



ORIGINAL ARTICLE

Retinoblastoma tumor suppressor protein is not a risk factor for the location of condyloma acuminatum among adults

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Cite this article as: Primisawitri PP, Muliato N, Ellistasari EY, Poncorini E, Kariosentono H, Widhiati S, et al. Retinoblastoma tumor suppressor protein is not a risk factor for the location of condyloma acuminatum among adults. Univ Med 2025;44:328-334

Date of first submission, 5, August 8, 2025

Date of acceptance, December 1, 2025

Date of published, December 3, 2025

ABSTRACT

BACKGROUND

Condylomata acuminata (CA) are sexually transmitted infections primarily caused by low-risk HPV types 6 and 11. Retinoblastoma protein (pRb) interacts with HPV oncoproteins, leading to destabilization, genomic instability, and carcinogenesis. This study aimed to determine whether variations in pRb expression and sexual orientation are risk factors of the anatomical location of CA lesions and to compare differences in pRb expression by sexual orientation.

METHODS

A cross-sectional study was conducted among 33 patients aged >18 years with clinically and histopathologically confirmed CA. Demographic data, pRb expression assessed via excision biopsy and immunohistochemistry, and relevant risk factors were collected. Statistical analyses including Simple and multiple binary logistic regression were used to analyze the data, with model fit assessed using the Hosmer–Lemeshow test.

RESULTS

Weak pRb expression predominated in anal lesions (92.3%; $p=0.018$) and among homosexual/bisexual individuals (57.9%; $p=0.031$), while moderate expression was more common in genital lesions (66.7%; $p=0.018$) and heterosexual individuals (57.1%; $p=0.011$). Simple logistic regression analysis showed that HIV status ($OR=11.88$; $p=0.025$), sexual orientation ($OR=13.33$; $p=0.001$), and moderate-to-strong pRb expression ($OR=24.00$; $p=0.008$; $OR=12.00$; $p=0.048$) were associated with lesion location. In the multivariate model, pRb expression was excluded due to multicollinearity. Sexual orientation remained the only independent predictor of lesion location (Adj $OR=8.89$; $p=0.023$), with heterosexual individuals more likely to present with genital lesions.

CONCLUSION

The expression of pRb was not a risk factor of the anatomical location of CA, and differed between genital and anal CA. However, sexual orientation emerged as the dominant independent factor associated with lesion location.

Keywords: Condylomata acuminata; HPV infection; malignancy; retinoblastoma protein; sexual orientation

INTRODUCTION

Condylomata acuminata (CA), also known as genital warts, anal warts, or anal condylomas, are sexually transmitted infections caused by strains of the human papillomavirus (HPV), manifesting as skin-colored fleshy papules in the vulval, genital, and anal areas.⁽¹⁾ The global prevalence of CA is estimated at 10-20%. In Bali (Indonesia) between 2015 and 2017, the prevalence was 5.48%. CA affects genders differently, with genital warts affecting 72.3% of females and 27.2% of males, and anal warts affecting 2.1% and 14.95%, respectively.^(2,3) Risk factors for HPV infection include alcohol consumption, smoking, immunodeficiency, HIV/AIDS, uncircumcised sexual partners, a history of CA, and same-sex relationships with infrequent condom use.⁽⁴⁾ Condylomata acuminata increase the risk of anogenital cancer, especially anal CA with dysplasia as a precursor to anal squamous cell carcinoma. The most common risk for malignancy is associated with high-risk (HR) HPV types (16, 18, 31, 33, and 35). However, 90% of anogenital CA are caused by low-risk (LR) HPV types (6 or 11).⁽⁵⁾

The retinoblastoma gene (RB1) is the first identified tumor suppressor gene, while the retinoblastoma protein (pRb) plays a crucial role in cell cycle development. The function of RB in cell cycle control is mediated through its interaction with the E2F family of transcription factors.^(6,7) The human papillomavirus is associated with the destabilization of pRb-related proteins p107 and p130 by the E7 oncoprotein. This contributes to the development of malignancy by inducing genomic instability.^(2,8) Mutations in HR-HPV 16 and E7-mediated pRb degradation can be analyzed using immunohistochemical staining on CA.⁽²⁾ A preliminary study showed that pRb mutations could be a specific marker for HPV infection and could correlate with HPV types on CA.⁽⁹⁾ Human papillomavirus infections are associated with the suppression of pRb.⁽¹⁰⁾

It can be hypothesized that pRb expression may serve as a prognostic indicator and could correlate with HR-HPV or LR-HPV types, which are relevant for both genital and anal CA. Detecting pRb expression in CA is considered an

indicator of active HPV infection and a marker for a favorable prognosis.

