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Investigating the Levels of Shed Extracellular Domain of HER2 Protein in the Sera of Bladder Cancer Patients

Ali Ariafar*, Mehrnoosh S. Hasheminejad, Shabnam Abtahi**, Abbas Ghaderi**♦**

**Urology-Oncology Research Center, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
***Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

Abstract

Background: Receptor tyrosine-protein kinase ERBB2, also known as human epidermal growth factor receptor-2 (HER2), is heterogeneously expressed in a variety of human cancers, including bladder cancer. Based on previous studies that show its association with bladder cancer progression, HER2 has been included in novel multiplatform biomarkers for prediction of bladder cancer prognosis. However, the clinical significance of HER2 status remains underinvestigated and poorly linked to the patients' clinicopathological features. Here, we aim to scrutinize the levels of the extracellular domain of HER2 in the sera of bladder cancer patients and correlate these levels with clinicopathological features of the tumor.

Methods: In the present analytical cross-sectional study, we enrolled 60 pathologically confirmed bladder cancer patients along with 20 age-sex matched healthy controls, and compared their serum HER2 levels as measured by enzyme-linked immunosorbent assay.

Results: We observed no statistically significant difference when comparing the levels of HER2 in the sera of cases and controls (P>0.05). Interestingly, serum HER2 levels of controls were higher than bladder cancer patients who had lymph node metastasis (P=0.036). Serum levels of HER2 were also higher in controls than bladder cancer patients with perineural invasion (P=0.028). We observed significantly higher HER2 serum levels in transitional cell carcinoma patients in comparison to non-transitional cell carcinoma patients (P=0.016).

Conclusion: Our observations are suggestive of the absence of any association between bladder cancer prognostic factors and serum HER2 levels. To draw any definitive conclusion, further studies with larger sample sizes that examine the presence of neutralizing auto-antibodies against serum HER2, immunohistochemistry examination of HER2 in bladder tumor and lymph node samples, and urinary HER2 levels, along with measurement of its serum levels would be helpful.

♦**Corresponding Author:**
Abbas Ghaderi, PhD
Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, P.O. Box 71345-3119, Shiraz, Iran
Email: ghaderia@sums.ac.ir

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Introduction

Bladder cancer (BLC) is one of the most prevalent cancers, ranking as the fourth most common malignancy in men. Worldwide, more than 12 million new cases are diagnosed annually.[1] Transitional cell carcinoma (TCC) is the pathological diagnosis for BLCs in the majority of cases. Some known risk factors for BLC development are cigarette smoking, occupational exposures (working in the dye, rubber, petroleum, leather and printing industries), and genetic events.[2]

Numerous biochemical and biological studies have attempted to associate BLC with biomarkers. The results of these studies have been analyzed to determine prognostic indicators, or to develop agents for diagnostic and therapeutic applications. Human epidermal growth factor receptor 2 (HER2) is one of these biomarkers.[3,4] In 1990, Zhau and colleagues have published the first report, which suggested a possible association between HER2 gene amplification and human bladder carcinogenesis.[5] Since this publication, several investigators have attempted to link different aspects of HER2 gene expression to BLC formation, its clinicopathological characteristics, prognosis, and possible discovery of new treatment options.[6]

The HER2 (also known as ERBB2) gene is a proto-oncogene located at 17q12 and encodes a 185 kd transmembrane glycoprotein, which belongs to the family of tyrosine kinase growth factor receptors.[7] The four members of the HER protein family mediate major cellular functions that include proliferation, differentiation, motility, and survival.[8] Amplification of the HER2 gene and/or its overexpression have been found in many human cancers, such as meningiomas,[9] gastric, esophageal, endometrial, ovarian, BLC, and lung cancers.[7] Currently, HER2-targeted therapies are among the standard treatment options in HER2-positive breast and gastric carcinomas.[10,11] Although overexpression or gene amplification of HER2 has been shown in a fraction of BLCs, agents that target HER2 have not been shown to be practical in the treatment of these patients.[4]

