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Research Article

Comparison of Salivary and Plasma Antioxidant Levels in Oral SCC Patients and Healthy Subjects

Azizi A*, Dabirmoghadam P and Rima Hossein ZN

No 4-10, Neyestan Street, Pasdaran Avenue, Tehran, Iran

***Corresponding author:** Arash Azizi, No 4-10, Neyestan Street, Pasdaran Avenue, Tehran, Iran, E-mail: drarashazizi@yahoo.com

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Abstract

Introduction: Oral squamous cell carcinoma (oscc) is one of the most prevalent cancers. It has become more evident that cellular aging and decreased surveillance play role in the development of this disease. The aim of the present study was to evaluate the total antioxidant status (TAS) of saliva and serum in patients with oscc.

Materials and Methods: This study was designed as case-control. In total, 26 patients with oscc (14 women and 12 men) were enrolled as case group, and 28 self-admitted healthy people (14 women and 14 men) were selected as control group. Measurement of total antioxidant status was carried out in saliva and plasma.t-test was used for statistical analysis with significant level set at P<0.05.

Results: The mean ± SD of salivary TAS in case group was 0.72 ± 0.08, and in control group was 1.48 ± 0.1 mmol, respectively, and there were significant differences between groups (P<0.005). The mean ± SD of the plasma TAS in the case group and the control group was 1.02 ± 0.05 and 1.92 ± 0.21 mmol, respectively. There were significant differences between two groups (P<0.005).

Conclusion: Our results showed that salivary and plasma levels of total antioxidant status in oscc Patients were lower than those in healthy subjects. Keywords: Oral squamous cell carcinoma, plasma, Antioxidant, salivary gland.

Introduction

Oral squamous cell carcinoma (oscc) is one of the most prevalent cancers and is one of the 10 most common causes of death. Incidence of oral cancer is age related, which may reflect time for accumulation of genetic changes and duration of exposure to initiator such as physical irritants, viruses, hormonal effects, cellular aging, and decreased immunologic surveillance with aging [1]. There has been an increasing research interest in oxidation of biological systems including free radicals, oxidative stress, and antioxidant defense mechanisms in inflammatory and malignant diseases [2,3]. Reactive oxygen species (ROS) are involved in the etiology and the pathogenesis of a variety of diseases. Reactive oxygen species are generated in vivo by multiple mechanisms, including the respiratory redox chain in mitochondria, the respiratory burst of phagocytes, and the activity of various oxidases. The excess ROS can damage cellular lipids, proteins, or DNA inhibiting their normal function [3,4,5]. To defend such damage, the body possesses several antioxidant systems that are important in the prevention of oxidative stress, and some body fluids such as saliva contain such activity. Saliva is rich in several antioxidants, and the antioxidant systems of saliva are highly complex [3,6]. Saliva is the first biological fluid that encounters the irritants. The anticarcinogenic potential of saliva has been already demonstrated in a study where it was shown that saliva could significantly inhibit the initiation and progression of oral cancer in animal models. The salivary antioxidant system plays a very important role in the anticarcinogenic capacity of saliva [7]. As the oral cavity is the start of digestion system, substances like food and drinks encounter saliva in the first step. It has been shown that saliva includes many defensive mechanisms that is called salivary antioxidant system [3,8,9]. Investigation of the total antioxidant capacity of saliva could provide valuable information about deficiencies, and the problems in these systems may increase the risk of inflammatory disorders [3,10]. The balance and cooperation among the different antioxidants provides greater protection against oxidant agents. Antioxidant cumulative action and synergistic interaction of all antioxidants in body fluids are considerably important. It has been reported in a few studies that impaired antioxidant balance is responsible for the cause of scc. Some researchers showed that the amount of total antioxidant status in oral scc patients is lower than healthy subjects [11,12,13]. Beevi et al reported that enhanced lipid peroxidation with concomitant decrease in antioxidant is indicative of oxidative stress that provides evidence of the relationship between lipid peroxidation and oral cavity cancer [11]. Moreover, Agha-Hosseini et al. [13] reported that there was lower total antioxidant capacity in oral scc patients in comparison with healthy subjects. Azizi et al. [14] reported that salivary and plasma levels of total antioxidant status in erosive oral lichen planus patients that were lower than those in healthy subjects. Regarding the insufficient studies related to this issue, and important findings in etiological factors, especially in patients with oral scc, the aim of present study was to measure of total antioxidant status (TAS) in both saliva and serum of patients with oral scc and comparison it with healthy subjects.

