Evaluation of 8-Hydroxy-2'-Deoxyguanosine Concentration and Antioxidant Enzyme Activities in Bladder Cancer Patients

Mesane Kanseri Hastalarında 8-Hidroksi-2'-Deoksiguanozin Konsantrasyonu ve Antioksidan Enzim Aktivitelerinin Değerlendirilmesi

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ABSTRACT Objective: Oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer. The purpose of this study was to examine 8-hydroxy-2'-deoxyguanosine (8-OHdG) level which is a marker of oxidative DNA damage, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities as antioxidant enzymes, in serum of urinary bladder cancer, and to determine relations between measured parameters and tumor characteristics such as histological grade, local invasion and tumor size. **Material and Methods:** Forty patients with urinary bladder cancer were included in the study. Blood samples were collected just before the resection. Serum levels of 8-OHdG were measured with a competitive ELISA kit, SOD and GPx activities were measured by spectrophotometric kits, GST activity was determined by spectrophotometric assay. **Results:** There was no significant difference between patient and control groups for serum 8-OHdG level and GPx activity. However serum SOD activity was significantly lower (P< 0.001) and GST activity was significantly higher (P< 0.001) in the patient group as compared to control group. Tumor size found to be negatively correlated with GST activity (r: -0.43, P< 0.01). **Conclusion:** Data show that serum level of 8-OHdG does not have a prognostic potential for urinary bladder cancer. Antioxidant balance is disturbed in these patients however changes in antioxidant enzyme activities does not have a prognostic value. The most promising antioxidant enzyme is GST because of its negative association with tumor size.

Key Words: Glutathione peroxidase; superoxide dismutase; glutathione transferase; 8-hydroxy-2'-deoxyguanosine; urinary bladder neoplasms

ÖZET Amaç: Oksidan/antioksidan dengenin kanser oluşumu ve ilerlemesinde önemli bir faktör olduğu ileri sürülmektedir. Bu çalışmanın amacı mesane kanserli hastalarda serumda DNA oksidasyonu belirteci olan 8-hidroksi-2'-deoksiguanozin (8-OHdG) düzeyi ile antioksidan aktivite olarak süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx) ve glutatyon S-transferaz (GST) aktivitelerini ölçmek ve bu parametrelerin histolojik grade, lokal invazyon, tümör büyüklüğü gibi tümör karakteristikleri ile ilişkilerini belirlemektir. **Gereç ve Yöntemler:** Mesane kanserli 40 hasta çalışma kapsamına alındı. Kan örnekleri ameliyatın hemen öncesinde toplandı. Serum 8-OHdG düzeyleri yarışmalı ELISA kiti, SOD ve GPx aktiviteleri spektrofotometrik kitlerle, GST aktivitesi spektrofotometrik yöntemle belirlendi. **Bulgular:** Hasta ve kontrol grupları arasında serum 8-OHdG düzeyi ve GPx aktivitesi bakımından anlamlı fark yoktu. Ancak, kontrol grubu ile karşılaştırıldığında hasta grubunda SOD aktivitesi anlamlı olarak düşük (P< 0.001), GST aktivitesi anlamlı olarak yüksekti (P< 0.001). Tümör büyüklüğü GST aktivitesi ile negatif korele olarak bulundu (r: -0.43, P< 0.01). **Sonuç:** Bulgular mesane kanserli hastalarda serum 8-OHdG düzeyinin prognostik bir önemi olmadığını göstermektedir. Bu hastalarda antioksidan denge bozulmuştur fakat antioksidan enzim aktivitelerindeki değişikliklerin prognostik bir değeri yoktur. Tümör büyüklüğü ile negatif korelasyon göstermesi nedeniyle bu konuda en umut verici antioksidan enzim GST'dır.

