Immunohistochemical Expression Profile of P16 In Cervical Carcinoma Patients at Siloam Mrccc Cancer Hospital In 2022

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Abstract
Cervical carcinoma is a rapidly growing health issue, with global cases reaching 662,301 and causing 348,874 deaths in 2022. Almost all cervical carcinoma cases are associated with persistent Human Papillomavirus (HPV) infection. The cell cycle-dependent kinase inhibitor (p16) serves as a reliable surrogate biomarker for HPV-associated cervical carcinoma. This descriptive observational study analyzes the dataset from the academic file "Ani Oranda Panjaitan_Fajar Lamhot Gultom.docx", which examines the immunohistochemical expression profile of the p16 protein in cervical carcinoma patients at Siloam MRCCC Cancer Hospital in 2022. Out of 42 archived cases, squamous cell carcinoma (SCC) was the dominant subtype with 34 cases (81%), followed by adenocarcinoma and adenosquamous carcinoma with four cases (9.5%) each. From the 32 patients who met the strict inclusion criteria for immunohistochemical staining, 30 patients (93.75%) demonstrated positive p16 expression, confirming a high prevalence of HPV-associated cervical carcinoma. Conversely, two patients (one SCC and one adenocarcinoma) showed negative p16 results. These findings indicate that over 93% of cervical carcinoma cases at this facility are directly associated with HPV, with SCC remaining the predominant subtype. Further research is needed to expand the sample size and integrate PCR HPV genotyping to monitor viral presence.

Keywords: *Cervical Carcinoma, HPV, p16, Immunohistochemistry and Squamous Cell Carcinoma.*

I. NTRODUCTION

Cervical carcinoma is a growing global health concern and is currently ranked as the fourth most common cancer among women worldwide. In 2022, approximately 660,000 new cases were reported globally, with nearly 350,000 deaths attributed to this disease. According to the 2022 GLOBOCAN data, cervical carcinoma is the second most common cancer among women and the third most common cancer across all genders in Indonesia. The country reported 36,964 new cases, representing 9% of all cancer cases, with a high mortality rate of 20,708 deaths (8.5%). Furthermore, a review of data collected from 2002 to 2019 at a hospital in Makassar showed that cervical carcinoma was the second most common cancer among women, accounting for 629 of 7,824 analyzed cases. Global projections estimate that the number of new cervical carcinoma cases will increase to 908,612 by 2045.

Cervical carcinoma is a malignant and infiltrative neoplasm arising from epithelial cells of the uterine cervix, with the potential to invade surrounding tissues and metastasize to distant organs. Based on histological characteristics and cellular origin, cervical carcinoma is classified into several subtypes. Squamous cell carcinoma (SCC) is the most common subtype, originating from the squamous epithelial cells of the ectocervix. Adenocarcinoma is the second most common subtype, arising from glandular epithelial cells of the endocervix. When both squamous and glandular components are present within the same tumor, the lesion is classified as mixed carcinoma or adenosquamous carcinoma.

High-risk HPV (HR-HPV) genotypes, particularly HPV16 and HPV18, are the most prevalent oncogenic types worldwide. However, the prevalence of other HR-HPV genotypes, including HPV51 and HPV52, has been increasing in Indonesia. Pathologists evaluate p16 protein expression through immunohistochemistry (IHC) as a biomarker to identify the association between HR-HPV infection and cervical carcinoma development.

The p16 protein, also known as a cyclin-dependent kinase inhibitor, plays an important role in cell cycle regulation. It functions by inhibiting cyclin-dependent kinase 4/6 (CDK4/6) activity, thereby maintaining the activity of retinoblastoma protein (pRb) and preventing uncontrolled cell proliferation. Persistent HR-HPV infection leads to the inactivation of tumor suppressor proteins, particularly pRb and p53, through viral oncoproteins. The loss of functional pRb results in compensatory overexpression of p16 protein through a negative feedback mechanism. Disruption of these regulatory pathways promotes uncontrolled cellular proliferation, which may ultimately contribute to cervical carcinoma development.

