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Correlation Ki67 Expression with Peritumoral Budding Tumor Index in the Case of Squamous Cell Carcinoma Cervix NOS and Adenocarcinoma Cervix NOS

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ABSTRACT

Background
Cervical cancer is a neoplasm in the cervix due to abnormal cells that damage the surrounding tissue. Cervical cancer from squamous cells is called squamous cell carcinoma cervix NOS and that from glands is called adenocarcinoma cervix NOS. The prognostic of both can be assessed by tumor budding and Ki67 immunohistochemistry, tumor budding is a tumor bud which is ≤ 5 cells in a cluster that grows in front of the parent tumor and immunohistochemistry Ki67 is a labile nonhistone nuclear protein which is expressed in the G1, S, G2 and M phases of the cycle cell.

Method
This research is an analytical type of research that aims to find out the relationship between Ki67 expression and the peritumoral budding tumor index compared to cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS with 36 paraffin block samples diagnosed with squamous cell carcinoma NOS and adenocarcinoma NOS at HAM Hospital. Medan. Assessment method of Ki67 expression where low expression (<20%) and high expression (≥20%). Assessment of peritumoral budding tumors in both cases was said to be low buds if < 5 buds and high buds if ≥ 5 buds.

Results
This study provides a significant appearance (p-value 0.0001) which means that peritumoral budding tumors with HE staining can be accepted as a measuring tool for assessing cervical cancer in addition to assessment using immunohistochemistry Ki67 which is generally used in the prognostic assessment of cervical cancer.

Conclusion
Assessment in this case can consider peritumoral budding tumor assessment to determine prognostic in cases of squamous cell carcinoma NOS and cervical adenocarcinoma NOS, if Ki67 immunohistochemistry is not available.

Keywords: Squamous Cell Carcinoma Cervix NOS, Adenocarcinoma cervix NOS, Peritumoral budding tumor, Immunohistochemistry Ki67

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INTRODUCTION

Cervical cancer is one of the malignancies of the cervical epithelium which is the cause of death in women worldwide. This disease ranks fourth most frequently appearing in women. According to Global Cancer Statistics 2020 data, there are an estimated 604,000 new cases and 342,000 deaths worldwide. 1 Cervical cancer is a neoplasm found in the cervical organs that has abnormal cell growth that can damage surrounding tissue. Cervical cancer that originates from squamous cells is called squamous cell carcinoma cervix NOS and that originates from glands is called adenocarcinoma cervix NOS.[2] The prognostic of these two cervical cancers provides a different picture, where the prognostic of these two cancers can be assessed using immunohistochemical staining Ki67 for the proliferation index of tumor cells present in these two cases.[3] However, in several previous studies, such as the study conducted by Almangush, et al., who assessed tumor budding as an independent prognostic assessment of cervical cancer. Therefore, the authors are very interested in conducting further research by assessing peritumoral budding tumors in these two cervical cancers and comparing them with Ki67 immunohistochemical staining. The objective of this study is that tumor budding can be used as an independent prognostic determinant if Ki67 immunohistochemistry is not available.

Budding tumors are tumor buds with less than 5 cells in a cluster in front of the parent tumor. Budding tumors can grow peritumorally and intratumorally. Peritumoral budding tumors are tumor buds consisting of ≤ 5 cells in front of invasive tumors, while intratumoral budding tumors are tumor buds that grow between tumor cells.[3,4]

The general definition of this Ki67 immunohistochemical stain is a nuclear labile nonhistone protein that is expressed in the G1, S, G2 and M phases of the cell cycle, then is rapidly catabolized in late M phase and is undetectable in early G0 and G1 cells. Immunohistochemical staining of Ki67 will stain the nucleus in a cell. This can be used for the assessment of malignancy in various tumors.[5]

METHOD

This research is an analytical study with a cross sectional approach. The study was conducted at Haji Adam Malik General Hospital Center (RSUP) Medan from June 2022 to February 2023. The population for this study was all secondary data and paraffin blocks from patients who had been diagnosed histopathologically as squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS. who met the inclusion and exclusion criteria in the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of Haji Adam Malik General Hospital, Medan. The sample size in this study was calculated by looking at the proportion of incidence of cervical cancer cases in Indonesia that were found in cases of cervical cancer. The sample of cervical cancer cases was sourced from the Ministry of Health and Republic of Indonesia data and information center in 2018 of 10.69%. a sample of 18 cases, this variable can be referred to as the independent variable. Then peritumoral budding tumors used HE staining with a total sample of 18 cases in cases of squamous cell carcinoma cervix NOS and cervical adenocarcinoma NOS with a sample size of 18 cases, this variable can be referred to as the dependent variable.[6]

