

An investigation of 8-hydroxy-2'-deoxyguanosine and 8-iso-prostaglandin F_{2α} levels in patients with larynx carcinoma

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Abstract

Objective: 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α}) are indicators of oxidative stress in the human body. The aim of the present study was to investigate serum 8-OHdG and 8-iso-PGF_{2α} levels in patients with larynx carcinoma and to compare them with healthy controls.

Methods: A total of 50 patients, consisting of 25 patients with larynx carcinoma (study group) and 25 healthy subjects (control group), were enrolled in the present study. Serum 8-OHdG concentration was measured using an ELISA kit, whereas an enzyme immunoassay kit was utilized for the measurement of serum 8-iso-PGF_{2α} levels. TNM stages of the patients were recorded from the patient records in the study group.

Results: 8-OHdG and 8-iso-PGF_{2α} levels were significantly higher in patients with larynx carcinoma than the control subjects. 8-OHdG levels were significantly higher in patients with LN metastases than the patients without LN metastases in the study group.

Conclusion: The findings of the present study showed the presence of significantly increased serum 8-OHdG and 8-iso-PGF_{2α} levels in patients with larynx carcinoma, which may support the role of oxidative stress in the development laryngeal cancers.

Keywords: Larynx cancer, oxidative stress, 8-hydroxy-2'-deoxyguanosine, prostaglandin F_{2α}.

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Introduction

Larynx carcinoma represents approximately one-third of all head and neck cancers and is mostly observed in men between the ages of 50 and 70.^[1] Smoking, alcohol, exposure to environmental factors such as asbestos, polycyclic aromatic hydrocarbons and textile dust, dietary factors, HPV infection, advanced age, exposure to asbestos, gasoline fumes and radiation have been implicated in the pathogenesis of laryngeal carcinoma. Among these etiological factors, smoking and alcohol consumption represent the most significant risk factors, which have demonstrated a linear association with the development of laryngeal cancer.^[2]

Typically in the human body, there is a strict balance between antioxidants and free radicals. However, if this critical balance deteriorates due to pathological circumstances, oxidative stress and formation of excessive free radicals occurs.^[3] In tissues, metabolic and structural damage occurs due to the reaction of free radicals with different molecules such as deoxyribonucleic acid (DNA), proteins and membrane phospholipids.^[4,5] More than 20 products that contribute to carcinogenesis are formed due to DNA base damage. Guanine is the DNA base most susceptible to damage and 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the best known guanine product.^[6,7] 8-OHdG is accepted as an indicator of oxidant-induced DNA damage. It is reported to be increased with different cancers.^[8-10]

Lipid peroxidation caused by free radicals is an important pathological process involving the oxidation of polyunsaturated fatty acids in biological membranes.^[11] Oxidative stress through lipid peroxidation is considered to play an important role in the development of multiple diseases including carcinogenesis. Isoprostanes, a family of prostaglandin-like compounds, are the by-products of free radical-catalyzed oxidation of arachidonic acid. 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$), a major isoprostane,^[12] remains very stable in body fluids and tissues and therefore is considered as the most ideal index for detection of excessive chemical lipid peroxidation and the extent of free radical oxidation in humans.^[13]

In the present study, we aimed to investigate 8-OHdG and 8-iso-PGF $_{2\alpha}$ levels in patients with larynx carcinoma and to evaluate their possible association with tumor, node and metastasis (TNM) staging of patients.

Materials and Methods

Ethical approval for the present study was obtained from

the local ethics committee (11.04.2017-04). A total of 50 patients, consisting of 25 patients with larynx carcinoma (study group) and 25 healthy subjects (control group) were enrolled in the present study. None of the participants had any history of smoking or alcohol consumption in the last one year, previous or synchronous cancer, or chronic disease. TNM stages of the patients were recorded from patient files. Five milliliters of blood were collected from each participant after 8 hours of fasting and centrifuged at 3500 rpm for 15 minutes. The serum was subsequently frozen and stored at -70°C . 8-OHdG concentration was measured using an ELISA kit (Cell Biolabs, CA, USA) in accordance with the manufacturer's instructions. The absorbance values of 8-OHdG were measured at 450 nm using a microplate reader. 8-iso-PGF $_{2\alpha}$ was measured using an enzyme immunoassay kit (Cell Biolabs, CA, USA). Absorbance from the enzymatic reaction was detected at 412 nm. The values of serum 8-OHdG and 8-iso-PGF $_{2\alpha}$ were expressed as picograms per millilitre (pg/mL). Blood samples were collected after the histopathological diagnosis of larynx carcinoma but before the initiation of treatment of the disease in the study group.

