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RESEARCH ARTICLE

TOTAL ANTIOXIDANT CAPACITY AND CERTAIN CYTOKINES IN PATIENTS WITH BLADDER CANCER

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ABSTRACT

Objectives: Bladder cancer (BC) is one of the most widespread cancers distressing men and women and thus has a philosophical impact on health care. An extensive variability in the occurrence of BC reflects its multifactorial with polygenic etiology. Antioxidants play an essential role in protection of the cells from oxidative damage. Antioxidant defense can be affected in cancer. TNF-α is one of the major mediators of inflammation and has been linked to all steps involved in tumor genesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis**.** Tumor cells secrete their own TNF-α in anticrime manner which further enhance the expression of other growth factors such as transforming growth factor (TGF). The aim of this study was to evaluate the serum total antioxidant capacity**,** TNF-α**,** TGF-β levels in patients with bladder cancer.
Methods: fifty patients with bladder cancer and 20 controls were enrolled in the study. Serum TAC, TNF-α, TGF-β levels were determined.
Findings: Serum TAC, TNF-α and TGF-β were significantly elevated in BC over that of the control ($p = 0.0001$). Data analysis according to the grade of the disease show a significant difference among high grade, low grade & control groups with significant differences in TAC, TNF-α and TGF-β levels between high and low grade groups ($p<0.001$).
Conclusion: Our results suggest that increased TNF-α and TGF-β with decreased antioxidant levels may be, in part, play a role in the pathogenesis of bladder cancer and may help in diagnosis, treatment and follow up of bladder cancer patients in the future.

INTRODUCTION

Bladder cancer is the 10th most common cancer worldwide, with the highest rates reported in Europe, North America and Australia, and accounting for an estimated 261 000 new cases diagnosed and 115 000 deaths each year; by comparison, relatively low rates were found in the Far Eastern countries (Meliker and Nriagu 2007). Histologically, most cases of bladder cancer are transitional cell carcinomas (90 %); 70 % of these are superficial and papillary subtypes (Ahirwar *et al*., 2008). The less common types are squamous cell carcinoma (3 - 5 %); adenocarcinoma (0.5 to 2 %); small cell carcinoma (less than 0.5 %); and sarcoma, carcinosarcoma/sarcomatoid tumours, paraganglioma, melanoma and lymphoma (less than 0.1 %) (Dahm and Gschwend 2003). Haematuria, frequent urination and pain during urination, are the most common symptoms of bladder cancer (Zeegers *et al*., 2004, Shariat, *et al*., 2009). Tobacco smoking, occupational exposure to chemicals, radiotherapy, dietary factors, bladder schistosomiasis, chronic urinary tract infection, chemotherapy are the main risk factors for bladder cancer. (Witjes *et al*., 2013) Antioxidants play an essential role in protection of the cells from oxidative damage. They include several agents such as enzymes (glutathione peroxidase, superoxide dismutase, catalase), large molecules (ferritin, albumin), and small molecules (uric acid, glutathione, bilirubin, ascorbic acid, α tocopherol, and vitamin E). Their defense mechanism in biological system involves chain breaking (superoxide dismutase) and preventive (Vitamin E) mechanisms. Antioxidant defense can be evaluated by measurement of either individual antioxidants levels in cells and plasma or total antioxidant capacity. The latter can be estimated by measuring total reducing activity of body fluids such as serum and plasma (Kwak and Yoom 2007).

Cytokines were initially discovered as secreted proteins that control various immune functions. It is now clear that cytokine functions extend to many other aspects of biology, including cancer. (Lin *et al*., 2007) A long-suspected causative connection between inflammation and cancer has been mechanistically established. (Grivennikov *et al*, 2010) At least 20% of all cancers arise in association with infection and chronic inflammation and even those cancers that do not develop as consequence of chronic inflammation, exhibit extensive inflammatory infiltrates with high levels of cytokine expression in the tumor microenvironment. (Grivennikov and Karin 2010).

