Role of TGF-β1 in Urinary Bladder Carcinoma

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Abstract:

The present study aimed to evaluate sera TGF- β1 concentration in patients with urinary bladder carcinoma (UBC). All malignant of them was transitional cell carcinoma (TCC) type , patients with urinary bladder disorders (UBD) and healthy control , and to study the correlation between sera TGF-β1 levels and tumor stages and grades in UBC patients . A direct ELISA test was used to quantify the seraTGF-β1 concentrations in sera of 58 patients with urinary bladder carcinoma UBC of different grades (G) and stages (T) all malignant of them was transitional cell carcinoma (TCC) type , 15 from patients with UBD and 15 healthy subjects . Sera levels of TGF-β1 were elevated in patients with UBC and UBD compared to healthy ($P \leq 0.01$) .There was no association between tumor stages and serum level of TGF-β1 ($P>0.05$) . Whereas there was an association between serum levels of TFG-β1 and tumor grades ($p \leq 0.05$) . Increased serum level was found in high grades G≥2 of patient with UBC .

Key words: Urinary bladder carcinoma , TGF-β1

Introduction

The incidence of human bladder cancer has increased extensively through the last decades. Despite several attempts to apply immunotherapy to the treatment of this malignant disease, it is still difficult to predict tumor progression, optimal therapy, and finally the clinical outcome [1]. The immune system plays a significant role in preventing tumor development, and it has a central role of immune surveillance in the pathogenesis of cancer [2] . Likewise, human subjects with congenital or acquired immune suppression have an increased frequency of cancer[3]. A new comer in a cytokine family whose members regulate organism development, the regulatory cytokine transforming growth factor β (TGF-β) made its debut with the rise of the vertebrates. TGF-β evolved to regulate the expanding systems of epithelial and neural tissues, the immune system, and wound repair. Tied to these crucial regulatory roles of TGFβ are the serious consequences that result when this signaling pathway malfunctions, namely tumorigenesis. Virtually all human cell types are responsive to TGF-β [4]. TGF-β1 is a multifunctional regulatory polypeptide that virtually all cell types secrete controls many aspects of cellular function, including cellular proliferation, differentiation, migration, apoptosis, adhesion, angiogenesis, immune surveillance, and survival . TGF-β has important role in tumor suppression and tumor progression [5]. TGF-β is a potent regulator of tumorigenesis. In cancer, two distinctive behaviors of TGFβ have been reported as a tumor suppressor at early stage of the disease, and as a tumor promoter at later stages. it suppresses the activity of the

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immune system and induces regulatory T cells [6],[7].Transforming growth factor-β1 also plays an important role in angiogenesis by promoting proliferation and migration of endothelial cells at low TGF-β1 concentrations whereas high concentrations lead to cytostasis and promote vessel maturation [8] .

Schuster and Krieglstein [9] found that the TGF-β1 signaling pathway is proapoptotic in most cases and, TGF-β mediated growth inhibition and apoptosis can be correlated with its function as a tumor suppressor .

Recent reports have shown that phagocytosis of apoptotic cells by macrophages leads to release of TGF-beta. Monocytes may therefore increase their secretion of TGF-beta in the peripheral blood as a result of ingestion of increased numbers of apoptotic cells [10] , this potentially explains the findings of increased intracellular production of TGF-β by monocytes in bladder cancer .

The aim of this study was to evaluate sera TGF- β1 concentration of patients with urinary bladder carcinoma UBC and control subjects (UBD and healthy), and study the correlation between TGF-β sera levels in UBC patients and tumor stages and grades .

Materials and Methods:

Sera samples from (58) patients with urinary bladder carcinoma (UBC) all malignant of them was transitional cell carcinoma (TCC) type from Al-Yarmook Teaching Hospital in Baghdad, and Baghdad Hospital for Specialists Surgeries were included in this study. In addition, (15) patients with urinary bladder disorders (UBD) and 15 healthy subjects were as a control groups.

