ORIGINAL ARTICLE

The association of p16 protein expression with clinicopathology in cervical cancer: a cross-sectional study

P16 and Clinicopathology of Cervical Cancer

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ABSTRACT

Objective. This study aims to analyze the association between p16 protein expression and clinicopathological characteristics in cervical cancer.

Materials and Methods. This cross-sectional study was conducted at Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital in Makassar, from October 2023 to April 2024. Data were collected from 80 cervical cancer patients. Immunohistochemistry

was used to evaluate p16 expression, and the Allred score was applied for classification. Statistical analyses, including the Kruskal-Wallis and Mann-Whitney tests, were used to determine the significance of p16 expression across clinicopathological factors.

Results. Patients ranged from 30 to 82 years old, with the majority presenting with Stage II or III cervical cancer, metastasis, and squamous cell carcinoma histopathology. The analysis revealed a statistically significant correlation between p16 expression and the stage of cervical cancer ($p = 0.007$) as well as histopathology ($p = 0.019$), with higher expression in Stage IV and squamous cell carcinoma. However, p16 expression was not significantly associated with clinical symptoms or metastasis.

Conclusions. P16 expression is significantly associated with the stage and histopathological type of cervical cancer, particularly in advanced stages and squamous cell carcinoma. These findings support the potential use of P16 as a prognostic marker for cervical cancer. Further research is recommended to explore p16's relationship with other clinicopathological

Key words

Cervical cancer, P16 protein expression, Tumor Suppressor Gene

Introduction

Cervical cancer is the second most prevalent cancer among women in Indonesia. According to Globocan 2022, there were 36,964 new cases and 20,708 deaths among Indonesian women [1]. Unfortunately, most cervical cancer patients present with advanced-stage disease, and the one-year cumulative survival rate for advanced-stage cervical cancer patients is only 77% [2].

Numerous studies conducted over the past four decades have demonstrated the independent prognostic significance of various factors such as lymph node involvement, stage, histological type, tumor size, LVSI, stromal invasion, and parametrial invasion in patients with cervical cancer. These factors underscore the importance of accurate and timely diagnosis in determining the degree of dysplasia, which influences patient management and prognosis. Currently, there is an expectation for enhanced sensitivity and specificity in diagnostic practices, particularly through the use of immunohistochemical tests, particularly through p16 testing [3].

p16, encoded by the tumor suppressor gene (CDKN2A), is directly involved in the negative feedback regulation of the cell cycle, functioning to inhibit cell proliferation at the G1-S phase by inactivating the E2F transcription factor (elongation factor 2) [4].The p16 gene undergoes alterations, such as amplification, can occur following infection and viral genome integration with the host genome. There are more than 200 types of human papillomavirus (HPV), but the majority of cervical neoplasia cases are linked to ongoing sexually transmitted infections from high-risk strains, including HPV-16, HPV-33, HPV-18, HPV-31, HPV-45, HPV-52, and HPV-58 [5]. Furthermore, the persistence of human papillomavirus (HPV) also a primary risk factors that influence the likelihood of recurrence of cervical dysplasia [6]. In HPV-related cancers, such as cervical cancer, HR-HPV infects the tissue and releases E7 proteins that inactivate the pRb protein, leading to increased synthesis and accumulation of p16 protein in cells due to a negative feedback mechanism, which results in increased cell proliferation [7].

Wang and Zhao reported that p16 expression was significantly associated with tumor size, stage, and metastasis [8]. Ismail et alstated that the correlation between p16 expression and histological subtypes was near significant, with 82.5% of squamous histology being positive p16 compared to 70% in other histological subtypes [9]. However, other studies have found no association between p16, clinical symptoms/signs, and metastasis [10,11]. These findings underscore the inconsistencies regarding the relationship between p16 expression and the stage of cervical cancer, as well as metastasis. Furthermore, the connection between p16 expression and clinical symptoms or histopathological types remains very limited. Investigating the relationship between p16 expression and the clinicopathology of cervical cancer may position p16 as a potential biomarker for aiding in cervical cancer diagnosis and optimizing appropriate treatment. Therefore, this study aims to explore the relationship between p16 protein expression and clinicopathological factors in cervical cancer.

