Research Article

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Serum levels of growth factors in patients with urinary bladder cancer
Mesane kanserli hastalarda serum büyüme faktörleri düzeyleri

Abstract

Background: Altered signalling of human epidermal growth factor receptor-2 (HER-2/neu), insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF) have been shown to play important role in tumor development and progression in various cancers. Their serum levels may be reliable indicator for diagnosis and progression of cancer.

Objective: To examine the serum levels of soluble HER-2/neu (sHER-2/neu), IGF1 and EGF in patients with urinary bladder cancer (UBC).

Material and methods: Serum levels of sHER-2/neu, IGF1 and EGF were measured by enzyme-linked immune assay in newly diagnosed, untreated patients with UBC.

Results: In the patient group, sHER-2/neu level was found to be increased, IGF1 level was found to be decreased in comparison to those in the control group. Although serum level of sHER-2/neu was lower in the patients with Ta stage than that in the patients with T1 and T2 stages, this difference was not at a statistically significant level.

Conclusion: Serum level of sHER-2/neu is increased in patients with UBC. Despite the lack of a significant association between sHER-2/neu level and pathological pT stage, sHER-2/neu may be a promising marker for UBC but IGF-1 and EGF have not such a potential.

Keywords: Urinary bladder cancer; sHER-2/neu; Insulin-like growth factor 1; Epidermal growth factor.

Özet

Giriş: İnsan epidermal büyüme faktörü reseptörü (HER-2/neu), insülin benzeri büyüme faktörü 1 (IGF-1) ve epidermal büyüme faktörü (EGF) sinyalizasyonundaki değişikliklerin tümör oluşumu ve ilerlemesinde önemli rol oynadığı çeşitli kanserlerde gösterilmiştir. Bunun serumdaki düzeyleri kanserin tanı ve prognozunun belirlenmesinde anlamlı bir belirteç olabilir.

Amaç: Mesane kanserli hastalarda serum çözünebilir HER-2/neu (sHER-2/neu), IGF1 ve EGF düzeylerinin belirlenmesi.

Materiyal ve metodlar: Yeni tanı konmuş, henüz tedavi almamış mesane kanserli hastalarda serum sHER-2/neu, IGF1 ve EGF düzeyleri enzim-bağlı immun yöntemlerle ölçüldü.

Bulgular: Hasta grubunda kontrol grubuna kıyasla sHER-2/neu düzeyi artmış, IGF1 düzeyi azalmış olarak bulundu. Ta evreli hastalarda serum sHER-2/neu düzeyi T1 ve T2 evreli hastalardakine göre düşük olsa da, bu farklılık istatistiksel olarak anlamlı düzeyde değildir.

Sonuç: Mesane kanserli hastalarda serum sHER-2/neu düzeyi artmıştır. sHER-2/neu düzeyi ile patolojik pT evresi arasında anlamli bir ilişki olmasına da, sHER-2/neu mesane kanseri için umut verici bir belirteç olabilir fakat IGF-1 ve EGF böyle bir potansiyele sahip değildir.

Anahtar kelimeler: Mesane kanseri; sHER-2/neu; Insülin benzeri büyüme faktörü 1; Epidermal büyüme faktörü.
Introduction

Urinary bladder cancer (UBC) is one of the most common cancers worldwide. It is the fourth most common solid tumor in men and the ninth in women, and exhibits a significant morbidity and mortality [1]. Five-year survival rate is >90% for early detected UBC and early intervention is important to increase survival time. Cystoscopy and voided urine cytology are still gold standards for diagnosis of UBC. However, cystoscopy is an expensive, uncomfortable invasive procedure which may require anesthesia. Although urine cytology has a reasonable sensitivity for detection of high-grade bladder tumors, it lacks in sensitivity to detect low-grade tumors. The development of specific, accurate and non-invasive tools would provide great benefit to both patients and healthcare system. Urinary biomarkers with high sensitivity and specificity have been investigated for a long time. Unfortunately, a biomarker which has a combined sensitivity and specificity to replace the established cystoscopy and voided urine cytology has not been recognized so far [1, 2].

Growth factors regulate cell proliferation and differentiation. The human epidermal growth factor receptor-2 (HER-2/neu), referred as c-erbB-2, belongs to ErbB family of receptor tyrosine kinases. ErbB family consists of four members: epidermal growth factor receptor (EGFR), HER-2/neu, HER3, and HER4 [3, 4]. Activation of HER-2/neu pathway is presumably driven by heterodimerization of HER2/neu with HER1, HER3 or HER4 and the subsequent activation of the downstream pathway [3]. The extracellular domain of HER-2/neu is cleaved and then released from membrane. The level of circulating HER-2/neu, referred as soluble HER-2/neu (sHER-2/neu), has been suggested as a useful biomarker in detecting and following various types of cancer [3–9]. Amplification of HER-2/neu gene and subsequent protein overexpression has been demonstrated in a large variety of tumor cells, and has been considered as a poor prognostic factor for several types of cancer [10–15]. HER-2/neu gene has regarded as a tumor-specific target in molecular therapies [16].