The observed inconsistency remains elusive and attempts to link BLCs characteristics and HER2 expression have resulted in controversial and even paradoxical reports. Several studies found links between HER2 overexpression and poor prognosis of BLC patients,[12-14] whereas a few others reported trends toward improved overall survival in patients with HER2 positive tumors.[15,16]

Human epidermal growth factor receptor 2 proteolytic cleavage sheds soluble truncated HER2 molecules in the serum, which only include the extracellular domain (ECD-HER2) and can be measured by means of quantitative enzyme-linked immunosorbent assay (ELISA).[17] Reports on the association of the levels of ECD-HER2 in the sera of BLC patients with clinicopathological features are scarce.[18-20] The value of serum ECD-HER2 testing for clinical decision-making in BLC patients remains vague and deserves to be scrutinized more thoroughly. In the current study, we have investigated the ECD-HER2 levels in the sera of BLC patients and its association with different clinicopathological features of this disease.

Materials and Methods

The present analytical cross-sectional study included 60 patients diagnosed with BLC who were recruited from a hospital affiliated with Shiraz University of Medical Sciences in 2016 and 2017. We recruited 20 age-sex matched healthy non-smoker individuals as the control group. Bladder cancer was diagnosed according to the pathological examination of biopsy samples obtained during complete transurethral resection (TUR) or radical cystectomy. Staging of the tumors removed via radical cystectomy was determined in accordance with the American Joint Committee on Cancer (AJCC), also known as the TNM system.[21] Other data that included age, gender, smoking status, opium consumption, and histopathological features of the tumor such as pathological diagnosis, tumor size (largest diameter in cm), grade of tumor (according to

the WHO-ISUP grading system),[22] lymphovascular invasion, and perineural invasion were obtained from medical records. All subjects were newly diagnosed and received no prior treatment (chemotherapy or radiotherapy). The Ethics Committee of Shiraz University of Medical Sciences approved the study and all participants consented to be included in this study.

Each participant provided 5 ml of venous blood. The blood was centrifuged and the separated serum was collected and stored at -80°C until analysis. Levels of ECD-HER2 in the sera were measured by a quantitative ELISA kit (Sigma-Aldrich, USA) according to the protocols described by the manufacturer.

Statistical Package for Social Sciences version 16 (SPSS Inc, Chicago, IL, USA) was used for data analysis. Variables are presented as mean±standard error of mean (SEM) or as median. Frequencies are presented as percent. Non-parametric tests, including the Mann-Whitney U test and Kruskal-Wallis test were used to analyze the differences among the groups. Comparison of subgroup frequencies was performed by the chi-square and Fisher exact tests. Two-tailed *P* value <0.05 was considered statistically significant.

Results

A total of 60 individuals with newly diagnosed BLC along with 20 age-sex matched healthy individuals agreed to participate in this study. The mean age of patients was 64.93±1.71 years (range: 26-89 years) and most were males (68.3%; n=41). Pathological diagnosis of the majority of tumor specimens was TCC (88.2%; n=53); more than half of them were pathologically high grade (60.4%; n=32). The mean size of the resected tumor specimens was 4.68±0.45 cm, with 78.3% (n=47) greater than 3 cm in their largest diameter. There was metastasis in 4 (6.8%) of the tumors. Table 1 lists the clinical and pathological characteristics.

When comparing the levels of ECD-HER2 in the sera of cases and controls, we observed no statistically significant difference (*P*>0.05). Interestingly, serum ECD-HER2 levels of controls (1121.0 pg/ml) were higher than BLC patients with lymph node (LN) metastasis (891.2 pg/ml; N>0), as shown in figure 1A (*P*=0.036); however, there was no difference between ECD-HER2 serum levels of normal individuals and patients without LN involvement (*P*>0.05). Serum levels of ECD-HER2 were higher in controls (1121.0 pg/ml) than BLC patients with perineural invasion (849.0 pg/ml) as seen in Figure 1B (*P*=0.028);

