CLINICAL SCIENCE

Serum adenosine deaminase, catalase and carbonic anhydrase activities in patients with bladder cancer

Necip Pirinççi,[I] İlhan Geçit,[I] Mustafa Güneş,[I] Mehmet Bilgehan Yüksel,[II] Mehmet Kaba,[III] Serhat Tanık,[III] Halit Demir,[IV] Mehmet Aslan[V]

[I] Yuzuncu Yıl University, Faculty of Medicine, Department of Urology, Van, Turkey. [II] Celal Bayar University, Faculty of Medicine, Department of Urology, Manisa, Turkey. [III] Ministry of Health, Regional Training and Research Hospital, Clinic of Urology, Van, Turkey. [IV] Yuzuncu Yıl University, Faculty of Science and Art, Department of Chemistry, Van, Turkey. [V] Yuzuncu Yıl University, Faculty of Medicine, Department of Internal Medicine, Van, Turkey.

OBJECTIVES: The relationship between adenosine deaminase and various cancers has been investigated in several studies. However, serum adenosine deaminase activity and carbonic anhydrase and catalase activities in patients with bladder cancer have not previously been reported. Therefore, the aim of this study was to measure serum adenosine deaminase, carbonic anhydrase and catalase activities in patients with bladder cancer.

MATERIALS AND METHODS: Forty patients with bladder cancer and 30 healthy controls were enrolled in the study. Serum adenosine deaminase, carbonic anhydrase and catalase activities were measured spectrophotometrically.

RESULTS: Serum adenosine deaminase, carbonic anhydrase and catalase activities were significantly higher in patients with bladder cancer than controls (all significant, $p<0.001$).

CONCLUSIONS: These markers might be a potentially important finding as an additional diagnostic biochemical tool for bladder cancer.

KEYWORDS: Adenosine Deaminase; Bladder Cancer; Carbonic Anhydrase; Catalase.

Pirinççi N, Geçit I, Güneş M, Kaba M, Tanık S, Demir H, et al. Serum adenosine deaminase, catalase and carbonic anhydrase activities in patients with bladder cancer. Clinics. 2012;67(12):1443-1446.

Received for publication on August 20, 2012; **First review completed on** September 11, 2012; **Accepted for publication on** October 19, 2012

E-mail: necippirincci@hotmail.com

Tel.: 90 (432) 215 0473

INTRODUCTION

Bladder cancer is a common tumor of the urinary tract. It is the fourth most common type of cancer in men in the United States. The most common risk factors for bladder cancer are exposure to industrial carcinogens, cigarette smoking, male gender and, possibly, diet (1). The most common type of bladder cancer develops from the urothelium and is known as transitional cell carcinoma (2).

Some defense mechanisms in the body prevent the development of free radicals and the damage they cause. One of the most important antioxidant enzymes is superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion into hydrogen peroxide (H_2O_2), which is removed by catalase (CAT) and glutathione peroxidase (GSH-PX). CAT is a well-known plasma antioxidant enzyme (3).

Carbonic anhydrase (CA) is a member of the alpha-family of carbonic anhydrases of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to carbonic acid. CAs have recently become a target of intensive research into carcinogenesis and tumor invasion (4). The relationship between CA and bladder cancer has been investigated in several studies (5-8).

In the purine metabolic pathway, adenosine deaminase (AD) is an important aminohydrolase. AD catalyzes the conversion reaction of adenosine to inosine and deoxyadenosine to deoxyinosine (9). Its main physiologic activity is related to lymphocytic proliferation and differentiation (10). The relationship between adenosine deaminase and various cancers has been investigated in several clinical studies, but the results are conflicting. Although enzyme activity was found to be increased in some cancerous tissue (11-13) in several studies, it was typically decreased in various neoplastic tissues (14,15). In addition, Watanabe et al. showed the activity of curcumin in an animal bladder cancer model, which most likely acted via the regulation of nuclear factor-kappa B and p53. Curcumin decreased tumor cell proliferation. Therefore, curcumin is a good choice for treating superficial bladder cancer in clinical trials (16).

