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T. Ahmet Serel · Tahir Turan · Sedat Soyupek
Zafer Aybek · Hakki Perk

Urine and serum free IGF-1 levels in patients with bladder cancer: a brief report

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Abstract Insulin-like growth factor (IGF)-1, a mitogenic and anti-apoptotic peptide, can affect the proliferation of epithelial cells, and is thought to play a role in cancer development. The free IGF-1 represents the biologically active fraction of IGF-1. We hypothesised that there is a difference in free IGF-1 levels in the urine and serum from patients with TCC and normal subjects. Urine and blood samples were collected from 30 cases of superficial TCC and an equal number control subjects without malignancy. Free IGF-1 levels were measured in duplicate by radioimmunoassay. Specimens of bladder carcinoma were staged histopathologically using the Mostoffi grading system. Statistical analyses were performed using the Mann-Whitney U-test, Pearson correlation and covariate analysis. There was no significant difference in urine and serum free IGF-1 levels between the two groups. The correlation between urine and serum free IGF-1 levels and age was not significant. There was also no significant relationship between free IGF-1 levels and histopathological grading. The results of this pilot study reveal that the free IGF-1 level does not help predict tumour marker in the patients with bladder cancer.

Keywords Bladder cancer · Free insulin-like growth factor-1

Introduction

The insulin-like growth factor (IGF) system is widely involved in the carcinogenesis of different tumours. Evidence has accumulated that IGF-1 plays an important role in the progression of malignant tumours. The suppression of the IGF-1 receptor promoter by wild-type p53 has been shown to contribute to the anti-tumourigenic activity of this tumour suppresser gene [19]. A close correlation between circulating IGF-1 concentration and cancer risk has recently been reported by prospective epidemiological studies that specifically showed a positive relationship between high plasma IGF-1 and increased risk of prostate, lung and colon cancer [3, 15, 21]. Iwamura et al. [9] showed that over expression of the IGF-1 receptor might reflect the malignant potential of bladder cancer cells.

In the circulation, ≥99% of IGF-1 is found in binding complexes [8]. However, a small, but significant proportion (< 5%) of IGF-1 (free IGF-1) is not associated with IGF binding proteins [1, 11]. Zapf et al. [22] and Guler et al. [7] reported that free IGF-1 represented the biologically active fraction of IGF-1.

To our knowledge, no reports of urine and serum free IGF-1 concentrations in relation to TCC have been published. We therefore investigated patients with bladder carcinoma to determine the variation in the quantity of biologically active free IGF-1 in the urine and to compare it to that in patients with no malignancy.

Patients and methods

Urine and blood samples were obtained from 30 patients with bladder cancer (pTa-T1) who had been resected within the previous 3 years and had completed intravesical therapy at least 2 months before randomisation. An equal number patients were included with no known malignant or urological disease, e.g. kidney stones, hydrocele.

Blood samples (0.5 ml) collected in test tubes containing 50 μl EDTA (10 mg/ml), were centrifuged at 2,500 rpm for 5 min. Plasma and serum were separated and aliquots were stored at –20°C until analysis. Urine specimens (1 ml) were also centrifuged and aliquots were stored at –20°C until analysis. The DSL free IGF-1 kit (Diagnostic Systems Laboratories, Free IGF-1 DSL-9400, Webster, Texas, USA) was used in duplicated direct assay immunoradiometric (IRMA) tests, as described by Miles et al. [16],

T. A. Serel (✉) · S. Soyupek · H. Perk
Department of Urology, School of Medicine,
Süleyman Demirel University,
Anabilim Dalı 32040, Isparta, Turkey
E-mail: aserel@sdu.edu.tr
Fax: +90-246-2329422

T. Turan · Z. Aybek
Department of Urology, School of Medicine,
Pamukkale University, Denizli, Turkey

for the determination of the free IGF-1 concentration. IRMA is a non-competitive assay in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilised on the inside walls of the tubes. The other antibody is radiolabelled for detection. The analyte present in the unknowns, standards and controls is bound by both of the antibodies to form a "sandwich" complex. For the direct assay, the unaltered sample was added directly to the assay tube. Unbound and readily dissociable IGF-1 was then captured by the antibody coating, the remaining sample was washed away, and the IGF-1 bound to the tube was then detected using a radiolabelled antibody directed to a second epitope. The results were expressed as ng/ml. Tumour samples were graded histopathologically using the Mostoffi grading system. The results were tested by Mann-Whitney U-test, Pearson correlation and covariate analysis. All tests were two-tailed and $P < 0.05$ was considered to indicate a significant difference. Data were analysed using the Statistical Package for the Social Sciences (SPSS).

Results

The mean age of patients with cancer was 63 ± 1.2(SEM) years while for the controls it was 33.4 ± 1.2 years. Neither the urine nor the serum free IGF-1 values differed between groups (Table 1). There was no correlation between urine free IGF-1 level and age ($r = 0.13$, $P = 0.3$) (Fig. 1). The serum free IGF-1 level was not significantly correlated with age ($r = -0.04$, $P = 0.7$) (Fig. 2). Using covariate analysis, there was no significant relationship between either urine and serum free IGF-1 levels and age ($P = 1.23$ and $P = 1.21$, respectively). The tumour was grade I in 14 patients, grade II in ten and grade III in six.

In Table 2, the mean free IGF-1 levels in urine and serum are shown taking into account the histopathological grading in patients with TCC. There was no correlation between the mean free IGF-1 levels in urine and histopathological grade or between the histopathological grade and levels in serum ($r = 0.002$, $P = 0.99$ and $r = -0.11$, $P = 0.54$, respectively).

