPV is clinically relevant because persistent infection with oncogenic genotypes is associated with epithelial dysregulation and malignant transformation, particularly in immunocompromised individuals.[6,7] However, clinical characteristics such as patient demographics, lesion morphology, sexual behavior, and immune status cannot reliably distinguish LR-HPV–only cases from HR-HPV–co-infected cases. This limitation is especially important in resource-limited settings, where molecular HPV genotyping is not routinely available.[8,9]

This diagnostic gap has important clinical implications, as failure to recognize HR-HPV co-infection may delay appropriate surveillance and management in patients at risk for disease progression.[6,10] In addition to clinical and behavioral factors, anatomical location may also influence HPV-related disease behavior due to differences in epithelial structure and local immune environment. However, this aspect has not been extensively evaluated in CA.[1]

Histopathological evaluation remains a practical and widely accessible approach for assessing HPV-related lesions. Conventional histological features of CA include hyperkeratosis, parakeratosis, koilocytosis, and papillary epithelial proliferation.[8] While these findings reflect productive HPV infection, some parameters—particularly atypical mitotic figures and altered keratinization—have been suggested as possible indicators of HR-HPV–related epithelial dysregulation.[11] However, the relationship between specific histopathological features and HR-HPV co-infection in CA has not been clearly established.

Immunohistochemical markers may offer additional biological insight.[12,13] Overexpression of p16 is widely accepted as a surrogate marker of HR-HPV–mediated inactivation of pRb and is commonly applied in the evaluation of HPV-related squamous lesions.[14] In contrast, p62/SQSTM1, an autophagy-related adaptor protein, has been implicated in HPV infection biology, with emerging evidence suggesting differential expression patterns between productive LR-HPV infection and HR-HPV–associated lesions.[15]

Therefore, this study aimed to evaluate the association between histopathological features, p16 and p62 immunohistochemical expression, and HPV genotype groups in anogenital CA. By integrating routine histopathological assessment with immunohistochemical markers and molecular HPV genotyping, this study aims to clarify the clinicopathological spectrum of CA and identify practical clues for recognizing HR-HPV co-infection in routine diagnostic settings.

Methods

Study Design and Setting

This retrospective cross-sectional study was conducted at the Department of Anatomical Pathology, Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, using archived formalin-fixed, paraffin-embedded (FFPE) tissue specimens diagnosed as anogenital CA between January and December 2024. Clinical and histopathological data were retrieved from institutional records.

Sample Selection

A total of 153 histopathologically confirmed CA cases were identified. Of these, 81 cases with adequate FFPE tissue, complete clinical data, and sufficient DNA quality were included. Selection was based on tissue adequacy and data completeness, and the final sample appeared to reflect the general age and sex distribution of the source population. Cases with exhausted tissue blocks, inadequate DNA quality, or incomplete records were excluded. All eligible cases within the study period were included; therefore, a formal sample size calculation was not performed.

Clinical Data Collection

Clinical and sociodemographic data were retrospectively extracted from medical records using a standardized form. Variables of interest included sociodemographic characteristics (age, sex, marital status, education, and occupation), sexual behavior (age at sexual debut, sexual orientation, and number and type of partners), and lesion-related features (site, morphology, number, size, duration, and recurrence history). Immunological status, including HIV infection and other immunosuppressive conditions, was also recorded. All data were anonymized before analysis. Lesion location data were extracted from medical records and categorized into three groups: cutaneous, mucosal, and combined lesions. Cutaneous lesions referred to keratinized surfaces such as the penile shaft, vulva, and perianal skin, whereas mucosal lesions included sites such as the cervix, vagina, urethra, and anal canal. Combined lesions indicated involvement of both cutaneous and mucosal sites.

Histopathological Evaluation

All HE-stained slides were independently reviewed by two board-certified anatomical pathologists blinded to HPV results. Discrepancies were resolved by consensus. Evaluated features included lesion morphology, hyperkeratosis, parakeratosis, koilocytosis, atypical mitoses, and lymphocytic infiltration. Features were semi-quantitatively graded (0–3) based on intensity and distribution, using predefined criteria (Table 1). Uniform scoring was applied to improve reproducibility.

HPV Genotyping

DNA was extracted from FFPE tissue sections using the Zybio Viral Nucleic Acid Kit (Magnetic Beads Method) on an automated extraction system. HPV genotyping was performed using the Allplex™ HPV28 real-time PCR assay (Seegene Inc., Seoul, South Korea), which simultaneously detects 28 HPV genotypes—19 HR-HPV types (HPV 16, 18, 26, 31, 33,

Table 1 Semi-Quantitative Scoring Criteria for Histopathological Features in Condyloma Acuminata

Feature	Category/Score	Definition
Lesion morphology (categorical)	Flat	Flat surface, no papillary projection
	Exophytic	Papillary epithelial proliferation with fibrovascular cores
Hyperkeratosis	0	No significant keratin thickening
	1	Thin keratin layer
	2	Distinct thickening of the stratum corneum
	3	Very thick, compact keratin layer covering most of the epithelial surface
Parakeratosis	0	None
	1	Focal retained nuclei in keratin layer
	2	Frequent retained nuclei in stratum corneum
	3	Widespread nuclear retention with disorganized keratinization
Koilocytosis	0	Absent
	1	Focal koilocytes (< 25% of epithelial cells)
	2	Multifocal koilocytes (25–60% of epithelial cells)
	3	Diffuse koilocytes (> 60% of epithelial cells)
Atypical mitoses	0	None
	1	1–2 atypical mitotic figures per 10 HPF, limited to basal layer
	2	3–5 atypical mitotic figures per 10 HPF, extending to suprabasal layers
	3	>5 atypical mitotic figures per 10 HPF, involving multiple epithelial layers
Lymphocytic infiltration	0	Absent
	1	Mild perivascular lymphocytic infiltration
	2	Moderate perivascular ± intraepithelial infiltration
	3	Dense, band-like lymphocytic infiltration with intraepithelial clusters

