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HIGH SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR-A IS ASSOCIATED WITH ADVANCE BLADDER CANCER STAGES.

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ABSTRACT

Background: Angiogenesis and lymphangiogenesis play key role in bladder cancer progression. Vascular endothelial growth factor (VEGF) is extensively studied and could be used either as target therapy or prognostic biomarker. Objective of this study was to assess relationship between serum VEGF-A and staging of bladder cancer. **Methods:** This cross sectional study was conducted in Sumatera Utara, Indonesia between February to September 2019. Vascular endothelial growth factor-A was determined by using enzyme-linked immunosorbent assay. Staging of bladder was assessed by computed tomography scan and histopathology. **Results:** Thirty patients with bladder cancer were included. Median age of subjects was 56.5 years old. Dominant gender and comorbidity were male and hypertension, respectively. Serum VEGF-A level was significantly higher in patient with more advance primary tumor stage T3/4 (p=0.029) and both lymph node stage N1-3 (p=0.015) and distant metastasis (p=0.001). **Conclusion:** VEGF-A is associated with advance bladder cancer stage and may be use as a prognostic biomarker and a target for future therapy.

Keywords: Biomarkers, Bladder carcinoma, TNM Staging, Vascular endothelial growth factor A.

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Background: Angiogenesis and lymphangiogenesis play key role in bladder cancer progression. Vascular endothelial growth factor (VEGF) is extensively studied and could be used either as target therapy or prognostic biomarker. Objective of this study was to assess relationship between serum VEGF-A and staging of bladder cancer. **Methods:** This cross sectional study was conducted in Sumatera Utara, Indonesia between February to September 2019. Vascular endothelial growth factor-A was determined by using enzyme-linked immunosorbent assay. Staging of bladder was assessed by computed tomography scan and histopathology. **Results:** Thirty patients with bladder cancer were included. Median age of subjects was 56.5 years old. Dominant gender and comorbidity were male and hypertension, respectively. Serum VEGF-A level was significantly higher in patient with more advance primary tumor stage T3/4 (p=0.029) and both lymph node stage N1-3 (p=0.015) and distant metastasis (p=0.001). **Conclusion:** VEGF-A is associated with advance bladder cancer stage and may be use as a prognostic biomarker and a target for future therapy.

Keywords: Biomarkers, Bladder carcinoma, TNM Staging, Vascular endothelial growth factor A.

INTRODUCTION

Bladder cancer is one of the most common malignancies that occur in the practice of urology, second only to prostate cancer. It accounts for 3% of all cancer and rank 10th most common cancer in the world.[1] Highest incidence was observed in high human development index country such as countries in Southern Europe, Western Europe and North America.[2] Around 70% of patients with bladder cancer present with superficial tumor (stages Ta, T1, or carcinoma in situ).[3] The rest suffer from muscle-invasive bladder cancer (MIBC) and associated with higher risk of progression into metastatic disease and death.[3,4]

Recently, studies on pathogenesis of bladder cancer revealed several factors contributing to invasiveness of bladder cancer.[4] Vascular endothelial growth factor (VEGF) is among the most studied factor in bladder cancer in many countries. [4-7] VEGF regulates endothelial cell proliferation, migration, vascular permeability, secretion and other endothelial functions.[8] Each type of VEGF has different mechanism of action. The main member of VEGF is VEGF-A (also known as VEGF). VEGF-A binds to VEGF receptor (VEGFR)-1 and VEGFR-2 which induces

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angiogenesis.[8]

In Indonesia, bladder cancer is the 12th most common bladder cancer and its incidence increased around 15% per year. Due to lower awareness and tendency to seek alternative treatment in Indonesia, patients tend to clinically present at more invasive stage.[9] However, similar study is lacking in Indonesia. Moreover expression of VEGF has been reported to vary in different stage of bladder cancer. Therefore, aim of this study was to assess relationship between serum VEGF-A and staging of bladder cancer in Medan, Indonesia.