To the best of our knowledge, serum AD, CA, and CAT activities in patients with bladder cancer have not previously been reported. Therefore, the aim of this study was to simultaneously investigate serum AD, CA, and CAT activities in patients with bladder cancer.

No potential conflict of interest was reported.

MATERIALS AND METHODS

Subjects

This prospective cross-sectional study included 40 male patients who were newly diagnosed (preoperative) with bladder cancer (mean age of 65.32±3.4 years) between January 2011 and October 2011 and treated at the Department of Urology at the University Hospital of Yuzuncu Yil.

All patients were lifetime non-smokers and free of drug, alcohol, or antioxidant supplement consumption and any metabolic disease. None of the patients had any other significant disease or malignancies except for bladder cancer, and only newly diagnosed patients (preoperative) with no prior chemotherapeutic treatment were included in this study.

Controls consisted of 30 healthy males (mean age of 60.82±5.2 years) randomly selected from a group of healthy non-smoking volunteers with no history of previous disease or drug or alcohol consumption.

The patient and control groups were of similar socio-economic status. Regarding tumor staging, 30 patients were diagnosed with a non-invasive tumor (Ta–T1), and 10 patients were diagnosed with an invasive tumor (T2–T4). Patients were classified into three groups with respect to tumor grading: 24 patients had well-differentiated tumors (G1), 10 had intermediate tumors (G2), and six had poorly differentiated (G3) tumors. The superficial urothelial papillary tumors were graded according to the 2004 World Health Organization (WHO) grading system as low-grade and high-grade papillary neoplasms.

The study protocol was performed in accordance with the Helsinki Declaration as revised in 1989. All participants were informed about the study protocol, and written consent was obtained from each participant.

Blood collection

Blood samples were obtained in the morning after 12 h of fasting. Blood samples were collected into empty tubes and immediately stored on ice at 4 ℃. The serum was then separated from the cells by centrifugation at 3,000 rpm for 10 min. Serum samples used for the measurement of AD, CA, and CAT levels were stored at -20 ℃ until they were used.

Measurement of adenosine deaminase activity

Serum AD activity was estimated spectrophotometrically by the method of Giusti, which is based on the direct measurement ammonia formation, which occurs when AD is in the presence of excess adenosine (17). The results were expressed as units per liter (U/L).

Measurement of carbonic anhydrase enzyme activity

Serum CA activity was assayed by CO_2 hydration. The hydration of CO_2 was measured using the method of Rickli and Wilbur-Anderson, with bromothymol blue as the indicator (18). The results were expressed as units per liter (U/L).

Measurement of catalase activity

Serum CAT activity was measured using H_2O_2 as a substrate (19). The degradation of H_2O_2 was monitored at 240 nm for 5 min using a spectrophotometer, and enzyme activity was expressed in units per liter of serum (U/L) at 25 ℃.

Table 1 - Demographic characteristics of the two groups in this study.

Parameters	Control (n = 30)	Patients (n = 40)	*p*-value
Age (years)	60.82±5.2	65.32±3.4	ns
Body mass index (kg/m^2)	22.56±2.43	23.12±1.62	ns

Values are the mean±SD;
ns = non-significant.

Statistical analysis

The results are expressed as the mean±standard deviation. Continuous variables were compared using Student's *t* test. Nonparametric continuous variables were compared using a Kruskal–Wallis one-way analysis of variance with a Mann–Whitney U-test post-hoc analysis. The results were considered to be statistically significant when the *p*-value was less than 0.05. The data were analyzed using the SPSS® for Windows computing program (version 11.0).

RESULTS

The demographic and clinical data of the bladder cancer and control groups are shown in Table 1. There were no statistically significant differences between bladder cancer patients and controls with respect to age and body mass index ($p>0.05$) (Table 1).

