The Correlation Between Expression Of Programmed Death-Ligand 1 With Nasopharyngeal Carcinoma Non-Keratinizing Squamous Cell Carcinoma And Keratinizing Squamous Cell Carcinoma Subtypes

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Abstract:
Background: Nasopharyngeal carcinoma (NPC) is a malignancy arising from nasopharyngeal mucosa that shows squamous differentiation. Data from Global Burden of Cancer (GLOBOCAN) 2018, nasopharyngeal carcinoma has accounts for 129,000 new cases. The high mortality rate indicates that NPC management through surgery, radiotherapy, and chemotherapy have not been satisfactory. Recently, Programmed Death-Ligand 1 (PD-L1) inhibitor has developed as immunotherapy for cancer. High expression of PD-L1 in various types of malignant tumors were associated with poor prognosis.
Objective: This study to assess correlation between expression of PD-L1 to nasopharyngeal carcinoma non-keratinizing squamous cell carcinoma (NKSCC) and keratinizing squamous cell carcinoma (KSCC) subtypes.
Material and Methods: This study was analytic, which was enrolled 46 of NPCs that stained by PD-L1 antibody, and assessed by Histoscore. Expression of PD-L1 negative is 0-99, and positive is 100-300.
Result: Male more than female, with the highest age group at 41-60 years old, and the most common histologic subtype are NKSCC. Positive PD-L1 42 (91,3%) and negative 4 (8,7%). On NKSCC, positive PD-L1 33 (89,2%) and negative 4 (10,8%). On KSCC, positive PD-L1 9 (100%) and negative 0 (0%). Fisher's Exact test was performed with p-value 0,571 ($p \geq 0,05$).
Conclusion: There was no correlation between expression of PD-L1 to nasopharyngeal carcinoma NKSCC and KSCC subtypes.

Keywords: Nasopharyngeal carcinoma, Programmed Death-Ligand 1, NKSCC, KSCC

I. INTRODUCTION

Nasopharyngeal carcinoma is one of the head and neck epithelial malignancies, and it is a serious threat to human health.[1] Data Global Burden of Cancer (GLOBOCAN) in 2018, the incidence of NPC accounts for 129,000 new cases.[2] Based on previous study report, the estimated incidence of NPC in China is up to 60.6 per 100,000 population, and mortality rate of up to 34.1 per 100,000 population.[3]

Histologically, nasopharyngeal carcinoma divided into three subtypes, include non-keratinizing squamous cell carcinoma (NKSCC), keratinizing squamous cell carcinoma (KSCC), and basaloid squamous cell carcinoma (BSCC). KSCC subtype have prognosis poorer than NKSCC subtype.[4] Radiotherapy or chemotherapy has not been satisfactory to treat NPC.[5] Recently, anti-Programmed Death-1 and anti-Programmed Death-Ligand 1 were in agreement with Food and Drug Administration (FDA) for the treatment of Head and Neck Squamous Cell Carcinoma (HNSCC) was being studied.[6] PD-L1 is a member of the Ig superfamily with the chromosomal location of 9p24.2, which encodes a 290-amino acid type I transmembrane protein, including an extracellular portion with IgV and IgC-like domains.[7]

The correlation between PD-L1 expression with histologic subtypes has been analyzed in lung carcinoma. Janzic et al. showed there was a significant correlation between PD-L1 with squamous cell carcinoma and adenocarcinoma in the lung carcinoma.[8] NKSCC and KSCC subtypes in nasopharyngeal carcinoma have different microscopic appearances and prognosis.[4] Therefore, researchers were interested to analyze the correlation between PD-L1 expression to nasopharyngeal carcinoma NKSCC and KSCC subtypes.

II. MATERIAL AND METHODS

Sample selection

This analytic research with retrospective approach was conducted in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and Anatomical Pathology Unit, Haji Adam Malik General Hospital. This study was done from October 2020 until May 2021, after receiving approval from Health Research Ethics Committee, Medical Faculty, Universitas Sumatera Utara.

These study samples were 46 paraffin-embedded tumor tissues, and histopathological diagnosed as NPC fulfilling inclusion and exclusion criteria. Samples were gathered by using consecutive sampling technique. Inclusion criteria were all adequate paraffin blocks and having medical record data include age, and gender. Exclusion criteria for this study was diminished or damaged paraffin blocks which can't be cut back.

Immunohistochemistry

Each sample was stained with PD-L1 antibody *Prediluted (Clone MD21R* from Medaysis). PD-L1 expression were interpreted using Histo-score system. Interpretation was done by adding proportion score and staining intensity. The intensity of staining was evaluated according to the following scale: 0, no staining; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. The proportion of all tumor cells found to express PD-L1 was determined and then multiplied by the staining intensity score to obtain a final semiquantitative H score (maximum value of 300 corresponding to 100% of tumor cells positive for PD-L1 with an overall staining intensity score of 3). It was categorized as negative expression if the final score ranges 0-99, and positive expression for score 100-300. All immunohistochemical images were evaluated by two experienced observers who were unaware of the identity of the specimens.

