RESEARCH ARTICLE

Ischemia Modified Albumin Levels and Oxidative Stress in Patients with Bladder Cancer

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Abstract

Background: Impaired oxidative/antioxidative status plays an important role in the pathogenesis of many diseases like cancer. The aim of this study was to evaluate the levels of the novel marker ischemia modified albumin (IMA) and albumin adjusted-IMA (Adj-IMA) in patients with bladder cancer (BC) as well as its association with total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI). **Materials and Methods:** Forty male patients with BC (mean age, 67.4±12 years) and forty age-sex matched healthy persons (mean age 56.0±1.7 years) were included in this study. Serum levels of IMA, TAS, TOS were analyzed and Adj-IMA and OSI was calculated. **Results:** Serum IMA, TOS and OSI values were significantly higher in patients with BC compared to controls (p<0.0001, p=0.01 and p=0.01, respectively), whereas TAS was significantly lower in BC patients (p=0.04). There was no significant difference for serum albumin-adjusted IMA levels between groups (p=0.4). **Conclusions:** In this study, it was found that there was an impaired oxidative/antioxidant status in favor of oxidative stress in BC patients. This observation was not confirmed by Adj-IMA calculation. There is no published report about serum concentrations of IMA in patients with BC. Further studies are needed to establish the relationship of IMA and oxidative stress parameters in BC and the significance of IMA to other cancers.

Keywords: Bladder cancer - ischemia modified albumin - oxidative stress - total antioxidant status - total oxidant status

Introduction

In recent years, different studies have described the role of IMA as a new marker for diseases related to inflammation. The generation of reactive oxygen species (ROS) and free radicals can transiently modify the N-terminal region of albumin and produce an increase in the concentration of IMA (Christenson et al., 2001; Roy et al., 2006). IMA may show a sensitive biochemical marker, especially for the diagnosis of myocardial ischemia (Sinha et al., 2004). Several reports proved that ischemia induces a cascade of proinflammatory reactions that lead to the production of ROS (Kotur-Stevuljevic et al., 2007; Vassalle et al., 2008). IMA is indicated a marker of ischemia and oxidative stress originating as a consequence of tissue hypoxia (Can et al., 2006; Senes et al., 2007; Lippi et al., 2009). There have also been studies describing the relationship between cancer and inflammation. This relationship may include both an extrinsic and an intrinsic pathway. The extrinsic pathway is related to inflammatory circumstances, which increase cancer risk, whereas the intrinsic pathway is maintained by genetic changes that cause inflammation, which may also induce oncogenesis (Mantovani et al., 2008).

BC is the eight most common cancer in the world. The incidence is variable between countries, being high in North America, Europe and Northern Africa, and low in Asia (Ferlay et al., 2010). There exists a different choice for males, largely due to differences in smoking habits and occupational exposure to carcinogens. Despite of several factors, the induced result in the human body is common: oxidative stress. Traditional wisdom holds it difficult to think of a disease in the etiology of which free radicals would not be involved. In recent years investigators raced to confirm "the impaired oxidative/antioxidative balance" was the absolute impact in the pathogenesis of many, perhaps every human disease, including the cancer (Crawford et al., 2012).

Serum levels of different oxidant species can be measured separately in laboratories. These measurements are time-consuming, expensive to perform, and require complicated equipment. Recently, serum lipid peroxidation

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levels were observed by determining total oxidant status (TOS) (Erel, 2005). Furthermore, total antioxidant status (TAS) is a useful indicator of the activity of antioxidants in serum (Erel, 2004). Therefore, measurements of TAS and TOS may show data about body's overall serum oxidative stress index (OSI), which may include antioxidants and oxidants that are not yet known or easily measured (Harma et al., 2003).

IMA levels are higher in many inflammatory and oxidative stress-associated diseases (Roy et al., 2006). Also, serum IMA levels increased in patients with gastric, prostate, soft tissue cancer and neuroblastoma (Mastella et al., 2009; Stachowicz-Stencel et al., 2011; Fidan et al., 2012). There is no published report about serum concentrations of IMA in patients with BC. Due to the fact that cancer and oxidative stress belong to an associated event, and based on the fact that albumin may be modified in situations associated with oxidative stress, the aim of this study was to evaluate the levels of the novel marker IMA in patients with BC as well as its association with TAS, TOS and OSI.

