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ORIGINAL PAPER

The association between serum hypoxia inducible factor-1α level and urothelial bladder cancer: A preliminary study

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Summary

Introduction: *We aim to evaluate the association between serum hypoxia inducible factor (HIF)-1α level and stage and grade of urothelial bladder cancer (UBC).*

Methods: *A case-control study was conducted at Haji Adam Malik Hospital Medan, Indonesia. Inclusion criteria for case group was subject aged 18 years or older and diagnosed with UBC based on histopathological examination. Control group consisted of gender and age matched healthy subjects. Serum HIF-1α level was determined using ELISA method. Data was analyzed with chi square, Mann Whitney, and independent T tests.*

Results: *A total of 80 subjects were enrolled and divided into case and control groups equally. Most subjects were males with mean age of 69.65 years for case group and 68.25 years for control group. Most subjects had advanced primary tumor and lymph node stages. Only 30% subjects had metastasized UBC. Higher serum HIF-1α level was observed in case group ($p < 0.001$). Serum HIF-1α level was strongly associated with metastasis stage ($p < 0.001$), followed by lymph node ($p = 0.005$) and primary tumor ($p = 0. 013$) stages. Serum HIF-1α level was not associated with grading ($p = 0.134$).*

Conclusions: *Serum HIF-1α level is associated with staging but not grading of UBC.*

KEY WORDS: HIF-1α; Grade; Stage; Urothelial bladder cancer.

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INTRODUCTION

Urothelial bladder cancer (UBC) is the 7th and the 17th most common cancer globally in men and women, respectively (1). This subtype is responsible for more than 90% bladder cancer cases (2, 3). Annually, there are 110.500 men and 70.000 women diagnosed with new UBC cases worldwide. The disease is more frequent in developed countries (1). Every year, 38.200 cases were diagnosed in *European Union* and 17.000 subjects died due to UBC (1, 2). Smoking is the most important risk factor for UBC. Exposure to chemical compounds, particularly aromatic amines and polycyclic aromatic hydrocarbons, parasitic infection, along with genetic predisposition are also considerable risk factors (1, 4).

Approximately 25% of newly diagnosed UBC are invasive, requiring radical surgery or radiotherapy (1, 2, 5). Unfortunately, the disease outcome is still poor despite systemic therapy (1). Five-year *overall survival* (OS) and *disease-specific survival* (DSS) rates for UBC are 57.2% and 77.3%, respectively (2). Early detection of UBC is important to improve patient's outcome, since the treatment can be delivered aggresively. *Hypoxia inducible factor* (HIF)-1α is a regulatory protein produced in hypoxic microenvironment that consists of two subunits. Its expression is found in several solid tumors including UBC, lung, breast, ovary, prostate, and kidney cancers. The binding of HIF-1α with its receptor initiates cell proliferation, migration, and invasion in UBC (3, 6, 7). Additionally, HIF-1α is also closely related to angiogenesis (3, 7-9). In this study, we aimed to evaluate the association between HIF-1α and stage and grade of UBC. Our findings may provide insight regarding earlier diagnosis, prompt management, improved outcome, and possible therapeutic method for UBC.

METHODS

This was a case-control study conducted in January-December 2022 at *Haji Adam Malik Hospital Medan, Indonesia*. The inclusion criteria for case group was subject aged 18 years or older and diagnosed with UBC based on histopathological examination. Beside diagnosing UBC, biopsy specimen from case group underwent hematoxylin and eosin staining to determine stage and grade of disease. All histopathological examinations were conducted at *Department of Pathology of Universitas Sumatera Utara*. Control group included healthy subjects who came to the hospital for general check-up or healthy hospital employers. Exclusion criteria was previous history of

malignancy, bladder lesion due to methastasis from distant primary cancers, patients receiving systemic therapy for badder cancer, patients with diabetes mellitus, chronic kidney disease, and cerebrovascular disease. We did gender and age mathcing between the two groups. All subjects received explanation regarding this study and were asked to sign informed consent. Subjects unwilling to participate in this study were excluded.

Serum sample was obtained from each subject in case group. Evaluation of serum HIF-1α level was conducted at *Research and Esoteric Laboratory Jakarta, Indonesia*.

We used HIF-1α Human ELISA kit (*Thermo Fisher Scientific Inc., Waltham, USA*) to determine serum HIF-1α level in this study. Data was analyzed using *Statistical Package for Social Science* (SPSS) software. Categorical data was presented in frequency and percentage while numerical data was presented in median and range if it was not normally distributed. Otherwise, it was presented in mean and standard deviation. Chi square test was utilized to determine relationship between categorical variables while Mann Whitney and independent T tests were used to determine the relationship between categorical and numerical data. All statistical analyses were conducted at confidence interval of 95%. A p value of < 0.05 was considered significant.

Results

A total of 80 subjects were enrolled in this study. All subjects were divided into the two groups equally. In case group mean age of subjects was 69.65 years and males subjects were prevalent. Most subjects had advanced primary tumor and lymph node involvement. Only 30% subjects in the case group had metastasized UBC. Significantly higher serum HIF-1α level was observed in case group compared to control group (Table 1).

From statistical analysis, we found that serum HIF-1α level was strongly associated with metastatic UBC (p < 0.001), followed by UBC with lymph node involvement (p = 0.005) and primary tumor (p = 0. 013) stage.

Table 1.
Baseline characteristics of subjects.

