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Original Article

Alterations in oxidative stress markers in laryngeal carcinoma patients

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Abstract

Background: Data describing how laryngeal cancer affects oxidative stress markers and antioxidants are limited. This study investigated serum antioxidant enzyme activities and oxidative stress markers before and after laryngectomies in patients with laryngeal cancer.
Methods: A total of 29 patients with laryngeal cancer and 25 healthy control subjects were enrolled. Serum malondialdehyde (MDA) levels and superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT), paraoxonase (PON), and arylesterase activities were measured spectrophotometrically. Blood samples were obtained from each patient just before surgery and 1 month after a laryngectomy.
Results: The serum PON, arylesterase, CAT, SOD, and GSHPx activities were significantly decreased (all $p < 0.001$) and serum MDA levels were significantly increased ($p < 0.001$) in patients with laryngeal cancer, compared with control subjects. In laryngeal cancer patients, the serum GSHPx and arylesterase activity levels increased significantly following laryngectomies (both $p < 0.001$), whereas the MDA levels decreased significantly ($p = 0.007$).
Conclusion: In patients with laryngeal cancer, the oxidant/antioxidant balance shifted toward oxidative stress. In addition, following laryngectomies, laryngeal cancer patients had increases in serum antioxidant enzyme activities and decreases in oxidative stress markers.

Keywords: Antioxidants; Laryngeal cancer; Laryngectomy; Oxidative stress; Paraoxonase activity

1. Introduction

Laryngeal carcinomas account for 2−5% of all malignant diseases that are diagnosed annually throughout the world.[1] Most laryngeal cancers are epidermoid cancers/squamous cell carcinomas. They occur five times more frequently in men than in women and most often in patients aged in their 40s−60s.[1] Tobacco use is reportedly the most significant risk factor for laryngeal cancer.[1]

In the human body, the balance between oxidants and antioxidants is important. When this balance is disturbed and oxidants become more prevalent, cells are disrupted and many pathological changes occur. Antioxidants act as a defense system to prevent the harmful effects that can result from free radical formation.[2] There are both enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx), whereas non-enzymatic antioxidants include vitamin E, vitamin C, vitamin A, selenium, transferrin, and lactoferrin. Paraoxonase (PON) and arylesterase also have antioxidant activities and play a role in lipid metabolism.[3]

Oxidative stress and free radicals reportedly play crucial roles in carcinogenesis.[4] Pro-oxidant molecules modulate genes that are related to differentiation and cell growth and may cause structural DNA changes, which trigger carcinogenic processes. Therefore, oxidants may play crucial roles in both the onset and progression of cancer.[4] One important

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indicator of oxidative stress is malondialdehyde (MDA), the end product of lipid peroxidation.[5]

Data describing how oxidative stress markers and antioxidants, including SOD, GSHPx, and CAT enzyme activities, are affected by laryngeal cancer are limited. This study investigated the PON1, SOD, GSHPx, and CAT enzyme activities together with the serum arylesterase and MDA levels in laryngeal cancer patients before and after laryngectomies. Using this method, we sought to determine the role of the oxidative system in the development and treatment of laryngeal cancer.

2. Methods

2.1. Study design

This study was performed in accordance with the Helsinki Declaration, as revised in 2000. Our local ethics committee approved the study protocol, and informed consent was obtained from each subject. This prospective study was performed in the Department of Otorhinolaryngology, Van Yuzuncu Yil University, Van, Turkey. The study included 29 patients (4 females and 25 males) with laryngeal cancer and 25 healthy subjects (6 females and 19 males).

The clinical diagnosis of laryngeal cancer was confirmed by the microscopic examination of biopsy material. The stage of each disease was determined using computerized tomography scans and diagnostic endoscopy. The control subjects were asymptomatic with unremarkable medical histories, and their physical examination results were normal. The enrolled participants were not receiving regular antioxidant vitamin supplements such as vitamin E or C. Patients who had an acute infection, an underlying systemic disease, or who were receiving any drugs were excluded.

2.2. Blood collection

Blood samples were obtained on the morning of, and 1 month after, surgery. The blood samples were collected in empty tubes and immediately placed on ice at 4 °C. The serum was then separated from the cells by centrifugation at 5000 rpm for 10 min. The serum samples were stored at −20 °C until the antioxidant enzyme activities and MDA levels were measured.

2.3. Serum oxidant and antioxidant measurements

Serum SOD activity was measured in accordance with the method of Sun et al.[6] The activity level of serum CAT was determined using hydrogen peroxide as a substrate. A total of 0.1 mL of enzyme solution was placed in a tube and sterile water was added to a second tube. Substrate and buffer solutions were added simultaneously to the two tubes and mixed using a vortex mixer. After 3 min, the absorbance at 240 nm was measured to determine enzyme activity.[7] Serum PON1 and arylesterase activities were determined using a kit developed by Erel et al.[8,9] The results are expressed as units/liter (U/L), which is equal to the hydrolysis of 1 μL of substrate in 1 min. Serum GSHPx activity was measured in accordance with the method of Beutler et al.[10] MDA is one of the peroxidation products formed when fatty acids and free radicals react. MDA levels were measured following the addition of thiobarbituric acid to form a colored product.[11]

2.4. Statistical analysis

The data were analyzed using SPSS (ver. 11.0; SPSS, Inc., Chicago, IL, USA). Parameters between groups were compared using Student's *t*-test. Paired sample *t*-tests were used to compare the levels of oxidant and antioxidant markers before and after laryngectomies. Qualitative variables were assessed using χ^2 tests. A p-value < 0.05 was considered statistically significant.

3. Results

The staging system used most often for laryngeal cancer is that described by the American Joint Committee on Cancer. Of the 29 laryngeal cancer patients, 10 had T1, 7 had T2, 5 had T3, and 7 had T4 stage disease. In total, 21 patients had no lymph node metastasis, 3 patients were categorized as N1, and 5 were N2. Distant metastasis data were not documented. Fourteen patients had moderately differentiated, five had poorly differentiated, and ten had well-differentiated squamous cell carcinomas.

Following the diagnoses, only the 12 patients with T3 or T4 disease had surgery; therefore, postoperative evaluations were performed only on these patients. Total laryngectomies were performed in all 12 subjects. Blood samples were also obtained 1 month after laryngectomy to analyze the levels of oxidative stress markers present. The remaining 17 patients with T1 or T2 disease were treated using radiotherapy. The demographic and clinical data for the study participants are shown in Table 1. No statistically significant differences between the patient and control groups were found with respect to age or sex ($p > 0.05$; Table 1). All patients and control subjects were active smokers, but none of them drank alcohol. The serum PON, arylesterase, CAT, SOD, and GSHPx activity levels were significantly lower in the patient than in the control group (all $p < 0.001$), whereas the serum MDA levels were significantly higher in the patient group ($p < 0.001$; Table 2).

Serum oxidative stress markers were also compared in the 17 patients with T1 or T2 disease and the 12 patients with T3 or T4 disease, and no significant differences were observed between these two groups (Table 3). Preoperative and postoperative serum antioxidant enzyme activities and oxidative stress levels were recorded for 12 patients as shown in Table 4.

Table 1
Demographic data for the study participants.

Parameters	Controls ($n = 25$)	Patients ($n = 29$)	p
Age (years)	60.32 ± 9.42	63.14 ± 9.54	0.281
Sex (females/males)	6/19	4/25	0.336
Smokers	25	29	1.00
Alcohol drinkers	0	0	1.00

Values are expressed as means ± SD or number of subjects.

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