Notes: Lesion morphology was analyzed as a binary categorical variable (flat vs exophytic), whereas all other histopathological features were assessed using a semi-quantitative ordinal scale (0–3). The atypical mitosis score was determined based on both the number and epithelial distribution of abnormal mitotic figures per 10 consecutive HPF, thereby providing an estimate of epithelial proliferative activity.

35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82) and nine LR-HPV types (HPV 6, 11, 40, 42, 43, 44, 54, 61, and 70). Thermal cycling was performed using a CFX96™ Real-Time PCR Detection System (Bio-Rad, USA) according to the manufacturer's protocol. Amplification results were analyzed using Seegene Viewer software. Samples were categorized into two genotype groups: LR-HPV-only and HR-HPV co-infection, based on the detected genotypes. Positive and negative controls were included in each run.

Immunohistochemical Scoring

Immunohistochemical staining for p16 and p62/SQSTM1 was performed on representative FFPE tissue sections using standardized protocols. p16 was scored on a four-tier scale (0–3) based on the extent and pattern of nuclear and cytoplasmic staining. p16 status was categorized as block-positive (strong, continuous staining involving ≥70% of epithelial thickness) or non–block-positive (negative, focal, or discontinuous staining). p62 was graded from 0 to 3 according to the intensity and distribution of cytoplasmic granular staining and categorized as high (moderate–strong) or low (negative–weak) expression. Nuclear p62 staining was not included, as cytoplasmic accumulation is considered more biologically relevant in HPV-related lesions.

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, and ordinal variables as medians with ranges. Group comparisons were performed using Chi-square, Fisher's exact, or Mann–Whitney *U*-tests as appropriate. Variables with $p < 0.05$ in bivariate analysis were entered into multivariate logistic regression. Odds ratios (ORs) with 95% CIs were reported. A two-sided $p < 0.05$ was considered significant. Immunohistochemical markers were analyzed separately to avoid collinearity.

Results

All 81 cases harbored at least one low-risk HPV genotype. HR-HPV co-infection was detected in 48 cases (59.3%), while 33 cases (40.7%) showed LR-HPV–only infection (Table 2). As multiple genotypes may be detected in a single specimen, the total number of detected genotypes exceeded the number of patients. HPV-11 was the most common LR genotype (96.3%). HPV-16 was the most frequent HR genotype (30.9%), followed by HPV-18, 33, 51, 52, 66, 73, and 82.

The associations between clinical characteristics and HPV genotype groups are presented in Table 3. Most socio-demographic and lesion-related variables did not differ significantly between groups. However, HIV infection was more common among patients with HR-HPV co-infection and showed a significant association with HR-HPV status. No significant differences were observed for age, sex, marital status, lesion number, lesion location, lesion size, or sexual orientation.

Bivariate analysis demonstrated significant associations between several histopathological features and HPV genotype groups (Table 4). As shown in Supplementary Table 1, the association between histopathological features and the expression of p16 and p62 was evaluated in detail. Hyperkeratosis and atypical mitoses were more frequently observed in lesions with HR-HPV co-infection, whereas parakeratosis and koilocytosis were more common in LR-HPV–only lesions. Lesion morphology and lymphocytic infiltration did not differ significantly between groups. Representative photomicrographs illustrating the grading and distribution of hyperkeratosis, parakeratosis, koilocytosis, and atypical mitoses are presented in Figures 1–4.

Neither p16 block positivity nor high p62 expression differed significantly between HPV genotype groups (Table 5). However, a significant association was observed between p16 block positivity and high p62 expression (Table 6). In multivariate logistic regression (Table 7), several histopathological features remained independently associated with HR-HPV co-infection. Hyperkeratosis and atypical mitoses were positively associated, whereas parakeratosis and koilocytosis were inversely associated.

Table 2 Distribution of Detected HPV Genotypes (Multiple Infections per Case Allowed)

Group	Genotype	n (%)
LR-HPV only	HPV 6	57 (70.4)
	HPV 11	78 (96.3)
	HPV 40	11 (13.6)
	HPV 42	6 (7.4)
	HPV 43	4 (4.9)
	HPV 44	1 (1.2)
	HPV 54	3 (3.7)
	HPV 61	0 (0.0)
	HPV 70	0 (0.0)
HR-HPV co-infection	HPV 16	25 (30.9)
	HPV 18	18 (22.3)
	HPV 26	4 (2.5)
	HPV 31	1 (1.2)
	HPV 33	4 (4.9)
	HPV 35	1 (1.2)
	HPV 39	2 (2.5)
	HPV 45	0 (0.0)
	HPV 51	4 (4.9)
	HPV 52	4 (4.9)
	HPV 53	2 (2.5)
	HPV 56	3 (3.7)
	HPV 58	2 (2.5)
	HPV 59	2 (2.5)
	HPV 66	5 (6.2)
	HPV 68	3 (3.7)
	HPV 69	0 (0.0)
	HPV 73	4 (4.9)
	HPV 82	5 (6.2)

Notes: All cases harboured at least one low-risk HPV genotype. Percentages represent the proportion of total cases (N=81) in which each genotype was detected. Because multiple HPV genotypes may be present in a single case, percentages are not mutually exclusive.