Data analysis

Data collected in this research were processed by using statistical software, statistical package for the social sciences (SPSS22) version (SPSS Inc., Chicago), and presented in tables. Statistical tests used in this study were Chi-square, Mann Whitney U, and Fisher's Exact test.

III. RESULTS

In this study, 46 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);

Table 1. Distribution of the characteristic of samples

Characteristic	Total (n=46)	Percentage (%)
Sex;		
• Male	31	67,4
• Female	15	32,6
Age (years);		
• <20	3	6,5
• 21 – 40	5	10,9
• 41 – 60	30	65,2
• >60	8	17,3
Histopathology;		
• NKSCC	37	80,4
• KSCC	9	19,6
Expression of PD-L1 staining;		
• Positive	42	91,3
• Negative	4	8,7

From table 1. we can see that in this study shown 31 of the samples were males and 15 samples were females, with most common group of age 41-60 years are 30. NKSCC are predominant in histopathology subtypes. The expression of PD-L1 are 42 samples with positive expression and 4 samples with negative expression.

Table 2. Characteristic of sex in NKSCC and KSCC subtypes

Histopathology Subtype	Sex		p-value*
	Male	Female	
NKSCC	26	11	0,398
KSCC	5	4	

*Chi-square

From table 2. in this study shown in NKSCC subtype consists of male 26 samples and female 11 samples. In KSCC subtype consists of male 5 samples and female 4 samples. Chi-square test revealed p-value 0,398 (p>0,05).

Table 3. Characteristic of age in NKSCC and KSCC subtypes

Histopathology Subtype	Age (years)				p-value*
	<20	21-40	41-60	>60	
NKSCC	3	4	25	5	0,196
KSCC	0	1	5	3	

*Mann-Whitney U

From table 3. this study shown both of NKSCC and KSCC subtypes consist of predominant age are 41-60 years old. Mann-Whitney U test revealed p-value 0,196 (p>0,05).

Table 4. Correlation of PD-L1 expression in NKSCC and KSCC subtypes

Histopathology Subtype	PD-L1 expression				p-value*
	Positive		Negative		
	n	%	n	%	
NKSCC	33	89,2	4	10,8	0,571
KSCC	9	100	0	0	

*Fisher's Exact

From table 4. this study shown expression of PD-L1 in NKSCC 33 (89,2%) positive, and 4 (10,8%) negative. In KSCC 9 (100%) positive, and 0 (0%) negative. Fisher's Exact test revealed p-value 0,571 (p>0,05).

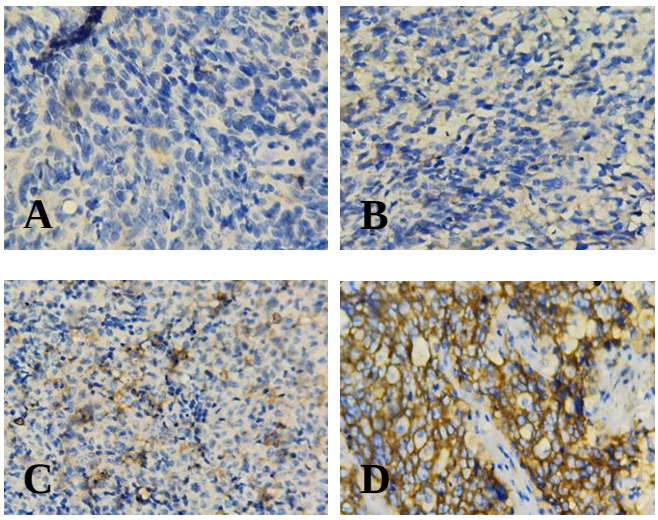

Figure 1. Expression of PD-L1 in this study. No staining, 0 (**A**). Weak staining, 1+ (**B**). Moderate staining, 2+ (**C**). Strong staining, 3+ (**D**).

IV. DISCUSSION

NPC arises from the epithelial lining of the nasopharynx, has remarkable ethnic and geographic distributions, with a particularly high prevalence in southern China, southeast Asia and northern Africa. Data from GLOBOCAN 2020, nasopharyngeal carcinoma in Indonesia has accounts for 19.943 new cases.[9] Nuraini *et al*. found 65 cases in Haji Adam Malik General Hospital for 2018 year. Incidence rates in more male than female.[10]

In the present study, there were 46 samples from nasopharyngeal carcinoma NKSCC and KSCC subtypes, which was fulfil the inclusion criteria. It was dominated by male 31 (67,4%) samples. The ratio for comparison between male and female was 2:1. In line with research by Purba *et al*. found sample of NPC with more male than female.[11] A study by Salehiniya et al showed that the incidence of nasopharyngeal carcinoma for male was 2 to 3 times higher compared to female.[12] This difference of sex characteristic might be caused by the difference in lifestyle (tobacco consumption).[13] In addition, 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.[14] It did analyze sex characteristic be correlated with NKSCC and KSCC subtypes, Chi-square test revealed p-value 0,398 (p>0,05), it's mean there was no correlation between sex with nasopharyngeal carcinoma NKSCC and KSCC subtypes. It is in line with study of Munir revealed there was no significant correlation between sex with histology subtypes in nasopharyngeal carcinoma.[15]

