Kulak Burun Bogaz Ihtis Derg
2004;12(1-2):16-22

ORIGINAL ARTICLE

Adenosine deaminase, xanthine oxidase, superoxide dismutase, glutathione peroxidase activities and malondialdehyde levels in the serum of patients with head and neck carcinoma

Baş-boyun kanserli hastaların serumlarında adenozin deaminaz, ksantin oksidaz, süperoksit dismutaz, glutatyon peroksidaz aktivitesi ve malondialdehit seviyesi

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Objectives: Clinical and epidemiological findings have provided evidence supporting a role of free radicals in the etiology of cancer. Scavengers and inhibitors of free radical processes have been demonstrated to prevent or delay the neoplastic process.

Patients and Methods: Adenosine deaminase, xanthine oxidase, superoxide dismutase, and glutathione peroxidase activities and malondialdehyde levels were measured in the sera of 35 patients with head and neck cancers and compared to those of healthy control subjects.

Results: Serum adenosine deaminase activity was found to be significantly increased in the patient group ($p<0.001$). Compared to the control group, glutathione peroxidase and xanthine oxidase activities and malondialdehyde levels were slightly higher and serum superoxide dismutase activity was slightly lower in the patient group, with none reaching statistical significance.

Conclusion: The results indicate that serum adenosine deaminase activity may be helpful in the diagnosis and follow-up of head and neck cancers. Further studies with a larger cohort of patients are needed to clarify the exact mechanism of adenosine deaminase elevation.

Key Words: Head and neck neoplasms/blood; tumor markers, biological/blood; adenosine deaminase; superoxide dismutase; glutathione peroxidase; xanthine oxidase; malondialdehyde.

Amaç: Klinik ve epidemiyolojik bulgular kanser etyolojisinde serbest radikallerin üstlendiği muhtemel rolü destekleyici kanıtlar sunmaktadır. Serbest radikal yokediciler ve inhibitörlerinin kanser sürecini ertelediği ya da engellediği gösterilmiştir.

Hastalar ve Yöntemler: Baş-boyun kanserli 35 hastanın serumlarında adenozin deaminaz, ksantin oksidaz, süperoksit dismutaz ve glutatyon peroksidaz aktiviteleri ve malondialdehit düzeyi ölçülerek sağlıklı kontrol grubu ile karşılaştırıldı.

Bulgular: Serum adenozin deaminaz aktivitesi, kanserli hastalarda kontrol grubu ile karşılaştırıldığında azalmış bulundu ($p<0.001$). Glutatyon peroksidaz ve ksantin oksidaz aktiviteleri ile malondialdehit düzeyi kontrol grubuna oranla hafif artmış bulunmakla birlikte arada anlamlı farklılık yoktu. Serum süperoksit dismutaz aktivitesi ise kontrol grubuna oranla hafif azalmış bulundu.

Sonuç: Bu sonuçlar, serum adenozin deaminaz aktivitesinin baş-boyun kanserlerinin tanı ve takibinde yararlı olabileceğini göstermektedir. Baş-boyun kanserli hastalarda adenozin deaminaz düzeyindeki artışın nedenleri üzerinde geniş tabanlı çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Baş-boyun neoplazileri/kan; tümör markerleri, biyolojik/kan; adenozin deaminaz; süperoksit dismutaz; glutatyon peroksidaz; ksantin oksidaz; malondialdehit.

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◆ *Received - July 29, 2003 (Dergiye geliş tarihi - 29 Temmuz 2003). Request for revision - January 21, 2004 (Düzeltme isteği - 21 Ocak 2004). Accepted for publication - January 29, 2004 (Yayın için kabul tarihi - 29 Ocak 2004).*

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Cancer is a multispectral disease process. The complex series of cellular and molecular changes that occur through the development of cancers are mediated by a diversity of endogenous and environmental stimuli.[1] Reactive oxygen species (ROS) and other free radicals have long been known to be mutagenic. It has been suggested that carcinogenesis might be induced by ROS.[2] On the other hand, clinical and epidemiologic findings have provided evidence supporting a role for free radicals in the etiology of cancer.[1] It has been demonstrated that scavengers and inhibitors of free radical processes prevent or delay the neoplastic process.[1]

Glutathione peroxidase (GSHPx), and superoxide dismutase (SOD) are primary scavenger enzymes of free radicals. Adenosine deaminase (ADA) is an aminohydrolase that catalyzes the deamination of adenosine to inosine. Xanthine oxidase (XO) plays an important role in the conversion of purine bases to uric acid.[3] Malondialdehyde (MDA) is an end-product of lipid peroxidation, indicating the degree of oxidative stress. It is also released as a result of toxic effect of active oxygen, which destroys unsaturated fatty acids in the cell membrane.[4]