Discussion

Overview of Main Findings

This study demonstrates that specific histopathological features are independently associated with HR-HPV co-infection in anogenital CA. Among the evaluated variables, atypical mitotic activity emerged as the most robust predictor, followed by hyperkeratosis, supporting their role as key morphological indicators of HR-HPV–related epithelial dysregulation.[16] Conversely, parakeratosis and koilocytosis showed inverse associations, suggesting that their predominance in lesions without HR-HPV involvement reflects their association rather than a protective effect.[17]

The present findings provide clinically meaningful evidence of the frequent occurrence of HR-HPV co-infection in anogenital CA. Similar observations have been reported in previous studies, reinforcing the concept that condylomatous lesions may harbor oncogenic HPV genotypes despite their benign clinical appearance.[18] Variability in reported HR-HPV prevalence across studies may partly be explained by methodological differences in specimen sampling and molecular detection sensitivity. Previous studies have demonstrated that optimized sampling techniques significantly influence HPV detection and genotype diversity in genital wart lesions.[19,20]

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Table 3 Association Between Clinical Characteristics and HPV Genotype Groups

Variable	HPV Genotype		OR (CI 95%)	*p-value*
	HR-HPV Co-Infection n=48	LR-HPV Only n=33		
Age group (years)				0.075
≤25	25 (52.1%)	14 (42.4%)	Ref	
26–35	18 (37.5%)	9 (27.3%)	0.893 (0.318–2.510)	
≥36	5 (10.5%)	10 (30.3%)	3.571 (1.016–12.555)	
Sex				0.379
Male	32 (66.7%)	25 (75.8%)	0.640 (0.236–1.734)	
Female	16 (33.3%)	8 (24.2%)	Ref	
Marital Status				0.567
Married	19 (39.6%)	11 (33.3%)	1.310 (0.519–3.310)	
Unmarried	29 (60.4%)	22 (66.7%)		
Number of lesions				1.000
Solitary	4 (8.3%)	3 (9.1%)	0.909 (0.190–4.358)	
Multiple	44 (91.7%)	30 (90.9%)	Ref	
Lesion locations				0.979
Cutaneous	17 (35.4%)	11 (33.3%)	Ref	
Mucosal	11 (22.9%)	8 (24.2%)	0.133 (0.015–1.198)	
Combined	20 (41.7%)	14 (42.4%)	1.280 (0.291–5.628)	
Lesion size				0.906
<1 cm	27 (56.3%)	20 (60.6%)	0.836 (0.387–2.319)	
>1 cm	21 (43.8%)	13 (39.4%)	Ref	
Sexual orientation				1.000
Heterosexual	26 (54.2%)	18 (54.5%)	Ref	
Homosexual	17 (35.4%)	11 (33.3%)	0.935 (0.355–2.460)	
Bisexual	5 (10.4%)	4 (12.1%)	1.156 (0.272–4.905)	
Immunological status				
HIV positive	24 (50.0%)	9 (27.3%)	2.667 (1.029–6.912)	0.041*
Chronic immunosuppressive disease	3 (6.3%)	1 (3.0%)	2.133 (0.212–21.451)	0.642
No immunosuppression	4 (8.3%)	0 (0.0%)	Ref	0.142

Notes: Odds ratios represent the likelihood of HR-HPV co-infection compared with LR-HPV–only infection. Categorical variables were compared using Chi-square or Fisher's exact test. A p-value <0.05 was considered statistically significant. *p <0.05.

Taken together, these findings provide an integrated clinicopathological perspective, highlighting the value of routine histopathological evaluation in identifying lesions with a higher likelihood of HR-HPV co-infection. In contrast, p16 and p62 immunohistochemical expression did not independently discriminate HR-HPV co-infection, although their co-expression reflected coordinated cellular response pathways.[11] Collectively, these findings suggest that histopathological assessment—particularly atypical mitotic activity—serves as the most reliable indicator of HR-HPV co-infection, while immunohistochemistry provides complementary biological context rather than functioning as the primary diagnostic discriminator.

Histopathological Indicators of HR-HPV Co-Infection

The present study shows that certain histopathological features provide meaningful insight into the presence of HR-HPV co-infection in anogenital CA. Among the evaluated parameters, atypical mitotic activity demonstrated the strongest association in both bivariate and multivariate analyses. Lesions with moderate-to-severe atypical mitoses were consistently associated with HR-HPV co-infection, highlighting the practical value of recognizing mitotic abnormalities in routine histopathological assessment.

