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Correlation Sal-Like Protein 4 (SALL4) Expression to Histopathological Subtypes in Nasopharyngeal Carcinoma at Haji Adam Malik Hospital 2020-2021

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ABSTRACT

Background
Nasopharyngeal carcinoma (NPC) is a malignant tumor in the head and neck region, which originates from nasopharyngeal epithelial cells. Management of NPC therapy is systemic therapy, surgery, radiotherapy, and chemotherapy. SALL4 is a carcinogenesis promoter gene and has been shown to play a role in modulating the effects of NPC radiation therapy.

Objective
This study aims to analyze the relationship between SALL4 expression and various subtypes of nasopharyngeal carcinoma at Haji Adam Malik Hospital in Medan in 2020-2021.

Method
Analytic descriptive study with a cross-sectional design on 33 histological specimens diagnosed with NPC from medical records/archives at the Adam Malik Haji Centre General Hospital, Medan, 2020-2021. Each specimen was stained with SALL4 immunohistochemistry. The Mann-Whitney U test (p ≥0.05) was used to assess the association of SALL4 expression with NPC histopathological subtypes based on WHO classification of head and neck tumours classification.

Result
The most gender is male 78.8% of the sample, the most age group is 41-50 years old (51.5%) of the sample. Differentiated NKSCC subtypes, strong SALL4 expression in 25% of samples, and weak expression in 75% of samples. The undifferentiated NKSCC subtype had strong SALL4 expression in 21.05% of the samples, and weak expression in 78.95% of the samples. The KSCC subtype had strong SALL4 expression in 16.7% of the samples, and weak expression in 83.3% of the samples. There was no relationship between SALL4 expression in various NPC subtypes. The Mann-Whitney U test was carried out on this variable and obtained a p-value of 0.7101 (p ≥0.05).

Conclusion
There is no correlation between SALL4's expression with subtypes in NPC.

Keywords: nasopharyngeal carcinoma, SALL4

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INTRODUCTION

Cancer in the head and neck region is a significant health problem for high-risk populations.[1] Based on GLOBOCAN 2020 data, nasopharyngeal carcinoma ranks fifth of all malignancies in Indonesia.[2] Nasopharyngeal carcinoma shows histopathological features in the form of squamous cell differentiation, which is divided into three subtypes: non-keratinizing squamous cell carcinoma (NKSCC), keratinizing squamous cell carcinoma (KSCC), and basaloid squamous cell carcinoma).[3]

Generally therapeutic management for NPC is currently carried out in the form of systemic therapy, surgery, radiotherapy, and chemotherapy.[4,5] One of the causes of failure of chemotherapy and radiation therapy is tumor heterogeneity which results in failure of tumor treatment. It is known that the KSCC subtype NPC has a low radiotherapy response and a worse prognosis compared to the NKSCC subtype.[6,7] Meanwhile, previous studies have stated that SALL4 has been shown to modulate the effect of radiation therapy on nasopharyngeal tumor cells.[8] However, studies on the presence of SALL4 on NPC have not been ever done in Medan. Thus, the author is interested in investigating how SALL4 expressed on NPC at the Adam Malik Haji Center General Hospital (RSUP) Medan in 2020-2021. This might contribute to clinicians knowing the expression of NPC samples to consider therapy with SALL4 inhibitors.

METHOD

This research is a descriptive analytic study at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara and the Anatomical Pathology Unit at the Central General Hospital (RSUP) Haji Adam Malik. This research conducted from June 2022 to January 2023 and got ethical clearance from the Health Research Ethics Committee, Faculty of Medicine, University of North Sumatra and RSUP Haji Adam Malik Medan. In this study, there were 33 NPC samples that met the inclusion and exclusion criteria.

The sample in this study was the entire accessible population using total sampling that met the inclusion and exclusion criteria. The inclusion criteria in this study were all slides derived from tissue biopsy and/or surgery that had been diagnosed histopathologically as NPC and had medical record data regarding age and sex. Exclusion criteria in this study were all paraffin slides and/or blocks that could not be reprocessed to serve as research samples.

Assessment of SALL4 expression by researchers by looking at NPC slides accompanied by two anatomical pathology specialists. Immunohistochemical expression of SALL4 (nominal scale) is by the presence of brownish stained granules in the nucleus of the tumor cells. Immunoreactivity was assessed semiquantitatively based on the multiplication of the area of positively stained tumor cells against the intensity of staining. The SALL4 expression assessment was categorized as follows percentage of area of positively stained cells (staining scope): 1=0-25%, 2=25-50%, 3=50-75%, 4=75-100%. Staining intensity (intensity): 0=negative, 1=weak positive; light yellow, 2=moderate positive; brown yellow. SALL4 expression=staining scope x intensity. SALL4 staining results: ≥6= strong expression, <6=weak expression.