Materials and Methods

Study population and clinical examinations

Forty male patients (mean age, 67,4±12 years) who had presented at the Urology Outpatient Clinic of Antalya Education and Research Hospital were prospectively included in the study. Age- and sex-matched healthy control subjects (40 male; mean age; 56.0±1.7 years) were also enrolled for comparison.

All subjects had full physical examination and were asked to complete a questionnaire and gave informed consent before the onset of study. All participants were of low socioeconomic status. The questions included: age, smoking, alcohol consumption, and detailed medical history. Consequently, blood pressure was measured manually with a sphygmomanometer. Body mass index was calculated as weight in kilograms divided by height in meters squared.

Those with a known past history of any major diseases like diabet, cardiac disease, renal, hepatic or endocrine disease were excluded. None of the participants in the present study were using drug medications including lipid lowering agents, vitamins or antioxidant drugs. All men were diagnosed for the first time to have BC (urothelial carcinoma) after pathologic review of transurethral resection (TUR) biopsies of the bladder. The control group consisted of volunteers without any significant urological medical history.

This study was performed in accordance with the ethical standards set by the Declaration of Helsinki and was approved by the local ethics committee. Informed consent for participation in the study was obtained from all subjects.

Blood samples were obtained after an overnight of fasting. Serum samples were then separated from cells by centrifugation at 3000 rpm for 10 min. Albumin levels were analyzed from fresh serum samples. Albumin concentrations were determined with a commercially available kit (Abott, USA) based on the bromocresol green method in our routine clinical biochemistry laboratory. Albumin level was expressed as g/dL. Serum portions were stored at −80 °C for analyzing IMA, TOS and TAS .

Analytical methods

Measurement of the ischemia modified albumin: Reduced cobalt to albumin-binding capacity (IMA level) was measured using the rapid and colorimetric method developed by Bar-Or et al. (2000). Briefly, 200 mL of patient serum was transferred into glass tubes and 50 mL of 0.1% $CoCl_2*6H_2O$ (Sigma-Aldrich Lot:S38901-248) added. After gentle shaking, the mixture was incubated for 10 minutes to ensure sufficient cobalt albumin binding. Then, 50 mL of 1.5 mg/ mL dithiothreitol (DTT) (Sigma-Aldrich Lot: D5545-1G) was added as a coloring agent. After two minutes, 1 mL of 0.9% NaCl was added to halt the binding between cobalt and albumin. A blank was prepared for every specimen. At the DTT addition step, 50 mL of distilled water was used instead of 50 mL of 1.5 mg/ mL DTT to obtain a blank without DTT. The absorbances were recorded at 470 nm with a spectrophotometer (Shimadzu UV1201). Color formation in specimens with DTT was compared with color formation in the blank tubes, and the results were expressed as absorbance units (ABSUs). The formula suggested by Lippi et al. was used for calculation of albumin-adjusted IMA (AdJ-IMA) levels expressed as individual serum albumin concentration/ median albumin concentration of the population x IMA value (Lippi et al., 2007).

The inter-assay variability of IMA method in our laboratory was calculated from serum samples of 20 healthy subjects and 20 patients with acute coronary syndrome. The within-day coefficient of variation was 1.23% for healthy subjects (mean 0.484, standard deviation: 0.006) and for acute coronary syndrome patients was 0.92% (mean 0.647, standard deviation: 0.006). All serum samples were analyzed within three days.

