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THE CORRELATION BETWEEN p16 AND p53 PROTEIN EXPRESSION WITH PATHOLOGICAL T STAGE (pT) AND LYMPH NODE METASTASIS IN PENILE SQUAMOUS CELL CARCINOMA AT PROF. DR. I.G.N.G. NGOERAH HOSPITAL

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ABSTRACT

Background: Penile carcinoma is a rare cancer worldwide. Approximately 95% of penile cancers are classified as penile squamous cell carcinoma (PSCC). Bali province has the highest incidence of PSCC in Indonesia. The prognosis of PSCC causes psychological trauma and becomes worse when the disease is diagnosed at advanced stages, making treatment more complex. The pathological stage and lymph node metastasis status are significant prognostic factors. The expression of p16 and p53 proteins plays a role in the classification and prognosis of PSCC. Various studies have shown that PSCC with positive p16 expression has a better prognosis compared to PSCC with abnormal p53 expression. Although the relationship between p16 and p53 expression and prognostic factors has been widely studied, research combining both p16 and p53 expressions remains limited. This study aims to evaluate the relationship between the expression of p16 and p53 proteins with pathological T stage and lymph node metastasis status in PSCC patients, as prognostic markers.

Methods: This study is an observational analytical research with a cross-sectional design. The sample consists of PSCC patients whose specimens were examined at the Anatomical Pathology Laboratory of Prof. Dr. I.G.N.G. Ngoerah Hospital from January 1, 2019, to June 30, 2025, according to the inclusion and exclusion criteria set by the researchers, with a sample size of 43 patients. Immunohistochemistry was performed using p16INK4A clone JC2 and p53 clone DO-7. The research results were analysed using Chi-square analysis to assess the relationship between p16 and p53 protein expression with pathological T stage and lymph node metastasis, with a significance level of $p<0.05$.

Results: The study found that the patients' age ranged from 32 to 97 years, with a mean age of 60.09±14.046 years. The most cases were at advanced stages (26 cases), and most patients showed no lymph node metastasis (31 cases). Immunohistochemical examination revealed that most cases had negative p16 expression (30 cases) and abnormal p53 expression (36 cases). Regarding the combination of p16 and p53 expressions, 26 cases showed negative p16 and abnormal p53, 10 cases showed positive p16 and abnormal p53, 4 cases showed negative p16 and normal p53, and 3 cases showed positive p16 and normal p53. However, based on Chi-square analysis, there was no statistically significant relationship between p16 and p53 protein expression and pathological T stage ($p = 0.493$) or lymph node metastasis ($p = 0.506$).

Conclusion: There is no significant relationship between p16 and p53 protein expression and pathological T stage or lymph node metastasis in SCCP.

Keywords: Penile squamous cell carcinoma, p16 expression, p53 expression.

INTRODUCTION

Penile carcinoma is a rare cancer worldwide, with a prevalence of 0.3-1/100,000 men in industrialized countries. However, the incidence of penile cancer varies across different populations globally.[1] According to data from the Global Cancer Registries (Globocan) in 2020, there were 36,068 new cases of penile cancer worldwide, with 13,211 deaths attributed to penile cancer. In Indonesia, there were 1,017 new cases and 347 deaths.[2] Bali is the province with the highest incidence compared to other provinces. In Bali, the peak incidence occurs in the 6th and 7th decades of life. Approximately 95% of penile cancer cases are classified as squamous cell carcinoma.[1] Among penile squamous cell carcinoma patients at Prof. dr. I.G.N.G. Ngoerah Hospital from 2016 to 2020, the majority were in the 51-60 age range.[3]