The data obtained in this study were processed computerized and the results will be presented in tabular form and an analytical test will be carried out using the Mann-Whitney U test on sex characteristics, the Kruskall- Wallis test on age characteristics, and the Mann-Whitney U test on expression correlation SALL4 with NPC histopathological subtype.

RESULT

This study used 33 samples from patients with NPC, non-keratinizing squamous cell carcinoma and keratinizing squamous cell carcinoma subtypes. The distribution of the characteristic of the samples in this study can be seen in the table below (Table 1).

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Table 1. Samples characteristic distribution.

Characteristic	Total (n= 33)	Percentage (%)
Gender		
Male	26	78.8
Female	7	21.2
Age		
<20 years old	0	0
21-30 years old	2	6.06
31-40 years old	2	6.06
41-50 years old	17	51.5
51-60 years old	6	18.8
>60 years old	6	18.8
Histopathology subtype		
NKSCC undifferentiated	19	57.5
NKSCC differentiated	8	24.2
KSCC	6	18.2
Basaloid	0	0
SALL4 imunohistochemical expression		
Strong	7	21.05
Weak	26	78.8
SALL4 imunohistochemical expression based on subtype		
NKSCC undifferentiated		
Strong	4	21.05
Weak	15	78.95
NKSCC differentiated		
Strong	2	25
Weak	6	75
KSCC		
Strong	1	16.7
Weak	5	83.3

From Table 1 we can see that in this study, 26 samples were male and 7 samples were female, with the majority of the samples was from 41-50 years old group of age (17 samples). Most histopathology subtype found was NKSCC undifferentiated. SALL4 immunohistochemical staining showed strong expression in 7 (21.2%) samples and weak in 26 (78.8%) samples.

From Table 2. The Mann-Whitney U test was carried out on sex characteristics, the p-value was 0.359 and the Kruskall- Wallis test was carried out on age characteristics, the p-value was 0.094.

From Table 3, the undifferentiated NKSCC subtype obtained strong SALL4 expression in 4 (21.05%) samples, and weak in 15 (78.95%) samples. The KSCC subtype obtained strong SALL4 expression in 1 (16.7%) sample, and weak in 5 (83.3%) samples. The Mann-Whitney U test was carried out on this variable and obtained a p-value of 0.7101.

Table 2. Characteristic of nasopharyngeal carcinoma patients based on histopathological subtype.

Characteristic	Histology subtype						*P-value**
	Differentiated		*Undifferentiated*		*KSCC*		
	N	%	N	%	*N*	*%*	
Gender							
Male	7	26.9	15	57.7	4	15.4	0.359*
Female	1	14.3	4	57.1	2	28.6	
Age							
21-30 years old	1	50.0	1	50.0	0	0	
31-40 years old	0	0	1	50.0	1	50.0	
41-50 years old	4	23.5	10	58.8	3	17.7	0.094**
51-60 years old	2	33.3	3	50.0	1	16.7	
>60 years old	1	16.7	4	66.6	1	16.7	

*Mann-Whitney U test
**Kruskall- Wallis test

Table 3. Correlation of NKSCC and KSCC histopathological subtypes with SALL4 immunohistochemical expression.

SALL4	histopathological subtypes						*P-value**
	Differentiated		*Undifferentiated*		*KSCC*		
	N	%	N	%	*N*	*%*	
Strong	6	28.1	15	57.7	5	19.2	0.7101
Weak	2	28.6	4	57.1	1	14.3	
Total	8		19		6		

*Mann-Whitney U test

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Immunohistochemical Expression of SALL4 in NPC Subtypes

