Expression of Programmed Death-Ligand 1 (PD-L1) Immunohistochemistry Staining in Nasopharyngeal carcinoma at Rumah Sakit Umum Pusat Haji Adam Malik Medan 2018

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is carcinoma arising from nasopharyngeal mucosa that shows light microscopic of squamous differentiation. Data from GLOBOCAN (Global Burden of Cancer) 2018, nasopharyngeal carcinoma ranks fifth based on the incidence of new cases from all malignancies in Indonesia. The high mortality rate indicates that NPC management through surgery, radiotherapy, and chemotherapy have not been completely satisfactory. Recently, Programmed Death-Ligand 1 (PD-L1) inhibitor has developed which is an effective cancer immunotherapy. Abnormal expression of PD-L1 in various types of malignant tumors is associated with invasion, decreased T-lymphocyte infiltration, poor prognosis, and survival time.
Objective: This study aimed to assess expression of Programmed Death-Ligand 1 (PD-L1) immunohistochemistry staining in nasopharyngeal carcinoma at Rumah Sakit Umum Pusat Haji Adam Malik Medan 2018.
Material and Method: This study is descriptive and cross-sectional, which enrolled 50 PD-L1 stained slides of NPC biopsy and assessed by Histo-score. Expression of PD-L1 negative is 0-99, and positive is 100-300.
Result: From 50 specimens in this study, we found that NPC patients were dominated by men (72%), with mean age 46,5±13,1 years-old. Positive expression of PD-L1 staining are 23 (46%) specimens, and negative expression of PD-L1 staining are 27 (54%) specimens.
Conclusion: Expression of PD-L1 immunohistochemistry staining in this study were found fewer positive expressions compared to negative expressions.

Keyword: nasopharyngeal carcinoma, immunotherapy, immunohistochemistry, Programmed Death-Ligand 1.

1. Introduction

Nasopharyngeal carcinoma is a malignancy that affects the nasopharynx that microscopically shows a differentiation of squamous cell, which is histopathologically divided into three entities; Non Keratinizing Squamous Cell Carcinoma (NKSCC), Keratinizing Squamous Cell Carcinoma (KSCC), and Basaloid Squamous Cell Carcinoma (Basalaoid SCC).[1] It was one of the epithelial cancers in the head and neck that became a serious thread for human health.[2] Based on the data from GLOBOCAN (Global Burden of Cancer) 2018, it was estimated that there were 129,000 new cases of nasopharyngeal carcinoma, with mortality rate of up to 73,000 cases.[3] Up to 80% of patients with nasopharyngeal carcinoma were observed in Asia, especially in South East Asia and South China. Based on previous study report, the estimated incidence for nasopharyngeal carcinoma in China is up to 60.6 per 100,000 population, and mortality rate of up to 34.1 per 100,000 population.[4] In Indonesia, according to GLOBOCAN 2018, nasopharyngeal carcinoma was the 5th top cancer based on incidence of new cases compared to all other malignancy in Indonesia.[3]

Current therapy for nasopharyngeal carcinoma was decided based on tumor stadium, either with radiotherapy or chemotherapy. In the last decade, prognosis of nasopharyngeal carcinoma is becoming better due to the advance in diagnostic imaging, radiotherapy and more widened use of systemic therapy. However, about 15-30% of these patients may experience metastasis to distant organs, in which most death caused by

nasopharyngeal carcinoma is associated with the tumor metastases rather than the primary tumor. This indicate that the management for nasopharyngeal carcinoma is still needs to be improved. Right now, an effective immunotherapy for cancer by using anti-Programmed Death-1 (PD-A) and anti-Programmed Death-Ligand 1 (PD-L1), which was in agreement with Food and Drug Administrarion (FDA) for the treatment of Head and Neck Squamous Cell Carcinoma (HNSCC) including nasopharyngeal carcinoma are being studied.[5,6]

In Indonesia the use of PD-L1 immunohistochemistry was new and studies that shows the PD-L1 staining pattern in nasopharyngeal carcinoma was also limited. Prior to that, the writer is trying to study about the PD-L1 staining pattern in patients with nasopharyngeal carcinoma in Rumah Sakit Umum Pusat Haji Adam Malik Medan.

2. Materials and Methods

This study is conducted using cross sectional method. Data was collected from Anatomical Pathology unit in Rumah Sakit Umum Pusat Haji Adam Malik Medan from January to December 2018, 65 population of patients diagnosed as nasopharyngeal carcinoma with histopathologic examination with hematoxylin-eosin (HE) staining was collected, and 50 samples that fulfil the criteria of inclusion to undergo immunohistochemistry staining with PD-L1 after requiring ethical clearance from the ethical committee for health study in Faculty of Medicine University of Sumatera Utara.