Penile squamous cell carcinoma (PSCC) is broadly classified into tumors associated with Human Papillomavirus (HPV) and those not associated with HPV.[4] PSCC associated with HPV is linked to the oncogenic properties of the E6 and E7 HPV genes, which possess transformative capabilities. Immunohistochemical demonstration of p16 overexpression serves as a surrogate marker for active high-risk HPV infection.[5] The pathogenesis of HPV-independent PSCC is associated with p53 mutations.[6] Pathological staging and metastasis to the lymph nodes (LN) are the most reliable prognostic factors for penile carcinoma.[7] Several studies evaluating p16 expression and p53 expression remain controversial. A study by Mohanti et al showed that positive p16 expression was more commonly found in PSCC with low-grade differentiation and early-stage pathology, while negative p16 expression was more frequently observed in PSCC with lymph node metastasis. Moreover, positive p16 expression was associated with a higher survival rate compared to PSCC with abnormal p53 expression, and dual-positive (p16 positive and p53 abnormal) PSCC exhibited a higher survival rate than dual-negative (p16 negative and p53 normal) PSCC.[8] A study by Trias et al found that PSCC with negative p16 expression and normal p53 expression had a low risk of lymph node metastasis. This study also demonstrated that PSCC with positive p16 expression and normal p53 expression had a favorable prognosis, whereas PSCC with negative p16 expression and abnormal p53 expression had a high risk of lymph node metastasis.[9] Research by Junior et al found that abnormal p53 expression was more commonly associated with advanced-stage PSCC, specifically pT3-pT4.[10]

To date, no studies have been conducted that evaluate the expression of p16 and p53 in penile squamous cell carcinoma (PSCC) in relation to pathological T stage (pT) and lymph node metastasis (LNM), particularly in the Bali region. This study aims to investigate the correlation between p16 and p53 expression and pathological T stage (pT) as well as lymph node metastasis in patients with penile squamous cell carcinoma treated at Prof. dr. I.G.N.G. Ngoerah Hospital.

METHODS

Study Subjects

This study employs a cross-sectional design. A total of 43 paraffin block samples will be selected using consecutive sampling. The samples will be obtained from patients diagnosed with penile squamous cell carcinoma who underwent penectomy and had their specimens histopathologically examined at the Anatomical Pathology Laboratory of Prof. dr. I.G.N.G. Ngoerah Hospital between January 1, 2019, and June 30, 2025.

Data on patients with penile squamous cell carcinoma will be obtained from the Management Information System of Prof. dr. I.G.N.G. Ngoerah Hospital. The inclusion criteria are as follows: paraffin block specimens derived from surgical materials (partial or total penectomy) with a confirmed histopathological diagnosis of penile squamous cell carcinoma (all subtypes), paraffin blocks containing sufficient tumor tissue, and patients who have not received radiotherapy or chemotherapy. The exclusion criteria include uncertain diagnoses, such as cases with differential diagnoses or mixed diagnoses involving other carcinoma types, and paraffin blocks that are damaged, moldy, or unavailable.

Clinicopathological Variables

The clinicopathological variables used in this study include pathological T stage (pT) and lymph node metastasis status. Pathological T stage is classified into early stages (pT1a, pT1b, and pT2) and advanced stages (pT3 and pT4). Lymph node metastasis status is categorized as follows: presence of lymph node metastasis (characterized by clinically palpable inguinal lymphadenopathy and confirmed histopathologically as metastatic lymph nodes) and absence of lymph node metastasis (inguinal lymph nodes are not palpable, or no inguinal lymphadenopathy is observed on ultrasonography, or inguinal lymphadenopathy is present but not confirmed on histopathological examination).

Immunohistochemical Staining

The expression of p16 and p53 proteins was assessed using immunohistochemical staining. 2. The expression of p16 and p53 was classified into four categories: positive p16 expression with abnormal p53 expression, positive p16 expression with normal p53 expression, negative p16 expression with abnormal p53 expression, and negative p16 expression with normal p53 expression. The p16 expression was evaluated using a monoclonal primary antibody, p16INK4A, clone JC2 from Sigma-Aldrich Cell