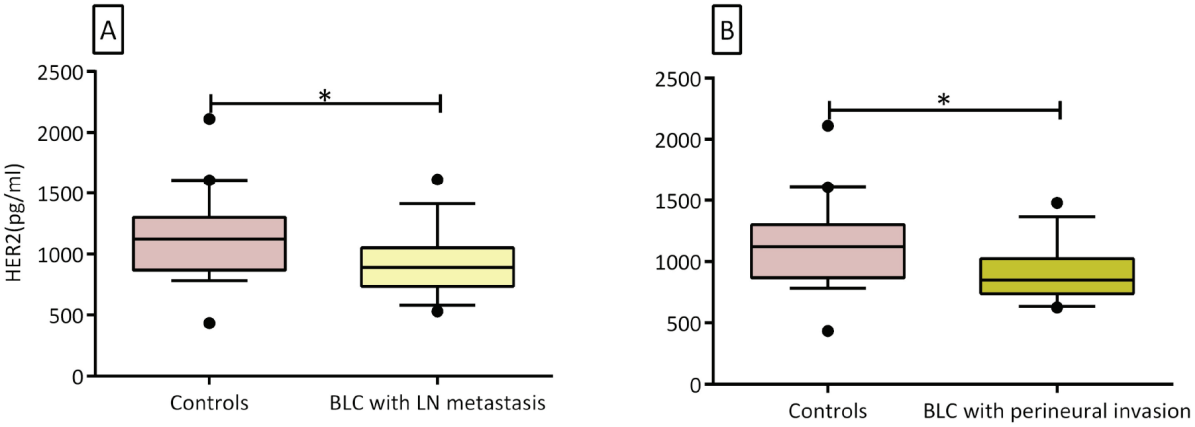

Figure 1. Box-and-whiskers diagram of serum human epidermal growth factor receptor 2 (HER2) levels. Outliers are plotted as individual points. The middle line of each bar represents the median serum HER2 levels. The bottom and top of the boxes are the first and third quartiles. The ends of the whiskers represent 10-90 percentiles. A. serum HER2 levels were significantly lower in bladder cancer (BLC) patients with lymph node (LN) metastasis than controls (1121.0 vs. 891.2 pg/ml; *P*=0.036). B. Serum HER2 levels were significantly lower in BLC patients with perineural invasion than controls (1121.0 vs. 849.0 pg/ml; *P*=0.028).

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

Variable		Number (valid percent)	HER2 serum levels (pg/ml)[1]	*P*-value
Gender	Male	41 (68.4)	980.1	0.189[2]
	Female	19 (31.6)	1054.0	
Smoker	Yes	24 (49)	1001.0	0.688[2]
	No	25 (51)	1017.0	
Pathological diagnosis	Transitional cell carcinoma (TCC)	53 (88.3)	1042.0	0.022[3]*
	Squamous cell carcinoma	5 (8.3)	735.2	
	Adenocarcinoma	2 (3.3)	1232.0	
Tumor size (cm)	<3	13 (21.7)	1205.0	0.075[2]
	>3	48 (78.3)	980.1	
T	Ta	11 (18.3)	1120.0	0.300[3]
	T1	6 (10.0)	1080.0	
	T2	15 (25.0)	986.4	
	T3	14 (23.3)	1040.0	
	T4	14 (23.3)	907.4	
N	0	20 (33.9)	987.4	0.713[3]
	1	5 (8.5)	735.2	
	2	3 (5.1)	980.1	
	x	31 (52.5)	1039.0	
Histological grade	PUNLMP[4]	13 (24.5)	1120.0	0.745[3]
	Low grade	8 (15.1)	1036.0	
	High grade	32 (60.4)	1030.0	
Lymphatic/ vascular invasion	Yes	19 (48.7)	1021	0.432[2]
	No	20 (51.3)	970.5	
Perineural invasion	Yes	13 (44.8)	864.1	0.645[2]
	No	16 (55.2)	977.8	
Tumor location	Diffuse infiltrative	8 (13.3)	935.5	0.128[2]
	Other sites	52 (86.7)	1024.0	

[1]Presented as median; [2]Mann-Whitney U test; [3]Kruskal Wallis test; [4]Papillary urothelial neoplasm of low malignant potential; *Statistically significant

however, there was no significant difference between ECD-HER2 levels in the sera of healthy controls and tumors without perineural invasion (P>0.05).

