European Archives of Oto-Rhino-Laryngology (2019) 276:1197–1204
https://doi.org/10.1007/s00405-019-05328-5

HEAD & NECK

An investigation of oxidative stress and coenzyme Q10 levels in patients with head and neck squamous cell carcinomas

Ahmet Aksoy[1] · **Senem Çengel Kurnaz**[2]

Received: 8 December 2018 / Accepted: 31 January 2019 / Published online: 7 February 2019

Abstract

Objective The purpose of this study was to determine the oxidative states of head and neck squamous cell carcinoma (HNSCC) patients by measuring their plasma levels of malondialdehyde (MDA), an indicator of lipid peroxidation, 3-nitrotyrosine (3-NT), an indicator of protein oxidation, and the coenzyme Q10 (CoQ10), an important antioxidant, and compare them with healthy individuals.

Materials and methods The plasma MDA, 3-NT and CoQ10 levels of 35 patients and 20 healthy individuals were measured with the high-performance liquid chromatography (HPLC) method. By comparing the patients' smoking habits, stage of the disease, size of the primary tumor and the presence of lymph nodes and the values of healthy individuals, the oxidative stress load of HNSCC patients was determined.

Results The mean plasma MDA levels of carcinoma patients were two times higher than those of healthy individuals ($p < 0.001$). When the mean plasma 3-NT levels of patients and healthy individuals were compared, no significant difference was found ($p > 0.05$). The mean plasma CoQ10 level of patients was low when compared with healthy individuals; however, no significant difference was detected ($p > 0.05$). In addition, as the stage and tumor size increased in HNSCC patients, their non-enzymatic antioxidant levels significantly decreased ($p < 0.05$).

Conclusions In HNSCC patients, lipo-oxidative damage increased while nitrosative stress did not change; however, antioxidant activity decreased which in turn increased both lipid peroxidation and oxidative stress. These findings support the contention that oxidative stress strongly reflects the health status of HNSCC patients.

Keywords Squamous cell carcinoma · Coenzyme Q10 · Malondialdehyde · 3-Nitrotyrosine · Oxidative stress

Introduction

Head and neck cancers, which develop in the upper aerodigestive tract, including the lips, oral cavity, paranasal sinus, pharynx and larynx, show similar biological behaviors [1, 2]. Epidemiological data show that cigarette and alcohol consumption are major risk factors for the disease [3]. HNSCCs are the cancer types most strongly associated with oxidative stress. Cigarette and alcohol consumption, which are known to cause the formation of reactive oxygen species (ROS), make a major contribution to the etiology of the disease [4].

Free radicals are substances that have one or more unpaired electrons in atomic or molecular structures. When they come in contact with other molecules in the cell they easily react, with the reaction products causing oxidative stress [5, 6]. Free radicals come into contact with a great number of simple and complex molecules such as membrane phospholipids, proteins, deoxyribonucleic acid (DNA) and carbohydrates. The reactions cause structural and metabolic changes in cells which result in the loss of function in many tissues and cells [6, 7].

Oxidative stress, as a result of the imbalance between ROS manifestation and inactivation, can play a role in the pathogenesis of many diseases, including cancer [8]. During carcinogenesis, lipid peroxidation and protein oxidation in the body increase and antioxidant systems are activated [9, 10].

✉ Ahmet Aksoy
aksoyahmet@ohu.edu.tr

[1] Department of Otorhinolaryngology, Faculty of Medicine, Nigde University, Fertek Mahallesi 51000 Merkez/Nigde, Turkey

[2] Department of Otorhinolaryngology, Faculty of Medicine, Bahcesehir University, Istanbul, Turkey

The main fatty acids that undergo lipid peroxidation in the cell membrane are the polyunsaturated fatty acids. Malondialdehyde (MDA) is a product of the peroxidation of fatty acids that contain three or more pairs of bonds [11]. MDA is an extremely reactive aldehyde that reacts with the free amino group of proteins, such as phospholipids and nucleic acids. It causes cytotoxicity, mutagenicity, membrane destruction and enzyme modification [12]. The MDA level is a commonly used indicator of the level of cell damage caused by oxidative stress [13].

Protein oxidation results from the covalent modification of proteins into ROS or oxidative stress products [6]. 3-Nitrotyrosine (3-NT) is produced as the stable end product of tyrosine nitrated by the peroxynitrite radical ($ONOO^-$) or as a result of the reaction of nitric oxide with other radicals. 3-NT is associated with many diseases and is an indicator of NO-dependent in vivo damage. Recently, it has also been reported as an indicator of inflammation [14, 15].