METHODS

This study was a cross-sectional study on bladder cancer patients that were admitted to Haji Adam Malik General Hospital, Medan in Indonesia. A total of 30 patients were enrolled in this study. Inclusion criteria were patients aged ≥ 18 years old patients, diagnosed with bladder cancer by cystoscopy and histopathology, and admitted to Department of Surgery, Adam Malik General Hospital, Medan between February and September 2019. Patients with history of other malignancy and bladder cancer caused by metastasis were excluded. This study was approved by local Health Research Ethical Committee, Faculty of Medicine, Universitas Sumatera Utara.

For all included patients, demographic data such as age, gender, co-morbidities and bladder cancer staging data were collected. Both diagnosis and staging of bladder cancer was determined clinically by computed tomography scan and pathologically by histopathology examination. Bladder cancer was classified with TNM staging, according to The American Joint Committee on Cancer (AJCC) staging system: "T' (T1-T4) represented primary tumor, while "N" (N0-N3) and "M" (M0-M1) represented lymph node involvement and distant metastasis.[10]

Serum VEGF-A level was then measured from each patient. Venous blood was drawn, allowed to clot for 30 minutes at room temperature, and centrifuged at 1,000 g for 15 minutes to obtain serum. Serum VEFG-A level was examined using enzyme-linked immunosorbent assay (ELISA) method (Quantikine® ELISA, Human VEGF Immunoassay R&D System Inc., Minneapolis, USA) as instructed by manufacturer.[11]

For statistical analysis, each variable in TNM staging was further classified into two categories for comparison. Primary tumor was classified into T1-T2 and T3-T4. Lymph node involvement was classified into N0 and N1-N3. Distant metastasis was classified into M0 and M1. Relationship between those variables and serum VEGF level was determined using univariate analysis with Mann-Whitney U test and multivariate analysis with linear regression test. Statistical analysis was done with SPSS version 22 at 95% confidence interval. A p value of <0.05 was considered significant.

RESULTS

Median age was 56.5 years old, with a range between 33-79 years old. Male patients accounted for 66.7% sample in this study. The most common comorbidity was hypertension (36.67%), followed by diabetes mellitus (30.00%) and chronic kidney disease (26.67%). All patients underwent transurethral resection of bladder tumor (TURBT). Characteristics of patients were tabulated in Table I. Sixty percent of patient presented with more advance primary tumor (T3-T4) but only 36.7% of patients had lymph nodes involvement (N1-3) and 20% had distant metastasis.

Serum VEGF-A levels were significantly higher in patients with more advanced primary tumor stage (Table 2: T1-T2=284 pg/ml [69-737.7] vs T3-T4=573.5 [100.4 – 2646]; *$p<0.001$*), advanced lymph node stage (Table

Table I: Patients demographics and tumour stage.

Characteristics	n (%)	p
Age (median, range)	56.50 (33-79)	N/A
Gender		
Male	20 (66.7)	0.068
Female	10 (33.3)	
Comorbidities		
Hypertension	10 (36.67)	N/A
Diabetes mellitus	9 (30.00)	
Chronic kidney disease	8 (26.67)	
Congestive heart failure	6 (20.0)	
Arrhytmia	2 (6.67)	
Primary tumor Stage (T)		
T1-T2	12 (40)	0.273
T3-T4	18 (60)	
Lymph node Stage (N)		
N0	19 (63.3)	0.144
N1-N3	11 (36.7)	
Metastasis Stage (M)		
M0	24 (80.0)	0.001
M1	6 (20.0)	

II: N0=414.8 [100.4 – 1782.6] vs N1-N3=1107.95 [556.8 – 2646]; *p<0.001*) and distant metastasis (Table II: M0=479.45 [100.4 – 2185.2] vs M1=1224.5 [717 – 2464]; *p<0.001*). On multivariate analysis, patients with advanced stage bladder cancers had VEGF-A levels which were 1.5 to 3 times significantly higher (Table II: Adj OR: T3-T4=1.54, *p=0.029;* N1-3=1.96, *p=0.015;* M1=2.97, *p=0.001*) than those with early stage bladder cancers (T1-T2, N0, M0).

