CORRELATION BETWEEN S100 PROTEIN EXPRESSION WITH CLINICAL STAGING NASOPHARYNGEAL CARCINOMA TYPE III

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Abstract

Introduction: Nasopharyngeal carcinoma is the most found head and neck cancer, which originated from a nasopharyngeal epithelial cell, and predilection site commonly at rosen muller fossa. S100 protein inflammatory mediators are involved in the regulation of cellular processes including inflammation and malignancy. S100 protein plays a central role in the proliferation, regulation of cell apoptosis and metastasis causing continuing growth of cancer cells through activation of STAT3 by IL-6, NF- κ B, ROS.

Objective: This study aimed to determine the correlation between S100 protein expression levels to the clinical stage of NPC WHO type III.

Method: This research is a cross-sectional analytic study. This study was held in the Anatomical Pathology Department of Hasan Sadikin Hospital from August until October 2015. The study was conducted using 29 pieces of secondary data, medical records and paraffin blocks anatomical pathology of NPC patients were examined S100 protein immunohistochemistry.

Result: This study was performed from 29 subjects (18 males and 9 females). There was a strong positive correlation between histoscore S100 protein expression with clinical staging $p < 0.05$. There is a significant correlation between S100 protein expression with the clinical stage of NPC WHO type III using double regression analysis ($F = 15.676$, $p = 0.000$).

Conclusion: There were significant correlation S100 protein expression levels to clinical stage nasopharyngeal carcinoma WHO type III.

Article Info

Keywords:

Nasopharyngeal carcinoma, S100 protein, clinical staging

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1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is the most found malignant tumor in the head and neck area originating from nasopharyngeal epithelial cells [1]. The highest incidence of NPC was found in Guangdong Province of South China with a frequency of 100 times more than the Caucasians which is 17.8 per 100,000 population per year. In Indonesia, NPC is in the fourth place of all malignancies and is ranked first in the field of Ear Nose Throat Health Head and Neck Surgery. The incidence of NPC in Indonesia is 6.7 per 100,000 populations per year with the highest age incidence of 4-5 decades, the ratio between men and women is 2-3: 1. The prevalence of NPC in the Department Otorhinolaryngology Head and Neck Surgery Dr. Hasan Sadikin Bandung during the period of 2010-2014 was 39.4% [2-5]. Nasopharyngeal carcinoma is still difficult to diagnose because the hidden nasopharyngeal location and initial clinical symptoms are not typical. Most NPC patients come with complaints of advanced neck lymph nodes so that the prognosis becomes worse [1-6].

The S100 protein is an inflammatory mediator released due to infection or inflammation as a danger signal. The high level of S100 protein is a sign of repeated infection and inflammatory reactions that can be found in tumor epithelium. Tsuji's research in 2006 stated that lymphocyte infiltration in NPC would cause an increase in S100 protein which is characterized by the distribution of Langerhans cells and reticulum [7, 8]. The infected nasopharyngeal epithelium VEB provides an increased response to interleukin-6 that involves the signal transducer activation activator of transcription 3 to promote growth and invasive factors [9]. The response is mediated by granulocyte activity involved in the process of NPC by inflammation. S100 protein is a protein found in granulocytes and macrophages. In the inflammatory process, reactive oxygen species are formed. Increased ROS levels will cause activation of transcription factors such as NF- κ B. Nuclear Factor-Kappa B has a role in cell immortality. An increase in NF- κ B is followed by an increase in IL-6 and S100 protein indicating a malignancy process. Besides, that ROS will also damage DNA

so that it promotes cell proliferation or suppresses apoptosis [10-13]. This process results in loose bonds. Some S100 proteins are affected by the process, so they are used as specific target proteins.

In the metastatic process of NPC, S100 protein is a mediator of p53 as a tumor suppressor gene. In NPC, high levels of p53 were found according to their stage so that they could predict an increase in expression of S100 protein found in tumor tissue and neck KGB of NFF18 patients. In a study in 2012, by Maletzki, expression of S100 protein was obtained from the presence of langerhans cells in carcinoma. Langerhans cells can be detected not only in the epidermis but also in the esophageal, tonsillar, nasopharyngeal, and lymphoid tissue epithelium. In NPC there is an increase in the number of Langerhans cells according to the tumor infiltration [7, 8, 10, 14].