CoQ10 is involved in the maintenance of membrane stability, energy conversion and biochemical processes such as ATP production. It is an antioxidant which removes free radicals and inhibits lipid and protein peroxidation. It is exposed to continuous redox processes. CoQ10 levels are generally reported to decrease in cancer patients. Reduced levels of CoQ10 have been found in colon, breast, lung, pancreas and prostate cancers [16].

The aim of this study was to measure the lipid peroxidation and protein oxidation levels (plasma malondialdehyde and 3-nitrotyrosine) and antioxidant activities (plasma coenzyme Q10) of patients diagnosed with HNSCC and to determine their oxidative states. The results were compared on the basis of the patients' smoking habits, stage of the disease, size of the primary tumor, the presence/absence of lymph nodes and with the values of healthy individuals and oxidative stress status of HNSCC patients.

Patıents and methods

Subjects

This study was conducted retrospectively with patients who attended the tertiary care center of Ondokuz Mayis University, Faculty of Medicine, Otolaryngology Department, between June 2012 and February, 2013, and who were diagnosed with HNSCCs. Ethics committee approval (2012/07) was received from Ondokuz Mayis University, Faculty of Medicine and financial support was provided by the scientific research project management office of Ondokuz Mayis University (TIP1904.12.030). The patients and a group of healthy individuals were informed in detail about the study and they each signed an informed consent form. Thirty-five patients (31 males/4 females) who were diagnosed with squamous cell carcinomas (SCCs) in their head–neck area for the first time and who had not had any treatment (surgical and/or radiochemotherapy) previously, and 20 healthy individuals (10 males/10 females) were included in the study. The ages of the patients ranged between 26 and 83 years (55.49 ± 12.72 years) and the ages of the control group ranged between 41 and 62 (46.3 ± 5.32 years). Patients who had been treated for head and neck cancer (HNC) previously (surgery, radiotherapy, chemotherapy), or who had a systemic disease (including diabetes mellitus, high blood pressure, coronary artery diseases, chronic obstructive lung disease or chronic renal failure), and those who had a cancer history other than the head–neck area, were not included in the study. Those in both groups who had received vitamin supplementation were also excluded from the study. Twenty-seven (77%) of the patients had a smoking history and 13 (37%) of them had a history of alcohol consumption. Until the day plasma samples were to be collected, the patients' medical histories, their cigarette and alcohol consumption habits, their head–neck examination findings, their endoscopic examinations and their imaging and biopsy results were recorded. All the patients had complete blood counts, detailed biochemical evaluation, electrocardiography, blood clotting tests, chest X-ray and blood-type assessment. The patients who were found to have metastasis, or were suspected of it in their neck examination, had neck ultrasonography or head–neck contrasted computerized tomography that included the primary lesion, or magnetic resonance imaging, based on the requirements. The patients for whom there was a likelihood of systemic metastasis had thoracic or abdominal scans. In addition, all of the patients had been recently diagnosed and they had not had any oncological treatment. Tumor staging for the patients was done according to the American Joint Committee for Cancer (AJCC) HNC tumor–node–metastasis (TNM) system that was published in 2010 [17]. The diagnoses were based on biopsies taken under local or general anesthesia from lesions detected during examination.

Blood sampling

Before the planned oncologic treatments (surgery and/or radiochemotherapy), 10 mL of venous blood was taken in tubes (containing EDTA) from the peripheral veins of patients diagnosed with HNSCC from biopsy results. Before samples were taken, the patients were asked if they had eaten and their blood samples were taken after they had eaten a meal. The blood samples were refrigerated. Total blood was centrifuged (Nüve, Turkey) at +4 °C and 3000 rpm for 10 min and the resulting plasma samples were kept in deep freezers (− 20 °C, Profilo, Turkey) until analysis.

Biochemical measurements

MDA analysis

For the analysis of the MDA level in plasma, the end product of lipid peroxidation, the method specified in Yoshioka et al. [18] (1979) and Eraslan et al. [19] (2007) was used and the method specified in Agarwal and Chase [20] (2002) and Chang et al. [21] (2007) was used for its analysis with HPLC (high-performance liquid chromatography). The measurements were made with a fluorescence detector (FLD) in a HPLC (Shimadzu prominence 20A, Japan) system. The absorbance of the pink color that arose as a result of the reaction between MDA and 2-thiobarbituric acid (TBA) at 515-nm excitation level and 553-nm emission level was recorded. Plasma MDA values were generated by calibration graphics from MDA standards and are stated in µM (µmol/L).

