

Correlation Index Peritumoral Budding Tumour with Grading Histopathology Cervical Squamous Cell Carcinoma NOS

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Abstract

Background: Cervical squamous cell carcinoma NOS cervix It is an invasive epithelial tumor with varying degrees of differentiation. The prognostic grading system based on WHO (2020) is assessed from nuclear pleomorphism, mitosis, tumor cell invasion, keratin, and necrosis. Tumor budding is an isolated tumor cell bud 5 cells.¹ This tumor budding phenomenon is considered the histological basis for the formation of metastases and further tumor invasion which is an independent prognostic, especially in cervical squamous cell carcinoma NOS.¹⁵

Research purposes: Analyze the relationship between tumor index peritumoral budding with grading histopathology in patients squamous cell carcinoma NOS cervix.

Materials and Methods: This research is an analytic research approach cross-sectional in 36 samples of paraffin blocks of patients diagnosed histopathologically with Cervical SCC. Then, the paraffin block was re-cut, followed by staining slides with H&E. Conducted an assessment of peritumoral budding tumor categorized as no buds (low grade) and 15buds (intermediate grade), also 15buds (high grade), rating relationship peritumoral budding tumor with grading histopathology squamous cell carcinoma NOS cervix as a prognostic independent on Cervical SCC statistically tested.

Results: Most were in the age group >51 years and a few were aged 31-35 years. Most parity 2x parturition, and most non-keratinizing squamous cell carcinoma compared keratinizing squamous cell carcinoma, the correlation between grading histopathology squamous cell carcinoma NOS cervix with age, there was no significant relationship. Relationship between grade with parity constant and unrelated.

Conclusion: Significant relationship between tumor index peritumoral budding with grading histopathology in cases squamous cell carcinoma NOS cervix

Keywords: tumor budding, peritumoral, grading histopathology, Cervical SCC

INTRODUCTION

Squamous cell carcinoma NOS cervix is a tumor invasive epithelium consisting of squamous cells with varying degrees of differentiation. Squamous cell carcinoma NOS cervix may be exophytic, papillary/polypoid, and endophytes that infiltrate the surrounding structures. Endophytic tumors are lined by normal epithelium or tumors located around the cervical canal.¹ Cervical carcinoma is the most common type of cancer worldwide. According to data from the Global Cancer Observatory (GLOBOCAN) in 2020, cervical carcinoma ranks fourth in the world and the leading cause of fourth cancer death in women. It can be estimated that 604,000 new cases and 342,000 deaths worldwide.² In Indonesia, cervical carcinoma is the second most common type of cancer in women with a total of 36,633 cases (9.2%) and a death count of 21,003 cases (9%).² Cervical carcinoma is ranked first with a ratio of 8/10,000 population. The estimated absolute number in North Sumatra is 4694 cases.³ Research conducted at Haji Adam Malik General Hospital Medan in 2016-2017, the number of patients with cervical carcinoma was found to be 262 people, of which the most patients were aged > 50 years (47.3%), followed by

patients aged 46-50 years (22.1%), 41-45 years (13.7%), 36-40 years (9.5%), 31-35 years (4.2%), and the least is patients with age <30 years (3.1%).⁴ The cause of cervical carcinoma is associated with the presence of infection human papillomavirus (HPV) type high risk (HR) persistent. HPV Types 16 and 18 are the main etiology of cervical carcinoma. In general, cervical carcinoma begins with infection HPV which then on the oncoprotein E6 or E7 provide changes in the maturation and differentiation of the cervical epithelium, giving rise to cervical carcinoma in women.⁵ Based on histopathological classification WHO 2020, this cervical squamous cell carcinoma can be divided into cervical squamous cell carcinoma (HPV) associated, cervical squamous cell carcinoma HPV independent and cervical squamous cell carcinoma HPV NOS.^{2,5}

Prognostic assessment of squamous cell carcinoma NOS cervix can be seen on grading system based on WHO by 2020 in assessing survival rates. Squamous cell carcinoma NOS cervix has several parameters in the assessment grading system. It can be in the form of growth pattern, differentiation, nuclear pleomorphism, mitosis, pattern, and depth of invasion of tumor cells in the stroma, and areas of necrosis.^{2,6}

