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Original Article / Özgün Makale

Serum prolidase activity, total oxidant/antioxidant, and nitric oxide levels in patients with esophageal squamous cell carcinoma

Özofagusun skuamöz hücreli karsinomu olan hastalarda serum prolidaz aktivitesi, total oksidan/antioksidan ve nitrik oksit düzeyleri

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ABSTRACT

Background: This study aims to assess the prolidase activity, nitric oxide levels, and oxidative status in patients with esophageal squamous cell carcinoma.

Methods: The study included 30 patients with esophageal squamous cell carcinoma (11 males, 19 females; mean age 61±3 years; range, 28 to 77 years) and 30 healthy controls (10 males, 20 females; mean age 58±5 years; range, 31 to 73 years). Serum prolidase activity, total antioxidant capacity, total oxidant status, and nitric oxide levels were measured. In addition, the oxidative stress index was calculated.

Results: Prominently elevated serum prolidase activity, oxidative stress index values, total oxidant status, and nitric oxide levels were detected in the patient group ($p<0.05$). Lower total antioxidant capacity levels were observed in the patient group ($p<0.05$).

Conclusion: Increased oxidant status with increased nitric oxide levels and prolidase activity were found in esophageal squamous cell carcinoma patients. Impairment of antioxidant mechanism with increased prolidase activity and nitric oxide levels may have a crucial role in the etiopathogenesis of esophageal squamous cell carcinoma.

Keywords: Esophageal cancer, nitric oxide, prolidase, total antioxidant capacity, total oxidant status.

ÖZ

Amaç: Bu çalışmada özofagusun skuamöz hücreli karsinomu olan hastalarda prolidaz aktivitesi, nitrik oksit düzeyleri ve oksidatif durum değerlendirildi.

Çalışma planı: Çalışmaya özofagusun skuamöz hücreli karsinomu olan 30 hasta (11 erkek, 19 kadın; ort. yaş 61±3 yıl; dağılım, 28-77 yıl) ve 30 sağlıklı kontrol (10 erkek, 20 kadın; ort. yaş 58±5 yıl; dağılım, 31-73 yıl) dahil edildi. Serum prolidaz aktivitesi, total antioksidan kapasitesi, total oksidan durumu ve nitrik oksit düzeyleri ölçüldü. Ayrıca, oksidatif stres indeksi hesaplandı.

Bulgular: Hasta grubunda belirgin şekilde artmış serum prolidaz aktivitesi, oksidatif stres indeksi değerleri, total oksidan durumu ve nitrik oksit düzeyleri saptandı ($p<0.05$). Hasta grubunda daha düşük total antioksidan kapasitesi düzeyleri gözlendi ($p<0.05$).

Sonuç: Özofagusun skuamöz hücreli karsinomu olan hastalarda artmış nitrik oksit düzeyleri ve prolidaz aktivitesi ile artmış oksidan durumu bulundu. Artmış prolidaz aktivitesi ve nitrik oksit düzeyleri ile antioksidan mekanizma bozukluğu, özofagusun skuamöz hücreli karsinomunun etyopatogenezinde önemli rol oynayabilir.

Anahtar sözcükler: Özofagus kanseri, nitrik oksit, prolidaz, total antioksidan kapasitesi, total oksidan durumu.

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Esophageal squamous cell carcinoma (ESCC) is the most commonly detected malignancy type of esophagus. This aggressive malignancy is characterized with poor prognosis and high metastasis rate.[1] The main treatment is surgical resection of the esophagus. Chemoradiotherapy can also be an alternative or adjuvant therapy. Smoking, drinking alcohol, and low fruit and vegetable intake have been claimed as risk factors in ESCC.[2] The chemicals in cigarette and alcohol may have important roles in the development of premalignant and malignant esophagus lesions.[2] These chemicals such as nitrosamine and acetaldehyde are prooxidants and they lead to generate reactive oxygen species and rise of oxidative stress.[3,4] Incremented oxidative stress were found to be related to the etiopathogenesis of esophagus cancer.[5]

Nitric oxide (NO) is a vasodilator molecule that derives from L-arginine. Nitric oxide, a neural transmitter, also has muscle relaxant and antiaggregant effects. Production of NO increases during inflammation. And chronic inflammation leads to increased NO synthesis that has a key role in carcinogenesis.[6] Nitric oxide leads to angiogenesis, inhibition of the apoptosis progression of the tumor, invasion, and metastasis in different tumors.[7] On the other hand, the inhibitory effect of NO on carcinogenesis and progression of tumor was reported.[7] The other effect of NO is phosphorylation of different proteins. Nitric oxide was demonstrated to increase prolidase activity via phosphorylation.[8]

Matrix metalloproteinases (MMPs) have important functions in collagen metabolism and remodeling of the extracellular matrix. The extracellular matrix is one of the important roadblocks for the tumor invasion. Activation of the MMPs leads to degradation of the extracellular matrix and this situation is the key point for tumor invasion and metastasis. Prolidase, a MMP, functions for cleaving proline and hydroxyproline from imidodipeptides.[9,10] Degradation of the collagen is an important pathway to obtain proline and hydroxyproline amino acids that are essential in other metabolic reactions and energy production. Prolidase degrades collagen to provide these amino acids.[10] Also, it was mentioned that prolidase promoted angiogenic signaling in tumors.[10] Many researchers claimed that prolidase activity may be related to tumorigenesis and this relationship was presented in different studies.[11-13] Thus, increased activity of prolidase was demonstrated in different malignancies.[11-13]

According to the literature, oxidative stress, NO levels, and prolidase activity are important factors in the development and spread of tumor. Hence, in this study, we aimed to assess the prolidase activity, NO levels, and oxidative stress status in patients with ESCC.