The epidermal growth factor (EGF) is the ligand for epidermal growth factor receptor (EGFR). EGF signalling pathway is responsible for stimulation of cell growth, tissue differentiation, and organogenesis. It has been shown to play important role in tumor development and progression [23–26].

In the present study, our aim was to determine serum levels of sHER-2/neu, IGF-1 and EGF in patients with UBC.

Materials and methods

A total of 37 patients with UBC (median age 54, 32 men and five women) diagnosed in Urology Department of Cerrahpaşa Medical Faculty Hospital were involved in the study. All patients were newly diagnosed with cancer and none of them received anti-cancer therapy prior to the collection of blood samples. Exclusion criteria are presence of serious or uncontrolled concurrent medical illness (lipid disorders, cardiovascular diseases, diabetes mellitus, chronic inflammatory diseases and infections) and a history of other malignancies. The control group was constituted by aged-matched 27 healthy volunteers (median age 52, 23 men and four women). All study subjects have normal thyroid, liver and kidney function. They have a similar lifestyle and eating habits, all of them are smoker. A detailed questionnaire was conducted to reveal lifestyle and eating habits of the individuals. The boundaries set for similarity are absence of workplace exposure to chemicals, absence of rural life and a diet including considerably amount of vegetable and fruit. They were not receiving any drug and antioxidant supplementation. The approval of the Cerrahpaşa Medical Faculty Ethical Committee was taken in accordance with the principles of Declaration of Helsinki and informed consent was obtained from all subjects.

Blood collection and laboratory analyses

Ten milliliters of venous blood samples were collected just before the surgical operation. After the centrifugation at 3000×g for 10 min, the serum was removed and kept at −80°C until the time of analysis. Serum level of sHER-2/neu was measured with a competitive enzyme-linked immunosorbent assay (ELISA) kit purchased from Bender MedSystems USA (catalog no: BMS207/BMS207CE). IGF-1 and EGF levels were measured with ELISA kits purchased from Assaypro, USA (catalog no: EE201I-1 and EI100I-1, respectively). Measurements were performed according to manufacturer’s instructions.
Statistical analysis

Statistical analysis was performed by SPSS software (version 15). As all measured parameters have abnormal distribution, sHER-2/neu, IGF-1 and EGF levels of the study groups were expressed as median (min-max) and comparisons between the groups were performed by non-parametric Mann-Whitney U test. Correlations between variables were examined by Spearman correlation coefficient. A value of $p < 0.05$ was considered as significant.

Results

Patient and tumor characteristics were shown in Table 1. Patients were classified according to tumor stage (by the UICC 2002 TNM staging system, for muscle invasiveness). Serum levels of sHER-2/neu, IGF-1 and EGF in the study groups were shown in Table 2. In the UBC group, serum level of sHER-2/neu was found to be increased ($p < 0.001$), serum level of IGF1 was found to be decreased ($p < 0.05$) in comparison to those in the control group. No significant difference was determined between the patient and control groups for serum level of EGF. Regarding the invasiveness, we compared the serum level of sHER-2/neu in the groups at stage Ta vs. T1+T2. Although serum level of sHER-2/neu was lower in the patients with Ta stage than that in the patients with T1 and T2 stages, this difference was not at a statistically significant level (Table 3).

Discussion

Primary risk factors for UBC include male gender, smoking and exposure to certain chemicals [27]. Early detection of disease and recurrences is a crucial factor for successful therapy and increased survival rate in patients with UBC. However, the options for early diagnosis and detection of recurrences are inadequate. In this context, there is a great need for new biomarkers that may help for early diagnosis and surveillance of UBC. Activation and subsequent overexpression of cellular oncogenes plays an important role in development of UBC. In malignant tumors, HER-2/neu overexpression is a direct result of gene amplification [13, 14]. The HER-2/neu overexpression has been investigated in a large number of malignant tumors [9, 15]. Several studies have revealed an association between increased HER-2/neu expression and advanced tumor stage, grade and poor patient survival [13, 14]. Recombinant humanized anti-HER2 monoclonal antibodies have been used in treatment of breast cancer [16].