While p16 and p62 expression were more frequent in lesions with HR-HPV co-infection, as shown in Supplementary Table 1, these markers did not reliably distinguish HPV genotype categories. This reflects the complex biological

Table 4 Association Between Histopathological Features and HPV Genotype Groups

Variable	HPV Genotype		OR (95% CI)	p-value
	HR-HPV Co-Infection n=48	LR-HPV Only n=33		
Histopathological Feature				
Lesion morphology			1.100 (0.229–5.273)	1.000
Exophytic	44 (91.7%)	30 (90.9%)		
Flat	4 (8.3%)	3 (9.1%)		
Hyperkeratosis			2.181 (1.124–4.231)	0.014*
Mild	12 (25.0%)	3 (9.1%)		
Moderate	11 (22.9%)	4 (12.1%)		
Severe	25 (52.1%)	26 (78.8%)		
Parakeratosis			0.242 (0.124–0.471)	<0.001**
Mild	10 (20.8%)	19 (57.6%)		
Moderate	11 (22.9%)	12 (36.4%)		
Severe	27 (56.3%)	2 (6.1%)		
Koilocytosis			4.028 (2.016–8.049)	<0.001**
Mild	24 (50.0%)	2 (6.1%)		
Moderate	7 (14.6%)	4 (12.1%)		
Severe	17 (35.4%)	27 (81.8%)		
Atypical Mitosis			0.050 (0.015–0.165)	<0.001**
Absent	2 (4.2%)	24 (72.7%)		
Mild	13 (27.1%)	8 (24.2%)		
Moderate-Severe	33 (68.8%)	1 (3.0%)		
Lymphocytic infiltration			1.483 (0.889–2.473)	0.141
Mild	25 (52.1%)	13 (39.4%)		
Moderate	11 (22.9%)	6 (18.2%)		
Severe	12 (25.0%)	14 (42.4%)		

Notes: Bivariate analysis was performed using the Chi-square or Fisher's exact test for categorical variables and the Mann–Whitney *U*-test for ordinal variables. Odds ratios (ORs) with 95% confidence intervals (CIs) are presented. A p-value <0.05 was considered statistically significant. *p <0.05; **p <0.01.

interplay between HPV genotype and cellular response, with histopathological features such as atypical mitoses and hyperkeratosis providing more reliable indicators of HR-HPV co-infection. Previous studies have shown that p16 and p62 expression, while informative, often do not predict HR-HPV co-infection, underscoring the importance of histopathological features in HPV-related lesions.[12,15]

From a biological perspective, atypical mitotic figures reflect disruption of cell-cycle checkpoints and genomic instability, which are hallmark features of HR-HPV–mediated oncogenic processes.[7,10] The E6 and E7 oncoproteins of HR-HPV inactivate p53 and pRb pathways, respectively, leading to uncontrolled cellular proliferation and aberrant mitotic activity.[21] Rather than indicating overt malignant transformation, these findings support the concept that atypical mitoses represent early morphologic manifestations of HR-HPV–related epithelial dysregulation, even in lesions clinically and histologically classified as benign warts.[7,21]

Hyperkeratosis was also significantly associated with HR-HPV co-infection, although less strongly than atypical mitoses. Increased keratin thickness may reflect altered epithelial differentiation in the setting of persistent viral–host interaction.[8,22] In contrast, parakeratosis and koilocytosis were more frequently observed in LR-HPV–only lesions, consistent with productive HPV infection characterized by preserved viral replication and epithelial maturation.[23,24]

Koilocytosis, a classic HPV infection cytopathic effect, showed an inverse association with HR-HPV co-infection in this cohort. This inverse association should not be interpreted as a protective phenomenon. It reflects the biological distinction between productive and transforming HPV infections.[24] LR-HPV infection typically results in prominent koilocytic changes due to active viral replication in the superficial epithelial layers, whereas HR-HPV–associated

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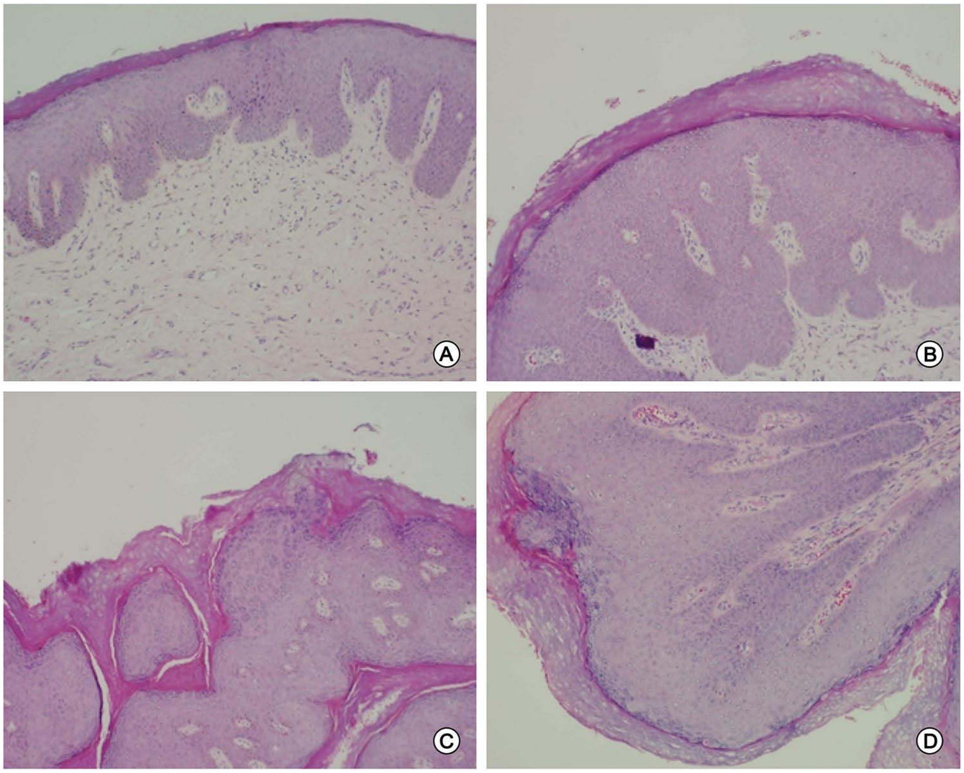