Marque. Immunohistochemical staining in tumor cells was observed in both the nucleus and cytoplasm of tumor cells, with a brown color ranging from weak to strong intensity. The p16 expression in tumor cells was evaluated based on the proportion of tumor cells stained relative to the total tumor mass. A positive result was defined as staining in ≥75% of tumor cells, and a negative result was defined as staining in <75% of tumor cells.[11] The p53 expression was assessed using a monoclonal primary antibody, p53, clone DO-7 from Leica. Immunohistochemical staining in tumor cells was observed in both the nucleus and cytoplasm, with a brown color ranging from weak to strong intensity. The p53 expression in tumor cells was evaluated based on staining patterns. Abnormal (mutant) p53 expression was considered positive if there was strong and continuous staining in the basal cell layer (basal overexpression pattern), strong and continuous staining in the basal cells extending into the suprabasal cells (diffuse overexpression pattern), staining in the cytoplasm with or without staining in the nucleus (cytoplasmic pattern), or if no staining was observed in the tumor cells but staining was found in adjacent skin, stroma, or inflammatory cells (null pattern). Normal (wild-type) p53 expression was considered positive if there was staining in the nuclei of a few cells in the basal and/or parabasal layers (scattered pattern), or moderate to strong staining in the nuclei of parabasal cells without staining in basal cells (mid-epithelial pattern).[12] The interpretation of p16 and p53 expression in tumor cells was performed by the researcher and two anatomical pathology specialists using a Leica DM 750 binocular light microscope. In case of discrepancies in the interpretation of p16 and p53 expression, a consensus agreement was reached through discussion.

Data Analysis

To assess the correlation between p16 and p53 expression and clinicopathological profiles (pT stage, lymph node metastasis), the chi-square test was used. If any cell in the contingency table had an expected value of <5, Fisher's Exact test was employed instead. 3. Conclusions were drawn based on a 95% Confidence Interval (CI) and a p-value < 0.05. All data analysis was performed using IBM SPSS Statistics 22.0.0.0 for Windows.

RESULTS

The characteristics of the study sample based on age showed that the youngest age in the study was 32 years, and the oldest was 97 years. The median age was 59 years, with a mean age of 60.09 ± 14.046 years. The distribution of cases based on clinicopathological characteristics is presented in **Table 1**. The table shows the distribution of cases by age, with most cases falling into the >55 years age group, totaling 28 cases (65.1%). Based on pathological T stage (pT), most cases were at an advanced stage, with 26 cases (60.5%). Regarding lymph node metastasis, most cases did not show lymph node metastasis, with 31 cases (72.1%) showing no metastasis.

Tabel 1. Case Distribution Based On Clinicopathological Characteristics

Sample Characteristic		Frequency	Percentage (%)
Age	≤ 55 year	15	34,9
	> 55 yaer	28	65,1
Pathological T Stage	Early	17	39,5
	Late	26	60,5
Lymph Node Metastasis	Positive	12	27,9
	Negative	31	72,1
p16 Protein Expression	Positive	13	30,2
	Negative	30	69,8
p53 Protein Expression	Abnormal	36	83,7
	Normal	7	16,3
p16 and p53 Protein Expression	p16 positive and p53 abnormal	10	23,3
	p16 positive and p53 normal	3	6,9
	p16 negative and p53 abnormal	26	60,5
	p16 negative and p53 normal	4	9,3

In the immunohistochemical examination, based on p16 protein expression, 13 cases (30.2%) showed positive p16 expression, while 30 cases (69.8%) exhibited negative p16 expression. **Figure 1** shows the positive and

negative p16 expression in the cases. Regarding p53 protein expression, 36 cases (83.7%) showed abnormal p53 expression, with the following patterns: 13 cases with a diffuse overexpression pattern, 16 cases with a basal overexpression pattern, and 7 cases with a null pattern. Seven cases (16.3%) exhibited normal p53 expression, with 5 cases showing a scattered pattern and 2 cases showing a mid-epithelial pattern. **Figure 2** shows both abnormal and normal p53 protein expression.