Table 1. Demographic and Clinical Data of Patients with BC and Controls (n=40)

Parameter (n=40)	Patients	Controls	p
Age, mean±SD	67.4±12	56.0±1.7	0.24
Gender	All men		
Smoker, (n %)	7 (16%)	8 (20%)	0.85
BMI (kg/m²), mean±SD	25.9±4.8	27.9±3	0.006*
Alcohol consumption, (n %)	0 (0%)	0 (0%)	
Albumin, mean±SD (g/dl)	3.50±0.49	4.41±0.27	<0.001*

*Statistically significant. The parameters were similar except the BMI, albumin levels

Table 2. Serum IMA, Alb-Adj IMA, TAS, TOS and OSI Levels of BC Patients Compared to the Controls (n=40)

Parameter (mean±SD)	Patients	Controls	p
IMA (ABSU)	0.588±0.07	0.474±0.04	<0.0001
TAS(nmol Troloks/L)	2.74±0.35	2.87±0.28	0.04
TOS(µmolH_2O_2 Equiv./L)	3.4 (1.2-7.1)	1.40 (1.01-1.68)	0.01
OSI	1 (0.4-3)	0.5 (0.4-0.6)	0.01
Adj-IMA (ABSU)	0.465±0.07	0.475±0.05	0.46

*The result values; for the normal distribution (mean±SD), for the anormal distribution (median) (95% CI for median), Alb-Adj IMA: albumin- adjusted IMA ABSU: Absorbance Unit

Table 3. The Patients were Divided Into Subgroups According to Grade of BC, and Presence or Absence of Muscularis Propria Invasion

Patient (N)	IMA	ADJ-IMA	TAS	TOS	OSI
Low grade (25) 95% CI	0.58 (0.53-0.63)	0.44 (0.42-0.49)	2.6 (2.4-2.9)	1.9 (0.90-7.6)	0.07 (0.03-0.3)
High grade (15) 95% CI	0.55 (0.54-0.64)	0.40 (0.37-0.48)	2.7 (2.3-3.1)	6.4 (1.97-20.5)	0.28 (0.06-0.66)
p	0.91	0.06	0.48	0.04	0.04
MP in. Neg (25) 95% CI	0.58 (0.53-0.62)	0.44 (0.41-0.48)	2.6 (2.5-2.9)	2.6 (1.1-7.4)	0.07 (0.04-0.3)
MP in Poz (15) 95% CI	0.55 (0.50-0.72)	0.40 (0.37-0.53)	2.7 (2.1-3.2)	7.3 (1.3-22)	0.28 (0.06-0.81)
p	0.48	0.24	0.83	0.07	0.09

*The only statistically significant difference was obtained in TOS and OSI levels being higher in high grade cancer. Median (95% CI: Confidence Interval), MP in (muscularis propria invasion)

Measurement of serum total oxidant status: Serum TOS levels were analyzed by using a novel automated colorimetric measurement method developed by Erel (2004). In this method, oxidants in the sample oxidize the ferrous ion–chelator complex to ferric ion which makes a colored complex with a chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu mol\ H_2O_2$ Equiv. /L).

Measurement of serum total antioxidant status: Serum TAS levels were analyzed by using a novel automated colorimetric measurement method developed by Erel (2005). In this method, antioxidants in the sample reduce dark blue-green colored 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. This method determines the antioxidative effect of the sample against the potent free radical reactions initiated by the produced hydroxyl radical. The results are expressed as micromolar trolox equivalent per liter.

Oxidative stress index: The percentage ratio of TOS level to TAS level was suggested as oxidative stress index (OSI) (Harma et al., 2003). For calculation, the resulting micromolar unit of TAS was changed to millimoles per liter, and the OSI value was calculated according to the following formula: OSI (arbitrary unit)=TOS (micromolar hydrogen peroxide equivalent per liter)/TAS (micromolar trolox equivalent per liter).

Statistical analysis

Statistical analyses were carried out using the statistical software version 11.5.1.0 (MedCalc, Mariakerke, Belgium). In normally distributed groups the results were presented with mean and SD, otherwise with medians. The significance of the differences between groups was determined by Student's unpaired t-test for normal distributions, and by the Mann–Whitney U-test in abnormal distribution. Pearson correlation coefficient and Spearman correlation coefficient were used to test the strength of any associations between different variables. P values less than 0.05 was accepted as the significance level.