3-NT analysis

Plasma 3-NT analysis was done using a commercial kit (Eureka 2010, LabDivision, Chiaravalle (AN) Italy) and was based on HPLC principles. A diode array detector (DAD) in an isocratic HPLC system was used for the measurements. The industrial kit's column and mobile phases were used for this measurement. The color intensity was measured by absorbance at 232 nm with a DAD detector. Plasma 3-NT values were generated by employing calibration graphics from 3-NT standards and are stated in µg/L.

KoQ10 analysis

The plasma KoQ10 level was measured with a commercial kit (ImmuChrom IC 1700, Heppenheim—Germany) and by using the HPLC principle. The measurements were made with a DAD detector coupled with an isocratic HPLC system. The industrial kit's column and mobile phases were used for this measurement. The color intensity was measured as absorbance at 275 nm with a DAD detector. Plasma KoQ10 values were generated by employing calibration graphics from KoQ10 standards and are stated in µg/mL.

Statistical analysis

All results are given as the mean ± standard deviation (mean ± SD) and median [minimum (min), maximum (max)] values. For statistical analysis and correlation analysis, independent-Student's *t* test, Mann–Whitney *U* test and Kruskal–Wallis variance analysis were used. The data collected was analyzed with "SPSS for Windows 15.0" (Statistical Package for the Social Sciences).

Results

In this study, the most frequent localization of primary tumor in the head and neck areas was the larynx with 18 patients (51%). Twelve of the larynx cancer patients (67%) had glottic localization while six (33%) had supraglottic localization. The tumor stage distributions of the HNSCC patients were as follows: 12, 14, 2 and 7 patients were in stages I, II, III and IVA, respectively (Table 1).

Table 2 shows the comparison of oxidative state and non-enzymatic antioxidant concentration levels between HNSCC patients and healthy individuals. Although the oxidative stress loads were higher and antioxidant capacity levels were lower in HNSCC patients with a smoking habit, no significant difference was detected ($p > 0.05$). Since the 3-NT levels in the plasma of 11 SCC patients and 8 healthy individuals were below the mean, their data were not included in the study.

Mean plasma MDA levels were significantly higher for Stage I, II and IVA patients than for the healthy control group ($p < 0.0001$). The level was significant for Stage III when compared with the healthy control group ($p < 0.05$). No significant differences were found between the CoQ10 and 3-NT levels for stages I, II, III and IVA, and the healthy controls ($p > 0.05$). The mean plasma MDA level increased as the stage of the disease progressed. The plasma MDA levels of both early and late stage HNSCC patients were significantly higher than those of the healthy individuals ($p < 0.0001$). In addition, the plasma MDA levels of late stage HNSCC patients were significantly higher than those of the early stage patients ($p < 0.01$). No significant differences were found between the 3-NT levels of early and late stage HNSCC patients and healthy individuals ($p > 0.05$). Furthermore, the plasma CoQ10 levels of late stage cancer

Table 1 Clinical TNM stages of HNSCC patients

Stages of the patients	Clinical staging (TNM)	Total
Stage I	T1N0M0	12
Stage II	T2N0M0	12
	T1N1M0 (nasopharynx)	2
Stage III	T3N0M0	1
	T3N1M0	1
Stage IVA	T4aN0M0	4
	T4aN1M0	1
	T4aN2aM0	2
Total		35

patients were significantly lower than those of the early stage patients ($p < 0.05$) (Table 3).

When the relationship between primary tumor size and MDA level was considered, the mean plasma MDA values for T3 and T4a were higher than the values for T1 and T2. The oxidative stress load was significantly higher for T1, T2 and T4a than for the control group ($p < 0.0001$). In addition, the oxidative stress level was significantly higher for T3 than for the control group ($p < 0.05$). When the mean plasma 3-NT values for all T stages and the control group were compared, no significant difference was found ($p > 0.05$). Regarding the relationship between the increase in primary tumor size and antioxidant enzyme level, antioxidant enzyme levels decreased as the tumor size increased. In addition, when the mean plasma CoQ10 levels were compared, those of the T3 and T4a groups decreased when compared with T1 and T2, and the control group, and the difference was significant ($p < 0.01$) (Table 4).

