Relationship of Immunohistochemical Expression of Protein 16 (p16) to Nasopharyngeal Carcinoma Subtype at Haji Adam Malik General Hospital Medan

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ABSTRACT

Background
Nasopharyngeal carcinoma is a malignant tumor that originates from the epithelial cells of the nasopharyngeal mucosa or gland and is a malignant tumor of the head and neck area. Immunohistochemistry Protein 16 is a tumor suppressor gene (TSG), which is a cyclin-dependent kinase (CDK) inhibitor that regulates tumor cells through the G1 phase of the often inactive cell cycle in cancer, Loss of protein 16 can indicate a loss of control over the cell cycle, resulting in low p16 expression and associated with a poorer prognosis. Research on the relationship of p16 expression to nasopharyngeal carcinoma has not been done much.

Methods
This study used 46 slide/block samples of paraffin in Nasopharyngeal carcinoma cases that had been histopathologically diagnosed at the Anatomical Pathology Unit of H. Adam Malik Hospital Medan from January 2022 to May 2024. All clinicopathology data is taken from medical records/pathology archives. p16 expression is assessed in the nucleus and or cytoplasm of tumor cells. The assessment of p16 expression was carried out semi-quantitatively based on the multiplication of area and intensity in the expressed tumor cells. Statistical analysis was carried out using the Eta test.

Results
Among the 46 nasopharyngeal carcinoma samples, 21 samples were found to have positive expressions with strong positive values in the NKSCC subtype as many as 14 samples (30.4%) and weak positive values in the NKSCC subtype as many as 7 samples (15.2%). Meanwhile, the results of immunohistochemical staining p16 were most negatively expressed, namely in the NKSCC subtype as many as 23 samples (50.0%) and in the KSCC subtype as many as 2 samples (4.3%). This variable Eta test was carried out and a p-value of 0.195 was obtained.

Conclusion
There was no relationship of p16 expression to the nasopharyngeal carcinoma subtype.

Keyword: nasopharyngeal carcinoma, immunohistochemical p16

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INTRODUCTION

Nasopharyngeal carcinoma is a malignant tumor that originates from the epithelial cells of the nasopharyngeal mucosa or gland and is a malignant tumor of the head and neck area.[1] Based on data from the Global Burden of Cancer (GLOBOCAN) in 2020, it is estimated that there are 133,000 new cases of nasopharyngeal carcinoma every year worldwide.[2] The high incidence rate of naphosaring carcinoma cancer in developing countries is usually caused by increasing age, lifestyle changes such as smoking habits, lack of physical activity and poor diet.[3]

The p16 gene plays an important role in the cell cycle and has an influence on cell proliferation through the gene product, the p16 protein. Mutations in the p16 gene result in gene inactivation so that p16 decreases or is not obtained. The mutation also affects the differentiation of nasopharyngeal epithelial cells by triggering normal nasopharyngeal epithelial cells to become mild dysplasia until the onset of nasopharyngeal carcinoma. Immunohisto-chemistry p16 is a tumor-suppressing gene that produces the p16 protein and functions as a CDK4/CDK6 binder so that it is able to inhibit the cell cycle.[4]

Low or missing p16 expression in nasopharyngeal carcinoma may be associated with a worse prognosis This is because the loss of p16 may indicate a loss of control over the cell cycle, which allows the tumor to develop faster. Conversely, normal or high expression of p16 could indicate more controlled cell division and, in some cases, may be associated with better clinical outcomes.[5]

Research conducted by Makitie showed that the non-expression of p16 showed poor predictors and prognosis of survival rates in patients with nasopharyngeal carcinoma.[6]

METHODS

This research is an analytical research with a cross-sectional approach. The research was conducted at the Haji Adam Malik Central Hospital, Medan. The population of this study consisted of all secondary data and paraffin blocks from patients diagnosed histopatho-logically with nasopharyngeal carcinoma that met the inclusion and exclusion criteria at the Anatomical Pathology Unit of Haji Adam Malik Central General Hospital, Medan. The sample in this study is 46 samples. The independent variable in this study was the immuno-histochemical expression of p16 and the bound variable in this study was the Subtype non-keratinazing squamous cell carcinoma (NKSCC), keratinazing squamous cell carcinoma (KSCC), Basaloid squamous cell carcinoma (BSCC).

