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Bladder cancer: total antioxidant capacity and pharmacotherapy with vitamin-E

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Abstract

Purpose Free radicals play an important role in the different complex course of carcinogenesis. Higher concentrations of reactive oxygen species are highly associated with the presence of tumors. The urinary bladder organ is also a target for many carcinogens. The major objective of this investigation was to measure the role of redox state or total antioxidant capacity (T-AOC) and antioxidant functions of vitamin E in patients with low-grade papillary cancer of the bladder (BC).

Methods The blood sample was used for measurement of the T-AOC by the Trolox-TAC assay kit. Thirty-five patients with BC and thirty-five healthy subjects that matched for age were entered in this study. The obtained data were analyzed using the Statistical Package (SPSS Inc, Chicago, IL, USA). The significance level was set at $p \leq 0.05$.

Results In healthy controls, the mean ± SD for T-AOC was 91.8 ± 16.6 (U/ml), that was significantly higher when compared to the mean value of 24.5 ± 28.9 (U/ml) in patients with BC ($p = 0.00$). The difference in concentration of T-AOC before and after prescription of vitamin E was encountered with a p value of 0.16.

Conclusions By reference to the significant difference between T-AOC in patients and healthy controls, our results strongly suggest a low level of T-AOC in patients with BC. The obtained changes in T-AOC before and after management with vitamin E recommended additional consideration associates with different stages and grade of tumor in patients with BC.

Keywords Vitamin E · Antioxidant capacity · Cancer · Bladder

Abbreviations

T-AOC	Total antioxidant capacity
BC	Bladder cancer
SD	Standard deviation
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
DNA	Deoxyribonucleic acid
Min	Minimum
Max	Maximum

Introduction

Bladder cancer (BC) is the most common urinary tract cancer in the world [1, 2]. In vitro and in vivo studies recommend that high levels of reactive oxygen species (ROS), reactive nitrogen species (RNS) and oxidative stress play a crucial role in human cancer. In fact, the presence of an unpaired electron results in certain common properties that are shared by most radicals. The most important free radicals are oxygen derivatives, particularly superoxide and the hydroxyl radical. In fact, ROS develop numerous biological properties and contribute to signaling events during physiological and pathological processes. Aggressive cancer cells depend on the elevated intracellular levels of ROS, could proliferate, self-renew, and metastasize [3–6]. In these reactions the key pathways were associated with mitochondrial electron-transport chain and other oxidizing agents such as; depurination, depyrimidination, single and double-stranded DNA breaks, base and sugar modifications and DNA–protein crosslinks. In these circumstances, there are not only the reactions of ROS and RNS with proteins, lipids, nucleic acid, but also there are reactions that could lead to

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genomic instability. Survival, proliferation, invasion, angiogenesis, inhibition of apoptosis, resistance to chemo- and radio-therapy of cancer cell promote by disruption of redox regulations, subsequent to systematic biological molecular damage. In such conditions, oxidation exceeds the control mechanism, and as a result of the rise in oxidative stress, ROS and RNS might be elevated. Therefore, in this situation within the human immune system, due to the disparity between capacities of total anti-oxidant and pro-oxidant there could be several types of damage to DNA [7–9]. Hunting these free radicals by exogenous or endogenous antioxidant molecules could alleviate oxidative/nitrosative stress or its consequences [3, 8]. In fact, the physiological role of antioxidant is to prevent damage to cellular components that might arise as a consequence of chemical reactions involving free radicals. There are associations between total antioxidant capacity (T-AOC) and is effectiveness in the early stage of carcinogenesis, as some reports stated that antioxidants could inhibit initiation and promotion associated with carcinogenesis [8–11].