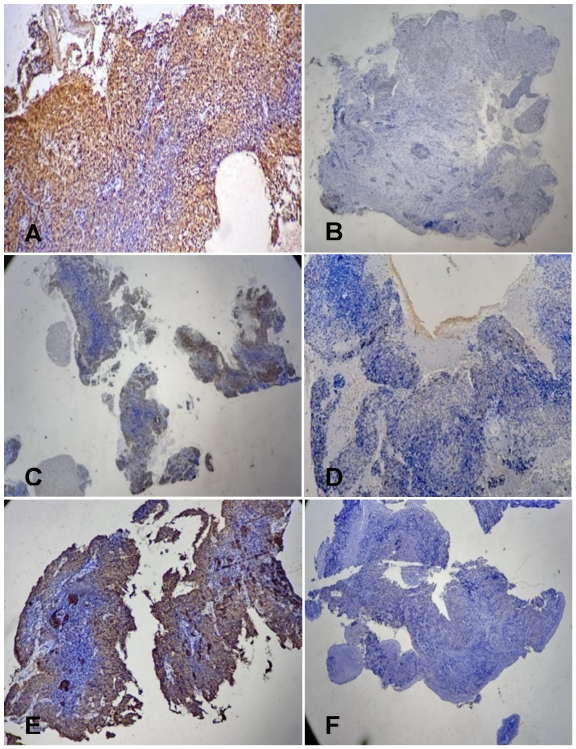


Figure 1. SALL4 expression of various NPC subtypes. A. High expression in differentiated NKSCC. B. Low expression in differentiated NKSCC. C. High expression in undifferentiated NKSCC. D. High expression in undifferentiated NKSCC. E. High expression in KSCC. F. Low expression on KSCC.

DISCUSSION

Nasopharyngeal carcinoma is a type of head and neck squamous cell carcinoma that has a unique geographic distribution, with a high incidence in China and Southeast Asia.[9] It is known that nasopharyngeal carcinoma the KSCC subtype has a lower radiotherapy response and a worse prognosis compared to the NKSCC subtype.[6] Meanwhile, previous studies have suggested that SALL4 has been shown to modulate the effect of radiation therapy on nasopharyngeal tumor cells.[7,10] There is evidence in several studies showing that SALL4 reappears in adult life can be associated with malignant transformation in tissues and also as a potential biomarker to assess cancer prognosis.[11,12]

Data from the Central General Hospital (RSUP) Haji Adam Malik Medan, there are 60 cases of nasopharyngeal carcinoma, in 2018 there were more male patients than female sufferers.[13] Research by Agus *et al* found the incidence of nasopharyngeal carcinoma at Haji Adam Malik General Hospital Medan in 2021 totaling 72 cases, with more cases occurring in males compared to women.[14]

The above is in accordance with what was found in this study, where out of 33 samples of patients with nasopharyngeal carcinoma who met the inclusion criteria were dominated by male sex by 78.8%, and the rest were female. If a comparison is made between the two sexes, the male to female ratio is 3:1. The difference between the number of incidents between men and women may be due to lifestyle habits (for example tobacco consumption) and is also thought to be related to the type of work men are more often exposed to carcinogenic which is a risk factor for developing nasopharyngeal carcinoma, such as exposure to dust, wood dust, formaldehyde, heat, and smoke.[6] Analysis of sex characteristics was carried out in association with histopathological subtypes of nasopharyngeal carcinoma, using the Mann-Whitney U test, the results obtained a p-value of 0.359 (p ≥0.05), indicating statistical test results that were not significant. There is a relationship between gender and subtypes of nasopharyngeal carcinoma. In line with previous research conducted by Agus in 2021, his research stated that there was no significant relationship between gender and histopathological type in nasopharyngeal carcinoma.[14] In this study, it was found that the most age distribution of nasopharyngeal carcinoma patients was in the age group of 41-50 years old, with the youngest being 24 years old and the oldest being 74 years old. This is in accordance with previous research conducted by Farhat *et al* at Haji Adam Malik General Hospital in 2017, stated that the incidence of nasopharyngeal carcinoma was more in the age group of 41-60 years old. From previous research Wu L *et al* in 2018 found patients with nasopharyngeal carcinoma can occur at all ages. In various countries, ages are described ranging from 4 to 91 years old.[15] Faiza *et al* also found that nasopharyngeal carcinoma can affect various ages, most commonly in the age range between

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40-60 years old.[16] In accordance with what is said in the literature, the various ages of nasopharyngeal carcinoma sufferers can be linked to the multifactorial causes of naso-pharyngeal carcinoma including strongly suspected due to poor healthy lifestyle such as smoking, alcohol, andlikes to eat preserved foods. Other risk factors including environmental factors such as exposure to dust, formaldehyde, heat, and chemical fumes are also likely to have contributed or been a risk factor for nasopharyngeal carcinoma, and are also associated because older age often decreases the body's resistance so that the body has difficulty fighting EBV or tumor antigens themselves.[6] Furthermore, an analysis of the characteristics of age associated with the histopathological subtype of nasopharyngeal carcinoma, using the Kruskall-Wallis test obtained a p-value of 0.094 (p ≥0.05), indicating statistical test results that were not significant, meaning that there was no relationship between age and histopathological subtype of nasopharyngeal carcinoma. This is in line with the research of Qu et al. who found no significant association between age and histopathological subtypes of nasopharyngeal carcinoma.[17]