This study revealed the distribution of nasopharyngeal carcinoma peaks at the group of age 41-60 years old. The youngest sample was 14 years old and the oldest sample was 70 years old. Farhat *et al*. got the highest incidence for nasopharyngeal carcinoma was group 41-60 years old in Haji Adam Malik General Hospital.[16] In line with the previous study by Adham *et al*. in 2012 which revealed the peak incidence for carcinoma in Indonesia was 40-49 years old group.[17] 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 Epstein-Barr virus (EBV) or the tumor antigen itself.[15] In addition, statistical analytic used to assess the age characteristic be correlated with NKSCC and KSCC subtypes, Mann-Whitney U test revealed p-value 0,196 (p>0,05), it's mean there was no correlation between age with nasopharyngeal carcinoma NKSCC and KSCC subtypes. In line with study of Qu *et al*. assessed that no significant associations between PD-L1 expression status age.[18]

One of the aims of this study to analyze the expression of PD-L1 in nasopharyngeal carcinoma NKSCC and KSCC subtypes. In the present study, we found samples more positive expression than negative expression. In line with Wang *et al*. detected positive-PD-L1 132 of 139 NPC's samples.[19] A number of studies have revealed high expression of PD-L1 included in breast carcinoma, renal cell carcinoma, and ovarian cancer.[20-22] The staining of PD-L1 immunohistochemical in the present study displayed brown color into membrane or cytoplasm of tumor cells. No difference displayed both of NKSCC and KSCC subtypes. The percentage of PD-L1 positive expression strongly depend in the functionality of the test kit and antibody.[23,24] In addition, statistical analytic used to assess correlation between PD-L1 expression to nasopharyngeal carcinoma NKSCC and KSCC subtypes, Fisher's Exact test revealed p-value 0,571 (p>0,05). It's mean there was no correlation between expression of PD-L1 with nasopharyngeal carcinoma NKSCC and KSCC subtypes. In contrast with Harahap *et al*. explained there was difference PD-L1 expression between lung squamous cell carcinoma (LSCC) and lung adenocarcinoma (LAC).[25] Jin *et al*. found significant correlation between PD-L1 expression and

subtypes of *non-small cell lung carcinoma (NSCLC)* from 1100 samples.[26] In the present study, it was no correlation between PD-L1 expression and nasopharyngeal carcinoma NKSCC and KSCC subtypes, may be caused by both of NKSCC and KSCC subtypes arising from similar origin cell. In addition, this study has limited samples, and abnormal distribution of samples. The mechanism through which PD-L1 is upregulated in NPC has not been fully elucidated.[24] Parsa *et al* reported loss of phosphatase and tensin homolog and resulting activation of the phosphoinositide 3-kinase pathway significantly upregulates PD-L1 in glioma.[27] Marzec *et al.* found constitutive activation of anaplastic lymphoma kinase induces PD-L1 expression via signal transducer and activator of transcription 3.[28] Fang *et al.* observed that high levels of PD-L1 expression in EBV-infected NPC cells were associated with latent membrane protein 1 (LMP1)-mediated oncogenic pathways and immune modulation via excretion of interferon-γ.[29] These results indicated that inhibition of the LMP1 oncogenic pathway and PD-1/PD-L1 checkpoints may provide a clinical benefit during the treatment of EBV-associated NPC.[24]

To date, a range of studies have confirmed that PD-L1 expression is associated with the prognosis of cancer patients. Shi *et al.* identified that high expression of PD-L1 in colorectal carcinoma was associated with the tumor-nodes-metastasis stage and prognosis.[30] Nomi *et al.* reported that pancreatic cancer patients with PD-L1-positive tumors exhibited a worse prognosis than those with PD-L1-negative tumors.[31] But contrast with meta-analysis study of Huang *et al*. indicated that higher/positive expression of PD-L1/PD-1 may not serve as suitable biomarkers for the prognosis of NPC, which was not in consistent with some previous studies about the prognostic value of PD-L1/PD-1 in other types of tumors.[32] Although based on literatures said that nasopharyngeal carcinoma KSCC subtype have prognosis poorer than NKSCC subtype, but statistical analyze in the present study revealed no correlation between PD-L1 expression with nasopharyngeal carcinoma NKSCC and KSCC subtypes.

This study was a retrospective and analytic, has limited on short time and abnormal distribution of samples. Therefore, researchers have ability to analyze a correlation between the variables.

V. CONCLUSION

In conclusion, our study revealed that there was no correlation between expression of PD-L1 to nasopharyngeal carcinoma NKSCC and KSCC subtypes. Long time and more samples are needed in further research to be able to use normal distributed samples of nasopharyngeal carcinoma NKSCC and KSCC subtypes for staining immunohistochemical PD-L1 antibody.

VI. COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

VII. ACKNOWLEDGMENT

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VIII. ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

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