Given the established association between p16 protein overexpression and persistent high-risk HPV infection, this study investigates the immunohistochemical (IHC) expression profile of p16 protein in cervical carcinoma patients. Additionally, this study evaluates the incidence and distribution of cervical carcinoma cases diagnosed in 2022 at Siloam MRCCC Cancer Hospital. The findings of this study are expected to contribute to a better understanding of p16 protein expression in cervical malignancies and may support increased public awareness regarding the importance of cervical carcinoma prevention.

II. METHOD

Given the established association between p16 protein overexpression and persistent high-risk human papillomavirus (HR-HPV) infection, this study investigated p16 protein expression using an immunohistochemical (IHC) approach in cervical carcinoma specimens.

This study employed a retrospective descriptive observational design. Cervical carcinoma cases diagnosed in 2022 at the Anatomical Pathology Laboratory of Siloam

MRCCC Cancer Hospital were selected. Cases with available paraffin-embedded tissue blocks were included. The original diagnostic slides were reviewed microscopically to identify specimens containing adequate tumor cells for further analysis.

The selected tissue blocks were processed using standard histotechnological procedures, followed by p16 protein immunohistochemical staining. A total of 42 patient records were identified. Of these, 32 samples containing adequate tumor cells were included for p16 protein staining analysis. The remaining 10 samples were excluded due to insufficient tumor cell representation, carcinoma in situ diagnosis, or lymph node specimen preparation.

Histotechnology procedure:

- Paraffin blocks were sectioned using a microtome at a thickness of 4 µm.
- The slides were placed in a water bath at 45–55°C for 15–30 minutes.
- Tissue sections were mounted on poly-L-lysine-coated slides.
- Each slide was labeled with specific code for identification

Immunohistochemical staining procedure:

Most IHC staining procedures were performed using the BenchMark GX IHC staining system (Ventana, 816695, Germany), while several steps were performed manually.

The staining procedure was conducted as follows:

- Slides were dried at 72°C for 4 minutes.
- Tissue pretreatment was performed using Cell Conditioning Solution 1 (CC1) for approximately 60 minutes.
- The p16INK4a primary antibody (Biocare Medical) was manually applied to cover the entire tissue section, followed by incubation for approximately 60 minutes.
- The Ventana OptiView DAB IHC Detection Kit was applied, containing a secondary antibody conjugated with horseradish peroxidase (HRP).
- The Ventana OptiView Amplification Detection Kit was subsequently applied to enhance the HRP signal, followed by the addition of 3,3′-diaminobenzidine (DAB) chromogen, resulting in brown staining of p16-positive cells.
- Hematoxylin II (Ventana Medical Systems, Inc.) was manually applied as a counterstain for nuclear staining.
- Bluing reagent (Ventana Medical Systems, Inc.) was subsequently applied.

The processing time for one batch of slides was approximately 2–3 hours, and the entire staining procedure required 2 days. The final interpretation of the 32 stained slides was performed by the researcher using an Olympus CX23 light microscope (1L82711) equipped with an Indomicro digital camera (Microscope Digital Camera, HDMI, 21M0402).

A slide was considered positive for p16 protein expression when strong and diffuse immunoreactivity was observed in both the cytoplasm and nuclei of tumor cells.

Positive staining was defined as continuous block staining involving a contiguous segment of tumor cells, with at least 10–20 consecutive positive cells. In squamous epithelial lesions, p16 block staining was considered positive when it involved the parabasal and basal cell layers.

III. RESULTS

Among the 42 slides examined, three histological types of cervical carcinoma were identified: cervical SCC in 34 cases (81%), cervical adenocarcinoma in four cases (9.5%), and cervical adenosquamous carcinoma in four cases (9.5%).

Tabel 1 Percentage Distribution of Cervical Carcinoma Subtypes in MRCCC Cancer Hospital

Hospital	**Squamous Cell Carcinoma (%)**	**Adenocarcinoma (%)**	**Adenosquamous Carcinoma (%)**
Siloam MRCCC Cancer Hospital (2022)	81%	9.5%	9.5%

Based on the established criteria, among the 32 slides stained for p16 protein expression, 30 cases were classified as positive and two cases were classified as negative. The two negative cases consisted of one cervical squamous cell carcinoma (SCC) specimen and one cervical adenocarcinoma speciments.

Fig. 1. (A) Cervical SCC in H&E staining at 100x magnification. (B) Cervical SCC showing negative p16 IHC staining at 100x magnification.

