Original Article
Middle East Journal of Cancer; October 2018; 9(4): 295-299

IL-35 Serum Levels in Bladder Cancer Patients: An Analytical Cross-sectional Study

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Abstract

Background: Bladder cancer is a prevalent disease with significant health care costs and high rates of recurrence. Results from numerous studies to associate bladder cancer with serum biomarkers have been analyzed for prognostic indicators, or to develop agents for diagnostic and therapeutic applications. Interleukin-35 is a suppressive cytokine that has a role in tumor immunity as a regulatory cytokine by suppressing T cell anticancer responses.

Methods: In the present study, we have investigated interleukin-35 serum levels in bladder cancer patients by ELISA, and compared these levels with a healthy comparison group, as well as among different clinicopathological subgroups.

Results: We observed no difference in serum levels of interleukin-35 in bladder cancer patients and healthy controls; however, bladder cancer patients diagnosed at lower stages (0a, I, II) had significantly higher levels of interleukin-35 in their sera compared to high stage (III, IV) patients (P=0.018).

Conclusion: Our results could indicate that interleukin-35 has no significant role in bladder cancer pathogenesis and progression. Interleukin-35 might not be a valuable biomarker for diagnosis or assessment of bladder cancer progression in clinical settings. However, further studies are needed in order to reach a definitive conclusion.

Keywords: IL-35, Bladder cancer, Serum biomarkers

Introduction

Bladder cancer (BLC) is one of the first cancers recognized to be immunogenic since Morales used bacillus Calmette–Guérin (BCG) to prevent its recurrence in 1976.[1] This cancer is the ninth most common malignancy worldwide with more than 12 million new cases that occur annually.[2] Transitional cell carcinoma (TCC) is the pathological diagnosis for the majority of BLC. Known risk factors for BLC development are cigarette smoking, occupational exposure (working in chemical, dye, rubber, petroleum, leather, and printing industries), and genetic events.[3]

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Received: November 4, 2017; Accepted: January 3, 2018

Numerous biochemical and biological studies have attempted to associate BLC with serum biomarkers. The results of these studies have been analyzed to determine prognostic indicators, or to develop agents for diagnostic and therapeutic applications. Of these biomarkers, a number of cytokines, such as interleukins, play critical roles in regulating immune responses and the pathogenesis of BLC.[4]

Interleukin-35 (IL-35) is a suppressive cytokine mostly produced by Foxp3+ regulatory T cells (Tregs) that is required for Treg-mediated immunosuppression.[5] This cytokine induces naive T cells and B cells to convert into Tregs and regulatory B cells (Bregs), respectively.[5-7]

Interleukin-35 plays a role in tumor immunity as a regulatory cytokine and suppresses the T cell anticancer response.[6] Wang et al. have observed that neoplastic cell-derived IL-35 induced myeloid cell accumulation and angiogenesis in the tumor microenvironment, and consequently tumor growth.[8] Elevated expression of IL-35 in tumor tissues and elevated plasma IL-35 levels have been shown to be indicators of poor prognosis in several malignancies, including pancreatic adenocarcinoma,[9-11] gastric cancer,[12] colorectal cancer,[13] prostate cancer,[14] breast cancer,[15] laryngeal squamous cell carcinoma,[16] acute myeloid leukemia,[17] hepatocellular carcinoma,[18] and non-small-cell lung cancer.[19]

The presence of IL-35 in the tumor microenvironment causes decreased lymphocytic infiltration and effector cell proliferation.[20] Furthermore, IL-35 within the tumor diminishes host memory responses, as demonstrated by Wang et al. in a metastatic lung B16 melanoma model.[8] Although expression of IL-35 in human cancers appears to be a fairly general phenomenon, the literature is currently devoid of clinical observations of IL-35 in human BLC.

In the present study, we investigated IL-35 serum levels in BLC patients, it's possible role in the pathogenesis of BLC, and association with clinicopathological features of this malignancy.