The period of the study from May-2011 to May-2012 were eligible for this study. The cases were diagnosed clinically by consultant urologists at Al-Yarmook Teaching Hospital, and Baghdad Hospital for Specialists Surgeons . The blood samples of 4-5 ml collected by venipuncture, using plastic disposable 5ml syringes, from all patients and control groups. Blood samples allowed to clot at room temperature, then centrifuge for 15 minutes at approximately 500 rpm to obtain of unhemolyzed cell-free serum. Serum samples stored aliquots at -20 ºC until use for the measurement of TGF-β1 levels [11]. TGF-β1 concentrations were quantitatively determined in sera of patients and healthy control subjects by means of ELISA (Enzyme Linked Immunosorbent Assay) using ready kits manufactured by USBiological company (USA) .

Ethical permission to conduct the research was obtained from these hospitals and from all participants in this study. Selections of the patients were accomplished with the assistance of surgeons in the hospitals .

Statistical Analysis

Statical analysis was performed with the statical package for social science SPSS 15.0. Univariate analysis using one-way analysis of variance (ANOVA) was performed to assess the differences in TGF-β1 levels between groups. When the ANOVA test demonstrated a significant value, post hoc least significant difference analysis was used to determine statistically significant differences between groups at significant level ≤ 0.05 .The data were presented in terms of means ±standard errors (S.E.).

Results and Discussion:

There was an elevated mean serum level of TGF-β1 in sera of UBC patients(1192.78 ±SE25.3 pg/ml) Figure (1), when compared to sera

TGF-β1 levels of UBD patients and healthy (786± SE 43.8 , 35.5 ±SE 2.5 pg/ml, respectively), with a high statistical significance(P = 0.00). The results showed also a high significant difference in sera TGF -β1 levels between groups, or within groups (F=275.157 , p =0.00) .

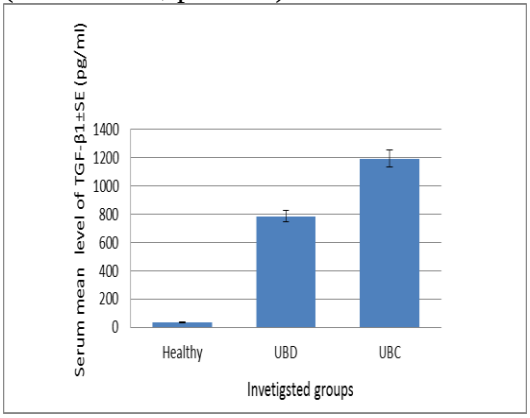

Fig.(1): Serum level of TGF-β1 in patients and healthy controls

Cancer cells are able to produce and secrete TGF-β, which is able to immunosuppress cancer patients in the absence of cytotoxic treatment [6]. Elevated levels of TGF-β have been shown to contribute to tumorogenesis through several different mechanisms. In addition to the loss of the anti-proliferative effects of the growth factor there are some data in the literature indicating that certain genetic mutations can lead to TGF-β becoming pro-mitotic for certain tumor cells [12]. Furthermore, malignant cells are able to produce large quantities of TGF-β. The consequence of over expression of TGF-β by cancer cells is an important factor for subsequent tumor progression. The excess amount of TGF-β promotes tumor angiogenesis and immune suppression [12] .

The results of this study compatible with the results of Shaker *et al*.[13] who found that TGF-β protein is overexpressed in bladder cancer .

The relationship between serum mean levels of TGF-β1 and tumor stages of patients with bladder cancer

Figure (2) showed the highest TGF-β1 levels were observed in sera of UBC patients with T3(1196.8 ±SE 49.4 pg/ml), then in sera of UBC patients with T2(1192.7±SE 30.5 pg/ml), and then T1(1188.8 ±SE 54.6 pg/ml). There is no statistical significant difference in sera TGF-β1 levels between groups, or within groups (F=0.007, P=.0.993). Although the difference sera TGF-β1 mean levels were observed between stages.