PATIENTS AND METHODS

This prospective study was conducted at Van Yüzüncü Yıl University Faculty of Medicine and Sakarya University Faculty of Medicine Thoracic Surgery Clinics between March 2013 and March 2017 and included 30 patients with ESCC (11 males, 19 females; mean age 61±3 years; range, 28 to 77 years) and 30 healthy controls (10 males, 20 females; mean age 58±5 years; range, 31 to 73 years). The definite diagnosis of ESCC was confirmed after investigation of the biopsy/surgery materials by a pathologist. Exclusion criteria were consumption of antioxidant or vitamin supplements, smoking or drinking alcohol. Patients with active infection or chronic systemic disorder or those treated with radiotherapy or chemotherapy were also excluded. The control group included individuals who had admitted to hospital for routine check-up. They had no symptoms and their clinical examination was totally normal. The exclusion criteria for the control group were the same as the patient group. The study protocol was approved by the Sakarya University Faculty of Medicine Ethics Committee. A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Blood samples were obtained after an overnight fasting and stored at 4°C. To separate the blood cells and the serum, the samples were centrifuged at 3000 rpm for 10 minutes and stored at -80°C. Afterwards, total antioxidant capacity (TAC), total oxidant status (TOS), prolidase activity, and NO levels were measured. Blood samples were taken from all patients before surgical treatment.

Serum TAC was counted by using an automatic method that was described by Erel.[14] The results were considered as mM Trolox equivalent per liter. Serum TOS was counted by using an automatic method that was described by Erel.[15] The oxidative stress index (OSI) was found by dividing the TOS level by the TAC level. The activity of prolidase was determined according to the Myara method and the results were considered as U/L.[16] The levels of NO were measured by using the Griess reaction, according to the method described by Tracey.[17] The results were considered as µmol/L.

Table 1. Demographic characteristics of study participants

	Patients (n=30)		Controls (n=30)		
Parameters	n	Mean±SD	n	Mean±SD	*p*
Age (year)		61±3		58±5	NS
Gender					NS
Female	19		20		
Male	11		10		
Body mass index (kg/m^2)		22.1±1.6		23.0±1.1	NS

SD: Standard deviation; NS: Non-significant.

Statistical analysis

The statistical evaluation was performed by using the SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The means and standard deviations of the results were determined. Normality of the distribution was determined with Shapiro-Wilk test. The changes between the groups were evaluated with Mann-Whitney U test for non-parametric factors and with Student's t-test for parametric factors. P values lower than 0.05 were accepted as statistically significant.

RESULTS

The demographic characteristics of study participants were demonstrated in Table 1. No distinct differences were observed between the patient and control groups with respect to age, gender, or body mass index ($p>0.05$).

All patients underwent surgical treatment while 16 patients were also treated with chemotherapy and/or radiotherapy. Grade 1 tumor was demonstrated in eight patients, grade 2 tumor in 12 patients, and grade 3 tumor in 10 patients. Submucosal tumor was observed in five patients, muscularis propria invasion in eight patients, and adventitia invasion in 17 patients. Lymph node metastasis was detected in 14 patients. There were three upper esophagus, 16 middle esophagus, and 11 lower esophagus tumors. Fourteen cases were in stage I+IIA, six cases were in stage IIB, and seven cases were in stage III. Three cases were inoperable because of aortic invasion (stage IV).

Prominently elevated prolidase activity, TOS levels, OSI values, and NO levels were observed in the patient group compared to the control group ($p<0.05$), whereas the TAC levels were found to be statistically significantly lower in the patient group than the control group ($p<0.05$) (Table 2).

Statistically significantly higher prolidase activity, TOS levels, OSI values, and NO levels were demonstrated in advanced stage patients compared to early stage patients ($p<0.05$) while TAC levels were decreased in advanced stage patients ($p<0.05$).

DISCUSSION

In this research, we found increased NO levels, prolidase activity, and TAC levels in patients with esophageal squamous cell carcinoma, while TOS levels were lower. Moreover, NO levels, prolidase activity, and TAC levels were found to be elevated in advanced stage patients compared to early stage patients.