Mastutik et al.⁽⁹⁾ demonstrated that pRb mutation mediated by p16INK4A may serve as a specific marker for HPV infection and could correlate with HPV type. Wei et al.⁽¹¹⁾ reported that the viral oncoproteins E6 and E7 inactivate several host tumor suppressor proteins, including p53, p21, and pRb, the latter often being referred to as the “guardian of the genome”. Another study observed that there was no statistically significant difference in pRb expression between cases of condylomata acuminata and cases of condylomata plana ($p=0.273$).⁽¹²⁾

In contrast to previous studies that primarily evaluated pRb expression using immunohistochemistry without distinguishing lesion sites, the present study uniquely investigated pRb expression in condylomata acuminata across two anatomical locations—anal and genital. This approach provided a novel perspective on how anatomical location may influence biomarker expression patterns in HPV-related lesions as the primary outcome. The secondary outcome observed was the variation in pRb expression according to sexual orientation.

METHODS

Research design

This study was of an analytical cross-sectional observational design and was conducted at the Dermatology and Venereology Clinic of Dr. Moewardi Hospital between June and August 2023.

Study subjects

A total of 31 patients clinically and histopathologically diagnosed with CA were included into the study. Inclusion criteria comprised patients aged >18 years with a diagnosis of CA, lesions located in the genital or anal region, lesion size <10 cm, and willingness to participate in the study by signing informed consent. Exclusion criteria included patients with malignancy and autoimmune disorders.

Measurements

Subjects who met the inclusion criteria were classified based on lesion location (genital or

anal). All participants underwent excisional biopsy under local anesthesia using 2% lidocaine HCl. The specimens were processed for immunohistochemical evaluation of retinoblastoma protein (pRb) expression using anti-phospho Rb (S249) antibodies, following standard deparaffinization, antigen retrieval, blocking, and staining procedures. pRb expression was assessed using the semi-quantitative Allred scoring system, in which the final score is obtained by multiplying the staining intensity and the proportion of positively stained cells.⁽¹³⁾ Staining intensity was categorized as 0 (no staining), 1 (weak/light brown), 2 (moderate/brown), and 3 (strong/dark brown). The percentage of stained cells was scored as 0 (<5%), 1 (5–25%), 2 (26–50%), 3 (51–75%), and 4 (>75%). The combined score was then grouped as follows: 0–1 (negative), 2–4 (weak), 5–8 (moderate), and 9–12 (strong). The measurement scale was ordinal.

Risk factor variables included gender, age, occupation, educational level, marital status, number of sexual partners, disease duration, HIV status, and sexual orientation. The secondary outcome was the variation in pRb expression in relation to sexual orientation.

Statistical analysis

The analysis was performed using SPSS version 22 software. A binary multiple regression analysis was used to analyze the data, with the p-value threshold of $p < 0.25$ being used as a screening criterion in a simple logistic regression to select candidate variables for inclusion in the final multivariable model.

Ethical clearance

The research received approval from the Ethics Committee of Dr. Moewardi Hospital/Faculty of Medicine, Sebelas Maret University (Ethical clearance No.1.525/VIII/HREC/2023). The study adheres to the Helsinki Declaration, and all research subjects provided informed consent for medical procedures.

RESULTS

The study included 33 patients with CA comprising 13 with genital warts and 20 with anal warts. Most patients were male (72.7%) and the remainder female (27.3%). The majority were aged 21–30 years (48.5%), with an age range of 17–63 years. Nearly half were employed in the

private sector (45.5%), had completed senior high school education (60.6%) and were single (54.5%). Regarding sexual orientation, the majority reported homosexual/bisexual 57.6% and heterosexual 42.4% (Table 1). Table 1 shows that most participants reported having less than 5 sexual partners (42.2%), while 24.2% had 6–10 partners.

Table 1. Clinicopathological characteristics of the subjects (n=33)

Variables	n	%
Gender		
Male	24	72.7
Female	9	27.3
Age (years)		
≤20	3	9.1
21 – 30	16	48.5
31 – 40	8	24.2
≥41	6	18.2
Occupation		
Laborer	2	6.1
Housewife	3	9.1
Self-employed	6	18.2
Student	6	18.2
Private sector	15	45.4
None	1	3.0
Educational level		
Elementary school	5	15.2
Junior high school	6	18.2
Senior high school	20	60.6
Diploma	1	3.0
Bachelor	1	3.0
Marital status		
Single	18	54.5
Married	11	33.3
Widower	4	12.2
Number of sexual partners		
1 – 5	14	42.4
6 – 10	8	24.3
>10	11	33.3
Location of the lesion		
Genital only	13	39.4
Anal only	20	60.6
Anogenital	0	0.0
Duration of the disease		
< 6 months	15	45.5
> 6 months	18	55.5
HIV status		
Non-reactive	6	18.2
Reactive	27	81.8
Sexual orientation		
Heterosexual	14	42.4
Homosexual/Bisexual	19	57.6
pRb expression		
Weak	13	39.4
Moderate	12	36.4
Strong	8	24.2