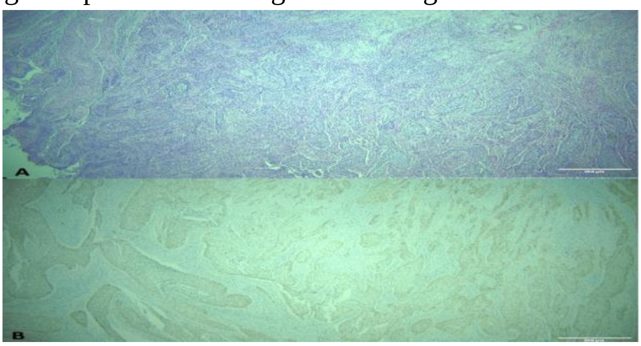


Fig. 2. (C) Cervical adenocarcinoma in H&E staining at 100× magnification. (D) Cervical adenocarcinoma showing positive p16 IHC staining at 100x magnification.

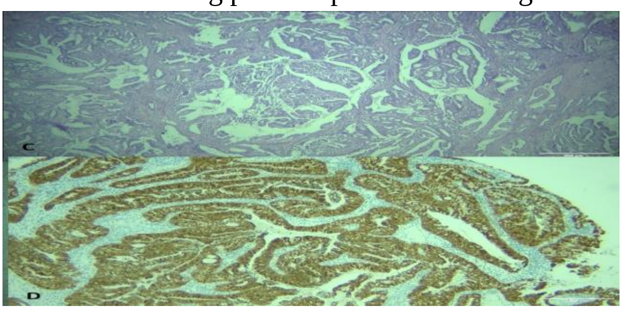
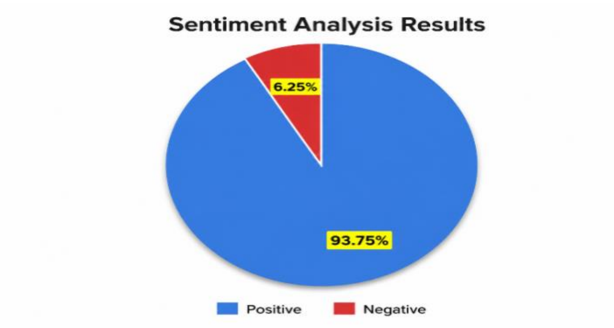


Fig. 3. Diagram of tp16 Protein Staining in Cervical Carcinoma at Siloam MRCCC Cancer Hospital in 2022



IV. DISCUSSION

According to the 2020 WHO Classification of Tumours Editorial Board, SCC represents the most common histological subtype cervical carcinoma, accounting for approximately 80–90% of cases, followed by adenocarcinoma (approximately 5%) and adenosquamous carcinoma (5–6%). These proportions are consistent with the findings of the present study, in which SCC was identified as the predominant cervical carcinoma subtype, representing 81% of cases at Siloam MRCCC Cancer Hospital in 2022.

A previous study conducted at Dr. Cipto Mangunkusumo Hospital, Jakarta, analyzed 678 cervical carcinoma cases diagnosed in 2013 and reported SCC as the most frequent subtype (74.2%), followed by adenocarcinoma (14%) and adenosquamous carcinoma (6.8%). The predominance of SCC observed in that study is comparable with the current findings, supporting the consistent observation that SCC remains the major histological subtype of cervical carcinoma. However, differences were observed in the distribution of adenocarcinoma and adenosquamous carcinoma. The current study demonstrated an equal proportion of adenocarcinoma and adenosquamous carcinoma (9.5% each), whereas the Dr. Cipto Mangunkusumo Hospital study reported a higher proportion of adenocarcinoma.

Similarly, a study conducted at Dr. Soedarso Regional Hospital, Pontianak, evaluating cervical carcinoma cases from 2017 to 2019, reported SCC as the dominant subtype (70%), followed by adenocarcinoma (23%) and adenosquamous carcinoma (7%). Although the proportion of SCC remained comparable with the present study, the distribution of adenocarcinoma was higher in the Pontianak study. Variations in the proportion of cervical carcinoma subtypes among different institutions may be influenced by several factors, including differences in study populations, referral patterns, geographical variation, sample size, and demographic characteristics of patients.