DISCUSSION

Assessment of bladder cancer in our study showed that most patients have more advance tumor progression when admitted to our center. Nevertheless, fewer patients had lymph node involvement or distant metastasis. This result is in concordance with previous report which stated that in Indonesia MIBC is more common than non MIBC (NMIBC) due to lack of awareness and tendency to seek alternative treatment despite accessible standard treatment.[9]

In this study, we found serum VEGF-A level was associated with primary tumor progression, lymph node involvement, and distant metastasis. This result is in concordance with current understanding that VEGF is among many factors involved in pathogenesis in bladder cancer, especially in enhancing angiogenesis. It facilitates the development of new capillary sprouts at the site of tumor. Thus it could increase the chance of tumor

Table II: Association of serum VEGF-A levels with TNM Staging in patients with bladder cancers.

Variables	Univariate analysis		Multivariate analysis	
	Median serum VEGF-A level, pg/mL (min-max)	p	Adj OR (95% CI)	p
Primary tumor Stage (T)				
T1-T2	284 (69 – 737.7)	<0.001	1.54 (1.1-2.48)	0.029
T3-T4	573.5 (100.4 – 2646)			
Lymph node Stage (N)				
N0	414.8 (100.4 – 1782.6)	<0.001	1.96 (1.31-4.35)	0.015
N1-N3	1107.95 (556.8 – 2646)			
Metastasis Stage (M)				
M0	479.45 (100.4 – 2185.2)	<0.001	2.97 (1.3-7.61)	0.001
M1	1224.5 (717 – 2464)			

VEGF – vascular endothelial growth factor.
Statistical testing performed based on univariate and multivariate linear regression analysis.

progression and metastasis.[12] To our knowledge this is the first study assessing association between VEGF and bladder cancer staging in Indonesia. This could be one step to determine national guideline for bladder cancer therapy especially in MIBC which is more commonly seen in Indonesia.

VEGF was known to be higher in patient with bladder cancer cell than healthy individual.[13] However previous studies assessing serum VEGF level and bladder cancer were conflicting. Miyake et al. found no difference between serum VEGF level of superficial and advance bladder cancer.[14] Bernadini et al. found significantly higher serum VEGF level in higher tumor grade and stage, vascular invasion, and metastasis.[15] On the contrary, Beecken et al found higher serum VEGF level in superficial transitional bladder carcinoma and well-differentiated tumor,[16] as did Szarvas et al. who stated non-invasive bladder cancer tend to have higher serum VEGF level although result was not significant.[17] Koprapu et al. demonstrated higher VEGF expression in NMIBC than MIBC by both immunohistochemistry and mRNA expression assessment.[18] Most recent study with larger sample size by Benoit et al. found that VEGF-A was elevated in invasive bladder cancer.[6] In current study, serum VEGF-A level was significantly elevated in more locally advance bladder cancer T stages, in those with lymph nodes involvement and with distant metastasis. This result confirmed current knowledge that VEGF-A is associated with tumor progression, therefore it should be elevated in more advance bladder cancer staging.

In this study, we also found VEGF-A was also associated with lymph node involvement. Role of VEGF-A to enhance lymphnode metastasis is unclear since it cannot bind to VEGFR-3.[8] This study is in concordance with Shariat et al. which observed higher VEGF expression in advance stage, lymphovascular invasion, and metastasis.[19] Previous animal study by Hirakawa et al. stated that VEGF-A also induced sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis by an unknown mechanism. At least three publications showed that only the expression of VEGF-D was associated primarily to lymph node metastasis in bladder cancer.[6,7,20,21] Suzuki et al. and Miyata et al. found expression of VEGF-C was also associated with tumor staging and lymph node involvement in bladder cancer, but result was conflicting to those reported in other studies.[6,7,21-23] With only the result of the study, we could not be sure about exact role of VEGF-A for bladder cancer's lymphangiogenesis, since other family member of VEGF was not assessed.

There were limitations that need to be addressed in future study. There were many confounding factors that could influence serum VEGF level. Comorbidities should be controlled in future study. Lastly, sample size must be larger to represent better reliable result and true epidemiological pattern with more variables used such as cancer therapy and prognosis.

CONCLUSION

Higher serum VEGF-A level is associated with advance stage of bladder cancer. Hence serum VEGF-A could be used as a biomaker target for future cancer therapy and also for prognostic purposes in bladder cancer. Future study with bigger sample size, stricter inclusion criteria, and assessing various VEGF type should be conducted to confirm the result of this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest and financial conflict.

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