Several studies investigated the activities of the above-mentioned enzymes in tissues in different diseases including head and neck carcinomas. Some of these enzymes have been studied in a few studies in head and neck carcinoma. However, serum levels of MDA and the activities of SOD, GSH-Px, ADA, and XO have not been reported, in concert, in a considerable number of patients with head and neck cancer. This study was designed to investigate *(i)* the changes in serum antioxidant enzyme activities and lipid peroxidation products, *(ii)* serum activities of purine catabolizing enzymes and *(iii)* possible relationships between the parameters studied in patients with head and neck cancers. These parameters were also studied in patient subgroups according to the origin and the stage of the cancer.

PATIENTS AND METHODS

This study was carried out with the serum samples of 35 patients with head and neck squamous cell carcinoma prior to treatment and of 20 healthy individuals as controls. The cancers were localized in the larynx (n=25), oral cavity (n=5), oropharynx (n=3), and hypopharynx (n=2). The mean ages were 63 years (range 37 to 80 years) and 56 years (range 30 to 72 years) in the study and control groups, respectively. The clinical and radiological data of each case were evaluated. To avoid interferences with the measurements of enzyme activities, patients who had received any previous treatment for the current carcinoma were excluded, including surgery, radiotherapy, chemotherapy, or immunotherapy.

Primary squamous cell carcinoma was histopathologically confirmed following surgical treatment of the patients. The stages of the tumors are shown in Table I.

Preoperative nutritional status and the hematologic values of the patients were found within normal ranges. Those presenting with any signs of an infection were excluded. Most of the patients were heavy smokers, and some of them had a history of alcohol usage, as well.

Ten milliliters of venous blood samples were collected from the patients and controls after their informed consent was obtained.

Assay of the enzymes activities: Blood samples were drawn into dry glass tubes during routine blood sampling for biochemical and hematological analyses. After immediate centrifugation (1000 x g for 10 min at +4°C), serum samples were stored frozen at -30 °C.

Serum ADA (EC 3.5.4.4) activity was estimated spectrophotometrically according to the method of Giusti,[5] which is based on the direct measurements of ammonia formation produced when ADA acts in excess of adenosine. The results were expressed as units per liter serum (U/L).

Serum XO (EC 1.2.3.2) activity was measured spectrophotometrically by the formation of uric acid from xanthine, through the increase in absorbance at

TABLE I

STAGES OF THE HEAD AND NECK TUMORS

Stages	No. of patients
I	3
II	15
III	13
IV	4

TABLE II

ACTIVITIES OF SUPEROXIDE DISMUTASE (SOD), GLUTATHIONE PEROXIDASE (GSH-Px), ADENOSINE DEAMINASE (ADA) AND XANTHINE OXIDASE (XO) ENZYMES AND MALONDIALDEHYDE (MDA) LEVELS MEASURED IN THE SERA OF PATIENTS AND HEALTHY CONTROLS

	SOD (U/mL)	GSH-Px (U/L)	ADA (U/L)	XO (U/L)	MDA (mmol/L)
Control group (n=20)	1.88±0.15	32.09±2.89	20.05±1.81	0.120±0.013	0.720±0.068
Study group (n=35)	0.88±0.12	35.04±2.59	40.51±2.97*	0.170±0.015	1.350±0.071

*p<0.001 compared to the control group.

293 nm.[6] A calibration curve was constructed with the use of 10-50 mU/mL concentrations of standard XO solutions (Sigma X-1875). One unit of activity was defined as 1 µmol uric acid formed per minute at 37 °C, pH 7.5. The results were expressed in units per liter serum (U/L).

The principle of estimating the total SOD (EC 1.15.1.1) activity is based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by O_2^- generated by the xanthine/xanthine oxidase system.[7] Activity was assessed in the ethanol phase of the serum after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of the serum and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as units per milliliter serum (U/mL).

Glutathione peroxidase (EC 1.6.4.2) activity was measured by the method described by Paglia and Valentine.[8] An enzymatic reaction was initiated by the addition of H_2O_2 to the reaction mixture containing reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and glutathione reductase. Changes in the absorbance at 340 nm were monitored by a spectrophotometer. Activity was expressed in units per liter serum (U/L).

The basic principle of the TBARS (thiobarbituric acid reactive substances) method is based on the reaction of TBARS with thiobarbituric acid (TBA) at +95 °C.[9] The fluorescence in the upper n-butanol phase was measured as an arbitrary unit by a spectrofluorometer (Hitachi Model F-4010, Japan), excitation at 525 nm and emission at 547 nm. The results were expressed as micromoles per liter serum (mmol/L).