The tumor budding assessment refers to a study conducted by Almangush, et al., with conditions at 200x magnification. This research was carried out manually using an Olympus CX23 microscope and with one researcher and two anatomical pathologists. This study also uses a two-tier system as a cut-off in assessing tumor budding criteria, namely it is said to be low buds if < 5 buds/5 HPF and said to be high buds if ≥ 5 buds/5 HPF using HE staining. Then the assessment of Ki67 immunohistochemical staining can be stained in the cell nucleus with 400x magnification. In both cases of cervical cancer, according to the WHO 5th edition, breasts with cut off were divided into 2 provisions, it was said to be low expression if the Ki67 immunohistochemistry was positively stained in the nucleus <20% and it was said to be high expression if the Ki67 immunohistochemistry was positively stained in the nucleus ≥ 20%.[7,8]

Data were analyzed using statistical software which is useful for assessing the association of Ki67 expression with peritumoral budding tumors in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS. This study used the Chi-Square test and Fisher's Exact test. The p-value <0.05 was stated to be statistically significant.

RESULT

Of the 42 samples with hysterectomy and biopsy procedures in this study, who were diagnosed with squamous cell carcinoma cervix NOS and cervical adenocarcinoma NOS from

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2017 to 2020 at Haji Adam Malik General Hospital Medan, out of 42 samples only 36 samples met the inclusion criteria.

Table 1. Characteristics of the study sample based on the distribution of Ki67 expression from cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS with peritumoral budding tumor distribution from cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS.

Characteristic	Total (n=36)	Percentage
Ki67 expression		
Low expression	19	52.8
High expression	17	47.2
Tumor Budding		
Low buds	19	52.8
High buds	17	47.2

Table 1. In this study, shows the two cases consisting of cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS with Ki67 expression divided into low expression and high expression, where low expression has a total of 19 cases (52.8%) and high expression has a total of 17 cases (47.2%).

This study also showed that peritumoral budding tumors were seen in both cases, namely cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS with low buds with a total of 19 cases (52.8%). Then the high buds with a total of 17 cases (47,2%).

Table 2. Correlation between *squamous cell carcinoma cerviks NOS* case and *adenocarcinoma cerviks NOS* with *Ki67* expression.

Table 2. Relationship between Ki67 expression in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS.

Diagnosis	Ki67 Expression				P-value
	Low Expression		High Expression		
	n	%	n	%	
SCC NOS (n=18)	10	55.6	8	44.4	0.738
Adenocarcinoma NOS (n=18)	9	50.0	9	50.6	
Total	19		17		

*Chi Square Test

Table 2. This study shows that there is no significant relationship between Ki67 expression in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS (with a value (p-value 0.738)). In cases of squamous cell carcinoma cervix NOS with low expression of Ki67 there were 10 cases (55.6%), high expression of Ki67 in 8 cases (44.4%). For cases of cervical NOS adenocarcinoma with low Ki67 expression there were 9 cases (50%), high grade Ki67 expression there were 9 cases (50.6%).

Table 3. Correlation between squamous cell carcinoma cerviks NOS case and adenocarcinoma cerviks NOS with tumor budding peritumoral.

Diagnosis	Tumor Budding				p-value*
	Low Buds		High Buds		
	n	%	n	%	
SCC NOS (n=18)	10	55.6	8	44.4	0.738
Adenocarcinoma NOS (n=18)	9	50.0	9	50.6	
Total	19		17		

*Chi Square Test

Table 3. This study shows that there is no significant relationship between peritumoral budding tumors in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS (with a p-value of 0.738). In the case of squamous cell carcinoma cervix NOS, there were 10 cases (55.6%) of low peritumoral budding tumors, 8 cases (44.4%) of high peritumoral buds tumors. In cases of adenocarcinoma of the cervix NOS with peritumoral low buds budding tumors there were 9 cases (50%), high buds peritumoral budding tumors there were 9 cases (50.6%).

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Table 4. Correlation between Ki67 expression with budding peritumoral tumor index in cases of cervical squamous cell carcinoma NOS and cervical adenocarcinoma NOS.

Ki67 Expression	Tumor Budding				p-value*
	Low Buds		High Buds		
	n	%	n	%	
Low Expression	n	100	0	0	0,0001
High Expression	10	0	8	100	
Total	0		8		

Table 4. This study provides a significant and interconnected picture of Ki67 expression and peritumoral budding tumors in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS (with a p-value of 0.0001). Both cases had 19 samples with low expression Ki67 having low grade tumor budding, similarly 17 samples with high expression Ki67 having high grade tumor budding. Based on this study, it can be concluded that the lower the expression of Ki67, the lower the grade of tumor budding and conversely, the higher the expression of Ki67, the higher the grade of tumor budding in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS.