From a immunohistochemical examination of S100 protein, we can identify Langerhans cells in both mature and immature forms. The picture of Langerhans cells at various stages in NPC shows tumor infiltration according to their progression. Progressivity was assessed based on the clinical stage, and the degree of expression of S100 protein that appeared by Langerhans cells from immunohistochemical examination [7, 8, 10, 14]. S100 protein examination by immunohistochemistry has advantages over other examinations, which are easier, not invasive, and cheaper.

2. MATERIAL AND METHODS

This research is a cross-sectional analytic study and was held in Anatomical Pathology Department of Hasan Sadikin Hospital from August until October 2015. The study was conducted using 29 pieces of secondary data, medical records and paraffin blocks anatomical pathology of NPC patients were examined S100 protein immunohistochemistry.

3. RESULT

The results of examination of S100 protein expression in WHO type III NPC patients who performed immunohistochemistry based on table 1, the distribution of 51-80% as many as 2 people (6.9%), and 27 people (93.1%) in distribution >80%. In the assessment based on intensity, there was a weak intensity of 5 people (17.2%). Moderate intensity was found in 8 people (27.6%), and a strong intensity of 16 people (55.2%).

Table 1. Results for Examination of S100 Protein Expressions in WHO type III NPC patients

Expressions	Results	%
Distribution		
50-80 %	2	6.9
80%	27	93.1
Intensity		
Weak	5	17.2
moderates	8	27.6
strong	16	55.2
Hystoscor		
Mean : 9.2 (3.07)		
Median: 9		
range: 4-12		

Table 2. Distribution of S100 proteins according to WHO type III NPC stages

Distribution	Stadium				Results	P
	I	II	III	IV		
<20%	0	0	0	0	0	0.791
20-50%	0	0	0	0	0	
51-80%	0	0	2	0	2	
>80%	2	6	8	11	27	

Spearman test $P < 0.005$

Table 2 shows the amount of S100 protein distribution according to the stage. From the data obtained in stage I there were 2 subjects with S100 protein distribution >80%. In stage II there were 6 subjects with S100 protein distribution >80%. Stage III there are 2 subjects with a distribution of 51-80% and 8 subjects distributed >80%, and at stage IV distribution of S100 protein >80%. With the Spearman statistical test, the value of $p = 0.791$ showed that there was no relationship between the distribution of S100 protein and the clinical stage.

Table 3. S100 protein intensity in WHO type III NPC patients

Intensity	Stadium				Results	P
	I	II	III	IV		
Weaks	2	3	0	0	5	0.000
Moderates	0	2	4	2	8	
Strong	0	1	6	9	16	

Spearman test $P < 0.05$

Table 3 shows the S100 protein intensity according to the stage. In stage I there were 2 subjects with weak intensity. Stage II there are 3 subjects with weak intensity, 2 subjects of moderate intensity, and 1 subject with strong intensity. Stage III there are 4 subjects with moderate intensity and 6 subjects with strong intensity. In stage IV there are 2 subjects with moderate intensity and 9 subjects with strong intensity. With the Spearman statistical test, the intensity ratio based on the clinical stage obtained with a value of $p = 0.000$, there is a significant relationship between the comparison of S100 protein intensity to the clinical stage.

Table 4. Histoscore results

Histoscore	Stadium				Results	P
	I	II	III	IV		
Negatif	0	0	0	0	0	0.000
Weak	0	0	0	0	0	
Moderate	2	3	0	0	5	
Strong	0	3	10	11	24	

Spearman test $P < 0.05$

Table 4 shows histoscore results in WHO type III NPC patients. From the data obtained in stage I histories with moderate positive as many as 2 subjects. In stage II, moderately positive and strong positive histories were obtained for each of the 3 study subjects. In stage III there were 10 positive positive histories in the study subjects. With the Spearman statistical test obtained a comparison of the S100 protein histories based on the clinical stage with a value of $p = 0.000$, there is a significant relationship between the comparison of S100 protein histories to the clinical stage.