sHER-2/neu that is shed from membrane is found at a detectible level in peripheral circulation. sHER-2/neu testing is an additional approach to examine HER2/neu overexpression in tumor tissue. The serum sHER-2/neu concentration is low in healthy individuals but it is abnormally high in cancer patients [4]. Recently, serum sHER-2/neu level has been suggested as a usefull biomarker for diagnosis and follow-up of certain types of cancer [5, 6]. sHER-2/neu testing allows clinicians to monitor dynamic changes in HER2/neu status over the course of disease progression. sHER-2/neu level reflects patient’s response to anti-cancer therapy in breast cancer [28, 29]. The serum level of sHER-2/neu has been found to be increased in various types of cancer including breast [3–5, 29, 30], prostate [6, 9], colorectal [7] and stomach [8]. As far as we know, there is only one study that examines serum level of sHER2/neu in patients with UBC. In contrast to our findings, Kim et al. [31] have reported

### Table 1: Patient and tumor characteristics.

|                        | Urinary bladder cancer group (n=37) | Control group (n=27) |
|------------------------|-----------------------------------|----------------------|
| Age (years)*           | 54 (37–71)                        | 52 (35–67)           |
| Gender                 | Male/female 32/5                  | 23/4                 |
| pT stage               | Ta 27                              |                      |
|                        | T1 5                               |                      |
|                        | T2 5                               |                      |
| Tumor size             | >3 cm 4                            |                      |
|                        | ≤3 cm 33                           |                      |
| *Median (min–max).     |                                   |                      |

### Table 2: Serum levels of sHER-2/neu, IGF-1 and EGF in the study groups.

|                        | sHER-2/neu (ng/mL) | IGF-1 (ng/mL) | EGF (pg/mL) |
|------------------------|-------------------|---------------|-------------|
| Urinary bladder cancer group (n=37) | 8.40 (5.00–20.80)* | 32 (8–144)* | 67 (16–300) |
| Control group (n=27)   | 4.00 (1.00–12.80)  | 60 (12–136)  | 81 (28–220) |

*p < 0.001 and *p < 0.05 vs. control group.
that there is no significant difference between UBC and control groups for serum sHER-2/neu level. The contradiction may be due to different techniques that are used in sHER-2/neu measurements. In the present study, the sensitivity of the ELISA kit used for sHER-2/neu measurement (the limit of detection of human sHER-2/neu) is 0.06 ng/mL. The overall intra-assay and interassay coefficients of variation are 1.9% and 5.8%, respectively. Serum level of sHER-2/neu was found to be increased in UBC patients. No significant association was determined between serum level of sHER-2/neu and pathological pT stage. sHER-2/neu seems as a promising parameter for UBC, however, in order to consider serum level of sHER-2/neu as a tumor marker, this preliminary study should be confirmed by further studies in future.

Epidemiological studies have revealed that there is a close relation between cancer risk and IGF-1 level in peripheral circulation. Except for ovarian cancer [32, 33], higher circulating IGF-1 level is associated with higher risk of prostate, lung, gastric, breast, head and neck cancers [18–20, 22]. Regarding these data, the possibility of targeting the IGF-1 receptor in anticancer therapy has gained great interest. As far as we know, serum level of IGF-1 in patients with UBC has been examined by two groups, previously. Both of them have determined that there is no significant difference between the patient and control groups [34, 35]. In the present study, in contrast to previous studies, the serum level of IGF-1 was found to be lower in the patients with UBC in comparison to those in the control group. Actually, the serum level of IGF-1 is largely depend on concentration of IGF-Binding Proteins. As a limitation of this study, the concentration of IGF-1-specific IGF-Binding protein could also be measured to interpret serum level of IGF-1.

EGF is one of the candidates that is a marker for tumor growth in early stages of cancer. Many studies have indicated an association between EGF-EGF receptor signalling and cancer risk [24, 25, 36]. As far as we know, there is no clinical research examining the serum level of EGF in patients with UBC. In the present study, no significant difference was determined between the UBC patients and the control group for serum levels of EGF. As a limitation of this study, sample size may be too small to detect an alteration in serum level of EGF in patients with UBC.

In conclusion, serum level of sHER-2/neu may have potential to be a marker for UBC, but IGF-1 and EGF do not have this potential. Although sHER-2/neu findings are promising, it should be kept in mind that this was a pilot study with limited case size. Our findings should be confirmed by further large-scale studies in future. As another limitation of the present study, all of the cases have high-grade tumors, however HER-2/neu should be compared between patients with low-grade vs. high-grade tumors. Furthermore, largescale studies examining serum levels of IGF-1 and EGF in UBC patients will be helpful to reveal their possible clinical significance in UBC in coming years.

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