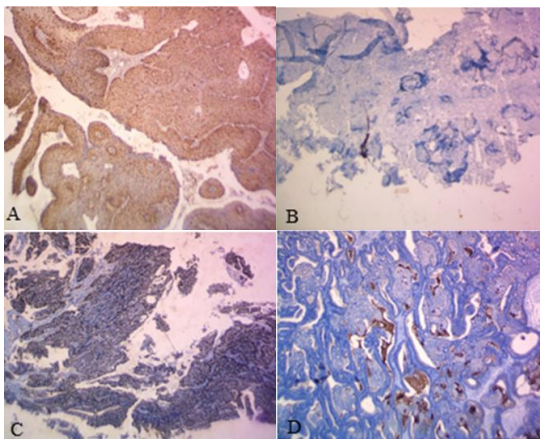

Figure 1. A. High expression of Ki67 squamous cell carcinoma cervix NOS case. B. Low expression Ki67 squamous cell carcinoma cervix NOS case. C. High expression Ki67 adenocarcinoma cervix NOS case. D. Low expression Ki67 adenocarcinoma cervix NOS case.

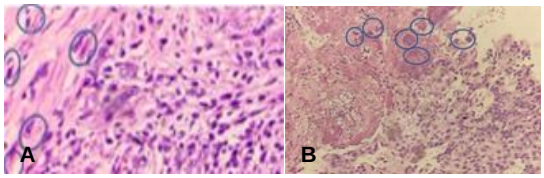

Figure 2. A. Budding peritumoral tumoron kasus squamous cell carcinoma cervix NOS case. B. Budding peritumoral tumor on kasus adenocarcinoma cervix NOS case.

DISCUSSION

In this study there was no significant relationship between Ki67 expression and cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS (p-value 0.738), which is in line with research conducted by Cambruzzi, et al., which linked Ki67 expression with SIL (squamous intraepithelial lesion) in the cervical organ where the relationship between the two is insignificant and not related to each other. 9 There was no significant relationship between peritumoral budding tumors and cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS (p-value 0.738), which is in line with research conducted by Koelzer, et al., in 2015, who tried linking peritumoral budding tumors with colorectal adenocarcinoma. This can be caused because each is not related to each other, the expression of Ki67 assesses the proliferation index of a tumor cell growth which indicates an assessment of a prognosis, while squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS is a malignancy of the epithelium in the cervical organ where the main cause is infection with high risk HPV (HPV 18) and low risk HPV (HPV 16).[10,11]

This study discusses the significant and interconnected features of Ki67 expression and peritumoral budding tumors in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS. This is in line with a study conducted by Piederson, et al., who tried to link Ki67 expression with tumor budding in cases of oral squamous cell carcinoma, which in this study showed a significant and interrelated relationship between Ki67 expression and tumor budding.[12] Expression Ki67 is an immunohistochemical stain that stains in the cytoplasm of cells and can be an assessment of the proliferation index of a neoplasm which is closely related to assessing the prognosis, this is closely related to the prognostic assessment of the presence of tumor budding which can be an assessment of the malignancy of a neoplasm, where there are more and more tumor budding the worse the prognosis as well as the expression of Ki67, the more it is expressed in a case, the worse the prognosis of that case will be.[13]

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This study discusses the significant and interconnected features of Ki67 expression and peritumoral budding tumors in cases of squamous cell carcinoma cervix NOS and adenocarcinoma cervix NOS (with a p-value of 0.0001). This research is in line with research conducted by Zheng, et al., in China in 2022, where researchers tried to link tumor budding with Ki67 expression in cases of squamous cell carcinoma of the cervix, both of which are interconnected and related to one another. Ki67 is a marker of proliferation and is known as a predictive factor in tumor growth. In principle, the more malignant a tumor, the higher the expression of Ki67, so that tumors that show a higher expression of Ki67 will have a worse prognosis when compared to tumors that show a lower expression of Ki67. can be one of the enforcement of prognostic factors based on the number of tumor buds that grow in front of an invasive tumor of less than 5 cells in 1 cluster, so if there are low budding tumors then the prognosis of both cases of cervical carcinoma will be better but vice versa if there is a tumor budding with high buds, the prognosis will be worse. [1,4,15]

The limitations in this study are in terms of assessing the tumor budding focus caused by the tumor cells being detached from their stem cells resembling fibroblast cells and inflammatory cells scattered in the stromal area, therefore additional examinations are needed in examining this tumor budding, so it is highly recommended to use pancytokeratin immunohistochemical examination to make it easier to differentiate between epithelium and fibroblast cells and inflammatory cells scattered in the stromal area.

CONCLUSION

The relationship between Ki67 expression and peritumoral budding tumors in the case of squamous cell carcinoma cervix NOS and cervical adenocarcinoma NOS has the assessment that the lower the expression of Ki67 is in line with the tumor budding low buds with the interpretation of having a good prognosis, and vice versa the high expression of Ki67 is in line with tumors budding high buds with interpretation has a poor prognosis in both cases of cervical carcinoma.

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