Statistical analysis was performed using SPSS version 16 Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). Independent-samples t test was used to compare the parametric conditions of the two groups and chi-square for comparison of categorical variables. A p value less than 0.05 was considered significant for all comparisons.

Results

All participants were male and the mean ages of the study and control group were 61.5 ± 7.7 and 60.9 ± 8.5 years, respectively ($p=0.136$). There were seven patients with LN metastases in the neck. 8-OHdG and 8-iso-PGF $_{2\alpha}$ levels were significantly higher in the study group than the control group ($p<0.001$) (Table 1). 8-OHdG levels were also significantly higher in patients with LN metastases than the patients without LN metastases in the study group ($p=0.015$), whereas 8-iso-PGF $_{2\alpha}$ levels were not significantly different between the patients with and without LN metastases (Table 2). Two patients had a distant metastasis in the study group, however, the number of patients with distant metastasis was not sufficient for a statistical comparison in terms of 8-OHdG and 8-iso-PGF $_{2\alpha}$. The mean values of 8-OHdG and 8-iso-PGF $_{2\alpha}$ levels according to the subgroups of T stages in the study group are shown in Ta-

Table 1. Mean values of 8-OHdG and 8-iso-PGF_{2α} levels in the study and control groups.

	Study group (n=25)	Control group (n=25)	p
8-OHdG (pg/mL)	0.394±0.065	0.134±0.098	<0.001
8-iso-PGF _{2α} (pg/mL)	461.1±53.0	221.2±59.2	<0.001

8-OHdG: 8-hydroxy-2'-deoxyguanosine, 8-iso-PGF_{2α}: 8-iso-prostaglandin F_{2α}, pg/mL: picograms per millilitre

Table 2. Mean values of 8-OHdG and 8-iso-PGF_{2α} levels in patients with and without LN metastasis in the study group.

	Lymph node Positive (n:7)	Lymph Node Negative (n:18)	P value
8-OHdG (pg/mL)	0.443±0.073	0.374±0.052	0.015
8-iso-PGF _{2α} (pg/mL)	480.0±35.4	453.8±57.7	0.278

8-OHdG: 8-hydroxy-2'-deoxyguanosine, 8-iso-PGF_{2α}: 8-iso-prostaglandin F_{2α}, LN: lymph node, pg/mL: picograms per milliliter

ble 3. Although the number of patients was insufficient for a statistical evaluation, we observed that 8-OHdG levels were increased as the T stage of the tumor increased in patients with larynx carcinoma.

Table 3. Mean values of 8-OHdG and 8-iso-PGF_{2α} levels according to the subgroups of T stages in the study group.

T stage	8-OHdG (pg/mL)	8-iso-PGF _{2α} (pg/mL)
T1 (n=3)	0.322±0.038	465.3±103.8
T2 (n=8)	0.366±0.053	439.2±60.6
T3 (n=8)	0.391±0.045	459.3±30.0
T4 (n=6)	0.472±0.045	490.8±30.6

8-OHdG: 8-hydroxy-2'-deoxyguanosine, 8-iso-PGF_{2α}: 8-iso-prostaglandin F_{2α}, n: number of patients, pg/mL: picograms per millilitre

Discussion

Oxidative stress indicators have been previously investigated in head and neck tumors including larynx carcinoma in different studies.^[10,14,15] Kumar et al^[10] measured the val-

ue of salivary total antioxidant capacity, glutathione, and free radicals including reactive nitrogen species and reactive oxygen species (ROS) in 100 patients with squamous cell carcinoma (SCC) of the head and neck. The findings of their study revealed an oxidant/antioxidant imbalance, which indicates a significant role of oxidative stress in SCC of the head and neck. Doğan et al^[14] found the values of total oxidative stress parameters to be significantly higher in malignant head and neck tumors than in benign tumors. Bozan et al^[15] demonstrated a significant decrease in serum antioxidants including superoxide dismutase, glutathione peroxidase, catalase, and paraoxonase with an increase of serum malondialdehyde in patients with laryngeal cancer. The authors concluded that the oxidant/antioxidant balance shifted toward oxidative stress in laryngeal cancer. In the present study, we aimed to investigate 8-OHdG and 8-iso-PGF_{2α} levels, which are important indicators of oxidant-induced DNA damage, free radical oxidation and lipid peroxidation. Serum 8-OHdG and 8-iso-PGF_{2α} levels were significantly higher in patients with larynx carcinoma than in healthy controls. Also, serum 8-OHdG levels were significantly higher in laryngeal cancer patients with LN metastases than in patients without LN metastases.