When the relationship between the lymph node stage, and lipid peroxidation and nitrosative stress was studied in HNSCC patients, N(+) patients had higher mean plasma MDA and 3-NT levels than N(−) patients; however, the difference between the means was not significant ($p > 0.05$). Furthermore, when the relationship between the lymph node stage and antioxidant levels was studied, N(+) patients had a lower mean plasma CoQ10 level than N(−) patients but the difference was not significant ($p > 0.05$). The oxidative stress load in HNSCC patients was not related to the presence or absence of lymph nodes ($p < 0.01$). When the relationship between the presence of lymph nodes and antioxidant levels was investigated, N(+) patients had lower antioxidant levels than the control group but the difference was not significant ($p > 0.05$) (Table 5).

Table 2 The comparison of mean plasma MDA, 3-NT and CoQ10 levels of head–neck SCC patients with the control group

Plasma	Patient group		Control group		*p* value
	Mean ± SD	Med (min, max)	Mean ± SD	Med (min, max)	
MDA (μM)	1.43 ± 0.58 (*n*: 35)	1.21 (0.69; 2.68)	0.75 ± 0.24 (*n*: 20)	0.68 (0.40; 1.52)	p < 0.001*
3-NT (μg/L)	51.40 ± 20.74 (*n*: 24)	56.22 (12.87; 84.38)	65.17 ± 18.90 (*n*: 12)	64.59 (35.37; 94.80)	*p* > 0.05
CoQ10 (μg/mL)	0.604 ± 0.327 (*n*: 35)	0.540 (0.130; 1.430)	0.624 ± 0.284 (*n*: 20)	0.597 (0.150; 1.310)	*p* > 0.05

*$p < 0.05$ was considered statistically significant in the analysis of the differences between means

Table 3 The comparison of early and late stage tumors and mean plasma MDA, 3-NT and CoQ10 levels of HNSCC patients

Plasma	Early stage (stages I + II)	Late stage (stages III + IVA)	Control group
MDA (μM) (Mean ± SD)	1.30 ± 0.52[a] (*n*: 26)	1.83 ± 0,59*[b] (*n*: 9)	0.75 ± 0.24 (*n*: 20)
3NT (μg/L) (Mean ± SD)	51.71 ± 20.80 (*n*: 20)	49.86 ± 23.58 (*n*: 4)	65.17 ± 18.90 (*n*: 12)
CoQ10 (μg/mL) (Mean ± SD)	0.677 ± 0.340 (*n*: 26)	0,394 ± 0.165**[c] (*n*: 9)	0.624 ± 0.284 (*n*: 20)

[a]$p < 0.0001$ early stage MDA levels when compared with control group

[b]$p < 0.0001$ late stage MDA levels when compared with control group

[c]$p < 0.05$ late stage CoQ10 levels when compared with control group

*$p < 0.01$ late stage MDA levels when compared with early stages

**$p < 0.05$ late stage CoQ10 levels when compared with early stages

Table 4 The comparison of tumor size and mean plasma MDA, 3-NT and CoQ10 levels of HNSCC patients

Plasma	T1–T2	T3–T4a	Control group
MDA (μM) (Mean ± SD)	1.31 ± 0.52[a] (*n*: 27)	1.85 ± 0.63*[b] (*n*: 8)	0.75 ± 0.24 (*n*: 20)
3-NT (μg/L) (Mean ± SD)	52.48 ± 20.58 (*n*: 21)	43.85 ± 24.84 (*n*: 3)	65.17 ± 18.90 (*n*: 12)
CoQ10 (μg/mL) (Mean ± SD)	0.675 ± 0.334 (*n*: 27)	0.366 ± 0.152**[c] (*n*: 8)	0.624 ± 0.284 (*n*: 20)

[a]MDA level of T1–T2 compared with the control group; $p < 0.0001$

[b]MDA level of T3–T4a compared with the control group; $p < 0.0001$

[c]CoQ10 level of Stages T3–T4a compared with the control group; $p < 0.05$

*$p < 0.05$; MDA level of Stages T3–T4a compared with Stages T1–T2

**$p < 0.01$; CoQ10 level of Stages T3–T4a compared with Stages T1–T2

Table 5 The comparison of lymph node and plasma mean MDA, 3-NT and CoQ10 levels of HNSCC patients when compared with the control group

Plasma	Lymph node (N0)	Lymph node (N+)	Control Group
MDA (μM) (Mean ± SD)	1.41 ± 0.60* (*n*: 29)	1.54 ± 0.50** (*n*: 6)	0.75 ± 0.24 (*n*: 20)
3-NT (μg/L) (Mean ± SD)	49.31 ± 20.30 (*n*: 22)	74.46 ± 9.28 (*n*: 2)	65.17 ± 18.90 (*n*: 12)
CoQ10 (μg/mL) (Mean ± SD)	0.635 ± 0.347 (*n*: 29)	0.456 ± 0.146 (*n*: 6)	0.624 ± 0.284 (*n*: 20)

