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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.

Table 2
Antioxidant enzyme activities and levels of oxidative stress markers in laryngeal cancer patients and control subjects.

Parameters	Controls ($n = 25$)	Patients ($n = 29$)	p
CAT (U/mL)	5.98 ± 1.43	0.15 ± 0.21	<0.001
SOD (U/mL)	31.34 ± 1.91	16.96 ± 0.37	<0.001
GSHPx (U/mL)	4.58 ± 0.69	0.57 ± 0.51	<0.001
Paraoxonase (U/L)	24.81 ± 1.92	6.25 ± 3.05	<0.001
Arylesterase (kU/L)	28.78 ± 1.11	7.06 ± 2.17	<0.001
MDA (μmol/L)	0.49 ± 0.16	1.50 ± 0.33	<0.001

Values are expressed as means ± SD. CAT = Catalase; MDA = Malondialdehyde; SOD = Superoxide dismutase; GSHPx = Glutathione peroxidase.

Table 3
Antioxidant enzyme activities and levels of oxidative stress markers in patients with T1 or T2 and T3 or T4 laryngeal cancer.

Parameters	T1 or T2 ($n = 17$)	T3 or T4 ($n = 12$)	p
CAT (U/mL)	0.19 ± 0.26	0.09 ± 0.06	0.13
SOD (U/mL)	16.95 ± 0.42	16.99 ± 0.32	0.81
GSHPx (U/mL)	0.52 ± 0.58	0.65 ± 0.41	0.50
Paraoxonase (U/L)	6.41 ± 3.76	6.02 ± 1.74	0.71
Arylesterase (kU/L)	7.60 ± 2.56	6.30 ± 1.17	0.11
MDA (μmol/L)	1.56 ± 0.35	1.42 ± 0.30	0.24

Values are expressed as means ± SD. CAT = Catalase; MDA = Malondialdehyde; SOD = Superoxide dismutase; GSHPx = Glutathione peroxidase.

Compared with preoperative levels, the serum GSHPx and arylesterase activities were significantly higher in patients with laryngeal cancer after laryngectomy (both $p < 0.001$), whereas the MDA levels were significantly lower ($p = 0.007$). However, there were no statistically significant differences in serum SOD, CAT, or PON activity after laryngectomy (all $p > 0.05$; Table 4).

4. Discussion

In this study, we found that the serum antioxidant enzyme activities, including CAT, SOD, GSHPx, PON, and arylesterase, had decreased in patients with laryngeal carcinoma, whereas the levels of MDA, a marker of oxidative stress, had increased. Additionally, 1 month after surgery, the GSHPx and arylesterase levels were significantly higher and the MDA levels were significantly lower than the preoperative values.

Reactive oxygen species (ROS) have been associated with many chronic diseases, including cancer. Previous studies have revealed that ROS increase the migration of tumor cells, and the risks of tumor invasion and metastasis.[4,12] Increased levels of ROS and increased sensitivity to ROS have been observed in cancer cells.[13] Moreover, antioxidant enzymes and molecules are potential treatment targets in malignant diseases.[14]

Data concerning oxidative system parameters in laryngeal carcinomas are limited and the results are controversial. Liu et al.[15] investigated plasma and tissue CAT, MDA, SOD, GSHPx, and nitric oxide (NO) levels in laryngeal carcinoma patients and compared the results with those from control subjects who were smokers. In the laryngeal cancer group, the CAT, MDA, and GSHPx levels increased significantly, whereas the SOD and NO levels decreased. Kacakci et al.[16] analyzed both blood and tissue samples from 30 patients with laryngeal carcinomas and reported that the plasma MDA levels were significantly higher in the patients, whereas the GSHPx, SOD, and CAT activity levels were significantly higher in the control subjects. In our study, we found increased oxidative stress and decreased antioxidant levels in patients with laryngeal cancer. However, we did not find differences in oxidative stress markers among patients at differing stages of the disease. One of the major strengths of our study is the large number of parameters we were able to study simultaneously. Moreover, to the best of our knowledge, oxidative stress data from laryngeal cancer patients following surgery are limited. Szuster-Ciesielska et al.[17] also reported that the ROS levels were higher in 16 patients with laryngeal carcinomas than in healthy control subjects, and the increases corresponded to different tumor stages. In addition, they found significant decreases in ROS production and serum CAT and peroxidase activities together with significant increases in serum SOD levels, following a partial or total laryngectomy. In contrast, Samir et al.[18] reported that lipid peroxidation products that reflect oxidative stress status increased significantly 30 days after surgery in 30 patients with laryngeal cancer; this may be associated with tissue damage.

GSHPx metabolizes hydrogen peroxide effectively and is important in preventing peroxidation.[19] A study by Mulder et al.[20] demonstrated that GSHPx activity was reduced in the laryngeal tissues of laryngeal cancer patients. Similarly, Canbay et al.[21] demonstrated decreased GSHPx activity in patients with laryngeal carcinomas compared with healthy control subjects. In this study, we also found that the serum GSHPx activity level was significantly lower in patients with

Table 4
Serum antioxidant enzyme activities and levels of oxidative stress markers before and after laryngectomies in patients with laryngeal cancer.