Materials and Methods

This study was conducted at Wahidin Sudirohusodo Hospital, Makassar Indonesia, between October 2023 to April 2024. The study received approval from the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University (531/UN.4.6.4.5.31/PP36/2023). Informed consent was obtained from all participants. The participants were enrolled into the study by consecutive sampling technique. The inclusion criteria were: 1) Data and paraffin blocks of patients diagnosed with cervical cancer based on clinical stage, histopathology, and lymph node metastasis, 2) No history of onother malignancies besides cervical cancer. The exclusion criteria were: 1) incomplete data.

The study sample consists of paraffin block data from patients diagnosed with cervical cancer and staining using the alfred Score (Figure 1). Samples were collected using purposive sampling, where all eligible members of the population at the study site who met the inclusion criteria were selected until the sample size was fulfilled. The minimum sample size was calculated based on a cross-sectional study design and population data from research by Izasi et al [12]. The sample size was determined using the following formula [13]:

$$n = \frac{Z^2_{1-\alpha/2} pq}{d^2}$$

$$n = \frac{(1{,}96)^2 x 0{,}75 x 0{,}25}{0{,}1}$$

$$n = \frac{3{,}84 x 0{,}187}{0{,}01}$$

$$n = 72{,}03 \approx 73$$

Description:

n = minimum sample size

$Z_{(1-\alpha/2)}$ =Standard normal distribution value at α (0.05) = (1.96)

p = Proportion of p16 expression in cervical cancer adenocarcinoma =75%[12]

q =1-p

d = Tolerable absolute error (0.1)

Based on this formula, the minimum sample size required is 73. To account for potential drop outs during the study, 10% was added to the minimum sample size, resulting in a total of 80 cervical cancer patients.

Statistical analysis was conducted using the SPSS 24.0 for Windows software (IBM, USA). Baseline characteristics were reported as frequencies and percentages. For normally distributed data, the results were reported as Mean ± SD and analyzed with an independent t-test. While in non-normally distributed data were reported as Median ± Interquartile Range (IQR) and analyzed using the Mann-Whitney test. The differences in baseline characteristics between the two groups will be analyzed using a chi-square test. A p-value of <0.05 was considered significant.

Results

This study collected data from 1,093 cervical cancer patients between October 2023 and April 2024 at Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital in Makassar. A total of 456 patients did not have anatomical pathology data, 326 lacked CT scan or MRI results, and 231 did not have paraffin blocks. Therefore, only 80 patients were eligible for this study (Figure 1). The characteristics of the study subjects are presented in Table 1.

Table 2 shows that cervical cancer patients did not have significantly different P16 scores based on clinical symptoms or metastasis, with p-values > 0.05. However, P16 scores were significantly different based on cancer stage and histopathology type, with p-values < 0.05. Specifically, the highest P16 score was observed in Stage IV, while the lowest was recorded in Stage I. Regarding histopathology type, the highest P16 score was found in squamous cell carcinoma, compared to cervical adenocarcinoma and adenosquamous cell carcinoma. In this study, immunoreactivity to P16 was classified based on the Allred score.

Discussion

To the best of our knowledge, this study is the first to investigate and clinicopatological cervical cancer in relation to clinicopathological features of Indonesian women**.** Most of participants in this study are aged between 45 and 59 years. This finding is consistent with the research conducted by Raju et al. (2022), which indicates that the age of cervical cancer patients ranges from 30 to 80 years, with an average of 54.3±12.0, and the maximum cases occur in the age range of 40 to 49 years, followed by those aged 60 to 69 years [7].

The risk of cervical cancer occurrence increases after the age of 25 years. Women living in high-income countries develop cervical cancer at an earlier age, usually around 40 years, whereas in low-income countries they are diagnosed at an older age, ranging from 50–69 years [14]. The Centers for Disease Control and Prevention (CDC) recommends the administration of the human papillomavirus (HPV) vaccine for preteens aged 11 to 12, particularly for those who are sexually active. Prophylactic vaccination, and more recently adjuvant vaccination, significantly reduce the risk of cervical cancer but do not eliminate [15]. It also reducing the incidence of lower genital tract dysplasia [16]. If the vaccines currently available provide long-term immunity, cervical cancer rates could potentially be reduced by 85% among individuals who were vaccinated prior to exposure to oncogenic HPV[17]. Screening procedures encompass with of Papanicolaou tests (Pap tests) and human papillomavirus (HPV) tests. Should the results of these tests be positive, they are followed by colposcopy and targeted biopsy. Early diagnosis is critical, as the stage at which the diagnosis is established serves as a significant prognostic indicator [15].