Table 2. Prolidase activity, nitric oxide, and oxidant/antioxidant levels in esophageal squamous cell carcinoma patients and controls

	Patients (n=30)	Controls (n=30)	
Parameters	Mean±SD	Mean±SD	*p*
TAC (mmol trolox equiv./L)	4.2±0.0	9.7±0.6	0.05
TOS (μmol H_2O_2 equiv./L)	20.2±4.4	8.6±2.5	0.05
OSI (arbitrary unit)	4.9±1.1	0.9±0.2	0.05
NO (μmol/L)	20.5±3.9	9.7±0.3	0.05
Prolidase (U/L)	44.9±4.6	24.2±3.5	0.05

SD: Standard deviation; TAC: Total antioxidant capacity; mmol Trolox Equiv./L: mM Trolox equivalent per liter; TOS: Total oxidant status; H_2O_2: Hydrogen peroxide; OSI: Oxidative stress index; NO: Nitric oxide.

We are exposed to many endogenous and exogenous stressors during the day. Oxidative stress leads to formation of free oxygen radicals. These radicals play role in deoxyribonucleic acid (DNA) damage which is important in cell cycle. Extracellular and intracellular antioxidant mechanisms are important to prevent the harmful effects of oxidant stress. Ensuring body's oxidant-antioxidant balance is essential for the continuing of homeostasis. Imbalance in oxidative stress may lead to increased DNA damage. Mutations related to DNA damage may be an initial factor in carcinogenesis.[18] Oxidative stress has been accepted as a predisposing factor in the development of many cancers.[18] Furthermore, the relationship between different malignancies with TOS and TAC levels was investigated in a great number of studies.[19-21] TOS and OSI index were found higher in esophageal cancer patients compared to controls.[21] Moreover, these biomarkers were reported to be candidates in the diagnosis of esophageal cancer.[21] On the other hand, authors demonstrated no correlation between stage of cancer and TOS levels.[21] This correlation was shown in breast cancer patients; the TOS and the TAC levels were presented as prognostic biomarkers in breast cancer.[19] In the current research, we found a correlation between stage and oxidative stress status in contrast with the other study related to esophageal cancer. This difference may be related to the different geographical and personal characteristics of the patients in the study groups.

Deteriorated antioxidant enzyme systems have been reported to be associated with cancer development. Low levels of antioxidants can activate tumor cells as well as elevate lipid peroxides in the body.[22] A study reported beneficial effects of antioxidant supplementation in the control of cancer development.[23] Another study demonstrated that a diet rich in fruits and vegetables may reduce oxidative stress and cancer risk.[24]

Collagen and extracellular matrix proteins are important in the maintenance of cell interaction and cell differentiation. Also, they have a significant function in tissue stabilization and preventing tumoral invasion. Matrix metalloproteinases have key roles in the spread of tumor by degrading collagen and extracellular matrix components.[25] Prolidase, a MMP family member, has functions in collagen turnover and remodeling of the matrix.[16] Distortion in collagen metabolism was demonstrated in stomach cancer tissue while higher prolidase activity was detected in tumoral tissue.[26] The importance of prolidase activity was investigated in several studies and increased activity was demonstrated in different cancer types.[11-13,27] Additionally, a positive correlation was detected between tumor volume and prolidase activity in gastric cancer patients.[11] A study related to breast cancer revealed that stage of the disease and tumor size were correlated with prolidase activity.[12] It was claimed that anti-prolidase activity agents may be crucial in the treatment of breast cancer.[27] We found higher serum prolidase activity in ESCC patients compared to healthy controls. Also, there was a positive correlation between stage of ESCC and prolidase activity. To our knowledge, this is the first study that investigated prolidase activity in ESCC patients.

Nitric oxide is known as a vasodilator molecule; however, it also enhances collagen metabolism by stimulating prolidase activity. It was reported that NO led to phosphorylation of the prolidase and phosphorylated prolidase was more active in collagen metabolism.[8] Angiogenic and proliferative effect of NO on tumor cells were demonstrated.[7] These effects are crucial in the spread of tumor. On the contrary, another report mentioned that NO had antitumor and antiproliferative effects.[28] A study on colon cancer showed that NO has an important role in the development of cancer, tumor invasion, and spread of the tumor.[29] Furthermore, NO were presented as an important factor in the development of gastrointestinal system tumors.[30] In an experimental study, foods that inhibit NO production were found useful in the prevention of the occurrence of esophageal tumor.[31] Many studies have presented higher NO levels in different malignancies such as head and neck, bladder, and gynecological cancers.[32-34] In the current research, elevated NO levels were detected in ESCC patients with respect to control subjects. In a study of Prestidge et al.,[35] NO levels in patients with esophageal cancer were significantly different than the control group and this situation played a role in the progression of esophageal cancer. Plasma NO levels might be used as biomarkers for the diagnosis of esophageal cancer.

This prospective study has some limitations. The sample size was limited because of the exclusion criteria related to smoking and drinking alcohol. Moreover, we did not measure NO or prolidase biomarkers in the tumoral tissue. Studies with larger sample sizes may provide further valuable information regarding these biomarkers in ESCC patients.

In conclusion, we investigated the prognostic significance of total oxidant status, oxidative stress index, total antioxidant capacity, nitric oxide levels, and prolidase activity in esophageal squamous cell

carcinoma patients. Increased prolidase activity, nitric oxide, total oxidant status, and oxidative stress index levels were detected in esophageal squamous cell carcinoma patients with decreased total antioxidant capacity levels. In addition, these values were correlated with the stage of the disease.

Declaration of conflicting interests

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