Angiogenesis, Inflammation, Platelets Count, and Metastatic Status as a Predictor for Thrombosis Risk in Nasopharyngeal Carcinoma Patients

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ABSTRAK

Tujuan: *untuk menilai penggunaan angiogenesis, peradangan, jumlah trombosit, dan status metastasis sebagai prediktor risiko trombosis yang diwakili oleh tingkat P-selektin pada pasien karsinoma nasofaring (NPC).* ***Metode:*** *studi potong lintang dilakukan pada pasien karsinoma nasofaring (NPC) di poliklinik Hematologi dan Onkologi Klinik Rumah Sakit Cipto Mangunkusumo, Jakarta, pada Mei - Oktober 2012. Data mengenai angiogenesis (CD105 dan VEGFR-2), peradangan (IL-6), jumlah trombosit, dan status metastasis dinilai pada saat pendaftaran, serta tingkat P-selektin larut untuk semua pasien yang memenuhi syarat penelitian. Analisis bivariat dilanjutkan dengan analisis regresi linier berganda untuk mengidentifikasi prediktor independen tingkat P-selektin larut.* ***Hasil:*** *dilakukan analisis pada 60 pasien NPC yang terdaftar dalam penelitian ini. Terdapat korelasi antara jumlah trombosit (r=0,389; p=0,002), IL-6 (r=0,595; p<0,001) dan jumlah metastasis (r=0,542; p<0,001) dengan tingkat P-selektin larut. Analisis korelasi menunjukkan bahwa ketiga variabel tersebut dapat memprediksi tingkat P-selektin larut yang disesuaikan dengan R-kuadrat 65%. Tidak ditemukan korelasi antara VEGFR-2 dan CD105 dengan tingkat P-selektin larut.* ***Kesimpulan:*** *jumlah trombosit, IL-6, dan status metastasis dapat digunakan sebagai prediktor tingkat P-selektin larut sebagai parameter risiko trombosis pada pasien NPC.*

Kata kunci: *karsinoma nasofaring (NPC), risiko trombosis, P-selektin larut.*

ABSTRACT

Aim: *to assess the use of of angiogenesis, inflammation, platelets count, and metastatic status as predictors for thrombosis risk represented by soluble P-selectin level in nasopharyngeal carcinoma (NPC) patients.* ***Methods:*** *a cross sectional study was conducted on NPC patients at the Hematology and Oncology Clinic of Cipto Mangunkusumo Hospital, Jakarta, during Mei to October 2012. Data regarding angiogenesis (CD105 and VEGFR-2), inflammation (IL-6), platelets count, and metastatic status were assessed at enrollment, as well as soluble P-selectin levels in all eligible patients. Bivariate analysis continued with multiple linear regression analysis were done to identify independent predictors for soluble P-selectin levels.* ***Results:*** *sixty NPC patients were enrolled in the study. There was correlation between platelet counts (r=0.389; p=0.002), IL-6 (r=0.595; p<0.001) and number of metastatic sites (r=0.542; p<0.001) with soluble P-selectin level, and a linear regression analysis showed that these three variables can predict soluble P-selectin levels with adjusted R-square 65%. There was no correlation between VEGFR-2 and CD105 levels with soluble P-selectin levels.* ***Conclusion:*** *platelet counts, IL-6 level, and number of sites of metastasis can be used as predictors of soluble P-selectin level as parameter of thrombosis risk in NPC patients.*

Key words: *nasopharyngeal carcinoma (NPC), thrombosis risk, soluble P-selectin.*

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an interesting cancer because of its defined geographic and racial distribution.[1-5] It is a highly prevalent malignant disease in several regions such as Southern China, especially in the Guangdong Province and has the highest prevalence in the world with 20-40 cases per 10^5 inhabitants. Recent data indicated intermediate incidence rates of NPC can be found in Southeast Asia, including Indonesia with 5.66 cases per 10^5 inhabitants. Nasopharyngeal cancer is frequently found in Indonesia, rating as the fourth most common cancer after cervical cancer, breast cancer and lung cancer.[6]

Cancer is frequently complicated by the development of thrombosis. The incidence of thrombosis in cancer patients is 20%, and several risk factors have been described in recent studies and include tumor type, tumor stage, age, sex, racial, year of diagnosis, surgery, chemotherapy, and the use of erythopoiesis-stimulating agents. The patients with metastatic cancer who have the highest incidence of thrombosis are i.e., pancreatic, ovarian, renal, lung, and brain cancers.[7-9] Recent studies showed the incidence of NPC with venous thromboembolism (VTE) to be 4.3%.