Statistical analysis: All the results were analyzed with the use of the independent samples t-test. The activities of the enzymes were expressed as means ± standard error of means.

RESULTS

Serum SOD, GSH-Px, AD, and XO activities and MDA levels obtained from the patients and controls are shown in Table II. The mean serum ADA level of the patients was significantly higher than that of the normal subjects (40.51±2.97 U/L *vs* 20.05±1.81 U/L; p<0.001). The mean serum SOD level was lower, and activities of GSH-Px and XO and MDA levels were higher in the patient group, but the differences did not reach significance (Table II).

The patients and controls were further analyzed in subgroups with respect to gender, the presence or absence of smoking, and alcohol usage. However, none of these significantly affected the biochemical parameters studied in the sera.

DISCUSSION

All cells and tissues of the human body contain numerous enzymatic and nonenzymatic antioxidants that protect them from oxidative stress.[4] Superoxide dismutase is one of these enzymes. It is widely distributed in oxygen-metabolizing cells. It catalyzes the removal reaction of toxic superoxide radical (O_2-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2).[10,11] Reactive oxygen species have an important role in the multistage events of chemical carcinogenesis.[12] Superoxide dismutase has been studied clinically and considerable interest has been focused on this enzyme in relation to the pathophysiology of various diseases, especially malignant tumors.[4,11,13-17] Most studies found decreased SOD activities in various types of cancer,[14,16-20] while some investigators found no meaningful differences[15,17] or increased SOD activity[13] in cancer tissues or sera of patients with some malignancies, suggesting that dis-

crepant results might arise from the differences in the tissue samples and from diverse conditions. As far as head and neck cancers are concerned, Yigitbasi et al.[14] reported a decreased activity of SOD, while Durak et al.[13] reported a significantly increased SOD activity in cancerous larynx tissues. Canbolat et al.[10] studied this enzyme in the sera of patients with larynx cancer and found an increased activity compared to non-cancerous tissues from healthy volunteers. Seven et al.[21] studied oxidative stress parameters in blood of patients with laryngeal carcinoma and reported no significant differences in the CuZn SOD activity as compared to controls. In our study, although patients with head and neck tumors exhibited higher levels of SOD activity (0.88±0.12 U/ml) than controls (1.88±0.15 U/ml), the difference was not significant. This decrease may result from the imbalances between the oxidant and antioxidant systems in head and neck cancer patients.

Glutathione peroxidase was another free radical metabolizing enzyme studied in our study. It catalyzes the reduction of organic hydroperoxide and hydrogen peroxide to water.[22] There are two major types of GSH-Px, one of which contains selenium (SeGSH-Px) and is with both hydrogen peroxide and organic hydroperoxides. The other GSH-Px consists of proteins that are not dependent on selenium (Se), and thus, is only active with organic hydroperoxides.[22] It has been studied in relation to the pathophysiology of various diseases, especially malignant tumours.[2,11,16,21,22] Some investigators reported an increased activity[2,23-25] while others reported a decreased activity[2,22] of this enzyme in different cancerous tissues. Mulder et al.[22] investigated both SeGSH-Px and total GSH-Px in head and neck cancerous tissues and reported higher degrees of SeGSH-px and total GSH-Px activities in laryngeal cancers. They also reported lower SeGSH-Px activities in primary oral/oropharyngeal and laryngeal tumors compared to control tissue samples. They concluded that head and neck cancers were mainly mediated by SeGSH-Px. In contrast, some investigators found conflicting results as to the GSH-Px activity in blood or plasma. Seven et al.[21] reported a lower GSH-Px activity in the blood of patients with larynx cancer compared to controls. Chiou and Hu[16] studied GSH-Px activity in erythrocytes of patients with myoma uteri and cervicitis. The activity was somewhat lower in myoma patients while it was significantly higher in cervicitis. In our study, the SeGSH-Px activity was 35.03±2.59 U/L in the patients with head and neck cancer and 32.09±2.89 U/L in the controls and the difference was not significant. Enzymatic systems including GSH-Px are very important for the elimination of free radicals, otherwise, they cause cellular destruction by interacting with subcellular structures such as nucleic acids, proteins, and enzymes.[26] The findings of our study suggest that hydrogen peroxide produced by several mechanisms may be decomposed without any problems in patients with head and neck cancers.