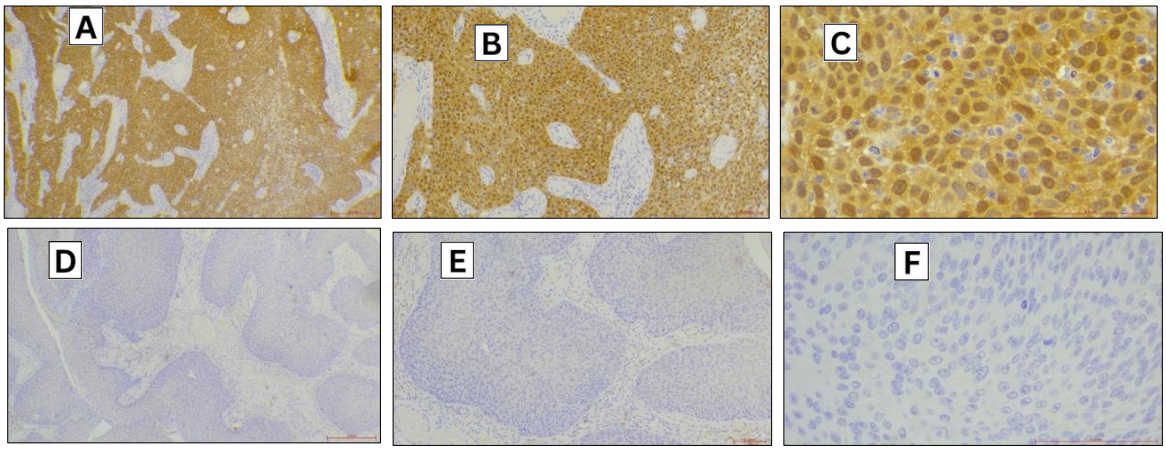

Figure 1. p16 Protein Expression

A-C. Positive p16 Protein Expression (p16 immunohistochemistry A.40x, B.100x, C.400x). **D-F.** Negative p16 Protein Expression (p16 immunohistochemistry D.40x, E.100x, F.400x)

When considering the combination of p16 and p53 expression, 10 cases (23.3%) showed positive p16 expression and abnormal p53 expression, 3 cases (6.9%) showed positive p16 expression and normal p53 expression, 26 cases (60.5%) showed negative p16 expression and abnormal p53 expression, and 4 cases (9.3%) showed negative p16 expression and normal p53 expression.

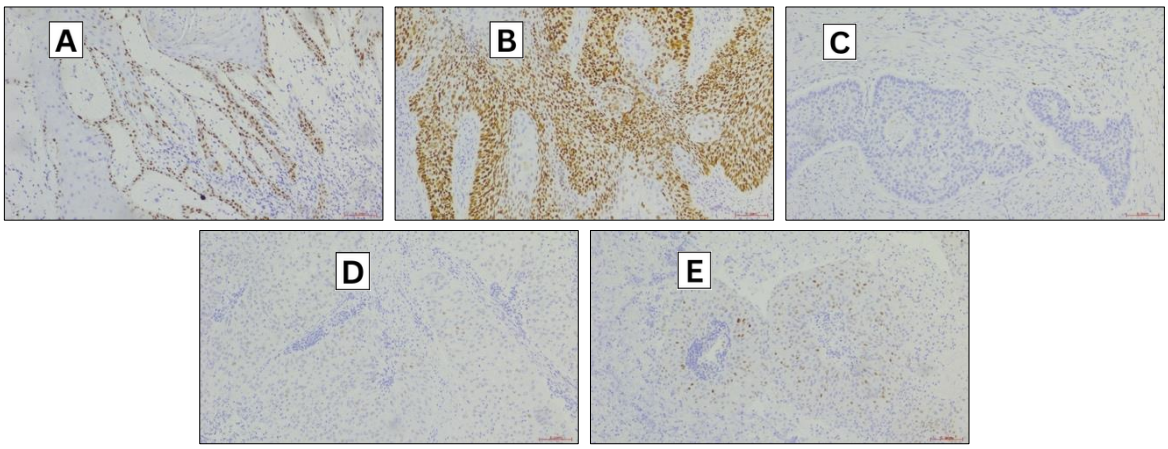

Figure 2. p53 Protein Expression

A-C. Abnormal p53 Protein Expression (p53 immunohistochemistry A.Basal Overexpression Pattern, B. Diffuse Overexpression Pattern, C.Null Pattern). **D-E.** Normal p53 Protein Expression (p53 immunohistochemistry D.Sacttered Pattern, E.Mid-Epithelial Pattern)