Figure 1 Grading of hyperkeratosis in condyloma acuminata. (**A**) Mild hyperkeratosis showing a thin keratin layer with preserved epithelial maturation. (**B**) Moderate hyperkeratosis is characterized by keratin thickening and partial surface compaction. (**C**) Severe hyperkeratosis with dense, compact orthokeratosis and focal parakeratosis. (**D**) Severe hyperkeratosis demonstrating pronounced keratin thickening and surface folding. H&E stain, original magnification ×100.

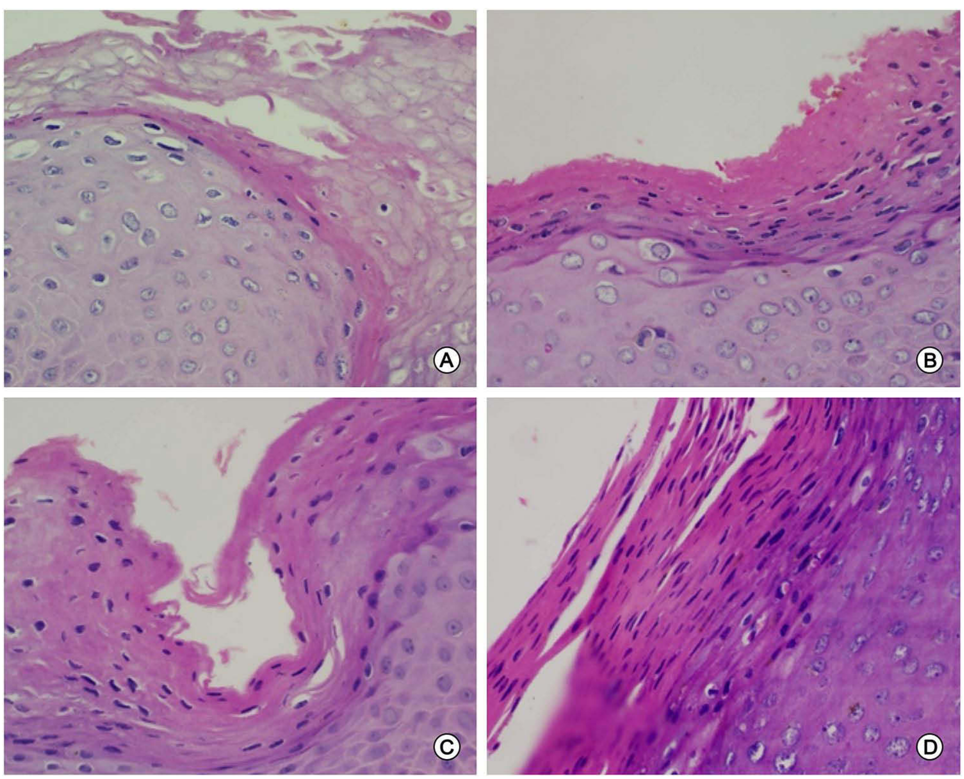

Figure 2 Grading of parakeratosis in condyloma acuminata. (**A**) Mild parakeratosis showing focal retained nuclei in the superficial keratin layer. (**B**) Moderate parakeratosis with frequent nuclear retention and mild granular layer disorganization. (**C**) Severe parakeratosis characterized by dense keratin with numerous retained pyknotic nuclei. (**D**) Severe parakeratosis demonstrating extensive nuclear retention and loss of the granular layer. H&E stain, original magnification ×400.