Adenosine deaminase is an important enzyme involved in purine and DNA metabolism. It catalyzes the conversion reaction of adenosine to inosine and deoxyadenosine to deoxyinosine following dephosphorylation. Lymphocyte-mediated immune responses are important host factors in cancer patients.[27] Adenosine deaminase may be required for proliferation and differentiation of lymphocytes, especially T-lymphocytes.[28] Its activity may be increased in patients with cancer, suggesting its involvement in stimulating cell-mediated immunity.[29]

Some studies examined the levels of ADA in different kinds of diseases, but the results were scant and inconclusive. In several studies, ADA activity was found to be increased in cancerous tissues or in sera of patients with cancer when compared to noncancerous samples,[10,30-33] although some researchers reported lower activities.[2,13,15,34] Three studies reported statistically significant or nonsignificant higher ADA levels in the sera of patients with cancer compared to control subjects.[10,33,35] In our study, ADA was measured as 40.51±2.97 U/L in the study group and 20.05±1.81 U/L in the control group and the difference was statistically significant ($p<0.001$).

Diagnostic enzymology or clinical enzymology is principally concerned with the changes in the activity of serum enzymes that are intracellular and that are normally present in the serum in low levels. By measuring the activities of these enzymes in diseases, it is possible to infer the nature of pathological changes occurring in the tissues of the body. There are several factors that affect serum enzyme activities and their amounts: leakage of enzymes

from cells, altered production of the enzyme, and proliferation of cells that secrete enzymes to the intercellular and intravascular space.[36] All the changes in the activities of the enzymes in the cell can easily affect the plasma levels or activities of the same enzymes in the intravascular environment. It is likely that increased serum ADA levels may be a result of the leakage or induction of the enzyme production from the primary tumor cells. The stage of the cancer and the immune response of the host may have a negative or positive influence in the release of ADA from tissue cells.[27] Further studies on the stage of the cancer and immune status of the host may help to understand increased ADA activity in cancer patients.

Xanthine oxidase is one of the DNA turn-over enzymes, which functions both in purine and free radical metabolism.[27] It is a regulatory enzyme of purine catabolism and also plays an important role in protein and amino acid catabolism.[3] It affects the rate-limiting step of purine catabolism, which converts xanthine and hypoxanthine to uric acid with formation of superoxide anion (O_2-) as a by product, which can further be used by SOD as a substrate.[15] Up to date, XO activity has been investigated in the cancerous tissues including head and neck region and in plasma or some other fluid such as bronchial washing fluid (BWF). To our knowledge, this is the first study reporting elevated XO levels in serum of patients with head and neck cancer. Xanthine oxidase activity was found to be decreased in cancerous tissues compared to healthy ones.[15,37] Durak et al.[15] suggested that decreased XO activity in cancer patients might result in an imbalance that favors the synthesis over the catabolic potential and might confer selective advantages to the cancer cells. The finding of an increased serum XO activity in this study is consistent with other studies.[13,27,30] When only serum studies are taken into account, there are few studies in the English literature with discrepant results. Alsabti[37] reported a significant fall in serum XO in patients with breast carcinoma. In our study, the XO level was slightly higher in the patient group compared to controls (p=0.076). Akyol et al.[27] studied XO activity in plasma and BWF in patients with lung cancer and found higher activity levels than the control group. They suggested that a high XO activity might reflect an attempt to lower salvage pathway activity for purines, which is vital for rapid DNA synthesis in cancerous lung tissues.

Malondialdehyde is an end-product of lipid peroxidation, indicating the degree of oxidative stress. Literature reports as to the lipid peroxidation in cancer patients are controversial. Some investigators reported lower MDA levels in cancerous tissues compared to controls.[38,39] Most studies did not find any difference in MDA levels, and some reported elevated MDA levels.[14,40-42] In our study, the mean MDA level was 1.35±0.07 mmol/L in the patient group compared to 0.72±0.07 mmol/L in the control group and the difference was not significant. Punnonen et al.[38] studied MDA levels in both cancerous tissues and sera and found that they were slightly decreased in cancerous tissues, but were increased in the sera of breast cancer patients. Serum studies on the MDA level reported elevated levels in cancer patients compared to controls, except for one which reported a decreased MDA level.[39] Consistent with our results, Torun et al.[41] found elevated MDA levels in serum of patients with head and neck cancers.

In conclusion, the results of our study suggest that increased serum ADA activity may be used as an additional diagnostic marker for the diagnosis and follow-up of the patients with head and neck cancers. Proliferation of the cancerous cells and the leakage of the enzyme into the circulation from abnormal cells may account for increased ADA activity in head and neck cancers. Further studies with a larger cohort of patients in specific groups are needed to clarify the exact mechanism of free radicals, such as ADA, and its elevation in the serum and to determine whether ADA may be used as a reliable diagnostic marker in head and neck cancers.

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