



International Journal of Research Publications

Correlation of Tumor-Stroma Ratio (TSR) With Primary Tumor Size and Lymph Node Involvement in Nasopharyngeal Carcinoma

Fiora Octrin Purba¹, Soekimin¹, T Ibnu Alferraly¹

¹Department of Anatomical Pathology, Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia

Abstract

Background: Nasopharyngeal carcinoma (NPC) is the fifth most common malignancy in Indonesia. Diagnosis of NPC is frequently established in late stage showing regional lymph node metastasis. Tumor-stroma ratio (TSR) has recently been identified as one of independent prognostic factor for several solid tumor, which low TSR (predomination of stromal component) correlates with unfavorable prognosis and increased risk of relapse.

Objective: This study aimed to investigate the correlation of TSR with tumor size and lymph node involvement in NPC.

Material and Method: A total of 68 hematoxylin-eosin stained histological slides of patients with NPC who underwent biopsy were reviewed. The percentage of tumor stroma was visually estimated with cut-off value of 50% stroma percentage. Patients with <50% stroma were categorized as high TSR group and those with $\geq 50\%$ stroma were classified as low TSR group. Correlations of TSR with tumor size and lymph node involvement were determined using Spearman correlation test.

Result: Based on data, of 68 patients; 36 (52,9%) had low TSR and 32 (47,1%) had high TSR. Tumor T2 had highest percentage in both low and high TSR (44,4% and 43,75%). The low TSR patients had higher number of positive N stage than high TSR. TSR was not significantly correlated with tumor size ($p=0,410$) and lymph node involvement ($p=0,507$). These results demonstrated that TSR itself could not clearly predict progression of T&N stage of NPC.

Conclusion: Stroma percentage was not correlated with tumor size and lymph node involvement in NPC.

Keyword: nasopharyngeal carcinoma, tumor-stroma ratio, tumor microenvironment

1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor originating from nasopharyngeal epithelial cells, and is most often found in the Rosenmüller fossa where tumor will invade the nearby structures. In some countries in the world, NPC is still rare (1.2 / 100,000 population per year), but in Inuit people, North African, and Chinese from Southeast Asia the NPC is more common. Based on data from GLOBOCAN-IARC 2018, in Southeast Asia, NPC ranks the tenth most cancer with an incidence of 5 / 100,000 population, frequently found in men than women (7.8: 2.4). While in Indonesia, 17,992 new cases of NPC were found in 2018 with incidence of 6.6 / 100,000 and is the third highest number of all cancers in men (10.5 / 100,000 men). Nasopharyngeal carcinoma is also the sixth highest cause of death from cancer with an annual incidence of 11,204 cases. Previous report showed a nasopharyngeal incidence in Indonesia of 5.66 / 100,000 population, with a male-female ratio of 2.4: 1 and the incidence in Medan receiving 4.3 / 100,000 population.

The relationship of neoplastic and stromal cells contributes to functional and structural support to tumor microenvironment, which plays role in tumor progression and metastasis. Aggressive tumor cells can induce changes in the phenotype and stromal function which will increase its aggressive potential. Tumor-induced stroma consists mainly of

mesenchymal cells, including fibroblasts and myofibroblasts, structural proteins and signaling molecules. Other populations in the tumor's microenvironment are endothelial cells, smooth muscle cells, and inflammatory cells or immune cells. These cells contribute to the accumulation of several types of cytokines and growth factors, angiogenic molecules, proteolytic enzymes which results in tumor growth and local invasion as well as metastasis. Therefore, an increase in the component of the stroma can increase the potential for tumor aggressiveness and poor clinical outcomes.

Tumor-stroma ratio (TSR) is a percentage of neoplastic cell relative to tumor stroma in tumor tissue, which low TSR value indicates a high proportion of stroma (rich stroma). Several studies regarding the assessment of tumor-stroma ratio (TSR) show that TSR can be an independent prognosis factor for some carcinomas. The tumor-stroma ratio (tumor-stroma ratio) in pathology specimens has been a concern for predicting tumor prognosis. Research by Zhang et al. concluded that NPC with a lot of stroma (rich stroma) is associated with a poor prognosis and an increased recurrence rate, so TSR can be a characteristic of the new histological prognosis in NPC.