Tabel 2. Percentage Distribution of Cervical Carcinoma Subtypes

Hospital	Squamous Cell Carcinoma (%)	Adenocarcinoma (%)	Adenosquamous Carcinoma (%)
Siloam MRCCC Cancer Hospital (2022)	81%	9.5%	9.5%
WHO	80-90%	5%	5-6%
Dr. Cipto Mangunkusumo Hospital (2013)	74.2%	14%	6.8%
Dr. Soedarso Pontianak Regional Hospital (2017–2019)	70%	23%	7%

The high proportion of p16-positive cases observed in this study supports the established role of p16 as a surrogate biomarker for high-risk human papillomavirus (HR-HPV)-associated cervical carcinogenesis. Diffuse and strong p16 immunoreactivity reflects the molecular consequences of persistent HR-HPV infection, particularly through disruption of the retinoblastoma (pRB) pathway by the viral E7 oncoprotein.

Under normal physiological conditions, the pRB protein regulates cell cycle progression by controlling the activity of E2F transcription factors. In HR-HPV-associated carcinogenesis, the E7 oncoprotein binds to and inactivates pRB, resulting in uncontrolled cell cycle progression. This mechanism triggers compensatory overexpression of p16INK4a protein, leading to the characteristic diffuse and strong block staining pattern observed in immunohistochemical examination. Therefore, p16 positivity in cervical carcinoma is considered an indicator of HPV-driven oncogenic transformation.

The high frequency of p16 positivity in this study is consistent with previous studies reporting strong p16 expression in the majority of cervical carcinomas, particularly in HPV-associated squamous cell carcinoma. The predominance of p16 expression further supports the concept that persistent HR-HPV infection represents the primary etiological factor in cervical carcinoma development. The findings also demonstrate the clinical utility of p16 immunohistochemistry as a practical biomarker, especially in settings where molecular HPV testing is unavailable or not routinely performed.

However, two cases in this study showed negative p16 expression, consisting of one cervical squamous cell carcinoma and one cervical adenocarcinoma case. Negative p16 staining in cervical carcinoma may occur due to several possible mechanisms. Although most cervical carcinomas are associated with HR-HPV infection, a subset of tumors may develop through alternative molecular pathways, resulting in absent or non-classical p16 expression patterns. In addition, technical factors, differences in antigen preservation, tumor heterogeneity, or variations in HPV genotype may influence p16 immunohistochemical results.

The negative p16 expression observed in one cervical adenocarcinoma case may reflect biological heterogeneity among glandular cervical carcinomas. Although HPV-associated adenocarcinomas commonly demonstrate p16 overexpression, some cases may show variable expression depending on their molecular characteristics and underlying pathogenesis. Similarly, the negative SCC case suggests that not all squamous carcinomas exhibit a typical HPV-associated molecular profile, emphasizing that p16 interpretation should be integrated with morphological evaluation and, when available, HPV molecular testing.

Overall, the findings of this study demonstrate that p16 immunohistochemistry shows a high positivity rate among cervical carcinoma cases at Siloam MRCCC Cancer Hospital. The results support the application of p16 as an important biomarker for identifying HPV-associated cervical carcinoma and highlight its value as an adjunctive diagnostic tool in routine histopathological evaluation.

V. CONCLUSION

This study demonstrated that squamous cell carcinoma (SCC) was the predominant histological subtype of cervical carcinoma among patients at Siloam MRCCC Cancer Hospital in 2022, consistent with the distribution reported in previous studies and the 2020 WHO Classification of Tumours.

Immunohistochemical evaluation showed a high proportion of p16 protein expression, These findings support the role of p16 as a surrogate biomarker for high-risk human papillomavirus (HR-HPV)-associated cervical carcinogenesis.

The results highlight the clinical utility of p16 immunohistochemistry as an adjunctive diagnostic tool in cervical carcinoma evaluation, particularly in identifying HPV-associated tumors through characteristic diffuse and strong block staining patterns. Although most cases showed p16 positivity, the presence of negative cases emphasizes the importance of integrating p16 immunohistochemical interpretation with histopathological assessment and, when available, HPV molecular testing for more comprehensive tumor classification.

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