Anahtar Kelimeler: Glutatyon peroksidaz; süperoksid dismutaz; glutatyon transferaz; 8-hidroksi-2'-deoksiguanozin; mesane tümörleri

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Recent studies have demonstrated the role of oxygen free radicals (OFR) in carcinogenesis. By interacting with genomic DNA, OFR have been suggested to damage specific genes which control cell growth and differentiation,[1] increase the activity of carcinogenic xenobiotics,[2] and stimulate faster growth of malignant cells.[3] OFR are highly reactive molecules because of unpaired electrons on the exterior orbitals. As a result of attacks by OFR to DNA many types of oxidized nucleoside have been determined. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most frequently detected and studied oxidative DNA lesion. 8-OHdG has a pro-mutagenic potential since it mispairs with A residues and leads to an increased frequency of spontaneous G:C→T:A transversion. This mutation is generally observed in mutated protooncogenes and tumor suppressor genes.[4] Upon DNA repair, 8-OHdG is excreted in the urine. Serum or urinary 8-OHdG level is considered as a biomarker of generalized cellular oxidative stress and is linked to degenerative diseases including cancer.[5]

Defense against OFR is provided by a system of enzymes and antioxidant compounds capable of preventing excess OFR production and neutralising OFR. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are enzymatic antioxidants which catalyze the detoxification of superoxide anion (O_2) and hydrogen peroxide (H_2O_2), respectively.[6] Glutathione S-transferases (GST) conjugate glutathione to various potentially carcinogenic compounds, facilitating their elimination from the body. Although GPx, SOD and GST are cellular enzymes that are available in the plasma at a detectable level.[7-9] increased oxidative stress under pathological circumstances may exhaust the antioxidant capability of cells and as a result, the susceptibility of target molecules to oxidative stress increases. Oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer.[10] We aimed to examine 8-OHdG level, SOD, GPx and GST activities in serum of urinary bladder cancer, and to determine relations between measured parameters and tumor characteristics such as histological grade, local invasion and tumor size. We hypothesized that serum level of 8-OHdG and activites of these enzymes may be prognostic markers which can be easily accessible for urinary bladder cancer.

MATERIAL AND METHODS

SUBJECTS

A total of 40 patient who admitted to Istanbul University, Cerrahpasa Medical Faculty, Department of Urology were included in the study (Table 1). Cerrahpasa Medical Faculty Ethical Committee approval was taken in accordance with the principles of Declaration of Helsinki and informed consent was obtained from the cases. Urinary bladder cancer was diagnosed according to pathology reports. None of the patients had undergone any previous treatments such as chemoterapy or surgery. The control group was constituted by 21 healthy volunteers. Both patients and controls were euthyroid and had normal liver and renal functions. Subjects with chronic inflammatory diseases were excluded. Subjects who had any inflammatory condition or infectious disease, and who were receiving drugs capable of interfering with oxidant/antioxidant system in previous six months were excluded from the study.

LABORATORY MEASUREMENTS

Eight ml of venous blood sample was collected before the operation. Following centrifugation at 2000 X g for 10 minutes, serum was removed and kept at the-80 ^{0}C until the time of analysis. Serum level of

TABLE 1: Patient and control characteristics.

	Urinary bladder cancer group (n= 40)	Control group (n= 21)
Mean age (years)	65 ± 10	61 ± 9
Gender		
Male	30	14
Female	10	7
Smoking (%)	82	75
Tumor Grade		
High (%)	52	
Low (%)	48	
Local invasion (%)	52	
Metastasis (%)	5	
Tumor size (cm)	2.5 ± 1.0	

8-OHdG was measured with a competitive ELISA kit obtained from Oxis (Portland, OR; USA).[11] Activity of SOD and GPx in serum were measured by spectrophotometric kits from Randox (Crumlin, UK).[12,13] GST activity was determined according to the method of Habig et al.[14] using 1-chloro, 2.4 dinitrobenzene as substrate. Formation of the S-conjugate was followed by its absorbance at 340 nm.