P16 expression is seen in the nucleus and/or cytoplasm of tumor cells which are characterized by a brown color.[7] The expression of p16 was calculated semiquantitatively, namely assessing the intensity (I) and area of the area (P). The results of the multiplication of the intensity and area of the area were grouped and scored into 3 groups. The intensity (I) is divided into 4 groups, namely, Score 0: unstained tumor cells (negative), Score 1: weak stained tumor cells, Score 2: moderately stained tumor cells, Score 3: strong stained tumor cells. The area of the affected area (P) is divided into 4 groups, namely, Group 0: negative, Group 1: positive tumor cells ≤10%, Group 2: positive tumor cells 11-50%, Group 3: positive tumor cells >50%. The results of the multiplication of the intensity and area of the affected areas were grouped and scored into 3 categories, namely, a score of 0 negative expressions, a score of 1-3, a weak positive expression, a score of 4-9, a strong positive expression.[6] Immunohisto-chemistry of p16 protein uses monoclonal antibody p16 antibody with dilution of 1:100). Positive staining is defined as the brown color of the nucleus and or cytoplasm. Assessment of the percentage distribution of stained cells using a light microscope (Olympus CX43) with 40 times, 100 times, and 400 times magnification (4x objective lens) across the field of view.[7]

The collected data will be analyzed using the SPSS statistical program. Data analysis will be carried out using the Eta correlation test. <0.05 is declared statistically significant. The p-value of <0.05 was declared statistically significant.

RESULT

In this study, 46 nasopharyngeal carcinoma samples were obtained at the Laboratory of the Anatomical Pathology Unit of H. Adam Malik Hospital, Medan.

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Table 1. Frequency distribution of characteristic nasopharyngeal carcinoma at H. Adam Malik Hospital Medan.

Characteristic	Value (n=46)	Percentage (%)
Gender		
Man	34	7.9
Woman	12	26.1
Age (years old)		
<21	1	2.2
21-30	3	6.5
31-40	7	15.2
41-50	13	28.3
51-60	17	37.0
>60	5	10.9
Histopathology subtypes		
KSCC	2	4.3
NKSCC	44	95.7
BSCC	0	0.0
Tumor infiltrating lymphocytes (Tils)		
Mild	12	26.1
Moderate	10	21.7
Severe	24	52.2
Lymphovascular invasion (LVI)		
Positive	0	0.0
Negative	46	100.0
Immunohistochemical expression of p16		
Negative	25	5.4
Weak Positive	7	15.2
Strong positives	14	30.4

Of the 46 research samples that were researched based on clinical data obtained from medical records or pathological archives, sex data were obtained for 34 male samples (73.9%) and female 12 samples (26.1%). The youngest age is 20 years old and the oldest age is 69 years old. The distribution of the age group of nasopharyngeal carcinoma patients in this study was 51-60 years old as many as 17 samples (37.0%), age 41-50 years as many as 13 samples (28.3%), age 31-40 years old was 7 samples (15.2%), age group <20 years old, as many as 1 sample (2.2%). The largest number and percentage of subtypes found were NKSCC which was 44 samples (80.4%), KSCC 2 samples (4.3%) and BSCC (0%). Tumor infiltrating lymphocytes (Tils) Most tumors showed severe lymphocyte infiltration, namely 24 samples (52.2%), followed by mild lymphocyte infiltration, namely 12 samples (26.1%), and at least moderate lymphocyte infiltration as many as 10 samples (21.7%). In this study, no lymphovascular invasion (LVI) was found from the 46 samples studied. The expression of immunohistochemical staining p16 was obtained by 21 samples with a strong positive value of 14 samples (30.4%) and a weak positive value of 7 samples (15.2%). The results of negative immunohistochemical staining expression were 25 samples (54.3%).

Table 2. Subtypes of histopathology by ages.