AD, CA, and CAT activities were detected in all samples. The mean activities of the enzymes are summarized in Table 2.

In the healthy controls, the serum AD, CA, and CAT mean values of activities were 16.4±0.4, 0.65±0.19, and 1.15±0.04 U/L, respectively (Table 2).

In patients with bladder cancer, the serum AD, CA, and CAT mean values of activities were 26.1±2.1, 0.96±0.20, and 1.85±0.07 U/L, respectively (Table 2).

The serum AD, CA, and CAT mean values of activities were significantly higher in patients with bladder cancer than in healthy controls (all significant; $p<0.001$) (Table 2).

DISCUSSION

In this study, we analyzed serum CA and CAT levels in patients with bladder cancer. In addition, we studied the activity of serum AD, which is an important aminohydrolase in purine metabolism, in patients with bladder cancer. Additionally, we aimed to determine the relationship between these enzyme activities in patients with bladder cancer.

We observed that serum AD, CA, and CAT levels were significantly higher in patients with bladder cancer than in healthy controls. To the best of our knowledge, no other studies have examined serum AD, CA, and CAT levels in

Table 2 - Serum adenosine deaminase, carbonic anhydrase and catalase activities in the two groups in this study.

Parameters	Control (n = 30)	Patients (n = 40)	*p*-value
Adenosine deaminase (U/L)	16.4±0.4	26.1±2.1	<0.001
Carbonic anhydrase (U/L)	0.65±0.19	0.96±0.20	<0.001
Catalase (U/L)	1.15±0.04	1.85±0.07	<0.001

Values are the mean±SD.
Significant, $p<0.001$.

patients with bladder cancer. Increased serum AD, CA, and CAT activities may play a role in the pathogenesis of bladder cancer. Therefore, we believe that serum AD, CA, and CAT levels may be an informative prognostic marker for bladder cancer. The activities of AD, CAT, and CA in patients with bladder tumors can also be measured in the urine. The follow-up to determine recurrence, particularly after the treatment of bladder tumors, may benefit from determining the activities of this enzyme before cystoscopy.

AD, which catalyzes the reaction in which adenosine is deaminated to inosine, has been accepted as an important enzyme in the maturation, proliferation, differentiation, and function of T lymphocytes (20). As an indicator of cellular immunity, the plasma activity of this enzyme has been suggested to be increased in diseases that cause a cell-mediated immune response, such as cancer (21).

Some studies have confirmed the value of estimating AD in the diagnosis and follow-up of patients with tuberculosis (22). Recent studies have focused on the diagnostic value of AD activity in typical (10,23) and atypical (24) pneumonia.

AD activity was increased in cancerous tissues and cells compared to noncancerous tissue in several studies (11-13). Some authors, however, found low lymphocyte AD activities in cancer patients (14,15). Sufrin et al. (13) found that the AD levels in lymphocytes from patients with bladder cancer were elevated with transitional cell carcinoma and correlated with stage, activity, clinical course, and tumor resection but not tumor grade. These researchers also found higher erythrocyte AD activities in the same patients and suggested that lymphocyte AD levels might be a sensitive indicator of bladder carcinoma. The high AD activity determined in cancerous bladder tissue reflected accelerated purine turnover and high salvage pathway activity (12). Similarly, Durak et al. (13) found increased AD activity in cancerous bladder tissues compared with adjacent cancer-free tissues and control bladder tissues. Our results are in agreement with those studies (12,13).

In contrast, Lal et al. (25) demonstrated that the increase in serum AD activity was directly related to the cancer stage, indicating that the increase was directly proportional to the primary tumor mass. Moreover, Nishihara et al. (26) reported that patients with lung cancer had elevated AD levels and that serum AD levels were significantly reduced in patients with lung cancer following surgery and radiotherapy. In contrast, Dasmahapatra et al. (14) and Kojima et al. (15) found low lymphocyte AD activities in head and neck cancer patients and gastric cancer patients, respectively. They suggested that low lymphocyte AD activities might be a more sensitive indicator of suppressed cellular immunity.