Materials and Methods

We conducted this analytical cross-sectional study to assess the serum levels of IL-35 in 60 BLC patients and 28 healthy, non-smoking, age-sex matched individuals. Bladder cancer was diagnosed according to pathological examination of biopsy samples obtained by complete transurethral resection (TUR) of the tumor or radical cystectomy specimens. Tumor staging was determined in accordance with the American Joint Committee on Cancer (AJCC), also known as the TNM system.[21] We obtained data that included age; gender; tumor size; histopathologic features of the tumor such as pathological diagnosis (tumor type); and tumor grade according to the WHO-ISUP grading system[22] from the patients' medical records. All participants were newly diagnosed and received no prior surgery, chemotherapy, or radiotherapy. We excluded individuals with additional neoplasms, autoimmune diseases, and lymphatic or lymphoproliferative disorders from the study. All participants provided written informed consent to participate. The Ethics Committee of Shiraz University of Medical Sciences approved this study.

Venous blood samples were drawn from 88 participants and collected in sterile, dry tubes. Sera were rapidly separated after coagulation and frozen at -70°C until the time of assay. Serum levels of IL-35 were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) method by a human IL-35 kit (MyBioSource, USA) according to the manufacturer's protocol.

Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL, USA) was used for data analysis. We used the Shapiro-Wilk test to determine if the variables had a normal distribution. Variables with normal distribution have been presented as mean± standard deviation (SD); otherwise, as median and interquartile range (IQR). Frequencies are presented as percent. Non-parametric tests that included the Mann-Whitney U test and Kruskal-Wallis test were used to analyze the differences among groups. A two-tailed *P*-value of less than 0.05 was considered statistically significant.

Table 1. Clinical and pathological characteristics of bladder cancer (BLC) patients.

Variable		Number (valid %)	IL-35 (pg/ml)[1]	*P*-value
Gender	Male	41 (68.3)	11.61	0.9302[2]
	Female	19 (31.7)	12.01	
Tobacco use	Yes	25 (51.0)	11.03	0.322[2]
	No	24 (49.0)	12.79	
Opium use	Yes	9 (18.4)	7.57	0.324[2]
	No	40 (81.6)	12.72	
Pathological diagnosis	TCC[4]	53 (88.3)	11.61	0.5652[2]
	Other[5]	7 (11.7)	13.57	
Stage	0a	11 (18.3)	13.83	0.1793[3]
	I	6 (10.0)	12.47	
	II	11 (18.3)	16.71	
	III	18 (30.0)	9.99	
	IV	14 (22.3)	9.79	
Grade	PUNLMP[6]	17 (32.7)	11.36	0.1873[3]
	Low	4 (7.7)	12.27	
	High	31 (59.6)	10.97	
N (lymph node invasion)	0	20 (33.3)	9.40	0.2623[3]
	1	6 (10.0)	12.50	
	2	3 (5.0)	7.44	
	Not known	31 (51.7)	--	
Tumor size	<3cm	19 (44.2)	16.02	0.4502[2]
	>3 cm	24 (55.8)	13.72	
Muscle invasion	Yes	43 (71.7)	10.84	0.1812[2]
	No	17 (28.3)	9.80	
Lymphatic/vascular invasion	Yes	16 (51.6)	10.84	0.202[2]
	No	15 (48.4)	7.70	
Perineural invasion	Yes	8 (50.0)	11.10	0.787[2]
	No	8 (50.0)	6.52	

1- Value presented as median; 2- Mann-Whitney U test; 3- Kruskal-Wallis test; 4- Transitional cell carcinoma; 5- Squamous cell carcinoma and adenocarcinoma; 6- Papillary urothelial neoplasm of low malignant potential