When comparing serum ECD-HER2 levels in different subgroups of patients, we observed significantly higher serum levels in TCC patients (1024 pg/ml) in comparison to non-TCC patients (784.5 pg/ml; P=0.016). There were no other significant associations between sHER2 levels and clinicopathological features of BLC observed.

Discussion

In this study, we investigated the ECD-HER2 levels in 60 BLC patients and 20 normal individuals. We observed no significant difference in the serum levels of ECD-HER2 between BLC patients and the normal control group. However, the serum levels of this molecule were significantly higher in healthy individuals compared to BLC patients with LN metastasis (P=0.036), as well as BLCs with perineural invasion (P=0.028). We observed significantly higher ECD-HER2 serum levels in TCC patients in comparison to non-TCC patients. No other significant associations between ECD-HER2 levels and clinicopathological features of the disease were found.

Although studies on HER2 expression in BLC tissue by means of immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are suggestive of its prognostic value,[6] our investigation along with other investigations have not demonstrated an association with serum ECD-

HER2 levels.[18,20] Consistent with our results, Kim et al. observed no significant difference in serum ECD-HER2 levels of BLC patients and a normal comparison group.[20] Their results were further supported by Arikan et al.[18] However, both studies observed higher levels of urinary ECD-HER2 in BLC patients in comparison to normal individuals.[18,20]

An interesting observation was reported by Fleischmann and colleagues, who demonstrated higher levels of HER2 amplification (IHC and FISH) in metastatic LNs compared to matched primary bladder tumors.[23] However, according to our results, such association appeared to be absent or even reversed with regards to serum ECD-HER2. We observed higher levels of this molecule in sera of normal individuals in comparison to BLC patients with LN metastasis, and observed no difference in serum levels of ECD-HER2 between the sera of LN positive and negative patients. Previously, we have observed similar results in meningioma patients, with higher ECD-HER2 serum levels in healthy controls than participants with meningioma.[9] There are several explanations for the observed discrepancy. We believe that the profile of HER2 tissue expression in BLC may not necessarily match its serum levels. Enzymatic digestion of the ECD-HER2 shed in the blood, which has been previously shown in breast cancer cases (authors' personal observation) might be one of the reasons. This digestion may produce a splice variant HER2 undetectable by the method we used. Furthermore, serum samples were kept at least 2 months before performing ELISA. Therefore, one could expect that the levels of HER2 in serum might not be in accordance with the fresh tissue analysis. On the other hand, it is presumable that higher expression of HER2 in these tumors can activate an immune response and trigger auto-antibody production against HER2 antigen[2,25] in the sera of BLC patients and, consequently, inability of ELISA to detect this antigen. The presence of HER2 autoantibodies has been demonstrated in the sera of breast cancer patients.[24-27]

Conclusion

Bladder carcinoma's depth of invasion, pathological grade, and LN metastasis are the three most important tumor characteristics that have a close relation to the prognosis of these patients.[6] Although, further studies are essential to draw any definite conclusion, our observations are suggestive of the absence of any association between these prognostic factors and serum ECD-HER2 levels in BLC. It seems that there is insufficient evidence to support the clinical use of serum ECD-HER2 testing in these patients. Limitations of this study include the low number of participants and resultant under-coverage bias, and not examining the past medical history of patients more thoroughly. Studies that measure the levels of serum and urinary ECD-HER2 coupled with IHC and FISH examination of HER2 in larger sample sizes are essential to further clarify the significance of ECD-HER2 serum levels and its association with pathological features. Examining the presence of neutralizing auto-antibodies against ECD-HER2 in BLC patients with HER2 over-expression would also help further clarify our observations.

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Conflict of Interest

None declared.

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