Parameters	Controls ($n = 25$)	Before the operation ($n = 12$)	After the operation ($n = 12$)	p_1	p_2	p_3
CAT (U/mL)	5.98 ± 1.43	0.09 ± 0.06	0.19 ± 0.21	<0.001	<0.001	0.161
SOD (U/mL)	31.34 ± 1.91	16.99 ± 0.32	16.68 ± 0.55	<0.001	<0.001	0.073
GSHPx (U/mL)	4.58 ± 0.69	0.65 ± 0.41	2.34 ± 0.70	<0.001	<0.001	<0.001
Paraoxonase (U/L)	24.81 ± 1.92	6.02 ± 1.74	4.80 ± 2.98	<0.001	<0.001	0.323
Arylesterase (kU/L)	28.78 ± 1.11	6.30 ± 1.17	17.91 ± 1.37	<0.001	<0.001	<0.001
MDA (μmol/L)	0.49 ± 0.16	1.42 ± 0.30	1.11 ± 0.08	<0.001	<0.001	0.007

Values are expressed as means ± SD. CAT = Catalase; MDA = Malondialdehyde; SOD = Superoxide dismutase; GSHPx = Glutathione peroxidase; p_1 = Results from comparing the control group with preoperative patient values; p_2 = Results from comparing the control group with postoperative patient values; p_3 = Results from comparing preoperative and postoperative patient values.

laryngeal cancer than in healthy subjects. However, after laryngectomy, the serum GSHPx activity levels increased significantly. Although there was still a significant difference in the activity levels observed in post-laryngectomy patients compared with those in control subjects, the elevations in serum GSHPx activity following surgery were encouraging.

SOD catalyzes the conversion of superoxide into less toxic compounds, and GSHPx protects against oxidative damage.[22] Liu et al.[15] and Kacakci et al.[16] reported decreased SOD activity in the laryngeal cancer group. Canbolat et al.[23] found no significant differences between the preoperative and postoperative SOD activity levels in patients with squamous cell laryngeal cancer. Conversely, decreased SOD activity was observed in breast, pancreas, and ovarian cancer patients.[24−26] In this study, the serum SOD activity levels were significantly reduced in laryngeal cancer patients compared with healthy control subjects. However, following laryngectomy, we found no significant alterations in serum SOD activity levels.

Hydroxyl radicals may be generated from hydrogen peroxide by the Fenton reaction.[27] However, this can be prevented by the antioxidant enzyme CAT. Kacakci et al.[16] reported decreased plasma CAT activity in patients with a laryngeal carcinoma. Dursun et al.[28] showed that the CAT activity levels were significantly lower in patients with esophageal cancer than in control subjects. We also observed that the serum CAT activity levels were significantly lower in patients with laryngeal cancer than in healthy subjects. However, no statistically significant changes in serum CAT activity were observed in patients following a laryngectomy.

PON1 has both PON and arylesterase activities.[29] Previous studies showed associations between PON1 and several cancers, including bladder, prostate, lung, and gastrointestinal cancers, and oral squamous cell carcinomas.[30−32] Karaman et al.[33] demonstrated that PON1 activity was significantly lower in laryngeal cancer patients than in control subjects. In this study, the serum PON and arylesterase activity levels were significantly reduced in patients with laryngeal cancer. After laryngectomy, serum arylesterase activity increased significantly, but there was no statistically significant change in serum PON activity levels.

MDA may act as a co-carcinogenic mediator.[30,34] Seven et al.[35] described how patients with laryngeal carcinomas had increased levels of lipooxidative damage. Similarly, Kacakci et al.[16] reported that patients with laryngeal carcinomas had increased plasma MDA levels compared with control subjects. Canbay et al.[21] also showed that laryngeal cancer patients had higher levels of MDA than control subjects. We observed that the serum MDA levels were significantly higher in laryngeal cancer patients than in healthy control subjects, but after the laryngectomies the serum MDA levels decreased significantly.

This study has some limitations. First, surgery was performed on only a few patients; therefore, the postoperative data are limited. Second, we did not measure the MDA levels, or GSHPx, SOD, and CAT enzyme activities, in cancerous laryngeal tissues. Some previous data have been obtained on oxidative stress markers in different tissues. Yigitbasi et al.[36] reported that the SOD activity levels in cancerous tissues were significantly lower than those observed in normal tissues, whereas no significant tissue-specific differences were observed in MDA levels or CAT activities. Kalayci et al.[37] found increased levels of GSHPx in cancerous tissues compared with adjacent cancer-free tissues; however, there were no significant differences in SOD activity. A study investigating epidermoid cancers of the head and neck region observed gradual decreases in tumoral tissue CAT and SOD enzyme activities as the diseases progressed.[38] These data are consistent with our results.

A recent study reported that supplementation with vitamin E, an antioxidant nutrient, was inversely correlated with the incidence of laryngeal cancer.[39] Antioxidants may have important roles to play in treating laryngeal carcinomas when the relationship between oxidative stress and pathogenesis is understood in greater detail.

Our findings suggest that in laryngeal cancer patients, oxidants increase and antioxidant levels decrease, resulting in a shift in the oxidant/antioxidant balance toward oxidative stress. Moreover, in laryngeal cancer patients, serum antioxidant enzyme activities increased, whereas oxidative stress levels decreased following laryngectomies. Increases in oxidants and decreases in antioxidants may play important roles in the etiopathogenesis of laryngeal cancer. Larger studies will be required to confirm and extend these results.

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