STATISTICAL ANALYSIS

The results of 8-OHdG level, SOD, GPx and GST activities are expressed as mean ± SD (standard deviation of the mean). Since majority of data were not normally distributed, statistical analysis of measured parameters were performed by nonparametric Mann Whitney U test. Differences between groups were considered significant at $P< 0.05$. Spearman correlation coefficient was used for correlation analysis.

RESULTS

There was no significant difference between patient and control groups for serum 8-OHdG level and GPx activity. Although 8-OHdG level was higher in the patient group, it did not reach a significant level. However, serum SOD activity was significantly lower ($P< 0.001$) and GST activity was significantly higher ($P< 0.001$) in the patient group as compared to the control group (Table 2). When the patient group was divided into subgroups with respect to local invasion, GST activity was found to be lower in the presence of local invasion (Table 3). With respect to the histological grade of tumor, no significant difference was found between low grade tumor group and high grade tumor group for any parameter (Table 4). Since a total of two patients had distant metastases, measured parameters were not analyzed with respect to metastasis. The relations of measured parameters (8-OHdG, SOD, GPx, and GST) and tumor grade and tumor size were examined. Tumor size found to be negatively correlated with GST activity (r: -0.43, $P< 0.01$).

TABLE 2: Measured parameters in the study groups.

	Control group (n= 21)	Urinary bladder cancer group (n= 40)
8-OHdG (ng/ml)	3.08 ± 1.06	3.72 ± 1.99
SOD (U/ml)	0.53 ± 0.25	0.32 ± 0.19[a]
G-Px (U/L)	2.44 ± 0.52	2.23 ± 0.62
GST (mU/L)	50 ± 19	96 ± 52a

[a] $P< 0.001$ versus control.

TABLE 3: Measured parameters in the patient group with respect to the local invasion.

	Local invasion (+) group (n= 21)	Local invasion (-) group (n= 19)
8-OHdG (ng/ml)	3.09 ± 1.13	3.49 ± 0.24
SOD (U/ml)	0.38 ± 0.26	0.30 ± 0.16
G-Px (U/L)	2.19 ± 0.73	2.21 ± 0.41
GST (mU/L)	78 ± 41	121 ± 67[a]

[a] $P< 0.01$ versus control.

TABLE 4: Measured parameters in the patient group with respect to the tumor grade.

	High grade (n= 21)	Low grade group (n= 19)
8-OHdG (ng/ml)	3.00 ± 1.10	3.56 ± 2.44
SOD (U/ml)	0.37 ± 0.25	0.30 ± 0.16
G-Px (U/L)	2.22 ± 0.70	2.21 ± 0.42
GST (mU/L)	90 ± 50	112 ± 56

DISCUSSION

In agreement with the hypothesis that oxidative stress is linked to cancer,[15] it was previously shown that 8-OHdG level in urinary bladder tumors was shown to be significantly higher when compared to neighboring non-cancerous tissues.[16] 8-OHdG levels were also found to be higher in leukocytes[17] of patients with urinary bladder cancer. In these previous studies, serum level of 8-OHdG was not measured and the relations between 8-OHdG level and tumor grade, stage, metastasis and tumor size were not examined. Exceptionally, Akcay et al.[17] reported that leukocyte 8-OHdG level was not correlated with the clinical grading and histology in the patients with bladder cancer. This is the first study investigating 8-OHdG level in serum. The aim of the present study was to investigate whether serum level of 8-OHdG had a predictive value in patients who underwent resection of for urinary bladder