Grading histopathology on squamous cell carcinoma NOS cervix can be divided by WHO female 2020, where grade 1 can be called well differentiated, grade 2 called moderately differentiated, grade 3 called poorly differentiated.^{7,8} Budding tumours a histopathological picture that can be identified using routine pathological examination. Budding tumor defined as a single cell or group of cells consisting of < 5 cells, which are located on the edge of invasive tumor front. This picture is found in 3 different types of malignancy which gives an overview of finger-like projections referred to as buds which can enter the surrounding tissue. During localized cancer growth, some of these clusters of cells detach from the main tumor body and invade the surrounding stroma. This phenomenon is considered to be the histological basis for the formation of metastases and further.^{9,10} Biological phenomenon budding tumour. the growth of tumor cell buds in front of invasive tumors has been described in various human tumors. Budding tumor have predictive and prognostic relevance. The phenomenon budding tumor until now is still not clear.¹¹ Therefore, researchers are interested in researching the assessment of budding tumor peritumoral as an independent prognostic indicator with grading on squamous cell carcinoma NOS cervix which can be used as information to clinicians in terms of assessing the aggressiveness of the carcinoma, without having to incur additional costs in terms of histopathological examination of malignancy in squamous cell carcinoma NOS cervix this.¹¹

MATERIAL AND METHODS

Analytical research with approach cross-sectional. This study

was conducted at the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra, Medan, and the Anatomic Pathology Unit, H. Adam Malik Hospital, Medan. This research was conducted from March 2021 to April 2022, after obtaining approval from the Health Research Ethics Committee, Faculty of Medicine, University of North Sumatra. The sample of this study was a paraffin block from patients who had been diagnosed histopathologically with *squamous cell carcinoma NOS cervix* that meets the inclusion and exclusion criteria. Samples were taken using *consecutive sampling*. Inclusion criteria included adequate clinical data in the medical record (includes age and number of parity) as well as preparations *slides* or representative paraffin block, derived from hysterectomy tissue results *TAH BSO* diagnosed histopathologically as squamous cell carcinoma *NOS cervix* with colored *H&E*. The exclusion criteria for this study were *slides* or paraffin blocks recorded according to data storage devices are not representative so they cannot be reprocessed and preparations *slides* or paraffin blocks derived from excisional biopsy tissue results. Each sample was recut and stained with *H&E*. The peritumoral budding tumor is the growth of cell shoots tumor in front of invasive tumor on histopathological preparations without ward appearance *H&E*. Evaluation budding tumor refers to a study that has been conducted by McCluggage, et al., in which the number of tumor buds was categorized as follows, if no buds (low-grade buds) and 15 buds (intermediate grade), also 15 buds (high grade).¹²

RESULT

This study, obtained 36 samples of cases of squamous cell carcinoma *NOS cervix* were conducted at the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra, and the Anatomic Pathology Unit at H. Adam Malik Hospital, Medan. Most age >51 years, with the least age 31-35 years. And with the highest number of parity 2x parturition (**Table 1**).

Table 1. Characteristics of the frequency distribution of the study sample by age, and the number of parity in patients with squamous cell carcinoma *NOS cervix*

Variable	f	%
age		
31-35 year	2	5.6
36-40 year	4	11.1
41-45 year	4	11.1
46-50 year	10	27.8
>51 year	16	44.4
Parity		
<2x partus	0	0.0
≥2x partus	36	100.0
Peritumoral budding tumour		
Low Grade	0	0.0
Intermediate Grade (<=15 buds)	19	52.8
High Grade (>15 buds)	17	47.2
Grade		
Grade 1	2	5.6
Grade 2	17	47.2
Grade 3	17	47.2

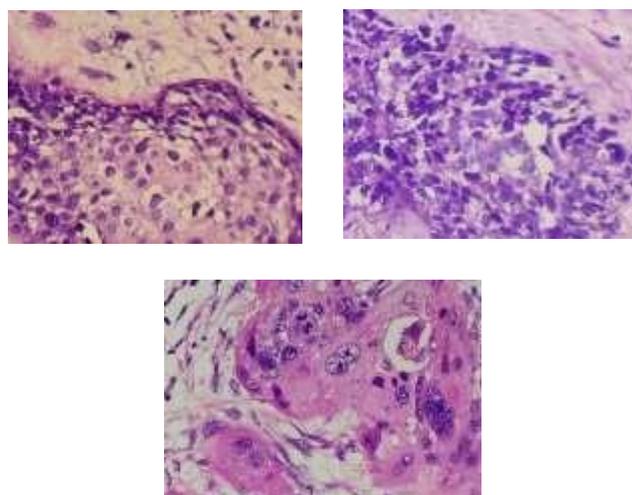


Figure 1. Low grade buds (H&E, 200X). B. Intermediate grade buds (H&E, 200X). C. High grade buds (H&E, 200X)