In the present study, we observed that serum AD activity was significantly higher in patients with bladder cancer than in healthy controls. Increased AD activity might provide a selective advantage for cancer cells to grow and develop more rapidly. We speculate that increased AD activity might be a result of the leakage of the enzyme from primary tumor cells.

CAT is highly expressed in some tissues and protects cells against the excessive formation of reactive oxygen species. CAT prevents the accumulation of H_2O_2 formed during oxygen transport. Although there is accumulating evidence that CAT activity is suppressed in cancerous tissues (13,27-29), some authors found unchanged or increased activity in some tumor tissues (30,31). Durak et al. (13) found decreased CAT activity in cancerous bladder tissues compared with adjacent cancer-free tissues and control bladder tissues. In contrast, Bayraktar et al. (31) recently reported that patients with bladder cancer had increased CAT levels. Several studies have shown decreased CAT levels in patients with lung cancer (27,28). Moreover, Corrocher et al. (29) reported decreased CAT activity in human hepatoma and suggested that the antioxidant defense system of hepatocellular carcinoma cells was severely impaired. In contrast, Bayraktar et al. (31) recently reported that patients with bladder cancer had increased CAT levels. The higher CAT activity observed in this study supports previous observations that the enzymatic antioxidant defense mechanism is not impaired in tumor cells and tissue, although this was not a universal characteristic of neoplastic cells.

In the present study, we found significantly increased CAT activity in the serum of bladder cancer patients. Our data clearly demonstrate that CAT activity is increased in patients with bladder cancer, and thus, our results are not in agreement with Durak et al. (13). However, our results are in agreement with Bayraktar et al. (31).

CAs are involved in several physiological and biological processes in humans. CA is not only a highly active enzyme but is also involved in cell-cell adhesion and cell proliferation (32), and it plays different roles in various tissues. Species can produce many different CA isozymes, some of which act in the cytosol, while others are membrane bound. For example, in humans, there are three cytosolic isozymes (CA-I, II, and III), five membrane-bound isozymes, and several related proteins that lack catalytic activity. CA-I is found primarily in erythrocytes. The human CA-II isozyme is widely distributed, and it has been identified in erythrocytes and the brain, eyes, and kidneys (4). Carbonic anhydrase IX (CAIX) is a hypoxia-inducible member of the carbonic anhydrase family that regulates intracellular pH, cell proliferation, cell adhesion, and tumor progression. Studies have demonstrated that high CAIX expression yields an aggressive tumor phenotype and a poor prognosis in many cancer types. However, with regard to bladder cancer, there have been only a few reports on a limited number of patients, and those reports produced inconclusive results (5,7,8). CAs are key enzymes that regulate acid-base homeostasis under both normal and pathological conditions (33). CA is abundantly expressed as a direct consequence of hypoxia in numerous cancers (34), and its activity has been documented in some cancerous tissues (5,7,8). However, there is limited information regarding serum CA activity in patients with bladder cancer (6). In the present study, we found significantly increased CA activity in the serum of bladder cancer patients.

The current study is the first to investigate serum AD, CA, and CAT activities in patients with bladder cancer. These markers might be potentially important as an additional biochemical diagnostic tool for bladder cancer. Further investigations in a larger cohort of patients with bladder cancer are needed to provide definitive data about the prognostic role of AD, CA, and CAT activities.

AUTHOR CONTRIBUTIONS

Pirinççi N conceived and designed the study and was also responsible for the draft of the manuscript. Geçit I was responsible for the data collection, critical revision and important intellectual content of the manuscript. Güneş M was responsible for the data collection. Yüksel MB was responsible for the statistical analysis. Kaba M and Tanık S were

responsible for the data collection. Demir H conceived and designed the study and was responsible for the critical revision and important intellectual content of the manuscript. Aslan M was responsible for the administrative support.

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