Characteristics	Case (n = 40)	Control (n = 40)	P
Mean age, years ± SD	69.65 ± 7.01	68.25 ± 7.74	0.400 [a]
Gender, n (%)			
Male	30 (75%)	32 (80)	0.592 [a]
Female	10 (25%)	8 (20%)	
Primary tumor stage (T), n (%)		NA	NA
T1+T2	10 (25%)		
T3+T4	30 (75%)		
Lymph node stage (N), n (%)		NA	NA
N0	18 (45%)		
N1	22 (55%)		
Metastasis stage (M), n (%)		NA	NA
M0	28 (70%)		
M1	12 (30%)		
Median HIF-1α level, pg/mL (range)	345 (142-587)	123 (94-234)	< 0.001 * [b]

*SD: standard deviation; [a] chi square test; [b] Mann Whitney test; * p < 0.05.*

Table 2.
Association between serum HIF-1α level and staging and grading of UBC.

Variables	Mean HIF-1α levels, pg/mL ± SD	P
Primary tumor stage (T)		
T3+T4	389 ± 126.66	0.013 *
T1+T2	273.4 ± 103.89	
Lymph node stage (N)		
N1	410.45 ± 115.4	0.005 *
N0	298.56 ± 123.42	
Metastasis stage (M)		
M1	477 ± 95.16	< 0.001 *
M0	310 ± 110.34	
Staging		
3+4	395.67 ± 124.11	0.008 *
1+2	273.4 ± 103.89	
Grading		
High grade	378 ± 131.42	0.134
Low grade	306.4 ± 117.04	

*SD: standard deviation; * p < 0.05.*

Overall, serum HIF-1α level was associated with UBC staging (p = 0.008) but not grading (p = 0.134) (Table 2).

Discussion

As most solid tumors grow, the need of oxygen for their metabolism is increased. This situation creates hypoxic condition (6, 10). Hypoxic condition upregulates several proteins including HIF-1α that it is important for adaptation of tumor, including UBC, in hypoxic condition. Angiogenesis or neovascularization is the end point of this adaptation (3, 4, 6), Hypoxia is also the culprit of treatment resistance in many cancers (8, 10) and HIF-1α is one of the underlying etiologies (11-13). Binding of HIF-1α with its receptor in the nucleus promotes cell proliferation, migration, and invasion. Overexpression of HIF-1α is associated with progression and recurrence of UBC (4, 6). The expression of HIF-1α in bladder cancer cells is also influenced by several other factors, such as elevated serum copper level and decreased serum zinc level (4).

In UBC, HIF-1α expression was higher compared to normal tissue (4, 8). The expression of HIF-1α in patients with bladder cancer was in line with the expression of *vascular endothelial growth factor* (VEGF) (r = 0.606). We know that VEGF is important in neovascularization and growth of malignant tissue (4). This finding was confirmed by *Theodoropoulos et al.* who found in their study that HIF-1α was positively associated with histological grade of UBC. This association was mediated by VEGF expression and *microvessel density* (MVD). Patients with high HIF-1α expression tended to have advanced disease and unfavorable outcome (8). *Badr et al.* also reported similar findings showing that HIF-1α expression is significantly higher in patients with bladder cancer despite its etiology. The level of urinary HIF-1α was also able to discriminate between malignant and non-malignant tumor with sensitivity and specificity of 82.1% and 63.3%, respectively. In contrast with our results, this study failed to demonstrate significant relationship between HIF-1α and UBC stage and grade (9).

Most patients with UBC expressed high HIF-1α. Tumor size, histological grade, tumor invasion, and recurrence of UBC were also associated with high HIF-1α expression. In line with previous study, this effect was linked to VEGF and MVD. *Disease free survival* (DFS) of UBC was independently influenced by HIF-1α (p = 0.011) (7). *Deniz, et al.* supported these findings with their study. Immunoreactivity of HIF-1α was in concordance with stage and histologic grade of UBC. Immunoreactivity of HIF-1α was also related to VEGF (p < 0.001) and MVD (p = 0.002) (14). Another study by *Theodoropoulos, et al.* in 2005 reported that HIF-1α expression is more common in high grade UBC. It was also positively correlated with increased proliferative activity, apoptotic rate, and MVD. However, they found no association between HIF-1α alone and prognosis of UBC. The prognosis of UBC was associated with both HIF-1α and mutation in p53 nuclear protein (5). A study conducted by Fus, et al. reported a contradictive result. They found that the expression of HIF-1α is significantly lower in high grade UBC. Negative correlation was also reported between the expression of HIF-1α and MVD (3).

We found that serum level of HIF-1α in case group is significantly higher compared to control group (p < 0.001). Serum HIF-1α was also higher in advanced UBC stage, including primary tumor, lymph node, and metastasis stage. Higher serum HIF-1α was also observed in advanced UBC grade but the difference was not statistically significant. There were several limitations in our study. We did not analyze risk factors for UBC other than gender and age. We also did not analyze variables that influence the level of serum HIF-1α. The kit we used to determine serum HIF-1α level was also different which may have given different result. Additional study, preferably a meta-analysis, is requested to determine the association between serum HIF-1α level and progression of UBC.

Conclusions

There was a significant association between serum HIF-1α level and staging of UBC. Serum HIF-1α level may aid in early diagnosis, prompt management, and improved outcome of patients with UBC.

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