*$p < 0.0001$; MDA level of N(0) compared with the control group

**$p < 0.01$; MDA level of N(+) compared with the control group

Discussion

Increasing numbers of empirical and epidemiological studies have reported that free radicals play a role in the pathogenesis of cancer development [22]. Since there is no 'gold standard' technique for the measurement of free radical activity, the determination of endogenous antioxidant levels, measurement of the level of products arising from the oxidation of macro-molecules and the direct measurement of free radical levels have been adopted as the three main approaches [23].

A great number of studies have measured the levels of plasma and cell antioxidants such as CoQ10 and the cellular activities of antioxidant enzymes to assess endogenous antioxidant capacity. The most important product of lipid peroxidation is MDA. The plasma MDA level is a measure of the lipid peroxidation level which in turn is an indicator of free radical-mediated damage [11]. In determining the extent of protein oxidation, 3-NT is a stable, reliable and commonly used marker of reactive nitrogen species (RNS) oxidant ($NO^{\cdot}$ and ONOO-) levels [23].

The purpose of this study was to measure the plasma MDA, 3-NT and plasma CoQ10 levels of patients diagnosed with HNSCC to characterize their oxidative states. The results were assessed in the context of the patients' smoking habits, stage of the disease, size of the primary tumor, the presence/absence of lymph nodes and compared with the values of healthy individuals to reveal the oxidative stress level of HNSCC patients. In the determination of plasma MDA, 3-NT and CoQ10 levels, the HPLC method was used because of its accuracy.

As well as nutritional factors, various etiological factors such as cigarette and alcohol consumption and oncogenic viruses (human papilloma virus, Ebstein–Barr virus) are known to be responsible for HNC [4, 24]. Canbay et al. [25] reported that HNC patients who smoked had higher lipid peroxidation product concentrations than non-smokers but no significant difference was found ($p > 0.05$). They also observed significantly lower antioxidant enzyme levels in the patients who smoked than in non-smokers ($p < 0.05$). The results of the current study support the results of the study of Canbay et al. [25] in that the MDA levels of the HNSCC patients who smoked and did not smoke were high but not significantly different ($p > 0.05$). Similarly, there was no significant difference in the CoQ10 levels of cancer patients based on smoking status ($p > 0.05$).

Rasheed et al. [9] reported that the MDA levels of smoking and non-smoking HNC patients were significantly higher than those of healthy individuals ($p < 0.001$). Furthermore, they found that the antioxidant levels (SOD, catalase, glutathione peroxidase, glutathione and ascorbic acid) of smoking and non-smoking HNC patients were lower than those of healthy individuals ($p < 0.001$). The current study found that the MDA levels of smoking and non-smoking HNC patients were significantly higher than those of the healthy individuals ($p < 0.001$). When the levels of the antioxidant CoQ10 of cancer patients and healthy individuals were compared on the basis of whether they smoked or not, no significant difference was found ($p > 0.05$).

MDA causes enzyme modification, cytotoxicity, membrane destruction, mutagenicity and carcinogenesis. It is an extremely reactive aldehyde, reacting with the free amino groups of proteins, such as phospholipids and nucleic acids, causing the effects mentioned.

Lipid peroxidation is a process involving the oxidation of polyunsaturated fatty acids which are basic components of biological membranes. Reactive electrophilic compounds are formed during lipid peroxidation. These compounds yield a number of adducts with DNA. In particular, adducts of deoxyguanosine with MDA, resulting from the reactions of DNA bases with epoxyaldehydes, are the most important group of adducts. The epoxyaldehydes are more reactive with DNA than the parent unsaturated aldehydes. The compounds resulting from lipid peroxidation mostly react with DNA, causing both genotoxicity and mutagenicity; among them, 4-hydroxynonenal is the most genotoxic and dMDA is the most mutagenic. DNA damage caused by the adduction of lipid peroxidation products with DNA may be repaired by the activity of glycosylases.