Age (years old)	Subtypes of histopathology							
	KSCC		NKSCC		BSCC		Total	
	n	%	n	%	n	%	n	%
<21	0	0.0	1	2.2	0	0	1	2.2
21-30	0	0.0	3	6.5	0	0	3	6.5
31-40	0	0.0	7	15.2	0	0	7	15.2
41-50	0	0.0	13	28.3	0	0	13	28.3
51-60	1	2.2	16	34.8	0	0	17	37.0
>60	1	2.2	4	8.7	0	0	5	10.9

This is in accordance with previous research conducted by Nuraini et al in 2021 and also found that the highest peak incidence of nasopharyngeal carcinoma patients occurred at the age of 41-60 years old. In line with research conducted by Adham et al, it was also found that the highest age peak in nasopharyngeal carcinoma starts from the age of 40 years old and above.[7,8]

The tendency of nasopharyngeal carcinoma sufferers to occur at an older age may be related to the immune system that has declined at that age, making it difficult for the body to eliminate EBV and tumor antigens on its own.[9] In addition, the process of carcinogenesis that takes time also affects the tendency to develop clinical manifestations of malignant cells in nasopharyngeal carcinoma, where to become an invasive carcinoma requires three triggers (EBV, genetics, and adverse environmental influences) that are interrelated to cause dysplasia in the nasopharynx epithelium, and high grade dysplasia will cause nasopharyngeal carcinoma that may arise in old age.[6,10]

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Table 3. Subtypes of histopathology by gender.

Gender	Subtypes of histopathology							
	KSCC		NKSCC		BSCC		Total	
	n	%	n	%	n	%	n	%
Man	1	2.2	33	71.7	0	0	34	73.9
Woman	1	2.2	11	23.9	0	0	12	36.1

Nasopharyngeal carcinoma patients are more commonly found in men with a ratio of 3:1 where this cancer is found three times more in men than women. In this study, the same results were also obtained where the male sex was more than that of women with 34 male samples (73.9%) and 12 female samples (26.1%) with the most nasopharyngeal carcinoma subtype being NKSCC. Differences between men and women may be due to different lifestyle habits (e.g. tobacco consumption) or biological differences.[10]

The research conducted by Utomo found that the most cases obtained from his research were men with the most subtypes being NKSCC.[10] In addition, the difference is suspected to be related to the type of work of men who are more often exposed to carcinogenic which is a risk factor for the onset of nasopharyngeal carcinoma, such as exposure to dust, wood dust, formaldehyde, heat, and smoke.[12] From the research conducted by Munir, there was no significant relationship between the histopathological subtypes of nasopharyngeal carcinoma and sex.[13]

Table 4. Analysis of the relationship between p16 expression and nasopharyngeal carcinoma subtype at H. Adam Malik Hospital Medan.

Expression p16	Subtypes of histopathology								p-value
	KSCC		NKSCC		BSCC		Total		
	n	%	n	%	n	%	n	%	
Negative	2	4.3	23	50.0	0	0	4	10.0	
Weak positive	0	0.0	7	15.2	0	0	7	15.2	0.195*
Strong positive	0	0.0	14	30.4	0	0	14	30.4	

In this study, the relationship between p16 expression and nasopharyngeal carcinoma subtype was analyzed, but only 2 subtypes were found in this study, namely KSCC subtype and NKSCC subtype, from this table, data was obtained on the total number of sample preparations with p16 immunohistochemical staining expression as many as 46 samples. Positive expressions were obtained from 21 samples with strong positive values in the NKSCC subtype as many as 14 samples (30.4%) and weak positive values in the NKSCC subtype as many as 7 samples (15.2%). Meanwhile, the results of immunohistochemical staining p16 were most negatively expressed, namely in the NKSCC subtype as many as 23 samples (50.0%) and in the KSCC subtype as many as 2 samples (4.3%). The Eta test was carried out, namely a correlation test between 2 variables when the data scale of the two variables is not the same where the first variable is a nominal data scale, while the second variable is an interval data scale and a p-value of 0.195 is obtained.

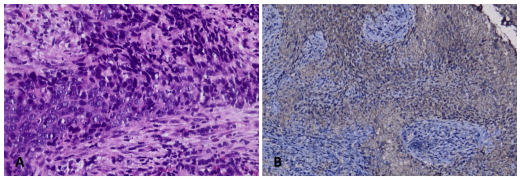


Figure 1. A. non-keratinazing squamous cell carcinoma nasofaring, Hematoxilyn and eosin staining 400 times magnification. B. non-keratinazing squamous cell carcinoma nasofaring, Immunohistochemical staining of P16 with 100 times magnification in the nucleus and/or cytoplasm.