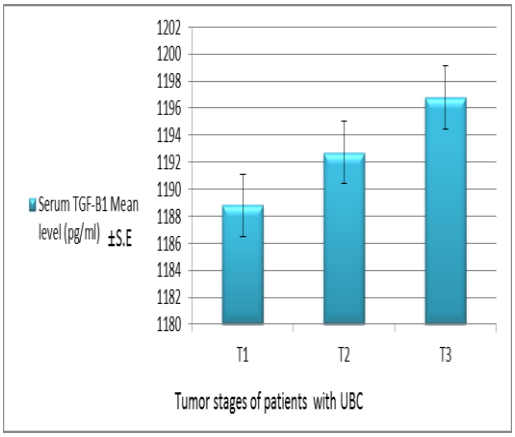

Fig.(2) : Sera level of TGF-β1 in tumor stages of patients with UBC

While ,the relationship between sera mean levels of TGF-β1 and tumor grades of patients with bladder cancer showed a high significant increased mean levels of TGF-β1 (1322.8 ±SE 50.4 pg/ml) in patients with G3 tumor as compared with patients with G1and G2 (1043.4 ±SE 22.9 , 1195.5 ±SE 29.5 pg/ml : P=0.00,P=0.005 respectively) Figure (3), and there was also a high significant difference in mean levels between G2 and G3 (P=0.014) .

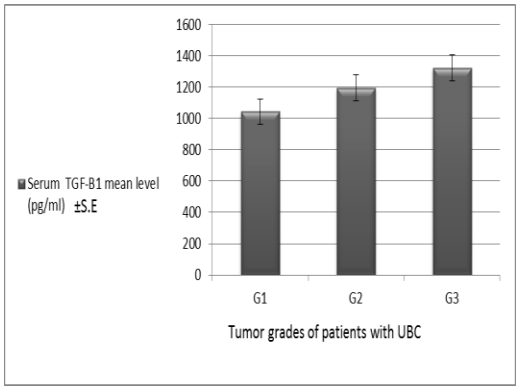

Fig.(3) : Sera level of TGF-β1 in tumor grades of patients with UBC

These result compatibles with Shaker *et al*. who found that TGF- mRNA is Over expressed in high grade TCC compared with low grade [13].

The results support evidence that TGF-β1 plays a role as a tumor suppressor in early disease and has pro-oncogenic effects in advanced tumor grade . Higher TGF-β1 protein expression is associated with increasing T-stage and metastatic disease, indicating that TGF-β1 is of importance in tumor progression [14] .

TGF-β1 production by tumor cells may affect the tumor environment via suppression of tumor-infiltrating immune cells and probably contributes to tumor cells aggressiveness through autocrine activation of Smad signaling [15].During tumorigenesis, TGF-β1 frequently stimulates the proteolytic activity of cancer cells by increasing the expression of matrix-degrading enzymes. Taken together, by decreasing the adhesiveness and increasing the motility and proteolytic activity of cancer cells, increased levels of TGF-β1 result in more invasive cancer cells, which may represent one of the tumor promoting activities of TGF-β1 [15].

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دور عامل النمو المتحول (TGF-β1) في سرطان المثانة

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الخلاصة :
هدفت الدراسة الحالية إلى تقدير مستوى تركيز عامل النمو المتحول TGF-β1 في أمصال مرضى سرطان المثانة البولية ومجموعتي (مرضى اضطرابات المثانة البولية والأشخاص الأصحاء)كعينات سيطرة ، و دراسة ارتباطه مع مراحل ومراتب مرضى سرطان المثانة البولية . تم استخدام اختبار الاليزا المباشر لتقدير مستوى تركيز البروتين في أمصال 58-مريض يعانون من سرطان المثانة البولية ضمن مراحل ورتب مختلفة معظم الاورام كانت من نوع سرطان الخلايا الانتقالي الحرشفي ،15مريض يعانون من اضطرابات المثانة البولية و15 أشخاص أصحاء . وقد وجدت الدراسة أن مستوى تركيز بروتين النمو المتحول TGF-β1 في المصل عالي احصائيا في مرضى سرطان المثانة البولية ومرضى اضطرابات المثانة البولية مقارنة بالأشخاص الأصحاء وكانت القيمة الإحصائية (P< 0.01) بين المجاميع . وبينت النتائج أيضا عدم وجود علاقة بين مراحل مرضى السرطان وتركيز البروتين وكانت ألقيمه الاحصائية (P> 0.05) في حين أن هناك علاقة بين مراتب مرضى السرطان وتركيز البروتين اذ كانت القيمة الاحصائية (P< 0.05) وأظهرت المراتب المتقدمة تركيز عالي للبروتين .