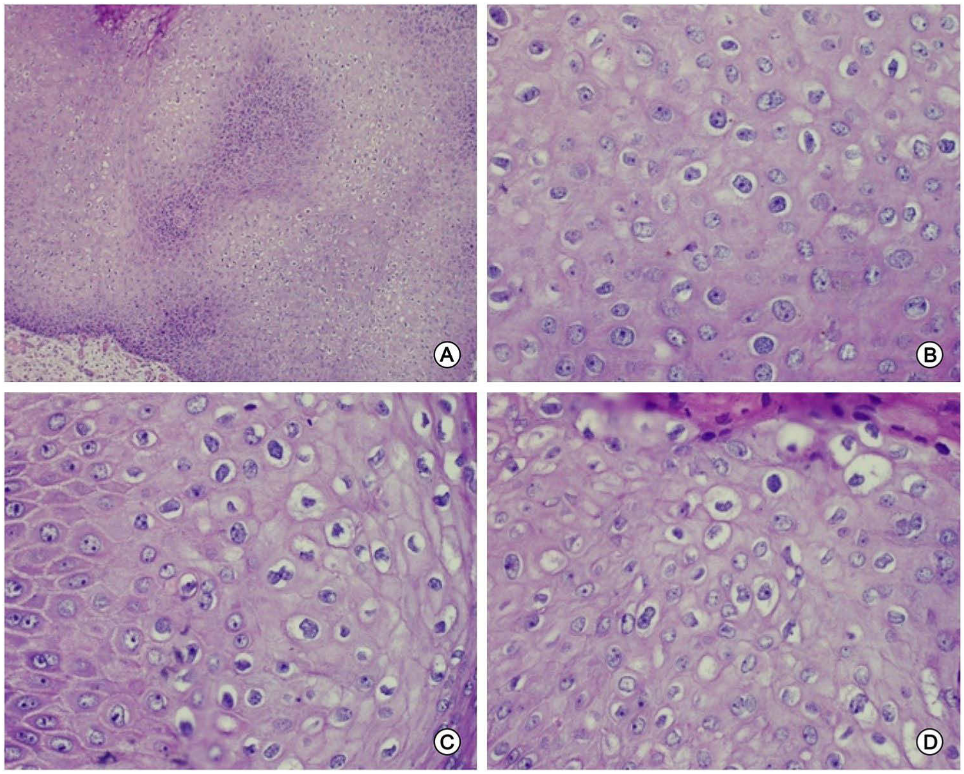

Figure 3 Representative morphologic variants of koilocytosis in condyloma acuminata. (**A**) Low-power view (H&E stain ×100) showing acanthotic squamous epithelium with koilocytic change in the upper epithelial layers. (**B–D**) High-power views (H&E stain ×400) demonstrating koilocytosis. (**B**) Koilocytes with perinuclear halos and mild nuclear enlargement. (**C**) Koilocytes with irregular nuclear contours and sharply defined perinuclear clearing. (**D**) Diffuse koilocytosis involving the upper epithelial layers with nuclear enlargement.

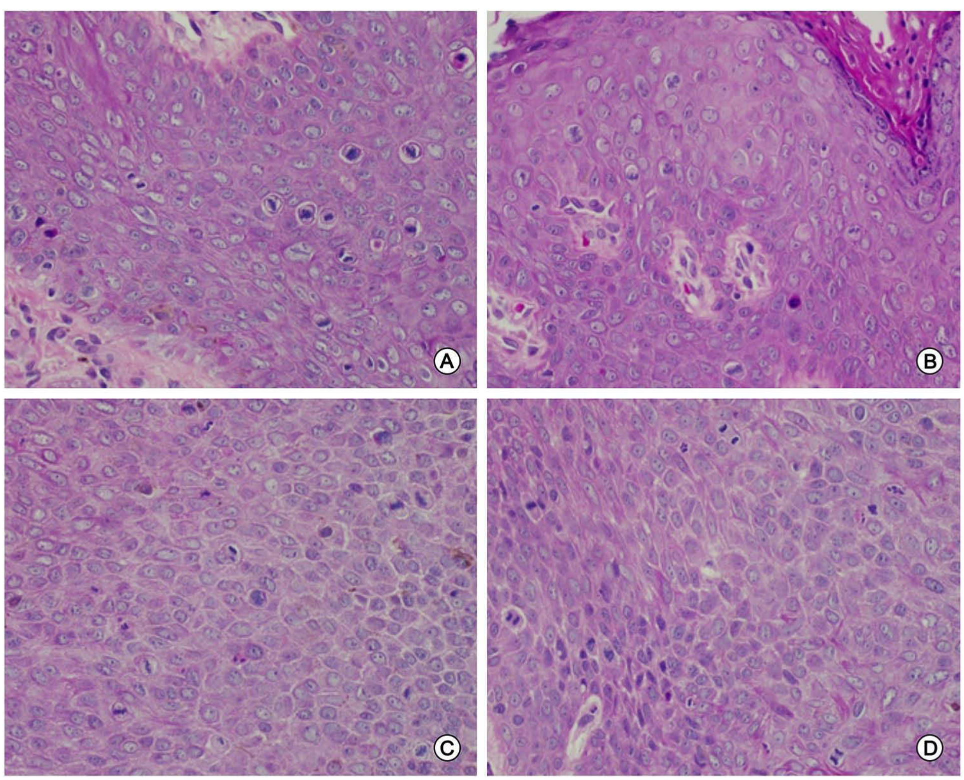

Figure 4 Atypical mitoses figure in condyloma acuminata. (A–D) Multiple atypical mitotic figures are observed within the epithelial layers. The nuclei show pleomorphism, hyperchromasia, and irregular chromatin condensation, with occasional multipolar and anaphase-bridging configurations. H&E stain, original magnification ×400.

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Table 5 Association Between Immunohistochemical Markers and HR-HPV Co-Infection

Variable	HPV Genotype		OR (95% CI)	p-value
	HR-HPV Co-Infection n (%)	LR-HPV Only n (%)		
P16				
Block-positive	32 (66.7)	20 (60.6)	1.300 (0.518–3.264)	0.576
Non-block-positive	16 (33.3)	13 (39.4)		
P62				
High	31 (64.6)	19 (57.6)	1.344 (0.541–3.335)	0.524
Low	17 (35.4)	14 (42.4)		

Notes: Categorical variables were compared using the Chi-square or Fisher's exact test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A p-value <0.05 was considered statistically significant.

Table 6 Association Between p16 and p62 Expression in Condyloma Acuminata

Variable	P62		OR (95% CI)	p-value
	High n (%)	Low n (%)		
P16			6.333 (2.327–17.237)	<0.001**
Block-positive	40 (80.0)	12 (38.7)		
Non-block	10 (20.0)	19 (61.3)		

Notes: Associations were analyzed using the Chi-square test. Odds ratios (ORs) with 95% confidence intervals (CIs) are presented. A p-value <0.05 was considered statistically significant. **p <0.01.