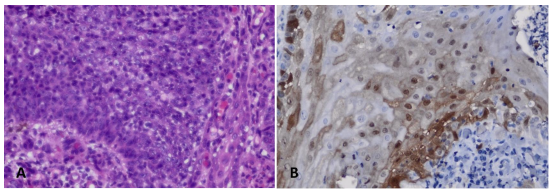


Figure 2. A. keratinazing squamous cell carcinoma nasofaring, Hematoxilyn and eosin staining 400 times magnification. Figure B. keratinazing squamous cell carcinoma nasofaring, immunohistochemical staining of P16 400 times magnification in the nucleus and/or cytoplasm.

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DISCUSSION

This study aims to analyze the expression of p16 immunohistochemistry against nasopharyngeal carcinoma. In this study, the expression of immunohistochemical staining p16 is characterized by a brown color that can be seen in the nucleus and or cytoplasm of nasopharyngeal carcinoma tumor cells.[14] In this study, the researcher used the second method in assessing the interpretation of p16 expression, then for the p16 antibody used is a monoclonal type of p16 antibody.

Recent research has said that p16 expression can be manifested in several types of tumors, namely skin tumors, head and neck tumors, lung tumors, cervical tumors, breast tumors, and penile tumors.[15] In this study, the researcher analyzed the expression of p16 immunohistochemistry on 46 samples of nasopharyngeal carcinoma and obtained more negative results of p16 immunohistochemical expression compared to positive results of p16 immunohistochemical expression. This is in line with the research conducted by Shimizu et al., namely from 81 nasopharyngeal carcinoma samples collected, where it was found that the percentage of negative p16 immunohistochemical expression was 72 samples and positive p16 immunohistochemical expression was 9 samples.[16]

Research conducted by Makitie et al found that negative p16 expression in nasopharyngeal carcinoma was associated with age, weight, and stage where some of these factors had a correlation with survival rates. Negative expression of p16 is also widely associated with hypermethylation of the p16 gene.[6] Another study also conducted by Xiang et al, the occurrence of negative expression of p16 can also be caused by the inactivation of the p16 gene, the results of the study prove that the inactivation of the p16 gene due to mutation has an important role in the occurrence of distant metastases in nasopharyngeal carcinoma.[17] Another literature search also showed that the p16 gene mutation affects cell differentiation. The effect of p16 gene mutation on nasopharyngeal carcinoma has started since the period of low-grade pre-invasive lesions. It is also said that the presence of EBV infection against cell differentiation begins during the period of high grade pre-invasive lesions. The combination of the influence of the p16 gene mutation and EBV infection further increases cell differentiation towards malignancy of nasopharyngeal carcinoma.[18] Research conducted by Hutajulu also stated that there is a link between the p16 gene mutation and EBV. In nasopharyngeal carcinoma type undifferentiated carcinoma, almost 100% of EBV infections are known.[19] The Epstein-Barr virus and its products further enhance the influence of p16 gene mutations in the process of cell differentiation and proliferation. The transformation into cancer cells is accelerating and cell proliferation is increasing, so the stage is increasing. The study of Hwang et al and Mäkitie et al found that negative expression of p16 due to inactivation of the p16 gene had a significant relationship with the early and advanced stages.[6.20] The hypothesis from other studies conducted by Lin et al that the unexpressed p16 of nasopharyngeal carcinoma may provide resistance to treatment in nasopharyngeal carcinoma.[21]

From the results of statistical tests in this study, the researcher found that there was no relationship between p16 expression and nasopharyngeal carcinoma subtype. Thus, the null hypothesis (Ho) in this study has no relationship.

CONCLUSION

A study on the analysis of p16 expression on the subtype of nasopharyngeal carcinoma has been carried out and the following conclusions have been obtained, the gender of the most patients with nasopharyngeal carcinoma is in males as many as 34 samples, the age of the

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most patients with nasopharyngeal carcinoma is at the age of 51-60 years old, the most histology subtype is NKSCC in 44 samples, the most Tils is severe in 24 samples, not found lymphovascular invasion in all existing samples, the most positive p16 expression was 21 samples in the NKSCC subtype. The age group of people with nasopharyngeal carcinoma is most commonly found in the NKSCC subtype while the age group of 51-60 years old and >60 years old is only found in 1 KSCC subtype. In this study, the most common gender of nasopharyngeal carcinoma patients was male in the NKSCC subtype. There was no association between p16 expression and nasopharyngeal carcinoma subtype.

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