MDA is commonly used in the assessment of lipid peroxidation because it is a good indicator of cell damage [12, 26, 27]. Many studies have reported that in HNC patients that as lipid peroxidation increases in the plasma and antioxidant levels decrease, oxidative stress increases. Siddartha et al. [2], Malathi et al. [28] and Nisha et al. [24] stated that plasma MDA levels increase significantly in head–neck cancer patients when compared with healthy individuals while total antioxidant capacity decreases ($p < 0.0001$). In

agreement with the results of previous studies, the current study reports a significant increase in the mean plasma MDA level of HNC patients when compared with healthy individuals ($p < 0.001$). In addition, the mean plasma CoQ10 levels of HNC patients were lower than in healthy individuals but the difference was not significant ($p > 0.05$).

Kaur et al. [26] investigated the effect of oxidative stress in oral cavity cancer who detected MDA levels were higher in patients with HNSCC compared to control group ($p < 0.005$). Mrowicka et al. [29] reported a high level of MDA in the blood of patients with HNC. The results of the current study are in agreement with those of these two studies.

Nisha et al. [24] studied the relationships between plasma MDA levels, TNM stage of cancer, lesion type and histopathology in HNC patients but they did not find any significant differences. In their study, Shariff et al. [22] reported that late stage HNC patients had higher serum levels of MDA than early stage patients. Separately, Salzman et al. [13] reported that although the MDA levels of stages T3 and T4 HNSCC patients were higher than those of stage T1 andT2 patients, they did not find any significant differences ($p > 0.05$). In the current study, the MDA levels of N0 patients were higher than those of the N(+) patients but no significant difference was found ($p > 0.05$). However, when the relationships between MDA and CoQ10 levels, and tumor size stage and disease stage of cancer patients were investigated, the differences were significant ($p < 0.05$). In contrast, no significant difference was found between lymph node stage, and MDA and CoQ10 levels ($p > 0.05$). As the stage of HNC patients increased, lipid peroxidation increased and antioxidant enzyme levels decreased. This supports the view that oxidative stress increases as the severity of the disease HNC increases and thus antioxidant balance is in support of oxidative stress.

Proteins modified by oxidation, which arise from the attack of free radicals on protein structures, are generally used as an indicator of oxidative stress [14].In the literature, there are a number of studies on nitric oxide products, another parameter used in the determination of protein oxidation levels in HNC patients. However, the number of studies on the 3-NT levels of HNC patients is more limited. It has been reported that 3-NT is not detectable in the plasma and tissues of healthy individuals [15]. Yaman et al. [15] used HPLC, an extremely precise method but were unable to detect 3-NT in the plasma of healthy individuals.

In some diseases, however, 3-NT levels were sufficient to be detected in plasma, urine and tissue samples [30]. Bahar et al. [31] assessed the nitric oxide products in the saliva of oral cavity cancer patients. Nitrosative stress is reported to increase and the antioxidant activity to decrease in cancer patients when compared with healthy individuals. Dahiya et al. [32] reported that the concentration of nitric oxide products increased in patients with HNC in comparison with healthy individuals. In the current study, when the mean plasma 3-NT levels of HNC patients and healthy individuals were compared, no significant difference was found ($p > 0.05$). In addition, the 3-NT levels in cancer patients did not differ significantly in terms of smoking habit, stage of the tumor, size of the tumor and lymph node ($p > 0.05$).

There are a number of studies in the literature on the mean plasma CoQ10 levels of HNC patients but no studies on the relationship between the CoQ10 levels of HNC patients and clinical parameters were found. The studies on the CoQ10 levels of cancer patients include lung, breast and digestive system cancers [33–35]. Folkers et al. [34] reported that CoQ10 levels decrease significantly in cancer patients when compared with healthy individuals. They determined the average CoQ10 levels of 116 patients with different cancer types and compared them with healthy individuals. Five of these 116 patients were HNC patients and their average blood level of CoQ10 was 0.78 ± 0.25 µg/mL. However, no significant difference was found when compared with healthy individuals ($p > 0.05$). In the present study, the average plasma CoQ10 level of HNC patients was lower than that reported by Folkers et al. [34] but no significant difference was found when compared with healthy individuals (0.604 ± 0.327 µg/mL, $p > 0.05$).

Nevertheless, the CoQ10 levels of late stage HNC patients were lower than those of early stage patients and healthy individuals in the present study, and the difference between them was significant ($p < 0.05$). In addition, CoQ10 levels decreased in HNC patients as the tumor size increased ($p < 0.05$). Normally cells are in a reduced state. In the case of oxidative stress, the balance of oxidative stress–antioxidant state shifts to an oxidated state which causes lower antioxidant levels. A relationship between carcinogenesis and low CoQ10 concentration has been reported [36].