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TNF-a is one of the major mediators of inflammation and has been linked to all steps involved in tumorgenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis (Sethi *et al*., 2008).Tumor cells secrete their own TNF-a in anticrime manner which further enhance the expression of other growth factors such as transforming growth factor-alpha (TGF-a) and epidermal growth factor receptor (EGFR), both of which mediate proliferation (Rama *et al*., 2012).

Transforming growth factor beta 1 (TGF-β1)is a potent inhibitor of proliferation in epithelial cells and acts as a tumor suppressor (Siegel and Massague 2003). Additionally, continuous expression of TGF-b by cancerous cells helps cancer to progress further. (Davis *et al*., 2008). Sharif-Afshar *et al*. (Sharif-Afshar *et al*., 2005) suggested a role of stromal TGF-b signaling with estrogens and androgens in bladder fibrosis. However, TGF- β 1 may facilitate tumor growth, as its over expression can enhance tumor metastases (Jun Guoa *et al*., 2011). The present study was performed to assess the serum total antioxidant capacity, TNF-α and TGF- in the selected patients with transitional bladder carcinoma. That results were compared to the results in control group.

Subjects and Methods

Fifty patients with transitional bladder carcinoma with age range from (31-85) years (mean±SD was 64.2± 11.6)) who were consecutively referred to Ghazi AL-HaririHospital for Specialized Surgery/Baghdad Medical City, from March 2013 to February 2014 were investigated. The diagnosis of patients was confirmed by a pathologist in the hospital referred to above according to 2004 WHO grading (Sauter *et al*., 2004). Those also divided to subgroups:-

A- Twenty eight (28) of them were patients with high grade transitional cell carcinomas of bladder with age range from(37-85) years.
B- Twenty tow (22) patients with low grade transitional cell carcinomas with age range from (31-80) years.In addition to twenty apparently healthy individuals whom age, sex and ethnicity matching that of the selected patients, were included as a control group.blood samples were obtained in the morning. Blood samples were collected into empty plain tubes and the serum was then separated from the cells by centrifugation at 3,000 rpm for 10 min.

To determine the serum total antioxidant, Total Antioxidative Capacity Fast Track kit (LDN, Germany) was utilized. The method is based on the reaction of peroxides with peroxidase followed by a colour reaction of the chromogenic substrate tetramethylbenzidine. Its blue colour turns to yellow after addition of the stop solution and can be measured photometrically at 450 nm. (Martinez *et al*., 2001). Transforming growth factor-beta was determined using Human TGF beta 1 ELISA kit (Creative Diagnostics, USA). This assay employs the quantitative sandwich enzyme immunoassay technique. (Miyazono 1992). Human TNF alpha ELISA Ready-SET-Go (eBioscience, Inc., USA) was used for the in-vitro quantitative determination of tumor necrosis factor in human serum (Tversky *et al*., 2008). The demographic and laboratory obtained data were analyzed by the SPSS software (version 20). Descriptive results were reported as mean (standard deviation). Independent sample t-tests, ANOVA test, and Chi-sequare were used to compare the results among groups. P value of <0.05 was considered as statistically significant.

RESULTS

Demographic characteristics of the studied subjects were presented in Table 1. The table showed no significant difference (P > 0.05) regarding the age group between patients and controls although higher percent (42%) of patients were over seventy years old. The table also showed no significant difference (P > 0.05) between males and females. Moreover, there was a significant difference (P < 0.0001) regarding to smoking between patients and controls suggesting smoking as a risk factor for BC.