Table 2. Comparison between pRb expression and lesion location

pRb expression	Location of the Lesion		p-value
	Genital	Anal	
Weak	1	11	0.018*
Moderate	8	4	
Strong	4	4	

Independent difference test: Mann–Whitney test (ordinal data); *significant at $p < 0.05$

The weak retinoblastoma protein (pRb) expression category was predominantly found in patients with anal lesions (92.3%). Moderate pRb expression was more common among patients with genital lesions (66.7%), while strong pRb expression showed an equal distribution between genital (50.0%) and anal (50.0%) lesion locations. Statistical analysis revealed a significant difference in pRb expression between anal and genital lesions ($p = 0.018$), indicating that anal lesions tended to exhibit weak pRb expression, whereas genital lesions were more likely to show moderate expression (Table 2).

Based on Table 3, duration (OR=1.75; $p = 0.435$) was not associated with lesion location ($p > 0.05$). However, HIV status (OR=11.88; $p = 0.025$), type of sexual relationship (OR=13.33; $p = 0.001$), moderate pRb expression (OR=24.00; $p = 0.008$), and strong pRb expression (OR=12.00; $p = 0.048$) showed significant associations with lesion location. HIV-reactive patients tended to have anal lesions, with an 11.88-fold higher risk.

Heterosexual relationships were more likely associated with genital lesions, while homosexual/bisexual relationships were more likely associated with anal lesions, with a 13.33-fold higher risk. Moderate and strong pRb expressions tended to be associated with genital lesions, with 24-fold and 12-fold higher risks, respectively (Table 3). Variables with $p < 0.250$ in the bivariate analysis were included in the multivariate analysis as follows.

In the model of Table 4, known HIV status, type of sexual relationship, and pRb expression were not significantly associated with lesion location ($p > 0.05$). This was likely due to multicollinearity among the independent variables, leading to unstable coefficient estimates. Therefore, logistic regression model 1 could not be used to predict lesion location. In this study, the retinoblastoma protein (pRb) expression variable was excluded to address multicollinearity, resulting in model 2 as an alternative.

Table 3. Simple logistic regression analysis of the relationship between duration, HIV status, sexual orientation, and retinoblastoma protein (pRb) expression with lesion location

Variables	Site of Lesion				OR	95%CI		p-value
	Genital		Anal			Lower	Upper	
	n	%	n	%				
Duration								
<6 months	7	46.7	8	53.3	1.75	0.43	7.17	0.435
≥6 months	6	33.3	12	66.7	Ref.			
HIV status								
Non-reactive	5	83.3	1	16.7	11.88	1.19	118.50	0.025*
Reactive	8	29.6	19	70.4	Ref.			
Sexual orientation								
Heterosexual	10	71.4	4	28.6	13.33	2.45	72.45	0.001*
Homosexual/Bisexual	3	15.8	16	84.2	Ref.			
pRb expression								
Weak	1	7.7	12	92.3	Ref.			
Moderate	8	66.7	4	33.3	24.00	2.25	255.94	0.008*
Strong	4	50.0	4	50.0	12.00	1.02	141.34	0.048*

Note: Chi Square/Fisher Exact test; OR= Odd ratio; CI= Confidence interval; * significant $p < 0.05$

Table 4. Multiple logistic regression analysis of the relationship of HIV status, sexual orientation, and retinoblastoma protein (pRb) expression with lesion location

Variables	OR	AOR	95% C.I.		p value
			Lower	Upper	
Model 1					
HIV	20.338	7E+08	0.000		0.998
Sexual orientation	1.022	2.78	0.37	21.03	0.323
pRb Expression					1.000
pRb Expression(1)	20.849	1E+09	0.00		0.998
pRb Expression(2)	0.000	1.00	0.13	7.57	1.000
Model 2					
HIV(1)	1.099	3.00	0.23	39.61	0.404
Sexual Orientation (1)	2.185	8.89	1.34	58.80	0.023*

Note : OR= Odds Ratio; AOR= Adjusted Odd Ratio; CI= Confidence interval; * significant at p<0.05

The analysis showed that sexual orientation (Adj OR=8.89; p=0.023) was the dominant variable significantly associated with lesion location (p<0.05), where heterosexual individuals had an 8.89-fold higher risk of genital lesions compared to homosexual/bisexual individuals. Based on these findings, it can be concluded that sexual orientation is the dominant variable significantly associated with lesion location.