The majority of participants in this study are patients diagnosed with cervical cancer, presenting with clinical symptoms that include vaginal bleeding, post-coital bleeding, lower abdominal pain, and leukorrhea. Notably, some patients exhibit no symptoms, which is considered a favorable condition. Cervical cancer is frequently identified at an advanced stage, often characterized by ambiguous clinical manifestations, such as abnormal vaginal bleeding. The prevalence of vaginal bleeding among women with cervical cancer ranges from 0.7% to 100%. The occurrence of vaginal bleeding can pose substantial challenges, leading to various adverse outcomes, including anemia resulting from recurrent episodes, which may necessitate blood transfusions, and in severe cases, can culminate in mortality due to significant hemorrhage [7,14].

Pain is one of the most common symptoms of advanced-stage cervical cancer. The pelvis is largely innervated by the sacral plexus and inferior gastric plexus. Therefore, neuropathic pain, which is often found in cervical cancer, is usually a result of infiltration of nerve endings and pelvic bones. Pain intensity can change over time as the cancer progresses [7,14].

In this study, it was found that the higher the stage, the larger the P16 expression. P16 expression independently relates to tumor stage because high p16 expression more frequently occurs in advanced FIGO stages [18]. In patients with squamous cell carcinoma, there is a significant statistical relationship between p16 staining intensity and tumor assessment as well as stage. Medium/strong staining intensity can predict stage 2B in patients with squamous cell carcinoma of the uterine cervix [19].

Cervical cancer is associated with HPV infection that can be identified by overexpression of p16 INK4a in premalignant and malignant lesions. Positive p16 INK4a expression increases with the degree of dysplasia, thus helping in the stratification of dysplastic and neoplastic lesions of the cervix [20]. In human neoplasms, p16 silencing occurs in three ways: homozygous deletion, promoter methylation, and point mutation. Homozygous deletion and promoter methylation are the majority of inactivation events in most primary tumors. P16 protein is an oncogene whose overexpression has been shown to result from the functional inactivation of HPV E7 protein, possibly reflecting cell cycle dysregulation induced by HPV. This inactivates the cyclin D - cyclin-dependent kinase protein complex (CDK) 4/6, preventing phosphorylation of the retinoblastoma protein (Rb) by CDK4/6, thereby placing Rb in growth inhibitory mode [21].

This study identified a relationship between the histopathological type of cervical cancer and P16. Notably, P16 expression was found to be highest in cases of squamous cell carcinoma. These findings are consistent with the study by Zuberi et al. (2021) that P16 expression is associated with the histological dysplasia grade of cervical cancer [22]. P16 was observed to increase from normal squamous epithelium to invasive cervical carcinoma, suggesting that it may serve as a valuable biomarker for predicting the risk of cervical cancer development in women [9].

P16 staining (indicating no observed expression) in HPV-related tumors can be caused by various mechanisms. Loss of heterozygosity is a common occurrence in several tumors and also in HPV-related carcinoma. Point mutations in P16, and more often, P16 gene silencing through promoter hypermethylation, have been reported in many human cancers, including HPV-related cancers. Negative P16 staining may also indicate false-positive results from HPV detection techniques. In this case, in other areas such as the vulva and head and neck, it has been proposed that HPV infection cannot be accurately diagnosed by detecting HPV DNA alone, and that a second test, such as P16 or E6/7 mRNA detection, is required to accurately classify tumors as definitively HPV-related. Moreover, there is evidence indicating that tumors exhibiting negative p16 expression may yield P16-positive staining results in

adjacent intraepithelial lesions, which strongly supports the notion of p16 overexpression loss due to mutation or gene silencing [23].