Table (2) revealed that Total Antioxidant Capacity (TAC) was significantly lower (P<0.0001) in TBC patients than controls. Serum TNF-α and TGF-β were significantly higher in TBC than control (p< o.ooo1) as demonstrated in same table. Data analysis according to the grades of the disease, show significant differences between high grade, low grade & control groups Table (3). Total Antioxidant Capacity (TAC) was significantly lower P< 0.05) in high grade and low grade groups when compared to controls with significant difference between high grade and low grade groups (P< 0.05). The analysis of data showed a highly significant difference among high grade, low grade and controls groups (P<0.0001). Serum Tumor Necrosis Factor Alpha (TNF-α) was significantly higher (P<0.05) in high grade and low grade groups when compared with that of controls with significant difference between high grade and low grade TBC patients (P< 0.05), and highly significant difference among high grade, low grade and controls groups (P<0.0001). There was a significant rise in the mean concentration of Transforming Growth Factor- beta in sera of high grade and low grade TBC patients when compared with that of controls (P<0.05) with significant difference between high grade and low grade TBC patients (P< 0.05) and highly significant difference among high grade, low grade TBC patients and controls (P<0.001).

DISCUSSION

Bladder cancer is a common tumor of the urinary tract (Macvicar 2000). It is one of the top five cancers in the eastern Mediterranean region, in which it ranks the 3rd in the order of incidence in Iraq (WHO,2009). The most common risk factors for bladder cancer are exposure to industrial carcinogens, cigarette smoking, male gender, and possibly diet (Wynder and Goldsmith 1977; Zeegers *et al*., 2004). Another major etiological factor is infestation by the parasite Schistosoma hematobium (Wynder and Goldsmith 1977). In the present study, the antioxidant defense was evaluated by measuring total antioxidant capacity (TAC) in serum. The measurement of different antioxidant molecules separately is labor-intensive, time-consuming and costly. Moreover, some investigators suggest that assessment of total antioxidant capacity of plasma may be more useful than measuring antioxidants individually

Table 1. Demographic characteristics of patients & control S

		TBC		High grade bladder cancer		Low grade bladder cancer		Controls		
Characters		No	%	No	%	No	%	No	%	P value
Age (years)	<60	13	26.0	4	14.3	9	40.9	5	27.8	
	60--69	16	32.0	11	39.3	5	22.7	10	55.6	0.144
	=>70	21	42.0	13	46.4	8	36.4	3	16.7	
Gender	Males	40	80.0	20	71.4	20	90.9	15	83.3	
	Females	10	20.0	8	28.6	2	9.1	3	16.7	0.378
Smoking	Smoker	37	74.0	23	82.1	14	63.6	3	16.7	
	Not	13	26.0	5	17.9	8	36.4	15	83.3	0.00002*

Table 2. Serum concentration of TAC, TNF-alpha, TGF-beta in TBC patients & controls expressed as Mean±SD

Serum markers	TBC Mean±SD No=50	Controls Mean±SD No=18	P-value
TAC (mmol/L)	0.756±0.353	1.597±0.372	o.ooo1*
TNF- α (pg/ml)	15.060±6.783	6.669±2.428	o.ooo1*
TGF-β (ng/ml)	31.635±8.459	22.783±10.024	o.ooo1*

*Significant using students-t-test for difference between two independent means at 0.05 level.
TAC: Total Antioxidant Capacity. TNF- α: Tumor Necrosis Factor.
TGF-β -: Transforming Growth Factor

Table 3. Serum concentration of TAC, TNF-alpha, TGF-beta in high grade TBC & low grade TBC patients compared with controls expressed as Mean±SD

Serum markers	High grade TBC	Low grade TBC	Controls	P-value (ANOVA)
TAC (mmol/L)	No=28	No=22	No=18	
	0.626±0.236[a,b]	0.921±0.409[a]	1.597±0.372	0.0001*
TNF- α (pg/ml)	18.227±7.000[a,b]	11.028±3.734[a]	6.669±2.428	0.0001*
TGF- β (ng/ml)	33.183±7.451[a,b]	29.666±9.399[a]	22.783±10.024	0. 001*