Table 4. Relationship of IMA and Adj-IMA with Oxidative Stress Markers

Parameter (n=80)	Albumin	TAS	TOS	OSI
IMA	r=-0.40	r=-0.24	r=0.32	r=0.32
	p=0.009	p=0.02	p=0.004	p=0.004
Adj-IMA	r=0.57	r=-0.09	r=-0.17	r=0.18
	p=0.0001	p=0.38	p=0.11	p=0.09

*Strong correlations between IMA and oxidative stress markers were not observed when IMA was replaced by Adj-IMA values

Results

There were no significant differences in blood pressure between patients and controls. There were smokers in both groups with a close ratio. No one in both groups reported routine alcohol consumption. Body mass index (BMI) was lower in BC patients (p=0.006). Nearly all BC patients reported unintentional weight loss, so the finding was thought to be due to cancer itself rather than life style. Demographic and clinical data obtained from BC patients and controls are summarized in Table 1.

Serum IMA, TOS levels and OSI index were significantly higher in patients with BC compared to controls (p<0.0001, p=0.01 and p=0.01 respectively), whereas TAS were significantly lower in BC patients (p=0.04). There was no significant difference for serum Adj-IMA levels between groups (p=0.46) (Table 2)

The final pathology reports of patients were evaluated, and subjects were divided into groups according to the tumor grades (high/low grade) and presence of muscularis propria invasion (present/absent). The only statistically significant difference was obtained in TOS and OSI levels being higher in high grade BC (Table 3)

There were negative correlations between serum IMA and albumin levels (r: -0.40, p=0.009), TAS and (r: -0.24, p=0.02) and positive correlation between IMA and TOS (r: 0.32, p=0.004), IMA and OSI (r: 0.32, p=0.004) levels. There was a positive correlation between IMA and albumin (r: 0.57, p=0.0001). There was no statistically significant correlation between Adj-IMA levels and TAS (r: -0.09 p=0.38), TOS (r: -0.17, p=0.11), OSI (r: 0.18, p=0.09) (Table-4)

Discussion

In this study, there was a significant increase in serum IMA, TOS levels and OSI index of BC patients compared to healthy subjects. Also there was a significant decrease for serum TAS levels. Unlike IMA, adj-IMA was similar

between BC and healthy controls. We found significant correlations between adj-IMA and other oxidative markers. Adj-IMA does not seem to be correlated with BC and oxidative stress. Correlation of IMA with oxidative stress markers does not seem to be clinically significant for BC.

The Albumin Cobalt Binding test or IMA was the first US FDA-cleared assay to detect myocardial ischemia. Condition of myocardial ischemia results in structural changes to the N terminus of the serum albumin, which reduces its binding capacity for cobalt cations. It has been suggested that these changes are related to the production of reactive oxygen species during ischemia and/or hypoxia, acidosis, and reperfusion (Bar-Or et al., 2001). However, increased IMA concentrations do not seem to depend purely on myocardial involvement. IMA may not be specific for cardiac ischemia. There are various data on IMA in patients with different states with ischemia of non-cardiac origin such as systemic sclerosis, peripheral vascular disease, skeletal muscle ischemia, glaucoma and diabetes mellitus (Roy et al., 2004; Montagnana et al., 2006; Piwowar et al., 2008). Also, serum IMA levels are increased in patients with gastric, prostate, neuroblastoma and soft tissue cancer (Mastella et al., 2009; Stachowicz-Stencel et al., 2011; Fidan et al., 2012).

In our study, serum IMA levels were significantly higher in patients with BC compared to controls, whereas serum albumin levels were significantly lower in BC patients. There was no statistically significant difference for serum Adj-IMA levels between groups. There are only a few studies evaluating the relationship between cancer and serum IMA levels. Fidan et al. (2012) showed that serum IMA levels were increased in patients with gastric cancer, but they did not evaluate the serum albumin levels. Mastella et al. (2009) found increased serum IMA levels in patients with prostate cancer, but this increase was not statistically significant. Also, in this study there was no comparision for serum albumin levels of patients and control group. Stachowicz-Stencel et al. (2011) found that serum IMA levels were statistically higher in pediatric patients with neuroblastoma and soft tissue sarcomas. They reported a weak negative correlation between serum IMA and albumin levels in participiants. In that study, serum albumin levels of patients and control group were in the reference intervals and there was no comparision between groups.

