

Expression of SOX2 Immunohistochemistry Based on Histopathological Subtype of Nasopharyngeal Carcinoma at H. Adam Malik Hospital Medan

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is a malignant tumor originating from the epithelial lining in the nasopharynx. Histopathologically, NPC is divided into non-keratinizing, keratinizing, and basaloid squamous carcinoma. NPC has 5-15% of local recurrence and 15-30% of distant metastases. SOX2 is one of the markers for cancer stem cells (CSC) and can be considered as targeting therapy in NPC.

Aim: Evaluating the immunohistochemical expression of SOX-2 based on nasopharyngeal carcinoma histopathological subtypes at Haji Adam Malik General Hospital Medan

Material and methods: Descriptive research was conducted on 43 patients with NPC, which data were taken from medical records and paraffin slides/blocks. Slides stained with hematoxylin and eosin were reviewed to assess the subtypes, tils and lvi. Furthermore, SOX2 immunohistochemical staining was performed and SOX2 expression was assessed based on the subtypes of KSCC, NKSCC and Basosquamous cell carcinoma.

Results and Conclusions: Of the 43 samples of NPC, 32 samples were found at the age of 41-60 years old (74.4%), male (58.1%), undifferentiated subtype of NKSCC in 31 samples (72.1%), intratumoral tils at low in 26 samples (60.5%) and stromal tils in the low in 24 samples (55.8%). Weak SOX2 expression in 23 samples (53.5%), Based on histopathology of SOX2 expression, most KSCC strongly expressed SOX2 in 4 cases (33.3%) and NKSCC weakly expressed SOX2 in 20 cases (66.7%).

Keywords : nasopharyngeal carcinoma; histopathological subtype; immunohistochemical staining; SOX2.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a type of head and neck malignancy originating from the nasopharynx epithelial lining and is commonly found in Asian countries, including Indonesia.[1] The latest data from GLOBOCAN (Global Burden of Cancer) 2018 shows an increased in the incidence to 129,100 cases per year with a mortality rate of 73,000 cases per year.[2] In Indonesia, NPC is the 5th most commonly found malignancy after breast, cervical, lung and liver cancer. It is known that the incidence rate is 17,992 cases and the mortality rate is 11,204 cases.[3,4]

NPC management based on tumor stage and radiotherapy options, is known to have improved the prognosis of NPC in recent decades. Nevertheless, 5-15% of patients still experience local recurrence, and 15-30% undergo distant metastasis.[5] For this reason, the treatment of NPC began to focus on identifying cellular and molecular mechanisms toward treatment resistance and metastasis which were the main target in treatment discovery and new drug design.

One of the targeting technologies for molecular biology therapy is the observation of SOX2 gene (sex-determining region Y (SRY) box 2), based on gene profiling. SOX2 is marker for cancer stem cells (CSC) in

various organs including NPC.[6] Most of the deaths in NPC are related to tumor metastases compared to the primary tumor itself.[7] Wang *et al.* in his experiment in China demonstrated strong SOX2 expression in NPC was significantly associated with poorer distant metastasis free survival (DMFS) compared to those with weak SOX2 expression.[8] Luo *et al.* in his study showed that strongly expressed SOX2 in NPC has a worse prognosis than underexpressed SOX2. Most NPC patients are sensitive to radiotherapy or chemotherapy, treatment failure remains high because local recurrence development and distant metastasis.[9,10]

SOX2 expression in NPC is promoted by transcription factors such as activating protein 2 (AP-2), homeobox protein 1 (Prox1) and Pax6. E2f3a cooperates with pRb p107 family member to suppress SOX2 expression, whereas E2f3b activates SOX2 expression by recruiting RNA polymerase II to its promoter. Cyclin-dependent kinase inhibitor p21 has also been found to directly bind to SOX2 enhancers and suppress SOX2 expression in NPC.[11,12] SOX2 also plays a role in identifying distant metastases from NPC tumors due to its rapid spread and high invasiveness. But, in some literature, the role of SOX2 in tumorigenesis is still controversial.[13]

Based on the background described previously, the researcher want to know how the immunohistochemical expression of SOX2 in histopathological subtypes of nasopharyngeal carcinoma at Haji Adam Malik General Hospital Medan.