Table 7 Multivariate Logistic Regression Analysis of Histopathological Predictors of HR-HPV Co-Infection

Variable	B	SE	Wald	*p*-value	aOR (Exp(B))	95% CI
Hyperkeratosis	1.457	0.707	4.250	0.039*	4.29	1.07–17.15
Parakeratosis	−1.421	0.639	4.942	0.026*	0.24	0.07–0.85
Koilocytosis	−1.406	0.663	4.498	0.034*	0.25	0.07–0.90
Atypical Mitosis	2.637	0.775	11.565	<0.001**	14.29	3.06–62.50
Constanta	−2.265	2.358	0.923	0.337	0.10	–

Notes: Variables with p <0.05 in bivariate analysis were included in the multivariate logistic regression model. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) are presented. A p-value <0.05 was considered statistically significant. *p <0.05; **p <0.01.

transforming infections tend to suppress late viral gene expression, leading to reduced koilocytosis despite increased proliferative activity.[11,24]

Lesion morphology and lymphocytic infiltration did not differ significantly between HR-HPV and LR-HPV groups. These findings suggest that architectural growth patterns and local inflammatory response alone are insufficient to discriminate HPV genotype categories and may be influenced by host immune status, lesion chronicity, or secondary inflammatory processes rather than by viral oncogenic potential.[25,26]

Role of p16 and p62 Immunohistochemistry in HPV-Associated Condyloma Acuminata

In this study, p16 and p62 immunohistochemical expression were not significantly associated with HR-HPV co-infection. Although both markers were numerically more frequent in HR-HPV–positive lesions, they did not reliably distinguish

HPV genotype categories. This indicates that immunohistochemical biomarkers alone are insufficient to predict HR-HPV co-infection in CA lesions.

p16 is widely recognized as a surrogate marker of HR-HPV–associated transforming infection because it is overexpressed following inactivation of the pRb pathway by E7 oncoprotein.[27] However, its diagnostic performance is highly context-dependent. In CA, which predominantly reflects productive HPV infection, p16 expression may exhibit heterogeneous, focal, or discontinuous staining patterns that do not meet the criteria for classic block positivity.[28] Previous studies of CA have shown that p16 expression typically presents as sporadic or focal staining, particularly in LR-HPV–associated lesions, whereas diffuse staining is less common and not uniformly present even in HR-HPV–positive cases.[12,28] This biological context likely explains its limited discriminatory value for HR-HPV status in this cohort.

Similarly, p62/SQSTM1 expression was not associated with HR-HPV co-infection. p62 is a multifunctional adaptor protein involved in selective autophagy, and its cytoplasmic accumulation has been linked to productive HPV infection, particularly in LR-HPV–associated lesions.[15] Experimental studies suggest that LR-HPV may induce autophagy during viral entry and replication, leading to p62 accumulation, whereas HR-HPV–associated transforming infections may alter or suppress autophagic flux. The absence of a direct association between p62 expression and HR-HPV co-infection in this study likely reflects the coexistence of productive and transforming viral processes within condylomatous lesions.[13,29]

Previous molecular studies have demonstrated dysregulation of autophagy pathways in CA, including increased expression of p62/SQSTM1 and LC3, mediated by altered microRNA regulation and autophagy-related gene expression. These findings support the biological relevance of p62 as a marker of epithelial stress and autophagy activation in HPV-infected tissue, rather than as a genotype-specific surrogate marker.[29] Our results extend this concept by demonstrating that p62 expression reflects biological dysregulation but is not associated with HR-HPV co-infection, highlighting the distinction between biological relevance and diagnostic discrimination.

The interaction between HPV genotype and immunohistochemical marker expression further supports this biological interpretation. In LR-HPV–dominant infections, viral replication occurs predominantly in the superficial epithelial layers, promoting productive infection with preserved epithelial differentiation and an active viral life cycle, which may lead to variable p62 accumulation without consistent activation of cell-cycle dysregulation pathways.[29,30] In contrast, HR-HPV–associated infections tend to disrupt host-cell regulatory mechanisms through E6/E7-mediated interference with the p53 and pRb pathways, promoting a transforming infection biology characterized by cell-cycle deregulation and cellular stress.[21,27] However, the manifestation of these processes is temporally and biologically heterogeneous, meaning that p16 and p62 expression may not increase synchronously or uniformly across lesions, even within HR-HPV–positive cases. This biological heterogeneity explains why HPV genotype does not translate into a linear or deterministic pattern of p16 and p62 expression, and why these markers reflect cellular response dynamics rather than viral genotype identity.[28,30,31]

Notably, p16 and p62 expression were strongly associated (Table 6). Lesions with p16 block-positive staining were approximately more than six times more likely to exhibit high p62 expression compared with non–block-positive lesions. This co-expression pattern suggests that p16 and p62 capture overlapping yet non-identical biological pathways related to epithelial proliferation and cellular stress responses, rather than functioning as direct surrogates for HPV genotype.[28,30]

Importantly, the presence of HR-HPV at the genotypic level does not necessarily translate into corresponding phenotypic manifestation at the tissue level. HR-HPV infection may be detectable at the molecular level before inducing overt biological and morphological changes in epithelial cells.[27] Consequently, lesions may be HR-HPV–positive by genotyping yet lack phenotypic markers of transformation, including p16 overexpression, p62 accumulation, or overt histopathological abnormalities. This genotype–phenotype dissociation may explain why HR-HPV co-infection does not consistently correlate with immunohistochemical marker expression, supporting the view that molecular infection often precedes phenotypic cellular transformation.[32]