2. Material and Methods

A total of 68 hematoxylin-eosin stained histological slides of NPC patients who underwent biopsy with clinical and radiology (CT-scan with TNM stage) data were reviewed. The data were collected from medical record in Haji Adam Malik General Hospital Medan Indonesia and patients who have been treated previously with chemotherapy, radiotherapy or combination were excluded. This study has been approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Sumatera Utara.

TSR assessment is done by determining the most invasive part of tumor with a low magnification (40x) and by using 100x magnification, this predetermined field of view must be found in the tumor cell and stroma component, and tumor cells must be found in minimal four field of view. Tissues around the stroma that do not contain tumors are considered to be unrelated to the tumor and are not included in the discussion. Stromal proportions were estimated in each sample. The cut-off value used is 50%, and divided into two categories: low stroma-high TSR (with stromal proportions <50%) and high-stromal / low-TSR (with stroma proportion $\geq 50\%$). TSR group. Correlations of TSR with tumor size and lymph node involvement were determined using Spearman correlation test.

3. Results

In this study, 68 cases of nasopharyngeal carcinoma patients that met the inclusion and exclusion criteria. All samples were obtained from nasopharyngeal biopsy. The distribution of the characteristics of sample can be seen in table 1.

Table 1. Distribution of the characteristic of samples

Characteristic	Total (n=68)	Percentage (%)
Sex		
) Male	49	72,1
) Female	19	27,9
Age (years); mean $\pm$ DS	46,5 $\pm$ 13,1	46,5 $\pm$ 13,1
Histopathology		
) NKSCC	62	91,2
) KSCC	6	8,8
) Basaloid SCC	0	0
Tumor size		
) T1	12	17,6
) T2	30	44,1
) T3	12	17,6
) T4	14	20,6
Lymph node involvement		
) N0	5	7,4
) N1	19	27,9
) N2	21	30,9
) N3	23	33,8
Tumor-Stroma Ratio		
) Low	36	52,9
) High	32	47,1

The mean age of patients in this study was 46.5 years with a standard deviation of 13.1 years, where the youngest was 14 years old and the oldest was 75 years. The most common age group was found in the age group 41-60 years, as many as 43 cases (63.2%). The distribution of tumor size of nasopharyngeal carcinoma based on the Tumor-stroma ratio (TSR) in this study can be seen in table 2.

Table 2. Distribution of tumor size based on tumor-stroma ratio (TSR)

Table 2: Distribution of tumor size based on tumor-stroma ratio (TSR)						
No	Tumor size	Tumor-Stroma Ratio				Total
		Low		High		
		n	%	n	%	
1.	T1	8	66,7	4	33,3	12
2.	T2	16	53,3	14	46,7	30
3.	T3	4	33,3	8	66,7	12
4.	T4	8	57,1	6	42,9	14
Total		36	52,9	32	47,1	68

The distribution of lymph nodes involvement in nasopharyngeal carcinoma based on the Tumor-stroma ratio (TSR) in this study can be seen in table 3.

Table 3. Distribution of lymph node involvement of nasopharyngeal carcinoma based on Tumor-Stroma Ratio (TSR)

Distribution of lymph node involvement of nasopharyngeal carcinoma based on Tumor-Stroma Ratio (%)						
No	Lymph node involvement	Tumor-Stroma Ratio				Total
		Low		High		
		n	%	n	%	
1.	N0	1	20,0	4	80,0	5
2.	N1	11	57,9	8	42,1	19
3.	N2	11	52,4	10	47,6	21
4.	N3	13	56,5	10	43,5	23
Total		36	52,9	32	47,1	68

Spearman correlation statistical test was performed to analyze the correlation between TSR and tumor size as well as TSR and lymph node involvement. The results concluded that the correlation of TSR with both of tumor size and lymph node involvement were not significantly correlated where p value was 0.410 ($p > 0.05$) and 0.507 ($p > 0.05$), respectively.

4. Discussion

In terms of sex characteristics, in 68 samples obtained men amounted to 72.1% with a ratio of men and women ranging from 2.57: 1. This is similar to the data obtained from the research of Adham et al. in which a male: female ratio of 2.4: 1. Several other studies elsewhere also reported that NPC were dominated by men compared to women, with an average ratio of 2-3: 1.5,16. This was thought to be related to living and work habits that caused men to be frequently exposed to the carcinogens that cause NPC (exposure to chemicals, smoke, dust, cigarettes etc.). According to the meta-analysis of Xue et al, smoking increases the risk of NPC by 1.6% .