In this study, the relationship between peritumoral budding tumor with histopathological grading in patients with squamous cell carcinoma *NOS cervix*. Peritumoral budding tumor most often found highgrade budding by 60.7%. The research shows a significant relationship between peritumoral budding tumor with grading histopathology in patients squamous cell carcinoma *NOS cervix* (p-value=0.0001) with a prediction that the higher high grade peritumoral budding tumor then it will be higher grading histopathology in patients squamous cell carcinoma *NOS cervix* compared low grade peritumoral budding tumor (PR=10.35) (**Table 2**). This study obtained 36 samples of cases of *squamous cell carcinoma NOS cervix* were conducted at the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra, and the Anatomic Pathology Unit at H. Adam Malik Hospital, Medan. Most age >51 years, with the least age 31-35 years. And with the highest number of parity 2x parturition.

This study shows the relationship between grade and age, where the relationship between the two is not significant, this can be seen from the results of the correlation analysis test between grade and age in cervical squamous cell carcinoma *NOS* patients (**Table 3**). The results of statistical test analysis showed that there was an insignificant relationship (p = 0.138).

This study shows the relationship between grade and parity (Table 4). Where the relationship between the two cannot be assessed, this can be seen from the results of the correlation analysis test between grade and parity in patients with cervical squamous cell carcinoma NOS. The results of statistical test analysis showed that the relationship between the two could not be assessed. The correlation between grade and parity in squamous cell carcinoma NOS cervix could not be assessed or run constant on the results of the analysis test.

squamous cell carcinoma as many as 116 cases in RSUP. H.

Table 3. The relationship between grade and age in patients with squamous cell carcinoma NOS cervix.

Variable	Age Category					Total f (%)	value	p*
	31-35 f (%)	36-40 f (%)	41-45 f (%)	46-50 f (%)	>51 f (%)			
Grade 1	0 (0,0)	0 (0,0)	0 (0,0)	2 (5,6)	0 (0,0)	2 (5,6)	6213	0,138
Grade 2	1 (2,8)	4 (11,1)	2 (5,6)	3 (8,3)	7 (19,4)	17 (47,2)		
Grade 3	1 (2,8)	0 (0,0)	2 (5,6)	5 (13,9)	8 (22,0)	17 (47,2)		
Total	2 (5,6)	4 (11,1)	4 (11,1)	10 (27,8)	16 (44,4)	36 (100,0)		

Table 4. The relationship between grade and parity in patients with squamous cell carcinoma NOS cervix.

Variable	Total Parity		Total f (%)	p*
	<2x parity f (%)	≥2x parity f (%)		
Grade 1	0 (0,0)	2 (5,6)	2 (5,6)	No value
Grade 2	0 (0,0)	17 (47,2)	17 (47,2)	
Grade 3	0 (0,0)	17 (47,2)	17 (47,2)	
Total	0 (0,0)	36 (100,0)	36 (100,0)	

DISCUSSION

The number of samples diagnosed as cervical squamous cell carcinoma NOS in this study was 36 samples, of which the most occurred at the age of >51 years as many as 16 cases, and patients with cervical squamous cell carcinoma at the age of 31-35 years at least 2 cases. The results of this study are not much different from previous studies. These results are in line with research conducted at Haji Adam Malik General Hospital Medan in 2016-2017 by Wardatan, *et al.*, where the number of patients with cervical carcinoma was found to be 262 people, then most patients were found to be >50 years old. Old people prove that because of the lack of screening facilities such as pap smears and the lack of public education about malignancy in women.^{4,13}

In this study, the results obtained in patients suffering from cervical squamous cell carcinoma NOS was based on histopathology, the highest histopathology were patients with non-keratinizing squamous cell carcinoma, which were 33 cases. This study is in line with the study of Leli, *et al.*, in 2010 which assessed the distribution of sufferers.¹²

Cervical carcinoma is based on histopathology, where the most common histopathology found was non-keratinizing

HPF there are 47 cases out of 125 with 49 cases intermediate

Table 2. The relationship between peritumoral tumor budding with histopathological grading in patients with squamous cell carcinoma NOS cervix.