This study showed that P16 is associated with both the stage and histopathological type of cervical cancer. Consequently, the P16 expression can yield valuable information related to patient prognosis so that it can be information for clinicians to explain to patients the state of the disease and the available therapeutic options to patients. Furthermore, this study contributes to the existing body of literature by providing evidence regarding the role of P16 in the pathophysiology of cervical cancer and its potential impact on patient outcomes.

Limitation

The cross-sectional nature of this study was a limitation. This study also only explored the correlation between P16 and aspects of cervical cancer clinicopathology, including clinical symptoms, stage, metastases, and histopathological type not as a prognosis factor.

Conclusion

Based on the research findings and discussion, it can be concluded that P16 expression is associated with the stage and histopathological type of cervical cancer. P16 is more commonly expressed in stage IV and in the squamous cell carcinoma histopathological type. However, P16 expression is not related to clinical symptoms or metastasis of cervical cancer. P16 can be useful as a prognostic marker for cervical cancer, particularly in relation to histopathology and cancer stage. Further research is recommended to explore the connection between P16 and other clinicopathological factors, such as cell differentiation.

COMPLIANCE WITH ETHICAL STANDARDS

Authors contribution

K.J.F.N: Conceptualization, data curation, investigation, formal analysis, validation, visualization. N.U.P: Data curation, supervision. I.S.:Data curation, supervision. U.M.: formal analysis,supervision. M.T.C.: formal analysis,supervision. S.A.: Writing – review & editing. L.L: Writing – review & editing.

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Study registration

N/A.

Disclosure of Interest

None of the authors have any conflict of interests related to this work.

Ethical approval

This study was approved by the Ethics Committee of Hasanuddin University of Medical Sciences

Informed Consent

Informed consent was obtained from all participants.

Data sharing

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

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Table 1. General Characteristics of Study Subjects