From table 1 it is known that the most common histopathology type of NPC is the non-keratinizing squamous cell carcinoma (NKSCC), which is 62 cases (91.2%) of the total 68 cases, while the keratinizing squamous cell carcinoma (KSCC) type is the least found, that is only 6 cases (8.8%). These results are in accordance with the literature which states that NKSCC is the most common type. Based on data from medical records, the histopathological diagnosis of nasopharyngeal carcinoma in this study still uses a previous WHO classification that uses undifferentiated carcinoma entities as distinguished from NKSCC. Basaloid SCC entities have not been commonly used because of their very rare incidence and after reviewing all the the samples, Basaloid SCC type was not found in this study. The results of this study are also in line with other studies conducted by Adham et al, Zhang et al, Tambunan NS, Asnir et al and Earnesty.

Regarding of tumor size, the most common was T2 amounted 30 cases (44.1%) of 68 samples, followed by T4 of 20.6% and the least were T1 and T3 of 12 cases respectively (17.6%). The results of this study are in line with studies conducted by Fasyah. However, this result differs from the study of Zhang et al. where in that study, the most common tumor size was T3 with a percentage of 37.6% and T2 was 31.2%. Likewise with research conducted by Chrestella et al. obtained the highest tumor size was T3 (32.5%). Nasopharyngeal carcinoma is difficult to diagnose at an early stage, because of its difficult anatomical location, which is hidden behind the nasal cavity, and early symptoms of Anpc are similar to other respiratory tract diseases so the patient comes in advance symptoms (expansion of tumor size and lymph node metastasis). In this study, we took samples of NPC patients who had just been diagnosed and had never received radiotherapy, chemotherapy or a combination of both. In addition, in Zhang's study, the TNM classification system used was still based on UICC / AJCC TMN staging system 6th Edition, while we used the 7th Edition, where there was a change in the T2a to T1 category in staging system.

For status of lymph node involvement, the highest was N3 which obtained in 23 cases (33.8%), followed by N2 (30.9% or 21 cases). This is not in line with the study of Zhang et al., who found that the highest percentage was N2, (43%), followed by N1 (32.3%). The results of this study are in line with research conducted by Fasyah where N3 was the most found,

amounting to 37.6%. This can also be seen from the main complaint of the most NPC patients ever reported is lump in the neck (81.6%) which causes discomfort to the patient so they come for treatment. The lump indicates the expansion / metastasis of the tumor to lymph nodes. NPC usually spreads through lymph vessels to the regional neck lymph nodes. Lymphatic flow from the Rossenmuller fossa flows into the Rouvier node to the retrofaringeal space and continues to the upper neck lymph nodes.

In this study, we assessed the value of tumor-stroma ratio (TSR) by measuring the proportions of tumor cells compared to its stroma, where the percentage of stroma was estimated at each tumor size (T) and each lymph node involvement (N). From this study it was found that the most TSR was low TSR which showed a greater percentage of stroma than tumor cells ($\geq 50\%$). A total of 36 cases (52.9%) of 68 study samples had low TSR values and 32 other cases (47.1%) had high TSR values (the percentage of tumor stroma was lower $<50\%$). This is not in line with the study of Zhang et al in China, where most of the NPC cases in his study (54.8%) had poor stroma (high TSR) and 45.2% had rich stroma (low TSR).

From all samples with low TSR values in this study, obtained T2 tumor size had the highest percentage in the range of 44.4% (16 cases) and T3 tumor size had the lowest percentage of 11.1% (4 cases). Whereas in the NPC case group with high TSR values, the most common tumor size was T2 with a percentage of 43.75% (14 cases) and T1 tumor size had the lowest percentage at 12.5% (4 cases). When compared with the study of Zhang et al. from 42 samples with low TSR values different results were obtained, in which in this study the tumor size with a low TSR value was found at T4 (33.3%) and the least was found in T1 tumor size, which was 11.9% (5 cases). Whereas in the group with a high TSR value (stroma poor) T3 tumor size obtained had the highest percentage of 45.1% (23 cases) and the lowest was T1 tumor size of 5.9% (3 cases).

Regarding the status of lymph node involvement in 36 samples with low TSR values in this study, the highest percentage was N3 in the amount of 36.1% (13 cases) and the lowest was N0 in 2.8% (1 case) where this is not in line with Zhan, et al. which showed the highest percentage in N2 (38.1%) but had similarities in the lowest percentage found in N0 (9.5%) of 42 samples. Whereas in the 32 NPC samples with high TSR values in this study, N2 and N3 had the same percentage of 31.3% (10 cases) respectively and the lowest was found in N0 which was 12.5% (4 cases). This result is slightly different from Zhang's research which found that the highest percentage was found in N2 (47.1%) and the lowest was found in N3 (7.8%) of 51 samples.