MATERIALS AND METHODS

The design of this study is a descriptive study using paraffin slide/block samples from 43 cases that have been histopathologically diagnosed as Nasopharyngeal Carcinoma (NPC) with various histopathological subtypes. Data was taken from the patient's medical record. Block samples that matched the inclusion and exclusion criteria were then cut and made immunohistochemical preparations using SOX2 antibody, with an outward pattern on the cell nucleus. Semiquantitative assessment of SOX2 expression by the researcher and two specialists in Anatomic Pathology.

RESULTS

From a total of 43 samples histopathologically diagnosed as NPC (Table 1), the following characteristics were found: the majority of patients were aged 41-60 years old in 32 cases (74.4%); the male gender was the highest with 25 cases (58.1%). Based on histopathological subtypes, the most cases were NKSCC, namely 36 cases (83.7%) with the majority being undifferentiated subtypes as many as 31 cases (72.1%) and the remaining 5 cases being differentiated (11.6%); Histopathological types of KSCC in this study were 7 cases (16.3%) and no basaloid squamous cell carcinoma (Basaloid SCC) type was found. The assessment of TILs in this study was based on intratumoral TILs and stromal TILs. High intratumoral TILs were found in 17 cases (39.5%) and low in 26 cases (60.5%). Meanwhile, high stromal TILs were found in 19 cases (44.2%) and low stromal TILs were found in 24 cases (55.8%). Location of SOX2 expression in this study are mostly found in cytoplasm of 39 cases (90.7%) and nucleus and cytoplasm in 4 cases (9.3%).

Table 1. Characteristic of Nasopharyngeal Carcinoma patient.

Characteristics	Total (n)	Percentage (%)
Age		
≤ 20 years	1	2.3
21-40	6	14
41-60	32	74.4
> 61 years old	4	9.3
Gender		
Man	25	58.1
Woman	18	41.9
Histopathological subtype		
NKSCC		
Undifferentiated	31	72.1
Differentiated	5	11.6
KSCC	7	16.3
Basaloid SCC	0	0
Intratumoral TILs		
High	17	39.5
Low	26	60.5
Stromal TILs		
High	19	44.2
Low	24	55.8
SOX2 Expression		
Nucleus	0	0
Cytoplasm	39	90.7
Nucleus and cytoplasm	4	9.3
Total	43	100

In this study, the distribution of SOX2 expression based on age was assessed (Table 2). SOX2 expression found at the age < 20 years was weak intensity 0%, moderate 0% and strong in 1 case (8.3%). At the age of 21-40 years, there were 3 cases of weak SOX2 expression (13%), moderate in 1 case (12.5%) and strong in 2 cases (16.7%). At the age of 41-60 years, 18 cases of weak SOX2 expression (78.3%), 7 cases (87.5%) and strong 7 cases (58.3%) were found. At the age of > 61 years, SOX2 expression was weak in 2 cases (8.7%) while in 0% and strong in 2 cases (16.7%).

Table 2. Distribution of SOX2 expression by age

Age	Weak	%	Currently	%	Strong	%
<20 years	0	0	0	0	1	8.3
21-40 years old	3	13	1	12.5	2	16.7
41-60 years old	18	78.3	7	87.5	7	58.3
> 61 years old	2	8.7	0	0	2	16.7

In this study, an assessment of the distribution of SOX2 expression by sex was carried out (Table 3). SOX2 expression in men with weak intensity were found in 11 cases (47.8%), moderate in 6 cases (75%) and strong in 8 cases (66.7%). In women, there were 12 cases of weak SOX2 expression (52.2%), moderate in 2 cases (25%) and strong in 4 cases (33.3%).

Table 3. Distribution of SOX2 expression by sex

Age	Weak	%	Currently	%	Strong	%
Man	11	47.8	6	75	8	66.7
Woman	12	52.2	2	25	4	33.3

The following is the distribution of SOX2 expression in NPC patients (Table 4). SOX2 was weakly expressed in 23 cases (53.5%), moderate in 8 cases (18.6%) and strong in 12 cases (27.9%).