DISCUSSION

The majority of patients was aged 21–30 years (48.5%), of male gender (72.7%), and worked in the private sector (45.4%). Most had completed high school education (60.6%). Lesions were predominantly located in the anal region (57.6%) compared to the genital region (42.4%). A high proportion of patients was HIV reactive (81.8%), and more than half had disease durations exceeding 6 months (54.5%). Immunohistochemical analysis of pRb expression showed weak expression predominating in anal CA (92.3%), whereas moderate expression was more common in genital CA (66.7%). Overall, pRb expression was undetectable in 67% of cases. A statistically significant association was found between lesion location and pRb expression.

This study found that condylomata acuminata (CA) patients were predominantly aged 21–30 years, aligning with previous epidemiological reports showing peak incidence in young adults aged 18–30 years.⁽¹⁴⁻¹⁶⁾ Male patients represented 67.31% of cases, higher than the 50% distribution reported by Puspawati et al.⁽³⁾ This discrepancy may be attributed to differences in health-seeking behavior, anatomical factors, and spontaneous regression of lesions in females. Lesions were

most commonly located in the anal region (57.6%), consistent with Rohanda et al.⁽¹⁷⁾ who found 64% of CA cases in the anal region, particularly in men who have sex with men. Regarding comorbidities, 81.8% of CA patients had reactive HIV, which is substantially higher than the 2.4–14% prevalence reported in high-risk groups.^(18,19) This reflects the strong association between HIV-related immunosuppression and CA persistence. In terms of molecular findings, this study showed that weak pRb expression was dominant in anal CA (92.3%) while moderate pRb expression was dominant in genital CA (66.7%). These findings are comparable to those of Soltani et al.⁽²⁰⁾ who reported absence of pRb expression in 68.1% of CA cases, supporting its role as a biomarker of HPV-driven oncogenic changes.

The study highlights the association between lesion location and pRb expression status. HPV oncoprotein E7 binds and degrades pRb, impairing its function as a tumor suppressor.⁽²¹⁾ Weak pRb expression in anal CA suggests higher oncogenic potential compared to genital CA. Since HPV-driven carcinogenesis involves evasion of growth suppression and deregulated cell cycle progression, lesion site may be an important predictor of malignant transformation risk. These findings provide evidence that anal CA, particularly in immunocompromised individuals, may represent a precursor state with higher malignant potential.

This study has several limitations, such as assessing pRb expression using a semi-quantitative method. More accurate quantitative methods, such as enzyme-linked immunosorbent assay (ELISA) or western blotting, are needed. Secondly, the study did not assess low- or high-risk HPV genotypes that could affect pRb

expression. Thirdly, the study only evaluated one type of tumor suppressor gene expression (pRb). Assessment of other protein expressions such as p16 or p53 can be conducted to support the role of pRb as a predictor of CA turning malignant. Fourthly, the study did not categorize HPV genotypes into HPV-HR and HPV-LR, so further research could classify HPV to identify genotypes that could predict CA turning malignant. Immunohistochemical assessment of pRb expression may serve as a cost-effective adjunct to histopathology for identifying CA lesions with higher malignant potential, especially in the anal region. This could aid clinicians in stratifying patients for closer surveillance and earlier intervention. Furthermore, given the high prevalence of HIV co-infection, routine HIV screening in CA patients is crucial for integrated care and prevention of HPV-related cancers.

Further research is warranted to employ more quantitative methods for measuring pRb expression, assess the expression of additional tumor suppressor proteins (e.g., p16, p53), and classify HPV genotypes to evaluate their correlation with malignant progression in condylomata acuminata (CA).

CONCLUSION

Sexual orientation emerged as the dominant factor associated with lesion location. The pRb expression was not a risk factor of the anatomical lesions of CA. Overall, sexual orientation was the strongest predictor of whether lesions occurred in the genital or anal region.

Conflict of Interest

No conflict of interest

Acknowledgments

The authors extend their gratitude to Dr. Moewardi General Hospital / Faculty of Medicine, Sebelas Maret University for their collaboration and support.

Author Contributions

Conceptualization: PPP, NM, and EYE; Methodology: PPP and MYY; Software: HK and SW; Investigation: PPP, NM, EYE, EP, HK, SW, BW and NAS; Data curation: BW and NAS; Writing – original draft preparation: PPP, NM, and EYE.; Writing – review & editing: PPP, NM, EYE, EP, HK, SW, and MYY; Supervision: NM,

EYE, EP, HK, and SW. All authors have read and approved the final manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Use of AI in Scientific Writing

No AI tools were used in the writing or editing of this manuscript.

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