Previous reports have showed that IMA levels are inversely related to serum albumin concentrations (Zapico-Muniz et al., 2004; Van der Zee et al., 2005; Refaai et al., 2006). The impact of serum albumin on IMA levels is still an important factor within the normal range (Van Rijn et al., 2008). Therefore correction for albumin concentrations is especially essential in populations with wide variations in albumin levels. It has been demonstrated that each 1 g/dL change in albumin within the physiologic range of albumin produces an opposite change of 2.6% in IMA levels (Zapico-Muniz et al., 2004). Also this proposes the need to evaluate IMA values together with those of albumin to avoid possible false-positive or -negative values in individuals with hypo- or hyper-albuminemia. This condition may lead to

serious concern about uncorrected IMA data in studies. In our study, this was clearly mentioned by correlation tests between serum IMA and oxidative stress markers. There were strong correlations between serum IMA and oxidative stress markers. On the contrary, there was no correlation between serum adj-IMA levels and oxidative stress markers. In the present study, we also analyzed adj-IMA levels and used a formula suggested by Lippi et al. (2007) according to individual and median population albumin levels. There were, however, no significant change between adj-IMA levels in the BC patients. Our results were in close agreement with those previously reported, and there were negative correlations between IMA levels and albumin concentrations within individual groups.

Cachexia and malnutrition in cancer patients are important problems due to a variety of mechanisms involving the tumor, the host response to the tumor, and anticancer therapies (von Meyenfeldt, 2005). Serum albumin shows a simple method of estimating visceral protein function. Malnutrition and inflammation suppress albumin synthesis (Yeun et al., 1998). In an adult the normal range of serum albumin is defined as 3.5-5.0 g/dL and levels <3.5 g/dL is called hypoalbuminemia (Di Fiore et al., 2007; Ishizuka et al., 2007). The inverse correlation between albumin synthesis and body weight index in cancer patients supports the possibility of a compensatory enhanced albumin synthesis in these metabolically affected patients. In the later stages of disease, malnutrition and inflammation suppress albumin synthesis (Ballmer et al., 1994). Depending on these changes in albumin, serum albumin levels should be considered in IMA studies.

Gecit et al. (2012), and Badjatia et al. (2010) showed increased oxidative stress and increased total anti-oxidant activity in bladder cancer patients. In our study, we found the difference of TAS, TOS and OSI between cancer patients and controls, statistically significant. The conflict may be ascribed to different methodology used in case of Badjatia et al. (2010) although, very disputably, we find our result more reasonable, as the state of disease must be the failure of the organism to show a proportional respond to the increase in oxidative status. Measurement of OSI is extremely valuable as it informs about those oxidative stress not yet recognized or not easily measured. On the other hand it is an unrefined, vague result, making it crucial to examine some sub-classes of anti-oxidants. In our study, we found that serum TOS levels and OSI index were significantly higher, while serum TAS levels were significantly lower, in patients with BC compared to the control group. Our results showed increased serum TOS levels and OSI index in patients with high grade bladder cancer. This findings suggest that serum TOS levels and OSI index are important factors for in disease progression.

In conclusion, increased serum TOS levels and OSI index with decreased serum TAS levels were found in patients with BC, and these findings strongly favor to impairment of oxidant-antioxidant balance. This observation was not confirmed by Adj-IMA measurement. However, our IMA vs. adj-IMA results must be considered to be a reminder that unadjusted IMA values might be quite deceptive, and may lead to inappropriate considerations.

One major limitation of the study is the small number of samples. Obviously larger studies are needed to establish the relationship of IMA and oxidative stress parameters in BC and relationship of IMA and other cancers.

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