In patients with BC, oxidative stress and the presence of inflammatory agents will increase xanthine oxidase activity and its' level by increasing the conversion of dehydrogenase to oxidase. Oxidative reactions and cellular damages could cause a significant increase in serum malodialdehyde concentration and significant decrease in T-AOC. In addition, antioxidant deficiencies in patients with BC could be a cause of a decrease in both; antioxidant intake and synthesis of endogenous enzymes, or an increase in antioxidant consumption. Therefore, due to high rates of recurrence in BC, a beneficial intervention strategy might be based on the prescription of vitamin C and vitamin E [11–14]. The relationship between BC and vitamin E also appears to be unclear with some studies that reported potential inverse associations and others no associations at all [15–20]. Measurement of T-AOC in biological fluids is believed to be a useful measure of the ability of antioxidant present (endogenous) in the fluids to protect against oxidative damage to membrane and other cellular components. Nonetheless, there are conflicting publications related to the correlation between T-AOC and early stage of carcinogenesis. Some reports indicated that antioxidants could inhibit initiation and promotion associated with carcinogenesis but others found that vitamin and antioxidant supplements have no preventive effect against BC. In addition to the useful present methods such as cystoscopy examination and biopsy, for early correction of carcinogenic state, giving consideration towards the status of oxidative stress by valuable screening test, such as measurement of T-AOC might be another advantageous clue in patients with BC [11, 21–25]. A talented strategy for targeting redox status of the bladder cells is to use readily available natural substances such as antioxidants that have already been identified to have chemopreventive potential, capable of intervening in carcinogenesis. To maintain optimal bladder function, based on the patient's clinical conditions, antioxidants detoxify the reactive intermediates and repair the resulting damage caused by oxidative stress [26–32]. Therefore, we investigated the difference in the level of T-AOC in BC and healthy adults, in addition to T-AOC alteration following vitamin E pharmacotherapy in BC.

Materials and methods

Study design and patient population

According to the previously published work [13, 14, 29] the sample size was calculated based on this formula;

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (P_1(1-P_1) + P_2(1-P_2))}{d^2}$$
$$= \frac{(1.96+0.84)^2((0.60\times 0.40)+(0.80\times 0.20))}{0.3^2} \approx 35,$$

n = sample size, $Z\alpha$ = level of confidence according to the standard normal distribution (for a level of confidence of 95%, $z = 1.96$, for a level of confidence of 99%, $z = 2.575$), p = estimated proportion of the population that presents the characteristic, d = tolerated margin of error.

The study included 35 patients with biopsy-confirmed transitional cell carcinoma of the bladder and 35 healthy age-matched controls. None of the patients have any other disease or malignancies except BC and only the newly diagnosed patients with no prior chemotherapeutic treatment were included in this study. Patients unable for treatment follow-up, those with nodal or metastatic disease were also excluded. The patients were supplemented daily by oral vitamin E 400 IU during 6 months [30, 31]. Patients were informed by senior clinical pharmacist associated with side effects of vitamin E such as; nausea, vomiting, diarrhea, headache, rash, fatigue or weakness, blurry vision, problems with the ovaries or testes. Due to interactions of vitamin E with aspirin, warfarin, tamoxifen and cyclosporine, patients with these drugs were excluded from the study. In accordance with the principles of the Declaration of Helsinki; the study was approved by the ethics committee of the Isfahan University of Medical Sciences, via the code number 296024.

Variables of interest and study outcomes

From healthy volunteers as control group, 5 ml blood sample was taken. From patients with BC, before and after

pharmacotherapy with vitamin E, 5 ml blood samples were withdrawn. Plasma or serum within the tube was covered with foil and stored at − 20 °C until analysis.

Description of the assay procedure

On the day of assay, samples were thawed at room temperature and measured by the quantitative colorimetric assay, using Human T-AOC Elisa Kit [31]. The Chroma of color and the concentration of the Human Substance T-AOC of samples were positively correlated with the redox state in patients and healthy subjects.

Statistical analysis and description of risk calculator development

Baseline patient characteristics were compared using Pearson's χ^2 test for categorical variables and the Wilcoxon rank-sum test for ordinal or continuous variables. Chi-square and student *T* test were used to compare two groups related to dissimilarity of redox state in patients with BC and healthy controls and association between vitamin E prescription and change in the redox state in patients with BC. Included in models were natural log-transformed T-AOC (in U/ml), age (by year), and pharmacotherapy with vitamin E. All statistical testing was two-sided with significance set at $p \leq 0.05$.

Results

There were not any significant differences associated with age between the treatment group and healthy subjects ($p = 0.32$). Figure 1 shows the state of redox by the distribution of T-AOC in the treatment group before-after and the control group. T-AOC distribution in the treatment group was not followed normal pattern before and after the prescription of vitamin E ($p = 0.00$). In the control group, the distribution of T-AOC was normal ($p = 0.724$).