CoQ10 supplementation of the diet has been reported to have positive results in various diseases and in cases where CoQ10 is lacking [37]. With CoQ10 supplementation of the diet of cancer patients, a protective effect of CoQ10 against tumoral tissue can be induced [16, 38]. Folkers et al. [33] reported that the administration of CoQ10 increased macrophage activity and increased the survival rate of cancer patients.

Conclusions

Lipo-oxidative damage increased in head–neck cancer patients but the nitrosative stress level did not change. In addition, antioxidant activity decreased which in turn increased both lipid peroxidation and the level of oxidative stress. These findings support the contention that oxidative stress plays a role in head–neck cancer patients. It, therefore,

is becoming increasingly obvious that oxidative damage plays a role in a great number of pathological conditions. It also plays a role in the initial and progressive stages of both ROS and RNS cancers. However, the underlying mechanism is not yet fully understood. Therefore, more empirical studies are needed on the role of free radical-mediated tumor development.

Acknowledgements This study was submitted as an oral presentation at the 35th Turkish National Otolaryngology—Head and Neck Surgery Congress on Novemder 2–6, 2013, in Antalya, Turkey. The authors thank Gregory Thomas Sullivan of the University of Queensland in Brisbane, Australia, for editing the English in an earlier version of this manuscript.

Funding This study was funded as a Ondokuz Mayıs University Scientific Research Projects (projectnumber - TIP1904.12.030).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict related to this research.

Ethical approval Ethics committee approval (2012/07) was received from Ondokuz Mayis University, Faculty of Medicine. All procedures performed in this study that involved human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Brockstein B, Masters G (eds) (2003) Headand Neck Cancer. Kluwer Academic Publishers, Dordrecht pp 1–295. ISBN 1-4020-7336-4
2. Siddartha SB, Kumari S, Rathan S, Babu S, Shetty S (2011) Status of serum sialic acid, antioxidant and lipid peroxidation in head and neck cancer patients. Res J Pharma Biol Chem Sci 2(1):1–5
3. Nagal MA (1999) Genetic alterations in head and neck squamous cell carcinomas. Braz J Med Biol Res 32:897–904
4. Chen YJ, Chang JTC, Liao CT, Wang HM, Yen TC, Chiu CC, Lu YC, Li HF, Cheng AJ (2008) Head and neck cancer in the betel quid chewing area: recent advances in molecular carcinogenesis. Cancer Sci 99:1507–1514
5. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C (2007) Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 121:2381–2386
6. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8):118–126
7. Sies H, Stahl W, Sundquist AR (1992) Antioxidant functions of vitamins; vitamins E and C, beta-carotene, and other carotenoids. Ann N Y Acad Sci 30(669):7–20
8. Gupta A, Bhatt MLB, Misra MK (2009) Lipid peroxidation and antioxidant status in head and neck squamous cell carcinoma patients Oxid Med Cell Longev 2(2):68–72
9. Rasheed HM, Beevi SS, Geetha A (2007) Enhanced lipid peroxidation and nitric oxide products with deranged antioxidant status in patients with head and neck squamous cell carcinoma. Oral Oncol 43:333–338
10. Taysi S, Uslu C, Akcay F, Sütbeyaz MY (2003) Malondialdehyde and nitric oxide levels in the plasma of patients with advanced laryngeal cancer. Surg Today 33:651–654
11. Dotan Y, Lichtenberg D, Pinchuk I (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res 43:200–227
12. Hoff HF, O'Neil J (1993) Structural and functional changes in LDL after modification with both 4-hydroxynonenal and malondialdehyde. J Lipid Res 34:1209–1217
13. Salzman R, Pacal L, Kankova K, Tomandl J, Horakova Z, Tothova E, Kostrica R (2010) High perioperative level of oxidative stress as a prognostic tool for identifying patients with a high risk of recurrence of head and neck squamous cell carcinoma. Int J Clin Oncol 15:565–557
14. Ischiropoulos H, Al-Mehdi AB (1995) Peroxynitrite-mediated oxidative protein modification. FEBS Lett 364:279–282
15. Yaman H, Unlu A, Karabıcak U, Cimen B, Balabanlı B, Erbil MK, Torkozkan N (2000) Measurement of 3-Nitrotyrosine by high performance liquid chromatography. T Klin J Med Res 18:26–30
16. Gurkan AS, Dundar OB (2005) Coenzyme Q10. J Fac Pharm Ankara 34(2):129–154
17. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A (2010) AJCC Cancer Staging Manual, 7th ed., Springer, New York, pp 21–100. ISBN 978-0-387-88440-0
18. Yoshioka T, Kawada K, Shimada T, Mori M (1979) Lipid peroxidation in maternal and cord blood and protective mechanism against actived-oxygen toxicity in the blood. Am J Obstet Gynecol 135(3):372–376
19. Eraslan G, Saygi S, Essiz D, Aksoy A, Gul H, Macit E (2007) Evaluation of aspect of some oxidative stress parameters using vitamin E, proanthocyanidin and *N*-acetylcysteine against exposure to cyfluthrin in mice. Pestic Biochem Physiol 88:43–49
20. Agarwal R, Chase SD (2002) Rapid, flurimetric-liquid chromatographic determination of malondialdehyde in biological samples. J Chromatogr B 775:121–126
21. Chang SI, Jin B, Youn P, Park C, Park JD, Ryu DY (2007) Arsenic-induced toxicity and the protective role of ascorbic acid in mouse testis. Toxicol Appl Pharmacol 218:196–203
22. Shariff AK, Patil SR, Shukla PS, Sontakke AV, Hendre AS, Gudur AK (2009) Effect of oral antioxidant supplementation on lipid peroxidation during radiotherapy in head and neck malignancies. Indian J Clin Biochem 24(3):307–311
23. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18:872–879
24. NishaSachdeva A, Lal H (2008) Some oxidative stress related parameters in patients with head and neck carcinoma. Indian J Clin Biochem 23(1):38–40
25. Canbay E, Celik K, Kunt T, Ertemur M, Canbay E (2002) An alteration in glutathione peroxidase activity and lipid peroxidation levels in patients with laryngeal carcinoma. Cumhuriyet Medical-Journal 24(4):175–178
26. Kaur J, Politis C, Jacobs R (2016) Salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitamin C, and vitamin E in oral pre-cancer and cancer: diagnostic value and free radical mechanism of action. Clin Oral Investig 20(2):315–319. https://doi.org/10.1007/s00784-015-1506-4. Epub 2015 Jun 17
27. Murugaiyan SB, Ramasamy R, Nakkeeran M, Rangdhol V, Srinivasan AR, Niranjan G (2015) Urinary 8-hydroxydeoxyguanosine as a marker of oxidative stress induced genetic toxicity in oral cancer patients. Indian J Dent Res 26(3):226–230. https://doi.org/10.4103/0970-9290.162880