Variable	Budding Tumor Peritumoral			Total f(%)	p
	Low f(%)	Intermediate f(%)	High		
Grade 1	0 (0,0)	2 (5,6)	0 (0,0)	2 (5,6)	0,0001
Grade 2	0 (0,0)	17 (47,2)	0 (0,0)	17 (47,2)	
Grade 3	0 (0,0)	0 (0,0)	17 (47,2)	17 (47,2)	
Total	0 (0,0)	19 (52,8)	17 (47,2)	36 (100,0)	

Adam Malik Medan.¹³ In the study of Wardatan, *et al.*, in 2014-2016 the same results were obtained with this study, namely the histopathological features of non-keratinizing squamous cell carcinoma were found, the most cases of cervical carcinoma as many as 142 people in RSUP. Haji Adam Malik Medan. The number of cases of non-keratinizing squamous cell carcinoma found based on the literature shows a malignant differentiation in cervical squamous cell carcinoma NOS and this possibility can be associated with RSUP. Haji Adam Malik Medan, which is one of the referral centers in North Sumatra, so many cases of cervical carcinoma malignancy are found, one of which is cervical squamous cell carcinoma with histopathological features of non-keratinizing squamous cell carcinoma.^{4,14}

This study on cervical squamous cell carcinoma NOS, also assessed the frequency of distribution based on the number of parities that can be found in RSUP. H. Adam Malik Medan with parity <2x parturition could not be found then parity number 2x parturition with a total of 36 cases (100%). According to research conducted by Vijendran (2008-2009) at Haji Adam Malik Hospital in Medan with the most parity history results obtained >3x parturition as many as 52-56 people between 2008- 2009.¹⁵ The same results were also obtained in Setyarini's research (2009) at Dr. Moewardi Surakarta with the highest number of cervical cancer patients with parity > 3, which was 22 cases.¹⁴ This difference is due to trauma during pregnancy and childbirth in women with higher parity rates and is closely related to the maturation level of the t-zone area and changes occur. epithelial cells in the cervix during pregnancy and this can increase the risk of cell transformation in the cervix resulting in persistent HPV infection.^{15,16}

Assessment of the characteristics of the frequency distribution of budding tumors in patients with cervical squamous cell carcinoma NOS in the anatomical pathology installation of RSUP. H. Adam Malik, can show the distribution of the most peritumoral budding tumor can be found in the intermediate grade peritumoral budding tumor (<15 /10 HPF) in cervical squamous cell carcinoma NOS has 19 cases, high grade peritumoral budding tumor (> 15 /10 HPF) in squamous cell carcinoma NOS cervix had 17 cases, low-grade tumor budding peritumoral (no buds) squamous cell carcinoma NOS cervix had no cases. This is in line with the study of Jessinghaus, *et al.*, which assessed tumor budding in cervical carcinoma, whereas the study of Jessinghaus, *et al.*, used a score. It is said to be low-grade tumor budding if there is no tumor budding /10

grade tumor budding $<15/10$ HPF, and a small number of cases in high-grade tumor budding $> 15/10$ HPF, 29 cases of 125 cases.¹⁵ Tumor budding is a sign of cancer cell budding and an early step in the metastatic process.¹⁶ This may be associated with the epithelial-mesenchymal transition (EMT). EMT is a transition process of epithelial cells into mesenchymal cells, where cancer cells are partially or completely released and will lose the characteristics of epithelial cells, due to the loss of E-cadherin which is a binder between cells, because E-cadherin is lost it will be separated from the surrounding epithelial cells and obtain the characteristics of mesenchymal cells so that they can migrate. The limitation of this study is that there is no uniform cut-off value for the number of budding tumors in cervical squamous cell carcinoma NOS. Therefore, the researcher used a tumor budding number assessment system based on research by McCluggage, *et al.*, which was used for the cut-off in this study, where the results of the tumor budding assessment were divided into three categories, namely low-grade tumor budding if not found. budding, then it is said to be intermediate tumor budding if $<15 /10$ HPF, and it is said to be high tumor budding $> 15 /10$ HPF.^{13,16,17}