The change in T-AOC before (24/2 ± 28/8; U/ml) and after prescription (24/7 ± 29.0; U/ml) of vitamin E was shown a *p* value of 0.16. Figure 2 shows that in 86% of patients with BC, the mean value of T-AOC was about 13.9 (U/ml), in 5% was around 45.2 (U/ml) and in 9% approximate to 110 (U/ml).

In the control group, the mean ± SD of T-AOC was (91.8 ± 16.6; U/ml) that was significantly higher when compared to the mean value (24.5 ± 28.9; U/ml) in patients with BC ($p = 0.00$). Figure 3 shows the detection of T-AOC in plasma versus serum in patients with BC.

Analysis of hematological and biochemical variables showed that the mean ± SD for platelet was 240,300 ± 194,505 with a minimum of 24,000 and a maximum of 538,000. Hemoglobin and hematocrit in the 9% of patients were lower than the others. Also in the 5% of patients, blood sugar with a mean value of 153.5 mg/dl was higher than others.

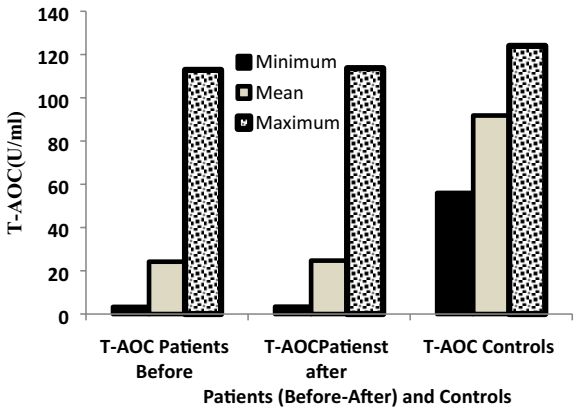

Fig. 1 Analyzed values of T-AOC in treatment and control group

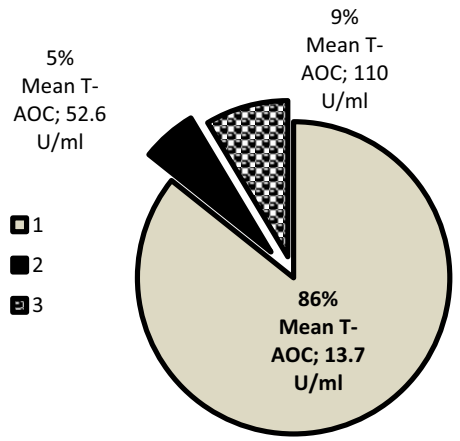

Fig. 2 Distribution of T-AOC in patients with BC

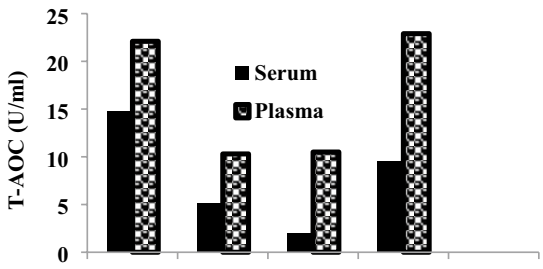

Fig. 3 T-AOC in plasma and serum of patients with BC

Discussion

In this study, we investigated the association between T-AOC in a cohort of 35 patients with low-grade papillary carcinoma of the bladder and 35 healthy volunteers. To scavenge free radicals and improve the patients' condition with oxidative/nitrosative stress and preventing BC recurrence, a 400 IU/day vitamin E during 6 months was administered and then the level of T-AOC was compared before and after intervention. Our results demonstrated that total capacities of endogenous antioxidants were significantly lower in patients with BC and strategy based on prescribing exogenous antioxidant such as vitamin E showed challenging regarding to the level of T-AOC. In agreement with the previous publication [22], high levels of free radicals and other potentially toxic, oxidizing species in the samples scavenge by the test solutions that result in lower level of T-AOC. Indeed, in disease condition any factor such as inflammation could affect the immune system. Subsequently, production of ROS, RNS and other molecules with unpaired electrons increases. The free electrons of these productions could bond to any prone part of the body such as the bladder and then create carcinogenic non-radical compound. In this study evaluation of redox state or antioxidant capacity in health and disease confirmed a lower antioxidant capacity in disease condition such as low-grade papillary cancer of the bladder. Bladder such as many organs within the human body is protected by powerful defense systems that help in response to oxidative stress challenges. In pathological condition, the higher dys-equilibrium between pro-oxidant processes and the antioxidant defense system results to decrease in T-AOC that might not be altered by the antioxidant effect of vitamin E [23–25]. Our findings potentially could have major clinical implications by an aim to challenge the current paradigm regarding earlier detection of BC by introducing simple blood sampling and plasma measurement of T-AOC [11]. It is well known that glutathionereductase, catalase, peroxidase, ascorbate, uricacid, albumin, transferrin and vitamin E is antioxidant especies. Therefore, prospective evidence regarding the efficacy of exogenous antioxidant such as vitamin E in patients with BC and its' association with redox state seems to be important. Measurement of the T-AOC in biological fluids obtained from patients with BC provided an indication of the overall capability to counteract ROS resist oxidative damage and combat oxidative stress-related diseases.