Discussion

We have shown that: (1) there is no clear association between free IGF-1 levels and the histopathological grading of superficial bladder carcinoma or age, and (2) high levels of free IGF-1 in the urine and serum can not be detected in cases of bladder carcinoma. The evidence that implicates IGF-1 in the aetiology of cancer of the bladder derives mostly from in vitro studies and pathophysiological considerations. There are two types of

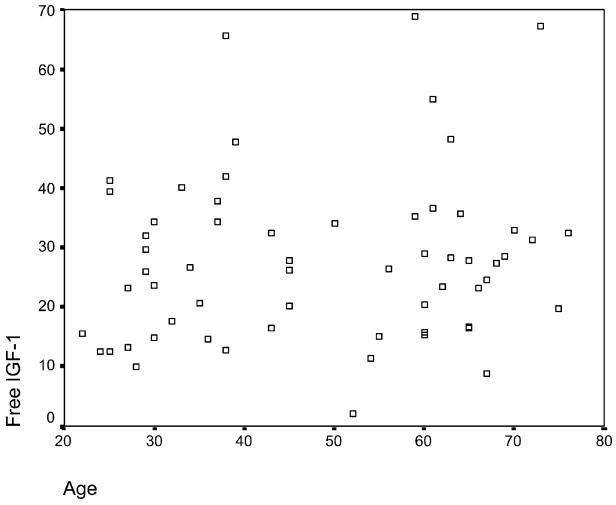

Fig. 1 Scatter diagram of urine free IGF-1 levels (ng/ml) and age (years)

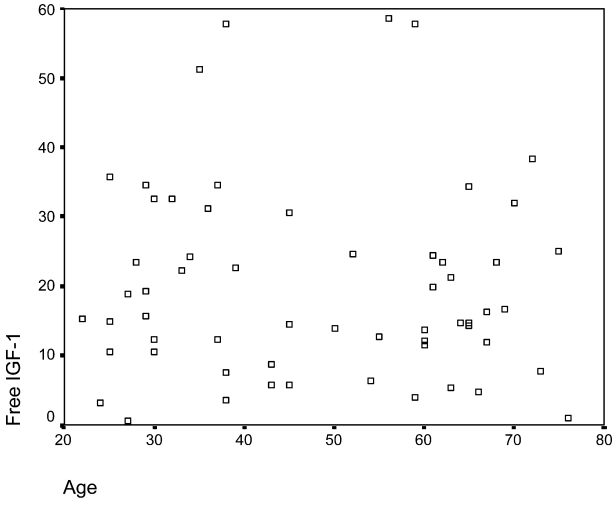

Fig. 2 Scatter diagram of serum free IGF-1 levels (ng/ml) and age (years)

Table 2 Mean(±SEM) free IGF-1 levels (ng/mlL) of in the urine and serum in of patients with TCC according to histopathological grading

Grade	Urine ($n = 30$)	Serum ($n = 30$)
I	28.,2 ± 4.9 SEM	25 ± 4.9 SEM
II	30.1 ± 5.1 SEM	16.6 ± 2.8 SEM
III	35.8 ± 8.2 SEM	17.4 ± 3.5 SEM

Table 1 Mean(±SEM) age (years), and free IGF-1levels (ng/mlL) of in the urine and serum inof distinguishing patients with TCC (Group A) from those without (Group B)

Variable	TCC ($n = 30$)	Control ($n = 30$)	p P value
Age (years)Mean age	63 ±1.2 SEM	33.4 ± 1.2 SEM	< 0.5
Urine fFree IGF-1 (ng/mLl)	28.1 ± 2.8 SEM	27.4 ± 2.3 SEM	> 0.5
Serum fFree IGF-1(ng/mlL)	19.7± 2.5 SEM	19.8 ± 2.5 SEM	> 0.5

receptors for the IGFs and the majority of the mitogenic effects appear to be mediated via the type 1 IGF receptor [2, 18]. IGF-1 receptors are very sensitive to stimulation by IGFs [4]. Iwamura et al. indicated that human bladder cancer cells possess functional binding sites for IGF-1 [9]. For malignant cells to be invasive, they must penetrate the basement membrane and migrate into surrounding tissues. Cellular proteolysis appears to play a central role in the processes of tumour invasion and metastasis. It is known that some growth factors have the ability to stimulate cellular proliferation and secretion of proteolytic enzymes that can degrade the basal membrane and the stromal components [13]. However, IGF-1 has not been found to regulate these enzymes. Nevertheless, several reports reveal that the number of IGF-1 receptors is a useful marker for the malignant potential of bladder cancer [14]. Our biochemical data suggest that the development of a bladder tumour is not driven by the rise of free IGF-1 levels in the urine and serum. In addition, many different factors e.g. alcohol use, fasting and puberty can alter circulating concentrations of IGF-1 [6, 10], and many diseases may also affect these levels [12, 20]. Thus, serum or urine free IGF-1 alone is unlikely to be a useful marker for bladder cancer.

The overexpression of IGF-1 receptors is found in several malignant tumours [5]. A positive correlation between IGF-1 receptor number and the malignant potential of the tumour, such as metastatic or invasive potential, has been reported [17]. In our study, there was no significant relationship between free IGF-1 levels in the urine and sera in patients and histological differentiation with superficial TCC. The results show that the free IGF-1 does not appear to correlate with histological differentiation of superficial bladder cancer. However, we believe that larger studies with more patients and controls are needed to determine the exact role of free IGF-1 in the development of bladder cancer.

In summary, this pilot study demonstrates that the free IGF-1 level in urine and sera does not differ between patients with bladder cancer and those without bladder cancer. Thus, free IGF-1 does not have a useful role as a marker for the presence of bladder cancer.

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