To assess the relationship between p16 and p53 protein expression and pathological T stage, a bivariate chi-square analysis was conducted. **Table 2** shows that from the 43 cases of penile squamous cell carcinoma (SCC) at Prof. dr. I.G.N.G. Ngoerah Hospital in Denpasar, there were 10 cases (23.3%) with positive p16 expression and abnormal p53 expression, 3 cases (6.9%) with positive p16 expression and normal p53 expression, 26 cases (60.5%) with negative p16 expression and abnormal p53

expression, and 4 cases (9.3%) with negative p16 expression and normal p53 expression.

In the cases with positive p16 expression and abnormal p53 expression, 5 cases (50%) were in early stages, and 5 cases (50%) were in advanced stages. In the cases with positive p16 expression and normal p53 expression, 2 cases (66.7%) were in early stages, and 1 case (33.3%) was in an advanced stage. In the cases with negative p16 expression and abnormal p53 expression, 8 cases (30.8%) were in early stages, and 18 cases (69.2%) were in advanced stages. In the cases with negative p16 expression and normal p53 expression, 2 cases (50%) were in early stages, and 2 cases (50%) were in advanced stages.

The chi-square analysis to evaluate the relationship between p16 and p53 protein expression and pathological T stage (pT) resulted in a p-value of 0.493, meaning that there was no statistically significant relationship between p16 and p53 expression and pathological T stage (pT).

To assess the relationship between p16 and p53 protein expression and lymph node metastasis, a bivariate chi-square analysis was conducted. **Table 3** shows that from the 43 cases of penile squamous cell carcinoma (PSCC) at Prof. dr. I.G.N.G. Ngoerah Hospital in Denpasar, there were 10 cases (23.3%) with positive p16 expression and abnormal p53 expression, 3 cases (6.9%) with positive p16 expression and normal p53 expression, 26 cases (60.5%) with negative p16 expression and abnormal p53 expression, and 4 cases (9.3%) with negative p16 expression and normal p53 expression. In the cases with positive p16 expression and abnormal p53 expression, 4 cases (40%) had positive lymph node metastasis and 6 cases (60%) had negative lymph node metastasis. In the cases with positive p16 expression and normal p53 expression, 1 case (33.3%) had positive lymph node metastasis and 2 cases (66.7%) had negative lymph node metastasis. In the cases with negative p16 expression and abnormal p53 expression, 7 cases (26.9%) had positive lymph node metastasis and 19 cases (73.1%) had negative lymph node metastasis. In the cases with negative p16 expression and normal p53 expression, 0 cases (0%) had positive lymph node metastasis and 4 cases (100%) had negative lymph node metastasis. The chi-square test analysis to assess the relationship between p16 and p53 protein expression and lymph node metastasis resulted in a p-value of 0.506, meaning there was no statistically significant relationship between p16 and p53 protein expression and lymph node metastasis.

Tabel 2. Correlation between p16 and p53 protein expression with Pathological T Stage (pT) at Prof. dr. I.G.N.G. Ngoerah Hospital

p16 and p53 Protein Expression	Stage		Total	p
	Early (pT1a, pT1b, pT2)	Late (pT3-pT4)		
p16 positive and p53 abnormal	5 (50%)	5 (50%)	10 (23,3%)	0,493
p16 positive and p53 normal	2 (66,7%)	1 (33,3%)	3 (6.9%)	
p16 negative and p53 abnormal	8 (30,8%)	18 (69,2%)	26 (60,5%)	
p16 negative and p53 normal	2 (50%)	2 (50%)	4 (9,3%)	

The significance level is set at p <0,05

Tabel 3. Correlation between p16 and p53 protein expression with Lymph Node Metastasis at Prof. dr. I.G.N.G. Ngoerah Hospital

p16 and p53 Protein Expression	Metastasis		Total	p
	Positive	Negative		
p16 positive and p53 abnormal	4 (40%)	6 (60%)	10 (23,3%)	0,506
p16 positive and p53 normal	1 (33,3%)	2 (66,7%)	3 (6.9%)	
p16 negative and p53 abnormal	7 (26,9%)	19 (73,1%)	26 (60,5%)	
p16 negative and p53 normal	0 (0%)	4 (100%)	4 (9,3%)	