[a] Significant difference as compared to controls (Student t- test P< 0.05)
[b] Significant difference between high & low grade groups (Student t- test P< 0.05)
* Significant difference among different groups (ANOVA test P< 0.05)
TAC: total antioxidant capacity. TNF- α: tumor necrosis factor.

since their synergistic interaction could be determined (Van Zoeren *et al*., 1997, Kampa *et al*., 2002). Our results revealed significant decreased levels of TAC in bladder cancer patients compared to healthy ones (p= 0.0001). also serum levels were significantly lower in high grade TBC and low grade TBC groups as compared with control group (p = 0.0001) these results came in accordance with previous study done by Ilhan Gecit • *et al*, 2012 which demonstrated a a significantly decreased serum TAC levels in bladder cancer patients than in control subjects.

It is known that the harmful effects of reactive oxygen species (ROS) are controlled by various cellular defense systems consisting of enzymatic (catalase, glutathione peroxidase, superoxide dismutase etc.) and non-enzymatic (vitamins E, C, glutathione etc.) components (Mates *et al*., 1999). Antioxidant depletion in the circulation may be due to the scavenging of lipid peroxides as well as sequestration by tumor cells (Sharma *et al*., 2007). However, if these systems are insufficient, severe metabolic malfunctions and oxidative damage to DNA may result, which, experimental studies in animals and in vitro have suggested, to be an important factor in carcinogenesis (Marnett 2000).

Tumour necrosis factor (TNF) cytokine, produced as the part of host defence against infection. This cytokine is involved in multiple inflammatory and immune responses and plays role in the pathogenesis of many autoimmune and infectious diseases. (Tsytsykova and Goldfeld 2002). In our study, markedly greater TNF level was found in TBC patients compared to control group (p= 0.0001) in addition to that, high grade TBC patients show significant increase in TNF level as compared with low grade TBC patients and control groups (p= 0.0001) and significant differences among high grade, low grade and controls(p= 0.0001) such results came in accordance with a previous studies by Maria Sofra ,*et al*,2013 when increase in TNF-α levels was also observed. It is important to note that increased TNF-α expression has been reported in recurrent, larger bladder tumors as well as in tumors that show progression in grade and stage (Feng *et al*., 2011; Maria *et al*., 2013).

TGF-b signaling is contextual, depends on the cell type, and has both positive and negative effects on cancer. Specifically, in cancer TGF-b exerts a perplexed role. Initially, it acts as a tumor suppressor since it induces apoptosis and inhibits the growth of cells (Massague 2008). However, changes in TGF-b signaling often correlate with tumor stage and rate of progression (Yang *et al*., 2010). At later stages of tumor progression, TGF-b acts as a tumor promoter. Seemingly at this stage cancer cells protect themselves and tend to acquire increasing resistance to ignore TGF-b growth inhibitory signals which is an important reason for the shift from being a tumor suppressor to a tumor promoter. Subsequently, cancer cells start secreting non-physiological levels of TGF-b in an autocrine and paracrine manner which may affect the differentiation of the tumor cells and the surrounding cellular environment, respectively, leading to development of the tumor

and metastasis in an immunosuppressive environment that is rich in TGF-b (Gorska *et al*., 2003). The present study showed that patients with bladder cancer had significantly higher serum TGF-b level than healthy controls, in addition to that, high grade TBC patients show significant increase in TGF-b level as compared with low grade TBC patients and control groups (P=0.0001), also there was significant difference between low grade level and control groups (P< 0.05), and highly significant difference among high grade, low grade TBC patients and controls (P<0.0001), this comparable to study done by *Hiroshi et al*. (1995) that demonstrated Transforming growth factor-beta 1 in bladder cancer was higher than that found in normal. Our results suggest that increased TNF-α and TGF-β with decreased antioxidant levels may, in part, play a role in the pathogenesis of bladder cancer and may help in diagnosis,treatment and follow up of bladder cancer patients in the future. It is believed that the administration of antioxidant vitamins such as A, C, and E may be useful in preventing and treating bladder cancer.

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