Thrombosis is promoted by angiogenesis and metastasis. Angiogenesis has the essential role in the formation of new vascular networks to support cancer growth, progression of cancer and metastasis in the head and neck cancer, including nasopharyngeal carcinoma (NPC). Angiogenesis induces activation of platelet and endothelial cell inflammation that stimulates the release of angiogenic factors and synthesis of interleukin-6 (IL-6) by macrophages and endothelial cells, increasing the incidence of thrombosis in cancer patients. Endothelial cells inflammation induce express tissue factors that is one of the key procoagulants, one of them being soluble P-selectin.[10-13] Recent studies showed that while the levels of the human soluble P-selectin are more than equal to 53.1 ng/ml, implying that P-selectin can be used as a predictor of venous thromboembolism in cancer patients.

Angiogenic factors also include activator and inhibitor molecules that influence the angiogenic process. Other proteins have been identified such as the angiogenic activator, including vascular endothelial growth factor (VEGF), transforming growth factor (TGF), and interleukin. The VEGF and their receptors includes VEGFR-2 and TGF and their receptors includes CD105 are receiving increasingly more attention in the field of angiogenesis. Based on these studies, the existence of VEGFR-2 and CD105 in the blood of cancer patients could be used as angiogenic parameters.[14]

To reduce the mortality and morbidity from NPC caused by thrombosis, it is therefore important to identify potential predictors for thrombosis in NPC. This study was aimed to study the correlation of angiogenesis, inflammation, and metastatic status with thrombosis in NPC patients. The specific purpose in this study was to determine correlation between parameters (CD105, VEGFR-2, IL-6, platelet count and metastatic sites) with soluble P-selectin and to observe any potential parameters that could be used as predictors for thrombosis in NPC.

METHODS

A cross sectional study was conducted among nasopharyngeal (NPC) cancer patients who visited the Hematology and Oncology Clinic of Cipto Mangunkusumo Hospital, Jakarta, from May to October 2012. Patients were included in the study if they have histologically confirmed diagnosis of NPC and underwent NPC staging (nasopharyngeal CT-scan, chest x-ray and CT-scan, and bone scan). Patients who recieved therapy before the enrollment, have systemic infection, active hemorrhagic, other malignant diseases, diabetes, end-stage renal disease, pregnant women, and were on antiplatelet and / or anticoagulants were excluded from the study. The study protocol has been approved by Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia.

Demographics data was confirmed by individual reports, while data regarding clinical, laboratory, radiographical, and histopathological examinations were obtained from patient's medical records. Detection of CD105, VEGFR-2, IL-6, as well as soluble P-selectin was done using ELISA. Platelet count was performed by Sysmex

Analyzer XE-2000.

Data regarding demographical and clinical characteristics was presented to describe study population. The correlations between CD105, VEGFR-2, IL-6 and platelets counts with soluble P-selectin were tested using Pearson's correlation test, while the correlation between number of metastatic sites with soluble P-selectin was tested using Spearman's correlation test in bivariate analysis. All predictors revealed p-value of <0.2 in bivariate analyses included in multivariate linear regression analysis to identify independent predictors for soluble P-selectin level. All statistical procedures were conducted using SPSS version 16.

RESULTS

Characteristics of Subject

During the study period, 60 patients met the eligibility criteria and were enrolled. Of the total number of patients, 45 (75%) of subjects were males with the mean age of subjects between 43.9 (standard deviation ±11.58) years old and most of them are in the 31-40 years age bracket. Demographical and clinical characteristics of study subjects are shown in **Table 1**.