Taken together, these findings indicate that p16 and p62 expression do not simply reflect the presence of specific HPV genotypes, but rather the biological response of epithelial cells to viral infection. Their expression levels are influenced by the phase of infection, the degree of cellular dysregulation, host immune control, and the balance between productive and transforming viral processes.[26,29,33] Consequently, p16 and p62 may remain low or heterogeneous in many lesions despite HR-HPV, particularly in early-stage or biologically controlled infections, and may increase only when cellular stress, cell-cycle disruption, and autophagy dysregulation become substantial.[34]

Clinical Implications and Practical Application

From a clinical perspective, the presence of moderate-to-severe atypical mitoses in CA may serve as a practical histopathological red flag prompting closer follow-up or consideration of HPV genotyping, especially in high-risk populations. The findings of this study have important implications for the routine diagnostic evaluation of anogenital CA. Although CA is traditionally regarded as a benign lesion driven by LR-HPV infection, the high prevalence of HR-HPV co-infection in this cohort underscores the need for more refined risk stratification beyond clinical impression alone.[18] HPV-related disease behavior has been reported to vary across different anatomical contexts, including cutaneous, mucosal, and combined lesion types, which differ in epithelial structure and local immune environment.[34]

In this study, lesion location was analyzed using this classification; however, no statistically significant association was observed with HR-HPV co-infection. These findings suggest that although anatomical variation is biologically relevant, lesion location alone may not be sufficient to discriminate HR-HPV co-infection in condylomatous lesions and may instead act as a modifying factor influenced by host, viral, and environmental interactions.[26] In many clinical settings, particularly in resource-limited environments, access to HPV genotyping remains limited, making histopathological assessment the most readily available and practical diagnostic tool.[35]

This study demonstrates that specific histopathological features, particularly atypical mitotic activity and keratinization patterns, provide clinically relevant information regarding HR-HPV co-infection. The strong association between atypical mitoses and HR-HPV co-infection suggests that careful evaluation of mitotic abnormalities should be emphasized in routine histopathological reporting of CA. Incorporating standardized semi-quantitative assessment of atypical mitotic activity and keratinization patterns into diagnostic workflows may help pathologists identify lesions warranting closer clinical surveillance or consideration for further molecular testing.[36]

In contrast, immunohistochemical markers p16 and p62, while biologically informative, did not independently predict HR-HPV co-infection. These findings indicate that p16 block positivity and high p62 expression should not be interpreted in isolation as surrogates for HPV genotype status in condylomatous lesions. Instead, their value lies in providing complementary insight into epithelial dysregulation and viral–host interactions.[29,37] The strong association between p16 and p62 expression observed in this study suggests that their combined immunophenotypic patterns may reflect transitional biological states between productive and transforming HPV infection, rather than definitive markers of HR-HPV presence.[17]

From a practical standpoint, a tiered diagnostic strategy may be justified. Histopathological features should remain the primary screening indicators for identifying CA lesions with a higher likelihood of HR-HPV co-infection. Immunohistochemistry may be selectively applied as an adjunct in morphologically ambiguous cases, while HPV genotyping should be reserved for lesions exhibiting high-risk histopathological features or occurring in clinically vulnerable populations, such as immunocompromised patients.[38] This integrated approach may help balance diagnostic accuracy with feasibility and cost considerations in routine pathology practice.

Limitations of the Study

This study has several limitations. Its retrospective design may lead to incomplete documentation and potential selection bias. HPV genotyping was performed on FFPE tissue, which may reduce detection sensitivity and lead to an underestimate of HPV genotype diversity, particularly in low-viral-load samples. Lesion location data were categorized for analysis; however, variability in clinical documentation may have affected classification precision and the ability to detect subtle associations. In addition, viral load, transcriptional activity, and integration status were not evaluated, and the absence of longitudinal follow-up limited assessment of clinical outcomes and lesion progression.

Conclusion

This study demonstrates that specific histopathological features, particularly atypical mitotic activity and keratinization patterns, are associated with HR-HPV co-infection in anogenital CA. In contrast, p16 and p62 immunohistochemistry did not independently predict HR-HPV status, although their expression patterns provide additional insight into epithelial dysregulation and viral–host interactions. Clinical characteristics were not significantly associated with HR-HPV co-infection, indicating limited discriminatory value of clinical features in identifying HR-HPV involvement in condylomatous lesions. Overall, these findings support the central role of routine histopathological evaluation in identifying CA lesions with a higher likelihood of HR-HPV involvement. Selective use of immunohistochemistry and molecular testing may complement morphologic assessment in clinically relevant cases.

Data Sharing Statement

The datasets generated and analyzed during this study are not publicly available due to institutional data protection policies, but are available from the corresponding author upon reasonable request. All molecular and histopathological records are securely stored in the Department of Anatomical Pathology at Universitas Padjadjaran, Bandung, Indonesia.

Ethics Approval

This study was reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia (No. 485/UN6.KEP/EC/2025; June 2, 2025). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Due to the retrospective nature of the study and the use of anonymized archival data, the ethics committee waived the requirement for informed consent.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in relation to this work.

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