The significance level is set at p <0,05

DISCUSSION

In this study, the results showed that the youngest patient was 32 years old and the oldest was 97 years old, with a median age of 59 years and a mean age of 60.09 ± 14.046 years. The age distribution of the cases in this study showed that the majority of cases were in the > 55 years age group, with 28 cases (65.1%). The age distribution of PSCC patients varied, but the majority were elderly. A review conducted by Thumma et al in 2024 found that the age range of PSCC patients was 20-90 years, with the most cases occurring in the 50-70 years age group.[13] A previous study by Lestari et al, conducted from 2004 to 2013, found the peak incidence occurred in the 6th and 7th decades of life.[1]

Most PSCC cases are found in older men, related to the long disease course which takes years to develop. The accumulation of risk factors over the years, such as chronic inflammation, lack of circumcision, HPV infection, and smoking, contributes to the higher incidence of PSCC in older age. These risk factors increase with age. Conditions like lichen sclerosus, which is common in older uncircumcised men, can be pre-cancerous and have a long-term relationship with the pathogenesis of PSCC. Poor hygiene also contributes to chronic inflammation. While HPV can be acquired at any age, its effects and the development of cancer take years to manifest. Another contributing factor is the delay in diagnosis. Patients may postpone seeking medical help due to embarrassment or lack of awareness of the symptoms, which can result in cancer being diagnosed at an advanced stage.[14]

This study found that based on the pathological T stage, most cases were in advanced stages, with 26 cases (60.5%). The distribution of penile squamous cell carcinoma (SCC) cases according to pathological T stage reflects the degree of invasion into surrounding tissues. These results differ from a study conducted by Mohanti et al, where 68% of cases were found in early stages (Mohanti et al., 2021), and a study by Trias et al., which found 80.3% of cases in early stages (Trias et al., 2024). However, this study is in line with a review conducted by Thumma et al., which found that in developing countries, the incidence of SCC cases in advanced stages was higher (Thumma et al., 2024). One of the main causes is the delay in diagnosis due to the lack of knowledge and patients' feelings of embarrassment.[14]

This study also found that based on lymph node metastasis, most cases did not show any lymph node metastasis, with 31 cases (72.1%). These findings are in line with a study by Mohanti et al, where 76.4% of cases did not show lymph node metastasis.[8] Lymph node metastasis is an important characteristic in penile squamous cell carcinoma, as it can affect the disease prognosis. According to Dorofte et al, most patients clinically did not show any lymph node enlargement, but up to 25% of those without clinically palpable inguinal lymph nodes had micrometastasis. Unfortunately, there is no radiological imaging method sensitive enough to detect micrometastasis.[15]

In this study, most cases exhibited abnormal p53 protein expression (83.7%). In the combination of p16 and p53 protein expression, the most common finding was negative p16 expression and abnormal p53 expression (60.5%). The finding of the most cases with abnormal p53 expression and the combination of negative p16 and abnormal p53 expression is consistent with studies by Mohanty et al in 2021 and Trias et al in 2024.

In this study, no statistically significant relationship was found between the expression of p16 and p53 proteins ($p = 0.493$) and pathological T stage (pT). These findings are in line with the study conducted by Trias et al. There are two possible explanations when the results of a study are not significant. The first possibility is that the null hypothesis is correct, meaning that in this study, there is no real relationship between the expression of p16 and p53 proteins and pathological T stage. The second possibility is that the proposed hypothesis in this study is indeed correct, but the existing data is not strong enough to support it. The second possibility could be because size being too small, meaning that there might be a relationship between the expression of p16 and p53 proteins and pathological T stage, but it is too small to detect. Another reason could be that the sample size is too small, meaning that the sample size in this study was not large enough to detect a relationship between p16 and p53 expression and pathological T stage.[16] Although no statistically significant relationship was found, this study is consistent with the research conducted by Trias et al in 2024, where in cases with negative p16 expression, advanced stages were more commonly found in cases with abnormal p53 expression.