Table 1. Characteristics of subject (N=60)

Characteristics	N (%)
Age (years), mean (SD)	43.9 ± 11.58
Age group	
- <20 years	2 (3.3%)
- 20 – 30 years	4 (6.7%)
- 31 – 40 years	17 (28.3%)
- 41 – 50 years	15 (25%)
- >50 years	22 (36.7%)
Stage	
- I – IVA	22 (36.8%)
- IVB	19 (31.7%)
- IVC	19 (31.7%)
Metastatic	19 (31.7%)
Hemoglobin levels (g/dL), mean (SD)	12.36 ± 2.04
Leukocyte count (per mm^3), mean (SD)	11.38 ± 8.36
Platelets count (per mm^3), mean (SD)	326.43 ± 108.47

Correlation between Angiogenesis, Inflammation, Platelet Counts, and Metastatic Status with Risk of Thrombosis

Table 2 shows the correlation between parameters of angiogenesis (CD105, VEGFR-2), inflammation (IL-6), platelet counts, dan metastatic status with risk of thrombosis (soluble P-selectin level) among study subjects.

Table 2. Coefficient correlation of predictors with soluble P-selectin

Predictors	Coefficient correlation	p-value
CD105	0.458	0.09
VEGFR-2	0.145	0.19
IL-6	0.595	<0.001
Platelet counts	0.39	<0.001
Number of metastasis sites	0.527	<0.001

Based on bivariate analyses above, we include all predictors in the multivariate linear regression analysis which revealed IL-6, platelet counts, and number of metastasis sites as independent predictors for soluble P-selectin level, with adjusted R^2 of 0.65.

DISCUSSION

The diagnosis and treatment of NPC in Indonesia are not without their challenges. First of all, a large number (**Table 1**) of patients who present themselves at the clinic are at their peak of their productivity – ages younger than 50 years – an age bracket which still has families to support. Secondly, another problem is the disease stage: more than 30% presented as metastatic disease. Stages I and II usually respond well to radiotherapy with / without chemotherapy but at present, current treatment of recurrent/metastatic disease has not been satisfactory.

Histopathologically, around 83.3% of cases proved to be undifferentiated carcinoma, or type III acoording to the WHO classification of NPC, with 15% WHO type II NPC tumors and 1.7% WHO type I NPC tumors. This is to compare with other studies in Singapore and China which reported NPC WHO type III as the most prevalent form.[15,16]

The result of radiology examination showed that most specific metastatic sites of NPC are bone metastasis, followed by lungs, non regional lymph tissue, liver and central nervous system (84.2%, 5% and 3.3% incidence for non regional lymph, liver, and central nervous system). A number of studies had reported the same results for metastatic sites of NPC. It showed that bone had the highest incident metastatic sites in a Tunisian and Singaporean study.[17,18] This study results showed around 52.6% of cases in bone metastasis was found in single bone metastasis sites followed by 47.4% incidence in multiple bone metastasis. NPC metastasis was more incidence in single organ and two organs with 13.3% incidence than in three organs only found 5% incidence. Metastatic sites was more found in NPC WHO type II and type III than NPC WHO type 1.

The aforementioned situation is compounded by the fact that cancer patients – NPC being one of them – have their treatment complicated by thrombosis. It is part of the cancer process and thrombosis in the form of lung embolism is the most common cause of death second only to the progression of the cancer.[8]

And not only are the types of cancer and stages are relevant in the thrombotic process, the cancer patient has also several risk factors predisposing to thrombosis its consequences, such as hospitalization and immobilization, the use of central catheters, and many comorbid conditions associated mainly in the elderly. It is thus very important that a marker – or set of markers – is found.

Using the ANOVA statistical test it was found that platelet count, interleukin-6 and metastatic status had a significant difference with sP-selectin. This result was the same as reported by Violetta et al where correlation between interleukin-6 and sP-selectin in the colorectal cancer patients had a significant difference. The regression linear test showed platelet count, interleukin-6 and metastatic status had a significantly influence sP-selectin with 67.1%. By using the same statistical test it was revealed that platelet counts influenced sP-selectin with 38.9% or r=0.389, different from that reported by Ay et al (r=0.19).[19]

Based on these result, we found that interleukin-6, an inflammatory parameter, platelet count and metastatic status could be used as potential predictors for thrombosis in NPC because of the correlation with the expression of sP-selectin in humans. Generally, the results of this study was good enough as it was able to collect 60 NPC patients and all of them were able to undergo testing for sP-selectin and platelet counts.