In many pathological conditions, T-AOC in plasma was introduced as a biomarker for investigation associated with the redox state condition. Plasma is a useful alternative due to its rapid processing time; therefore, in this study plasma samples were analyzed. Previous publications reported that improper design or use of blood sample collection strategies can adversely affect the accuracy of laboratory test results [23, 24]. In this study, there was a significantly higher amount of T-AOC in plasma when compared to serum in which corresponded to an approximate higher mean T-AOC of 169.3%. Ladenson et al. in 1974 confirmed that plasma, which contains fibrinogen and other clotting factors, has a higher viscosity and total protein content than serum [33]. Serum has a higher concentration of thromboglobulins, potassium, activation peptides and platelet factor 4 for coagulation factors [34–36].

After testing age for normality distribution, there was not any significant difference between the two groups regarding age ($p=0.32$). Distribution of T-AOC in patients with BC before and after pharmacotherapy with vitamin Ewas not followed a normal distribution ($p=0.00$). In the control group, the distribution of T-AOC was normal ($p=0.796$). There was a significant difference ($p=0.00$) between T-AOC in patients with BC ($24/5 \pm 28/9$; U/ml) and healthy controls ($91/8 \pm 16/6$ U/ml). Previous publications reported that in patients with BC, any pathogenic disorders could release inflammatory factors that resulted in carcinoma as an imbalance between redox state and T-AOC [36–41]. The significance of T-AOC in many diseases such as multiple sclerosiswas reported as well. The study showed that mean serum T-AOC in multiple sclerosis groups of patients were significantly lower when compared to the control group of subjects, suggested that oxidative stress plays an important role in the pathogenesis of multiple sclerosis and, therefore, recommended the addition of antioxidants in diet and therapy of patients [37].

Comparison of T-AOC before and after prescription of pharmacotherapy with vitamin E was controversial. In the present study, the function of vitamin E after prescription in a population of patients with BC could be discussed associated with its' role both as an antioxidant and as a component of biological membrane. By restoring an amphipatic balance vitamin E, stabilize membrane by the formation of complex with long-chain fatty acids and the hydrophilic products of phospholipase A, lysophopholipids. Therefore donation of the electron to peroxyl radical, inhibition of free radical formation and apoptosis of cancer cells by prescription of vitamin E showed challenging regarding not sufficiently change in bladder cancerous redox state or T-AOC by a p value of 0.16 [42]. Previous publications confirmed that the recurrence of BC may decrease by vitamin E intake [43] but regarding tumor development and growth, grade and stage of BC might affect redox imbalance and antioxidant activity [44]. In addition, in individual over fifty the quantification of nutrient intake was not an adequate predictor of plasma antioxidant capacity [45]. Another publication confirmed that enhancing immune response and modulating gene expression, intracellular antioxidant properties of vitamin E was

associated with lower incidence of prostate cancer. However, higher serum vitamin E level was correlated with a higher risk of prostate cancer [46].

Conclusion

This is the first study that measured the total antioxidant activity by Human T-AOC Elisa Kit in patients with BC. The distribution of T-AOC showed non-normal pattern in patients with BC. There was a significantly lower level of T-AOC in patients when compared to healthy controls. Change in antioxidant capacity before and after management with vitamin E was challenging and additional consideration should be given to intensification of measurement of redox state by T-AOC in patients with BC regarding two different stages and grade. Larger prospective studies are suggested to confirm these associations in the future.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

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