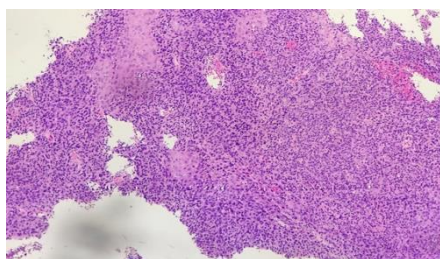
Table 4. Distribution of SOX2 expression in NPC patients

Age	Weak	%
Weak	23	53.5
Currently	8	18.6
Strong	12	27.9

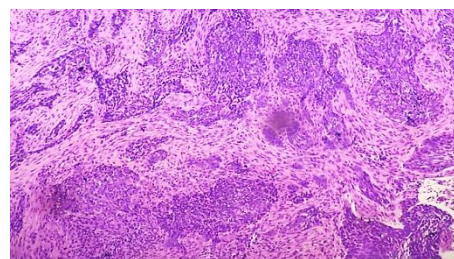
In this study, the distribution of SOX2 expression was assessed based on the histopathology of NPC (Table 5). SOX2 expression in NKSCC with weak intensity were found in 20 cases (87%), moderate in 8 cases (100%) and strong in 8 cases (66.7%). In KSCC, there were 3 cases of weak SOX2 expression (13%), moderate in 0 cases (0%) and strong in 4 cases (33.3%). SOX2 expression was not found in the Basaloid SCC type.

Table 5. Distribution of SOX2 expression based on histopathology

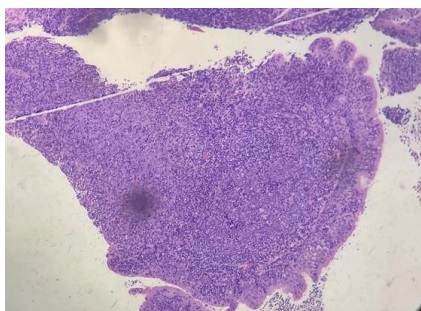
Age	Weak	%	Currently	%	Strong	%
NKSCC	3	13	0	0	4	33.3
KSCC	20	87	8	100	8	66.7
SCC Basaloids	0	0	0	0	0	0



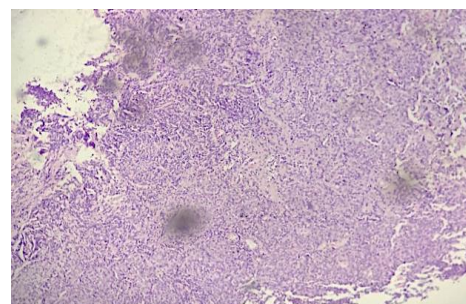
i-Tils: low, s-Tils: high.(HE 100x)



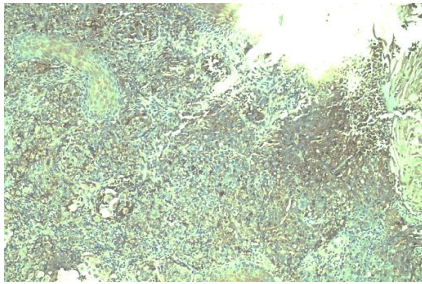
i-Tils: high, s-Tils: high. (HE 100x)



i-Tils: high , s-Tils: high(HE 100x)

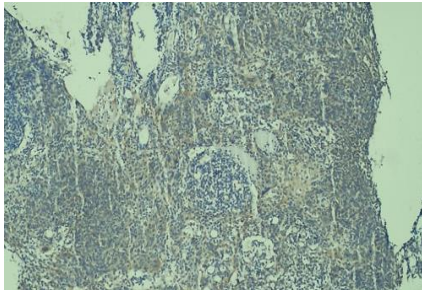


i-Tils: low, s-Tils: low (HE 100x)

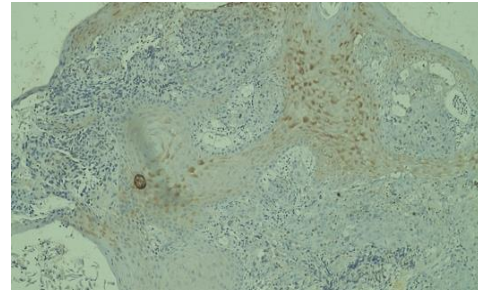


Strong SOX2 Expression

Strong SOX2 Expression



Moderate SOX2 expression



Weak SOX2 expression

DISCUSSION

In this study, most patients with NPC were found in the 41-60 year age group (74.4%). The results of this study are in line with the research conducted by Farhat *et al.*[14] According to literature, the incidence of NPC increases over the age of 30 years with a peak incidence of 40-60 years and then decreases. This situation is related to the function of DNA repair mechanisms (DNA repair) and immune system that has been decreased when experiencing mutations after the age of 40 years. Previous studies have shown that there are 3 main etiologies of NPC, namely genetic predisposition, prolonged exposure to chemical carcinogens, consumption of salted fish or fermented foods from young age and EBV infection.[15]