DNA mutation as a result of oxidative stress is a critical step in carcinogenesis, especially during the initiation

process. Increased levels of DNA by-products expressed during oxidative stress such as 8-OHdG have been demonstrated in various tumors.^[8-10,16] Kumar et al.^[10] found saliva 8-OHdG levels 1.6-fold higher in patients with SCC of the head and neck than in control subjects. Kaur et al.^[17] compared saliva 8-OHdG levels between patients with oral precancerous lesions, cancer and a control group of healthy age- and gender-matched individuals. The authors demonstrated a significant increase in saliva 8-OHdG levels in patients with oral SCC and precancerous lesions. The 8-OHdG levels were also investigated in patients with laryngeal tumoral lesions. In a study composed of 53 patients with benign vocal cord lesions and larynx SCC and 64 controls, the authors demonstrated that blood and urine 8-OHdG levels were significantly higher in patients with larynx SCC than in control subjects.^[8] Also, 8-OHdG levels were significantly higher in tumor tissues than in non-tumor tissue and benign vocal cord lesion tissues in that study. In the present study, serum 8-OHdG levels were significantly higher in patients with larynx carcinoma than the control group. Also, 8-OHdG levels were significantly higher in patients with LN metastases than in patients without LN metastases in the study group. These results may indicate an association between DNA damage induced by oxidative stress and laryngeal cancer. Furthermore, the higher 8-OHdG levels in patients with LN metastases may suggest a possible relation between regional metastasis and 8-OHdG, which should be evaluated with further studies including more participants. In the present study, we also observed that 8-OHdG levels increased as the T stages of the patients increased. However, this finding had no statistical evidence due to the low number of patients in T subgroups.

Although ROS act as signal molecules inducing cell growth, migration and differentiation at physiological concentrations in the human body, ROS can oxidize lipids and proteins, which can cause production of 8-isoprostane at higher concentrations.^[18,19] Higher urinary isoprostane levels were previously reported in different cancers such as prostate^[20] and lung cancer.^[21] Senghore et al.^[22] investigated 8-OHdG and 8-isoprostane levels in patients with potentially malignant oral disorders including submucous fibrosis or leukoplakia. The authors found a significant correlation between 8-isoprostane levels and an increased risk of potentially malignant oral disorders. In the present study, 8-iso-PGF_{2α} levels were found to be significantly higher in patients with larynx carcinoma than in healthy

controls, which may support the role of oxidative stress and lipid peroxidation in the development of laryngeal cancer.

Increased levels of 8-OHdG and 8-iso-PGF_{2α} in smokers have been shown in different studies.^[23,24] Mazlumoglu et al.^[8] reported that urinary and blood 8-OHdG values were slightly higher in smokers than in those who never smoked or in former smokers among patients with larynx cancer. However, the authors stated that this result may be due to the low number of participants in their study. In the present study, we included only patients who were non-smokers or who gave up smoking for at least one year to prevent the confounding effect of smoking on 8-OHdG and 8-iso-PGF_{2α} levels.

The major limitation of the present study was the absence of 8-OHdG and 8-iso-PGF_{2α} measurements in different sample types obtained from the participants. Since these markers can be detected in blood, tumoral tissue, saliva and urine samples, simultaneous measurements of 8-OHdG and 8-iso-PGF_{2α} values in these samples would provide additional data. Secondly, the present study had a limited number of patients, especially for demonstrating the difference of 8-OHdG and 8-iso-PGF_{2α} levels between patients with different T stages. Although 8-OHdG levels were found to be increased as the T stages increased, the number of patients in the subgroups of T stages was insufficient for statistical evaluation.

Conclusion

The findings of the present study showed the presence of significantly increased serum 8-OHdG and 8-iso-PGF₂ levels in patients with larynx carcinoma, which may support a role of oxidative stress in the etiopathogenesis of laryngeal cancers. However, the association between the levels of these markers and TNM stages should be investigated with further studies with larger numbers of participants.

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Ethics Committee Approval: The study protocol was approved by the local ethics committee (No: 11.04.2017-04).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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U.D.; Confirming the accuracy of the data and the analyses – Y.S.Ç., N.B.

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