The grading of squamous cell carcinoma NOS cervix based on the classification according to WHO female in 2020, is divided into three categories, grade I for well-differentiated cancers where the cancer cells are still similar to the original cells, grade II for moderately differentiated cancers.), grade III for cancer with poor differentiation (poorly differentiated), in which patients with cervical squamous cell carcinoma NOS based on histopathological grading of squamous cell carcinoma NOS cervix, and most commonly found in grade 2, which is 17 cases, followed by grade 3 in 17 cases and grade 3 found 2 cases, this is not in line with research from Latifah, *et al.*, which assessed as many as 12 cases from samples that had a good degree of differentiation (well differentiated) or grade 1, and 11 cases from samples had moderate degrees of differentiation. differentiated) or grade 2 and the most with a degree di poor differentiation (poorly differentiated) or grade 3 which is 22 cases, which can be seen a little difference in grade 2 which has a higher value than grade 3.¹⁷ Generally this is following the theory that the growth rate in cervical carcinoma is generally associated with the level of differentiation included in the grading system. This can be observed from the growth rate of cervical carcinoma based on the WHO female literature 2020.^{2,18}

This study also assessed the correlation between grade and age, where in this study the relationship between the two was not significant with $p = 0.138$, where the relationship between grade and age was mostly in the >51 year age category which on average was in grade 3 as many as 17 cases. This is in line with research in 2010 – 2013 at the Hospital. Dr. M. Djamil Padang conducted by Panji, *et al.*, were in this study grade 1 and grade 2 often occurred in cervical carcinoma patients aged 45-49 years, while grade 3 occurred in cervical carcinoma patients aged 50-54 years. Based on this data, it is illustrated that grade 1 and grade 2 are more common in women of productive age compared to grade 3, and grade 3 is more common in unproductive age, namely 50-54 years. In the study of cervical squamous cell carcinoma NOS at RSUP. Haji Adam Malik Medan is more often found at the age of > 51 years with grade 3, because the higher

the age, the faster the growth of cervical squamous cell

carcinoma NOS. This is supported by the literature which states that cervical carcinoma occurs in women. productive age with a peak incidence at about 45 years, about 10 to 15 years after the detection of the precursor.¹⁹

The correlation between the grade of cervical squamous cell carcinoma NOS and the number of parity was assessed to be more than 2x labor and was not found in the number of parity

<2x parturition for grades 1, 2, and 3. mutual influence between the two. Based on the description of the number of parties that have also been studied by Vijendran *et.al.*, and Setyarini *et.al.*, regarding the frequency distribution of the number of parties in cervical carcinoma, where the number of parity that is greater in cervical carcinoma is >3x parturition.¹³,¹⁵ This is because the higher the virginity, the higher the chance of getting cervical cancer because trauma to the t-zone will cause abrasion and persistent HPV infection, but for correlation with the grading system for cervical carcinoma, researchers have not found previous research. that connects the two, but in this study, it was found that the two did not influence each other.^{14,16,20}

The relationship between the tumor budding index and histopathological grading in cervical squamous cell carcinoma NOS showed a statistically significant test analysis result and there was a relationship between the two, it could be seen from the statistical analysis test with a value of $p=0.0001$ ($p<0.05$). This is in line with the study of Satabongkoch, *et al.*, who investigated the number of tumor budding relationships with other clinical and pathological features. The number of high tumor budding was significantly associated with grade 3 ($p<0.001$).²⁰ This study suggests that tumor budding can be an indicator to assess tumor aggressiveness in cervical carcinoma.²¹

Currently, there are still differences in the assessment of the grading system in reporting tumor budding. Some divide it based on the three-tier grading system, but some divide it based on the two-tier grading system.^{21,22} However, this grading system is not an absolute standard.²² Research on tumor budding has been carried out on several malignancies, namely colorectal, gastric, oesophageal, lung, head and neck, and breast.²³ Research on tumor budding has begun in recent years where the phenomenon of tumor budding is associated with adverse clinical outcomes.^{22,23,24,25}

CONCLUSIO N

After the research has been carried out, we highlight the following conclusions:

1. Frequency distribution of cervical squamous cell carcinoma NOS patients by age, getting the most assessments in the >51 year age group, namely 16 cases and the least being at the age of 31-35 years as many as 2 cases. The highest number of parity 2x labor which amounted to 36 cases, and more frequently found in non-keratinizing squamous cell carcinoma as many as 33 cases compared to keratinizing squamous cell carcinoma.

2. This study also found a correlation between the histopathological grading of cervical squamous cell carcinoma NOS and age, which was not significant, so the higher the grade, the higher the patient's age

category. The relationship between grade and parity is constant and unrelated.

3. There is a significant relationship between peritumoral tumor budding index and histopathological grading in cases of squamous cell carcinoma NOS cervix.

Acknowledgments

We acknowledgments to all staff and residents of the Department of Pathology Anatomics, Universitas Sumatera Utara / H. Adam Malik Hospital Medan for all your support and guidance.

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