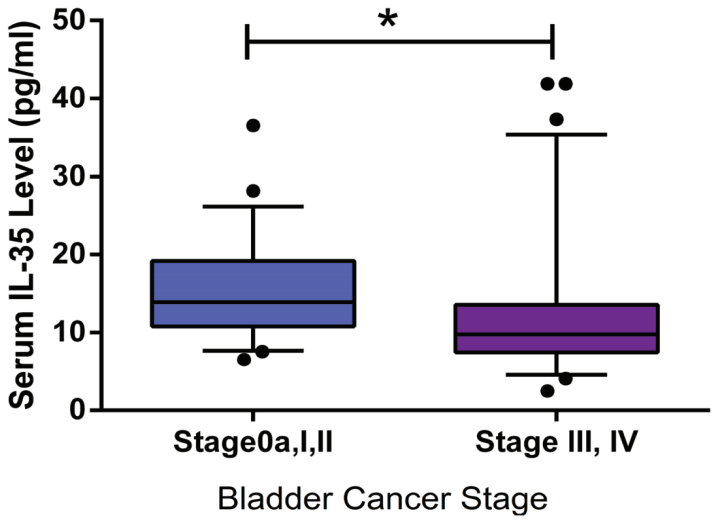

Figure 1. Box-and-whiskers diagram of serum IL-35 levels. Outliers are plotted as individual points. The middle line of each bar presents the median serum Il-35 levels. The bottom and top of the boxes are the first and third quartiles. The ends of the whiskers represent 10-90 percentile; Patients diagnosed at lower stages (0a, I, II) had higher levels of IL-35 in their sera compared to high stage patients (III, IV) (13.90 vs. 9.79 pg/ml; *P*=0.018).

Results

The mean age of patients at the time of diagnosis was 64.9±13.2 years. The majority were men (63.3 %). Pathological diagnosis of most tumor specimens was TCC (88.3%) and all were papillary. Most tumor samples were pathologically high grade (59.6%) and three had metastasized. Table 1 presents other clinical and pathological characteristics.

We observed no statistically significant difference between IL-35 serum levels in cases and controls (11.75 vs. 11.68 pg/ml; $P>0.05$). However, we noted that patients diagnosed at lower stages (0a, I, II) had higher levels of IL-35 (13.90 pg/ml) in their sera compared to high stage patients (III, IV) with 9.79 pg/ml ($P=0.018$), as seen in figure 1. There was no other significant correlation between IL-35 serum levels and other clinicopathological features of patients that included age, gender, cigarette smoking, opium addiction, tumor type, differentiation grade, and vascular and perineural invasion ($P>0.05$).

Discussion

Interleukin-35 is a member of the IL-12 superfamily. As with other members of this superfamily, it is a heterodimer composed of an α chain P35 and a β chain Epstein-Barr virus induced gene 3 (EBI3).[5] Recent studies have revealed two distinct roles in inflammatory/autoimmune diseases as well as cancers for this cytokine.[23]

In this study, we observed significantly higher IL-35 serum levels in patients diagnosed with lower stages of BLC (organ confined: 0a, I, II) compared to those diagnosed at higher stages (III, IV). Our results contrasted previous studies, which reported the association of higher IL-35 serum levels with worse prognosis and more advanced tumors.[8,10-12,20,24-26] However, consistent with our results, Lu and Yuan observed higher IL-35 levels in the sera of patients with thyroid adenoma compared to those with thyroid carcinoma.[27]

Congruous with our observations, Long et al. reported a role for IL-35 in suppressing cancer activity by inhibition of cancer cell growth and increased apoptosis sensitivity of human cancer cells.[28] They transfected different cell lines with the IL-35 gene and observed that IL-35 expression suppressed cancer cell growth via cell cycle arrest at the G1 phase and markedly increased apoptosis of these cells.[28]

Bladder cancer is a prevalent disease with significant health care costs and high rates of disease recurrence. Numerous studies have attempted to associate BLC with serum biomarkers. In the present study, we observed higher serum IL-35 levels in tumors confined to the bladder (stages 0a, I, II) compared to those that spread beyond this organ (stages III, IV). However, there was no difference in IL-35 serum levels in BLC patients and healthy controls; this could be indicative of the lack of a significant role by IL-35 in BLC pathogenesis and progression. Possibly, IL-35 would not be a valuable biomarker for the diagnosis or assessment of BLC progression in the clinical setting. Still, studies that enroll larger numbers of BLC patients with more detailed pathological and clinical aspects of the tumor, along with consideration of tumor infiltrating lymphocytes might further clarify the role of IL-35 in BLC formation and progression.

Acknowledgement

The present study was financially supported by a grant from Shiraz University of Medical Sciences, Shiraz, Iran (grant no. 93-01-01-8082).

Conflict of Interest

None declared.

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