One of the aims of this study was to analyze the relationship between SALL4 immunohistochemical expression and histo-pathological subtypes of nasopharyngeal carcinoma. In this study, we examined the expression of SALL4 from 33 nasopharyngeal carcinoma samples. The results showed that more samples had weak expression compared to strong expression. The undifferentiated NKSCC subtypes showed strong SALL4 expression in 4 (21.05%) samples, and weak in 15 (78.95%) samples. The KSCC subtype showed strong SALL4 expression in 1 (16.7%) sample, and weak in 5 (83.3%) samples. The Mann-Whitney U test was carried out on this variable and obtained a p-value of 0.7101 (p-value ≥0.05). In our study, the statistical test results were not significant, meaning that there was no relationship between immuno-histochemical expression and nasopharyngeal carcinoma subtype. This is in line with research conducted by Nie X *et al* have detected SALL4 expression in 131 nasopharyngeal carcinoma samples. In that study, it was stated that there was no relationship between SALL4 expression and histopathological subtypes of naso-pharyngeal carcinoma with a p-value of 0.214 (p-value ≥ 0.05).[8]

The transcription factor SALL4 is associated with embryonic cell pluripotency and has been shown to be a useful immunohistochemical marker for germ cell tumors. However, information about the distribution of SALL4 in normal human tissues and non-germ cell tumors is still limited. It is also said that SALL4 is more expressed in young cells (germ cells).[18] Currently, many studies have confirmed that SALL4 expression is associated with the prognosis of cancer patients. SALL4 activation also participates in the pathogenesis of tumor initiation and disease progression.[19,20] SALL4 expression also found in lung cancer, endometrial cancer, gastric cancer, liver cancer, and esophageal cancer. [36-40] He *et al* indicated that SALL4 may serve as an important marker for the prognosis and therapy of Esophageal Squamous Cell Carcinoma (ESCC).[21] Han *et al* concluded that high serum levels of SALL4 protein can be used as a biomarker to predict tumor recurrence and survival in hepatocellular carcinoma (HCC) patients.[22] In the head and neck region, SALL4 is positively expressed in oral squamous cell carcinoma (OSCC) and correlates with the grade of the tumor.[7] SALL4 has also been shown to modulate the effect of radiation therapy on nasopharyngeal tumor cells, where inhibition of the SALL4 gene will reduce proliferation, increase tumor cell apoptosis, and result in termination of the tumor cell cycle.[8]

It is known in several literatures including WHO Head and Neck fifth edition 2022 that nasopharyngeal carcinoma of the KSCC subtype has a low radiotherapy response and a worse prognosis compared to the NKSCC subtype. Meanwhile, previous studies have suggested that SALL4 has been shown to modulate the effects of radiation therapy on nasopharyngeal tumor cells. It is known in several literatures including WHO Head and Neck fifth edition 2022 that nasopharyngeal carcinoma of the KSCC subtype has a low radiotherapy response and a worse prognosis compared to the NKSCC subtype. Meanwhile, previous studies have suggested that SALL4 has been shown to modulate the effects of radiation therapy on nasopharyngeal tumor cell.[6,7,23,24]

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Radiotherapy with or without concomitant chemotherapy is the standard treatment for nasopharyngeal carcinoma patients, whose efficacy is limited in part by radioresistance factors. DNA methyltransferase 3B (DNMT3B) has been reported to participate in tumorigenesis through DNA methylation, but its role in mediating nasopharyngeal carcinoma development and radioresistance remains unclear. Radiation can induce DNMT3B, which may be one reason for radioresistance. DNA methyltransferases (DNMTs) are involved in transcriptional silencing and play an important role in malignant cancer DNA methylation. There are five DNMTs encoded by the human genome: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1, DNMT3A, and DNMT3B.[24,25] DNA methylation is an important feature of epigenetic gene modification, which regulates the transcriptional activity of specific genomic regions under conditions of constant DNA sequencing and controls gene expression.[26] SALL4 expression in nasopharyngeal carcinoma is highly correlated with promoter DNA methylation. The SALL4 and DNMT proteins co-occupy the same promoter site and their own transcription can be derived through recruitment of DNA methylation enzymes and histone deacetylation. mRNA expression of DNA methyltransferase 3 alpha (DNMT3A) in the SALL4 knockdown group was downregulated, indicating that DNMT3A may contribute to the regulatory effect of SALL4.[26,27]

CONCLUSION

This study concluded that there is no relationship between SALL4 expression in subtypes of nasopharyngeal carcinoma.

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