Samples for this study is a paraffin block with formalin fixation from the patient with nasopharyngeal carcinoma to make the samples and then stained with immunohistochemistry PD-L1, after that the samples will be evaluated with the Histo-score for every samples. The final score varies from 0-300. It was categorized as negative expression if the final score ranges from 0-99, and positive expression for score 100-300.[7]

3. Results

In this study, 50 samples that fulfil the criteria of inclusion was studied and undergo the immunohistochemistry PD-L1 staining. The characteristic of the sample distribution is as seen in the table below;

Table 1. Distribution of the characteristic of samples

Characteristic	Total (n=50)
Sex	
• Male	36
• Female	14
Age (years); mean ± DS	46,5 ± 13,1
Histopathology	
• NKSCC	44
• KSCC	6
• Basaloid SCC	0
Expression of PD-L1 staining	
• Positive	23
• Negative	27

From table 1 we can see that in this study it was shown that 36 of the samples were male and 14 samples were female, with the mean age (years) was 46.5 ± 13.1. on the data about the expression of the immunohistochemistry staining with PD-L1 we found 23 samples with positive expression and 27 samples with negative expression.

Table 2. Distribution of characteristic of samples based on age

Age (years)	Total (n)	Percentage (%)
≤ 20	3	6
21 – 40	4	8
41 – 60	35	70
> 60	8	16
Total	**50**	**100**

From table 2 we can see that the average age of patients with negative in this study was 46.5 years old with its standard deviation 13.1 years, in which the youngest patient was 14 years old and the oldest patient was 75 years old. The age group of negative in this study was mostly between 41-60 years old, which covers 35 (70%) samples, followed by group of 8 samples with age > 60 years old (16%), and group of 4 (8%) samples with age between 21-40 years old, and the less was for group of age ⩽ 20 years old which was 3 (6%) samples.

Table 3. Distribution of expression immunohistochemistry PD-L1

Expression of PD-L1	Total (n)	Percentage (%)
Positive	23	46
Negative	27	54
Total	**50**	**100**

More data with negative expression maybe seen compared to the positive expression in table 3. From all 50 samples there were 23 (46%) samples that shows positive expression from immunohistochemistry staining with PD-L1 and 27 (54%) samples with negative expression.

4. Discussion

There were 50 samples in this study with nasopharyngeal carcinoma that fulfil the criteria of inclusion which was dominated by male 72% and the rest are woman. The ratio for comparison between male and female was 6:2.3. A study by Salehiniya et al showed that the incidence of nasopharyngeal carcinoma for male was 2 to 3 times higher compared to female. This difference might be caused by the difference in lifestyle (i.e. in tobacco consumption). Also, this might also relate to the type of job for male which was more susceptible to carcinogenic substances which was a risk factors for nasopharyngeal carcinoma such as dust, sawdust, formaldehyde, heat, smoke, and chemical gasses.[1,8]

In this study we could also see that the distribution of nasopharyngeal carcinoma peaks at the group of age between 41-60 years old. The youngest sample was 14 years old and the oldest sample was 75 years old. This result was similar to the previous study conducted by Farhat et all in Rumah Sakit Umum Pusat Haji Adam Malik Medan in 2017, which shown that the highest incidence for nasopharyngeal carcinoma was between

41-60 years old group.[9] Also, similar result was shown in the previous study by Adham et all in 2012 which show that the peak incidence for carcinoma in Indonesia was between 40-49 years old group. The high prevalence for nasopharyngeal carcinoma in elder people might be related to the decrease immunity function, which makes it harder for the body to eliminate EBV or the tumor antigen itself.[10,11]

The percentage of PD-L1 positive expression strongly depend in the functionality of the test kit and antibody, number of samples examined, amount of tumor cell and immunity cell taken during the specimen obtainment process which strongly depend on the operator, and the cut-off value used.[12,13] The use of different clone of PD-L1 antibody may also contribute to the low expression of PD-L1 in previous studies. Other than technical difficulties of immunohistochemistry, temporal and spatial factor must also be considered when evaluating PD-L1 in cancer. In prior to that, interferon- γ secretion by lymphocytes dan infiltrate the tumor may increase the PD-L1 regulation in the tumor cell, which may cause induction of apoptotic T cell through PD-L1 interaction with PD-1. Collectively, the prognostic value of PD-L1 immunohistochemistry depends in the duration of biopsy which is related to the development of tumor and related to the previous therapy including chemotherapy and radiotherapy.[14] Deng et al in their study showed that the expression of PD-L1 was regulated after irradiation in mice.[15] Lee et al also in their study found that there was less positive PD-L1 expression compared to negative expression in patients with nasopharyngeal carcinoma after receiving radiative therapy.[13] This study was a retrospective and descriptive therapy. The limitation of this study is in its ability to analyze a correlation or to evaluate the prognosis of the nasopharyngeal carcinoma in the area in which this study was conducted.

5. Conclusion

More samples are needed in further research to be able to find an even distribution of nasopharyngeal carcinoma cases for immunohistochemistry PD-L1 staining. Further research is conducted using prospective analytic methods over a longer period of time.

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