carcinoma. In the light of previous data,[16,17] we had expected higher 8-OHdG level in the serum. We supposed that if 8-OHdG level was high in tumor tissue it might also increase in serum, and serum level of 8-OHdG might reflect the oxidative stress on DNA, and it might be related to the tumor characteristics. However, although serum 8-OHdG level was higher in the patient group, it did not reach a significant level. Considering that the only mechanism for the appearance of 8-OHdG in serum is the repair of 8-OHdG residues by DNA repair systems, unchanged serum 8-OHdG level makes one to suppose that DNA repair may be impaired during the carcinogenesis. Various defects in DNA repair result in different forms of cancer. Indeed, mismatch repair defects that have been determined in some kinds of gastric and colorectal cancer,[18,19] and uroepithelial cancers of the ureter (and bladder to lesser extent) has been suggested to share a number of characteristics of mismatch repair deficiency-driven tumorigenesis.[20] 8-OHdG level did not exhibit significant changes with respect to tumor grade and presence of local invasion. Serum level of 8-OHdG was not found to be correlated with tumor grade and size. Taken together, all those data show that serum level of 8-OHdG is not a prognostic marker for urinary bladder cancer. However, as limitation of the present study, tumor volume was heterogenous in the patient group and the study population was small. In order to clarify prognostic potential of serum level of 8-OHdG, further studies in larger groups are needed.

Antioxidant system is highly complex and multifactorial. It includes various enzymes and small molecules and they may not exhibit harmonious change. Scientific data for SOD activity in urinary bladder cancer patients is extremely limited and contradictory. As compared to those of cancer-free adjacent tissue, SOD activity in cancerous bladder tissue was found to be decreased by Durak et al.[21] and determined to be increased by Savic- Radojevic et al.[22] As far as we know, SOD activity in peripheral circulation has not been examined in urinary bladder cancer patients so far. Decreased serum SOD activity determined in the present study may be a reflection of poor antioxidant defence in urinary bladder cancer patients. In fact, expression of some genes can be altered during the malignant transformation. In urinary bladder cancer, the expressions of a number of genes may be down-regulated, and SOD may be one of them.

Increased GPx activitiy was determined in cancerous bladder tissues when compared to cancer-free adjacent tissues.[22,23] GPx activity was also measured in erythrocytes. Arıkan et al.[24] reported decreased GPx activity, Yalcın et al.[25] reported increased GPx activity in erythrocytes of patients with urinary bladder cancer as compared to controls. Serum GPx activity has not been examined in urinary bladder cancer patients previously. In the present study, serum GPx activity did not present a significant difference between urinary bladder patients and controls. The most interesting finding of this study was the increased GST activity in the patient group, and its negative association with tumor size. Enhanced GST activity in urinary bladder tumors as compared to cancer-free adjacent tissue has been suggested by early studies.[26,27] Five classes of soluble GSTs are known in humans, including alpha, mu, pi, theta and zeta.[28] GST mu1 (GSTM1) is involved in the detoxification of polycyclic aromatic hydrocarbons found in tobacco smoke and in some industrial chemicals which are known as carcinogenic agents. Majority of the studies investigating the relation between bladder cancer and GSTM1 have reported an increased risk when there is a lack of GSTM1 activity.[29,30] Increased GST activity in urinary bladder cancer patients may be a result of up- regulation of GST gene during the carcinogenesis. Alternatively, this increase may be a cellular adaptive response or defense mechanism against malignant transformation. Although GST activity was higher in the patients with low grade tumor, it did not reach a significant level. However, GST activity was significantly higher in the patients without local invasion as compared to patients with local invasion. Furthermore, GST activity was negatively correlated with tumor size. Taken altogether, these findings support our suggestion that increased GST activity is a defensive response to malignant transformation in early steps.

In conclusion, in the contrary to previous data obtained from cancerous urinary bladder tissues, serum 8-OHdG level is not significantly different from controls in urinary bladder cancer patients. Although antioxidant balance is disturbed, changes in antioxidant enzyme does not have a prognostic value. The most promising antioxidant enzyme as a prognostic marker is GST because of its increased activity and its negative association with tumor size in the urinary bladder cancer patients. Is increased GST activity spesific for urinary bladder cancer or a common condition in all cancers? This is of interest. Our syudy was a preliminary study with some limitations such as heterogenity of tumor stage and a small patient population. More detailed studies in larger and homogenous groups should be held to clarify the prognostic potential of serum level of 8-OHdG and SOD, GPx, GST activities in urinary bladder cancer patients.

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