In this study, NPC was more common in male than female. This is in accordance with Petersson *et al.* in the WHO classification of Head and Neck (2014) stated that the incidence of NPC is more common in men than women with a ratio of 3: 1.[16] This can be caused by several factors, due to the work environment free radicals exposure, lifestyle and diet.

Based on histopathological subtypes, NKSCC were found in 36 cases (83.7%). This is in line with the research conducted by Farhat *et al.* that shows the most commonly found histopathological subtype of NPC is NKSCC (79.4%).[14] Research conducted by Suta *et al.* and Almangus *et al.* show that undifferentiated carcinoma is the most commonly found NKSCC subtype.[17,18] Basaloid squamous cell carcinoma is not found in this study. WHO Head and Neck 2016 and some journals haven't shown any incidence of basaloid squamous cell carcinoma of NPC, especially in Asia.[16]

In this study, intratumoral and stromal TILs were also assessed where the highest intratumoral TILs were found to be low in 26 cases (60.5%) and the highest stromal TILs to low were 24 cases (55.8%). Wang *et al.* study also shows low TILs were found in 299 cases while high TILs were found in 292 cases.[10] Research by Almangush *et al.* in Finland on the degree of intratumoral TILs shows that TILs was mostly found in high degree (65.2%) and the degree of stromal TILs was also mostly found at high degree (73%). Almangush *et al.* also shows a significant relationship between EBV and TILs, where tumors with positive EBV had higher stromal and intratumoral TILs. Low intratumoral TILs were associated with poor overall survival.[18] The degree of intratumoral TILs in KSSC in this study was found to be low in 7 cases (100%). The results of this TILs study have propensity for poor overall survival.

In this study, 39 samples of SOX2 expression were expressed in the cytoplasm, and 4 samples were expressed in the cytoplasm and nucleus. This is in accordance with the literature which says that SOX2 in early embryonic development is expressed in the cell nucleus but at a later stage SOX2 can be expressed in the cytoplasm. Baltus *et al.* explained that the expression of SOX2 in the cytoplasm is closely related to the location of acetylation in the nucleus of cells that export SOX2 signals to the cytoplasm during embryonic cell differentiation.[19]

SOX2 expression in this study was found to be highest in men aged 41-60 years. Elmogy *et al.* study in oral patients SCC also found the that SOX2 expression was found to be the most in men aged 50 years, but in this study there was no significant relationship between SOX2 expression and no detailed explanation was obtained.[20]

SOX2 expression of NPC patients in this study were divided into 3 degrees of distribution, namely weak, moderate and strong. The highest number was found in weak expressions, namely 23 cases (53.5%). In the research of Wang *et al.* in China where the most cases of moderate positive expression were found in 40.7% and followed by a strong positive expression of 26.9% and a weak positive expression of 24.1% and it was stated that a strong expression of SOX2 in NPC was associated with distant metastasis-free survival. (DMFS).[14]

In this study, weak SOX2 expression was more common, which was expressed in the undifferentiated subtype of NKSCC in 20 cases (87%). This study is in line with the research of Wang *et al.* who found that SOX2 expression in NPC was higher in undifferentiated NKSCC.[21] This was presumably because undifferentiated NKSCC had poor differentiation with a high degree of mitosis resembling cells at the embryological stage.

CONCLUSION

Nasopharyngeal carcinoma (NPC) is more common in males in the 41-60 year age group. In this study, the expression of intratumoral TILs and stromal TILs was found to be more common in low grades. Weak, moderate, and strong SOX2 expression was more commonly found in the 41-60 year age group and the highest expression was found in weak expression. SOX2 expression based on gender was found to be varied, strong SOX2 was found mostly in males while weak SOX2 was found mostly in females. SOX2 expression either weak, moderate and strong were mostly expressed in NKSCC cases.

COMPETING INTEREST

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

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ETHICAL APPROVAL

The Health Research Ethical Committee, University of North Sumatra, Medan, Indonesia approved this study.

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