Mutations in the p53 gene directly affect the stage of cancer, as this gene plays a crucial role as a tumor suppressor. When the p53 gene is mutated, cancer cells lose their regulatory mechanisms and gain new abilities that drive cancer growth to more advanced stages. Mutations in p53 not only halt its normal function but can also trigger cancer-promoting (gain of function) activities that accelerate tumor progression. The normal p53 protein works by halting the cell cycle when DNA damage occurs, providing time for repair or triggering programmed cell death (apoptosis). A mutated p53 results in the loss of this function, allowing damaged cells to proliferate uncontrollably and accelerate tumor growth. Several studies have shown that p53 mutations are often found in tumors at later stages. This suggests that p53 mutations may be an event driving the progression of tumors from early stages to more aggressive forms. The loss of p53 function leads to greater genetic instability, allowing additional mutations in other genes. The accumulation of these mutations drives cancer cells to become more malignant and harder to control.[17] High expression of p53 in penile SCC is associated with pathological T stage, including invasion into the spongiosum, corpus cavernosum, urethral invasion, and a lower survival rate.[18] The study conducted by Mustasam et al supports the understanding that penile cancer cases

not associated with HPV tend to exhibit more aggressive behavior.[19]

In this study, no statistically significant relationship was found between the expression of p16 and p53 proteins (p = 0.506) and metastasis to the inguinal lymph nodes (LN). The non-significant result may be due to a small sample size.[20] Although no statistically significant relationship was found, this study aligns with research by Mohanty et al, Trias et al, and Lopes et al, where metastasis to the LN was more frequently found in cases with abnormal p53 expression, regardless of whether p16 expression was positive or negative. This is consistent with previous studies that found that patients with abnormal p53 expression have a 4.8-fold higher risk of developing metastasis compared to patients with normal p53 expression. Lymphatic embolism by neoplastic cells and p53 mutations are single predictive factors for lymph node metastasis.[21]

Mutations in the p53 gene cause cancer cell invasion through various mechanisms, primarily through a phenomenon known as gain of function (GOF). Mutated p53 proteins not only lose their normal tumor-suppressing function but also acquire new oncogenic abilities that actively drive invasion and metastasis. Normal p53 actively inhibits epithelial–mesenchymal transition (EMT), a process in which tightly bound epithelial cells transform into migratory and invasive mesenchymal cells. Normal p53 achieves this by increasing the expression of E-cadherin (a protein that binds cells) and inhibiting transcription factors that trigger EMT. Mutated p53 reverses this mechanism, leading to increased transcription factors that directly activate EMT programs, resulting in more invasive and migratory cells.[22] The most common p53 mutation is a missense mutation, which produces a protein that not only fails to function but can also interact with p63 and p73. This interaction inhibits the tumor-suppressing functions of p63 and p73, ultimately promoting the invasive potential of cancer cells.[23]

CONCLUSION

The conclusion of this study is that there is no correlation between p16 and p53 expression with the pathological T stage (pT) in PSCC patients at Prof. dr. I.G.N.G. Ngoerah Hospital, Denpasar (p=0.493). Additionally, there is no correlation between p16 and p53 expression with lymph node metastasis in PSCC patients at the same hospital (p=0.506). Further studies with a larger sample size are highly recommended, as well as prospective studies evaluating other prognostic factors such as lymphovascular invasion, perineural invasion, and tumor-infiltrating lymphocytes.

CONFLICT OF INTEREST

There are no conflicts of interest in this study.

ETHICAL APPROVAL

This study has received approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University/ Prof. dr. I.G.N.G. Ngoerah Hospital with ethical clearance number 2202/UN14.2.2.VII.14/LT/2025 and has been granted permission by Prof. dr. I.G.N.G. Ngoerah Hospital with letter number DP.04.03/D.XVII.2.2.2/62941/2025.

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AUTHOR CONTRIBUTIONS

All authors have made equal contributions to the writing of this research, from the development of the conceptual framework, data collection, data analysis, to the interpretation of the research results.

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