28. Malathi M, Vijay M, Shivashankara AR (2011) The role of oxidative stress and the effect of radiotherapy on the plasma oxidant-antioxidant status in head and neck cancer J Clin Diagn Res 5(2):225–249
29. Mrowicka M, Zielińska-Bliźniewska H, Pietkiewicz P, Olszewski J, Majsterek I (2015) Evaluation of selected indicators of antioxidant status in patients with head and neck cancer. Otolaryngol Pol 69(5):44–50
30. Herce-Pagliai C, Kotecha S, Shuker DEG (1998) Analytical methods for 3-nitrotyrosine as a marker of exposure to reactive nitrogen species: a review. Nitric Oxide Biol Chem 2(5):324–336
31. Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM (2007) Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 109(1):54–59
32. Dahiya K, Dhankhar R, Madaan H, Singh V, Arora K (2012) Nitric oxide and antioxidant status in head and neck carcinoma before and after radiotherapy. Ann Clin Lab Sci 42(1):94–97
33. Folkers K, Brown R, Judy WV, Morita M (1993) Survival of cancer patients on therapy with coenzyme Q10. Biochem Biophys Res Commun 192:241–245
34. Folkers K, Osterborg A, Nylander M, Morita M, Mellstedt H (1997) Activities of vitamin Q10 in animal models and a serious deficiency in patients with cancer. Biochem Biophys Res Commun 234:296–299
35. Portakal O, Özkaya Ö, İnal ME, Bozan B, Koşan M, İskender S (2000) Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. Clin Biochem 33(4):279–284
36. Trueba GP, Sanchez GM, Giuliani A (2004) Oxygen free radical and antioxidant defense mechanism in cancer. Front Biosci 9:2029–2044
37. Potgieter M, Pretorius E, Pepper MS (2013) Primary and secondary coenzyme Q10 deficiency: the role of therapeutic supplementation. Nutr Rev 71(3):180–188
38. Gaby AR (1996) The role of coenzyme Q_{10} in clinical medicine: part-I. Alt Med Rev 1(1):11–17