This study was as the first investigation aiming to measure sP-selectin, VEGFR-2, and CD105 in among NPC patients in Southeast Asia. As most patients were found at later stages of the disease, a larger sampling consisting of the whole spectrum of stages (I,II,II and IV) might provide better data and is on planning. It is hoped that future testing will include platelet activators and other factors contributing to the elucidation of sP-selectin, including leukocytes, lipid profiles and endothelial cells dysfunction.

CONCLUSION

In this study, there was statistically significant correlation between interleukin-6, platelet count, and metastatic status with sP-selectin. VEGFR-2 and CD105 had not a significant correlation with interleukin-6, sP-selectin, and metastatic status. So that, the combination of interleukin-6 (inflammatory parameters), platelet count and metastatic status have the potential to predict the occurrence of thrombosis in NPC patients. It is hoped that these findings will contribute to the prevention of thrombosis in NPC patients.

REFERENCES

1. Baizing NM, Morand P, Seigneurin JM, et al. Complementary determination of Eipstein-Barr virus DNA load and serum markers for nasophayngeal carcinoma screening and early detection in individual at risk in Tunisia. Eur Arch Otorhinolaryngol. 2012; 269(3):1005-11.
2. Bray F., Haugen M, Moger TA, Tretli S, Aalen OO, Grotmol T. Age incidence curves of nasopharyngeal carcinoma worldwide: bimodality in law-risk populations and aetiologic implications. Cancer Epidemiol Biomarkers Prev. 2008;17(9):2356-65.
3. Busson P, Keryer C, Ooka T, Corbex M. EBV-associated nasopharyngeal carcinoma: from epidemiology to virus targeting strategies. Trends Microbiol. 2004;12(8):356-60.

4. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev. 2006;15(10):1765-77.
5. Dymicka-Piekarska V, Matowicka-Karna J, Gryko M, Kemona-Chetnik I, Kemona H. Relationship between soluble P-selectin and inflammatory factors (interleukin-6 and C-reactive protein) in colorectal cancer. Thrombosis Res. 2007;120(4):585-90.
6. Adham M, Kurniawan AN, Muhtadi AI, et al. Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. Chinese J Cancer. 2012;31:185-9.
7. Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. JAMA. 2005;293(6):715-22.
8. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. Blood. 2008;11(10):4902-7.
9. Paneesha S, McManus A, Arya R, et al. Frequency, demographics and risk (according to tumour type or site) of cancer-associated thrombosis among patients seen at outpatient DVT clinics. Thromb Haemost. 2010;103(2):338-43.
10. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Nat Cancer Ins. 1990;82(1): 4-7.
11. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1:27-31.
12. Folkman J. Clinical applications of research on angiogenesis. N Engl J. 1995;333:1757-63.
13. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol. 2002;29(6):15-8.
14. Pan MH, Hsieh MC, Kuo JM, et al. 6-shogaol induces apoptosis in human colorectal carcinoma cells via ROS production, caspase activation, and GADD 153 expression. Mol Nutr Food Res. 2008;52:527-37.
15. Liu Q, Chen J-O, Huang Q-H, Li YH. Trends in the survival of patients with nasopharyngeal carcinoma between 1976 and 2005 in Sihui, China: a population-based study. Chinese J Cancer. 2013;32(6):325-33.
16. Shanmugaratman K, Chan SH, de-The G, et al. Histopathology of nasopharyngeal carcinoma: correlation with epidemiology, survival rates and other biological characteristics. Cancer. 1979;44(3):1029-44.
17. Khanfir A, Frikha M, Ghorbel A, Drira MM, Daoud J. Prognostic factors in metastatic nasopharyngeal carcinoma. Cancer Radiother. 2007;11(8):461-4.
18. Ong YK, Heng DM, Chung B, et al. Design of a prognostic index score for metastatic nasopharyngeal carcinoma. Eur J Cancer. 2003;39(11):1535-41.
19. Ay C, Pabinger I. Predictive potential of haemostatic biomarkers for venous thromembolism in cancer patients. Thromb Res. 2012;129(1):6-9.