TSR assessment was assessed in the area of the tumor front, where a stromal reaction to tumor cells will be seen. As with other TSR studies on solid organs such as colon, TSR assessment is carried out on the most invasive part of the tumor. Lymphocytes are one of the stromal components that are often found in NPC and are taken into account in TSR assessment in NPC. This makes it difficult to evaluate the percentage of stromal components in NPC, so that further research is needed by using additional examinations to be able to distinguish the percentage of stromal components and tumor cell components more clearly.

Spearman correlation test was performed to test the correlation between TSR and tumor size of NPC, p value = 0.410 ($p > 0.05$) was obtained which shows there was no significant correlation between TSR and tumor size. This result is in accordance with research conducted by Zhang et al. Regarding the correlation between TSR and lymph nodes involvement of NPC, p value = 0.507 ($p > 0.05$) was obtained. This result also shows that there was no significant correlation between TSR and lymph node involvement, this is in accordance with the research of Zhang et al.

References

- Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, et al. Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. *Chin J Cancer*. 2012;31(4):185–96.
- Chrestella J, Farhat F, Daulay ER, Asnir RA, Yudhistira A, Nasution IA. Cyclooxygenase-2 expression and its correlation with primary tumor size and lymph node involvement in nasopharyngeal carcinoma. *Maced J Med Sci*. 2018;6(11):2001–5.
- Chua MLK, Wee JTS, Hui EP, Chan ATC. Nasopharyngeal carcinoma. *Lancet* [Internet]. 2015;6736(15):1–13. Available from: [http://dx.doi.org/10.1016/S0140-6736\(15\)00055-0](http://dx.doi.org/10.1016/S0140-6736(15)00055-0).
- Earnesty G. Hubungan nilai mean platelet volume terhadap stadium klinis dan tipe histopatologi pada penderita karsinoma nasofaring di RSUP Haji Adam Malik Medan pada Tahun 2014 – 2016 [Internet]. Universitas Sumatera Utara; 2018. Available from: <http://repository.usu.ac.id/handle/123456789/12325>.
- Fasyah I. Response Rate Post Kemoradioterapi pada penderita KNF di RSUP.H.Adam Malik Medan Tahun 2014 - 2016 [Internet]. Universitas Sumatera Utara; 2018. Available from: <http://repository.usu.ac.id/handle/123456789/12325>.
- Jia W, Qin H. Seminars in Cancer Biology Non-viral environmental risk factors for nasopharyngeal carcinoma : A systematic review. *Semin Cancer Biol* 2012;22(2):117–26.
- Li J, Zou X, Wu Y, Guo J, Yun J, Xu M, et al. A Comparison between the Sixth and Seventh Editions of the UICC / AJCC Staging System for Nasopharyngeal Carcinoma in a Chinese Cohort. *PLoS One*. 2014;119:1–20.
- Petersson B, Bell D, El-Mofty SK, Gillison M, Lewis JS, Nadal A, et al. Nasopharyngeal carcinoma. In: El-Naggar AK, Chan JK, Grandis J, Takata T, Sliotweg PJ, editors. *WHO Classification of Head and Neck Tumours*. 4th Ed. Lyon: IARC; 2017. pp. 64–70.
- Tambunan NS. Hubungan ekspresi imunohistokimia Vascular Endothelial Growth Factor (VEGF) dan Tumor-infiltrating lymphocytes (TILs) dengan tipe histopatologi dan stadium klinis karsinoma nasofaring [Internet]. Universitas Sumatera Utara; 2016. Available from: [repository.usu.ac.id/bitstream/handle/123456789/67168/Chapter II.pdf?...4...%0A](http://repository.usu.ac.id/bitstream/handle/123456789/67168/Chapter%20II.pdf?...4...%0A).
- WHO. Indonesia [Internet]. IARC. 2019. Available from: [http://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact sheets.pdf](http://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact%20sheets.pdf).
- Zhang X, Zhang Z, Liu F, Cheng Y. The Tumor-stroma ratio is an independent predictor for survival in nasopharyngeal cancer. *Oncol Res Treat*. 2014;37:480–4.