Table 5. The relationship between age, sex, and expression of S100 protein with clinical stage

Variabel	Koefisien B	SE (B)	Value t	Value P	Anova test
Age	-.019	0.009	-2.086	0.047	-
Sex	0.438	0.226	1.939	0.064	F: 15.676
Protein S100	0.190	0.040	4.724	0.000	p: 0.000
konstanta	1.489	0.723	2.060	-	-

r^2 (%) = 65.3%; SE : Standard Error

Table 5 explains the relationship between age, sex, and expression of S100 protein with a clinical stage based on multiple regression analysis, with the results of the B coefficient S100 protein expression = 0.190, with a value of $t = 4.724$ and $p = 0.000$. With the ANOVA test, get the Fischer statistical value = 15.676 and a significant value of 0.000.

4. DISCUSSION

From the data of S100 protein expression, it was found that the distribution of 50-80% amounted to 6.9% and >80% as much as 93.1%. The amount of S100 protein distribution according to the stage, from the data obtained in stage I there were 2 subjects with S100 protein distribution >80%. In stage II there were 6 subjects with S100 protein distribution >80%. Stage III there are 2 subjects with a distribution of 51-80% and 8 subjects distributed >80%, and at stage IV distribution of S100 protein >80%. With the Spearman statistical test, the value of $p = 0.791$ showed that there was no relationship between the distribution of S100 protein and the clinical stage.

On examination of the S100 protein intensity, we found a weak intensity of 17.2%, moderate 27.6% and strong 55.2%. Based on the stage of the number of subjects S100 protein intensity in stage I, there were 2 subjects with weak intensity. Stage II there are 3 subjects with weak intensity, 2 subjects of moderate intensity, and 1 subject with strong intensity. Stage III there are 4 subjects with moderate intensity and 6 subjects with strong intensity. In stage IV there are 2 subjects with moderate intensity and 9 subjects with strong intensity. With the Spearman statistical test, the intensity ratio based on the clinical stage obtained with a value of $p = 0.000$, there is a significant relationship between the comparison of S100 protein intensity to the clinical stage.

Histoscore results were based on stages of WHO type III NPC patients, from data obtained in stage I histories with moderate positive as many as 2 subjects. In stage II, moderately positive and strong positive histories were obtained for each of the 3 study subjects. In stage III there were 10 positive histories in the study subjects. In stage IV the positive histories were 11 subjects, with a value of $p < 0.005$ with a mean of 9.2, median 9, and a range of 4-12. This shows a strong positive immunoreactive result.

The relationship between the expression level of S100 protein and clinical stage based on multiple regression analysis showed that the expression of S100 protein with a coefficient $B = 0.190$, $t = 4.724$ and $p < 0.005$. The regression equation is clinical stage = $1.489 - 0.019 \times \text{age} + 0.438 \times \text{sex} + 0.190 \times \text{protein S100}$ ($r^2 = 63.3\%$). In the Anova statistical test, the Fischer value of 15,676 and sig was obtained. The p-value 0.000 in the regression model means that this multiple regression analysis models are significant. In this study, it was found that there was a correlation between the levels of expression of S100 protein towards the clinical stage of WHO type III KNF which was significantly based on the multiple regression analysis tests. From histopathology a score of 9.2 was obtained which gave a strong positive result so it can be concluded that the correlation between the expression levels of S100 protein to the clinical stage of the KNF WHO type III was positively strong

These results are in accordance with Tsuji et al. research in 2006 regarding the effect of RAGE by LMP1 on metastatic GB patients with nasopharyngeal carcinoma. In this study, 42 paraffin blocks of NPC patients consisting of 3 patients without enlarged KGB and 33 patients with nodes enlargement (N1-3) were then analyzed and stained by immunohistochemistry. The result is RAGE expression >20% in 29 subjects with $p = 0.0093$ after Mann-Whitney test. In this study, it was concluded that there was a relationship between the expression of RAGE and metastatic nodes patients with NPC [11].

The study by Maletzki et al. In 2012 stated that the S100 protein was used as a diagnostic and prognostic factor in colorectal and hepatocellular malignancies where there was an increase in the expression of S100 protein from the preneoplastic phase until metastasis occurred. This was concluded after finding 161 articles over a 10-year study of the expression of S100

proteins in various malignancies related to poor differentiation, progression, advanced stage, and metastatic growth. Expression of S100 protein is considered a poor prognostic factor in some cancers [15].

5. CONCLUSION

There were significant correlation between S100 protein expression levels to clinical stage nasopharyngeal carcinoma WHO type III.

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