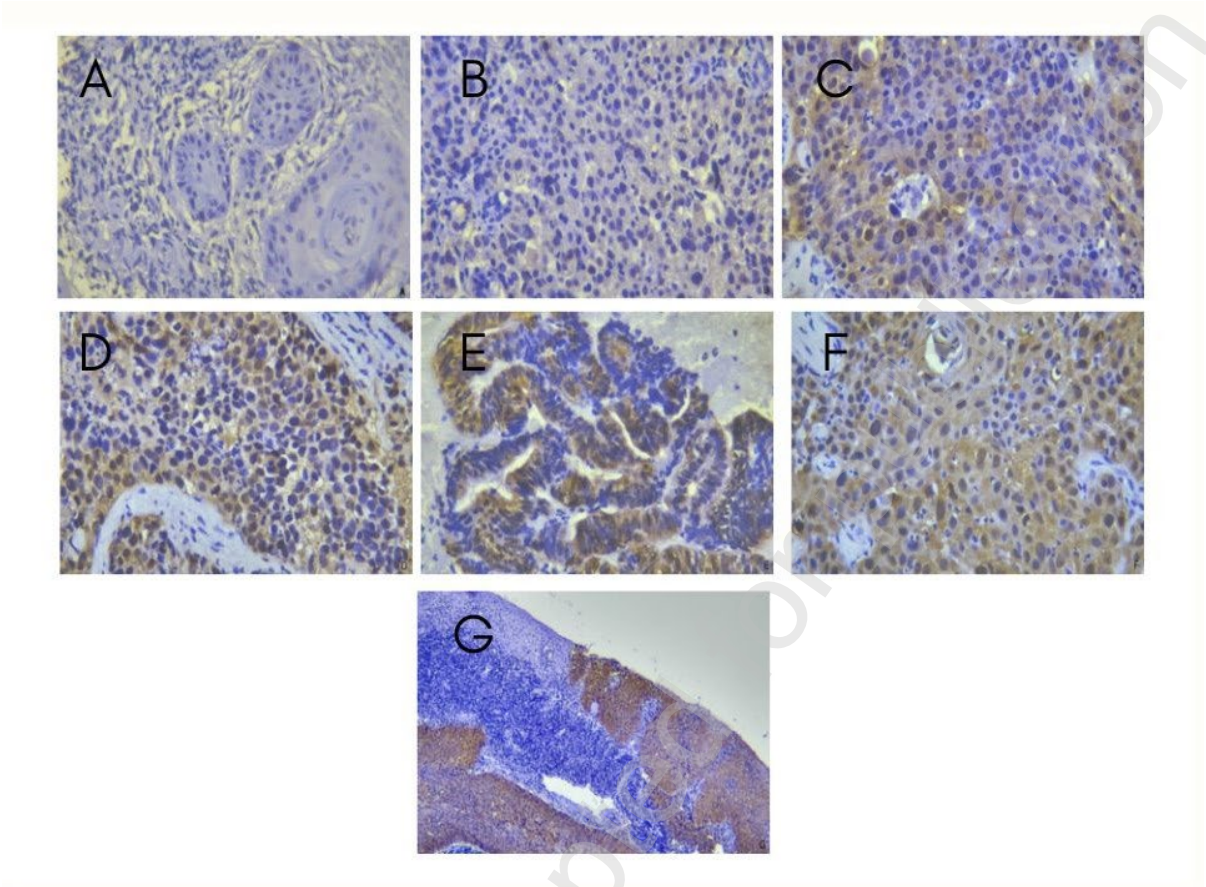
Characteristic	n (%)
Age	
30-44 years	31 (38.7)
45-59 years	40 (50.0)
≥ 60 years	9 (11.3)
Education	
No formal education	14 (17.5)
Elementary-Middle School	42 (52.5)
High School-College	24 (30.0)
Occupation	
Employed	13 (16.3)
Unemployed	67 (83.8)
BMI	
Underweight	3 (3.8)
Normal	29 (36.3)
Overweight	28 (35.0)
Obese	20 (25.0)
Parity	
Nulliparous	13 (16.3)
Primiparous	16 (20.0)
Multiparous	45 (56.3)
Grand multiparous	6 (7.5)
Clinical Symptoms	
Good	31 (38.8)
Vaginal bleeding	36 (45.0)
Post-coital bleeding	7 (8.8)
Lower abdominal pain	3 (3.8)
Discharge	3 (3.8)
Cervical Cancer Stage	
Stage I	2 (2.5)
Stage II	51 (63.7)
Stage III	15 (18.8)
Stage IV	12 (15.0)
Metastasis	
Positive	62 (77.5)
Negative	18 (22.5)
Histopathology	
Squamous cell carcinoma	63 (78.8)
Cervical adenocarcinoma	8 (10.0)
Adenosquamous cell carcinoma	9 (11.3)

Table 2. Comparison of P16 Score Based on Clinicopathological Aspects

Clinicopathology	P16 Score	p-value
	Median (Min-Max)	
Clinical Symptoms		
Good	6,00 (2,00-8,00)	
Vaginal bleeding	6,00 (0,00-8,00)	
Post-coital bleeding	7,00 (6,00-7,00)	0.129[a]
Lower abdominal pain	8,00 (4,00-8,00)	
Discharge	3,00 (3,00-5,00)	
Cervical Cancer Stage		
Stage I	4,00 (4,00-4,00)	
Stage II	6,00 (0,00-8,00)	0.025[a*]
Stage III	6,00 (0,00-8,00)	
Stage IV	8,00 (0,00-8,00)	
Metastasis		
Positive	6,00 (0,00-8,00)	0.092[b]
Negative	5,50 (4,00-8,00)	
Histopathology		
Squamous cell carcinoma	6,00 (0,00-8,00)	
Cervical adenocarcinoma	5,00 (4,00-7,00)	0.019[a*]
Adenosquamous cell carcinoma	5,00 (0,00-6,00)	

*Note: a Kruskal Wallis test, b Mann Whitney test, * significant at $p < 0.05$.

Figure 1. *Representative images of p16 immunohistochemistry staining in cervical cancer using the Alfred Score.*



A. Negative (Intensity 0, Frequency 0); B. Positive (Intensity 1, Frequency 1); C. Positive (Intensity 1, Frequency 2); D. Positive (Intensity 2, Frequency 3); E. Positive (Intensity 3, Frequency 4); F. Positive (Intensity 3, Frequency 5); G. Positive and Negative Staining