Materials and Methods

This was a case–control study that was conducted in the dental faculty of Azad university medical sciences and Amir Alam hospital in Tehran-Iran .Twenty-six patients with who referred to oral medicine department of Azad university and Amir Alam hospital enrolled as a case group, (14 women and 12 men), and 28 self-admitted healthy people (14 women and 14 men) were selected as control group. Age and gender were matched in both groups. Diagnosis of oral scc (oscc) was made by clinical examination and confirmed by histological examination. The most frequent presentation of oscc was chronic ulcers with ill- defined borders, exophytic masses with bleeding and pain. For definitive diagnosis, biopsy was always carried out. Histopathology features for diagnosis of oscc were dysplasia of cellular that involved epithelium and basement membrane. Some of them had inflammatory infiltration. All of specimens were diagnosed by two oral medicine and oral pathologists. Exclusion criteria in both groups included subjects who had received any systemic treatment such as systemic steroids or other immunosuppressive drugs as well as NSAIDS and intake of any supplementary vitamins, for the last 8 weeks, and topical medications for the last 4 weeks prior to sample collection. Also, patients with a history of trauma, any surgery 4 weeks

prior to sampling, and Patients with systemic and periodontal diseases (periodontal pocket more than 3mm) were not included. Smoking and alcohol drinking were matched in both groups. Matching of smoking was determined by question from patients. For example if there was nonsmoker subject in case group, we would have selected other nonsmoker subject with the same age and sex and other characteristic adjusted with exclusion criteria in control group. The pack-year is a unit to measure the amount a person has smoked over a long period of time. It is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. For example, 1 pack-year is equal to smoking 20 cigarettes (1 pack) per day for 1 year, or 40 cigarettes per day for half a year, and so on [14]. The Ethics committee of Islamic Azad University of medical sciences-dental branch approved the study protocol, and each subject signed a detailed informed consent form. Measurements of total antioxidant status were carried out in saliva and plasma. Fasting blood samples were obtained in both groups. After collection of blood samples into citrate containing glass tubes (3.5 mg citrate /ml blood), blood was centrifuged at 3000 g for 10 min at 4°C to obtain plasma, which was stored in small aliquots at) 4°C. Saliva samples were gathered in the morning following an overnight fasting at least for 8h. All of specimens gathered between 9-11 AM in comfortable place and out of stress. The patients were first asked to rinse their mouth using distilled water. After 5 min, we started to gather unstimulated samples. Then, the patients were told to sit comfortably and to spit into the plastic tubes five times per min for 5 min. Samples were centrifuged 4000 g for 10 min at 4°C; the upper parts were drawn and stored in small aliquots at)4°C. TAS of saliva and plasma were measured and is expressed in mmol. Measurement of TAS was performed using by an Aeroset 2.0 analyzer and a total antioxidant kit (Rel Assay Diagnostic, Turkey). With this kit the reduced ABTS (2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonate))molecule is oxidized to ABTS ,using hydrogen peroxide alone in an acidic medium (acetate buffer 30 mmol/L; PH 3.6). In the acetate buffer solution the concentrated (deep green) ABTS molecules remain more stable for a long time. While it is diluted with a more concentrated acetate buffer solution at high PH (acetate buffer 0.4 mol/L; PH 5.8), the color is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations. This reaction can be monitored spectophotometrically and the bleaching rate is inversely related to the total antioxidant capacity (TAC) of the sample. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assay, and the assays, and the assay results are expressed as mmol Trolox equivalent/L [15]. All values are expressed as mean ± SD. Student's t-test was used for some statistical analysis. $P<0.05$ was considered statistically significant.

Results

The mean and standard deviation (SD) age the case and control groups were 50 ± 3.7and 52.4 ± 3.3 respectively. There was no statistically significant difference between both groups. The mean ± SD of salivary TAS in case group was 0.46 ± 0.08, and in control group was 1.07 ± 0.10 mmol, respectively, and there was significant difference between groups (P=0.01). The mean ± SD of the plasma TAS in the case group was 0.8 ± 0.08 and 1.64 ± 0.34 mmol, respectively. There was significant difference between two groups (P=0.01). The mean ± SD time of involvement patients with this disease was 5 ± 2.3 month.9 patients had oscc in floor of mouth, 6 patients had oscc in ventral surface of tongue, 5 patients had oscc in buccal mucosa, 4 patients had oscc in alveolar ridge and 2 patients had oscc in gingiva. The mean ± SD of amount of saliva in case group was 2.2 ± 0.3 ml and in control group was 2.3 ± 0.5. There was no significant difference between two groups about amount of saliva.

Discussion

The purpose of this study was to compare TAS in patients with oral squamous cell carcinoma and healthy subjects. Our results showed that TAS was significantly lower in case group compared with the control group. Many factors that are considered as etiology of oral scc might have a direct impact upon oxidant⁄antioxidant system. Accumulation of genetic changes and duration of exposure to the initiators and promoters (these include chemical and physical irritants, viruses, hormonal effects), cellular aging, and decreased immunologic surveillance with aging. Evidence from long-term follow up of immunosuppressed patients after solid organ and hematopoietic stem cell transplantation shows that immunosuppression increase the risk of the development of scc [1]. Other factors that play a role in the progression of disease may include allelic loss at other chromosome regions, mutation of proto-oncogene or epigenetic changes such as deoxyribonucleic acid (DNA) methylation or histone deacetylation. Cytokine growth factors, angiogenesis, cell adhesion molecules, immune function, and homeostatic regulation of surrounding normal cells also play a role in the progression of disease [1]. The role of cytokines including epidermal and transforming growth factors may be relevant in oral scc [1]. Changes in cell surface receptors and major histocompatibility class I and class II antigens have been seen and may indicate that immune surveillance and immune function may be affected in patients with oral cancer [1]. Total numbers of T cells may be decreased in patients with head and neck cancer and mixed lymphocyte reaction is reduced in some patients, and a diminished migration of macrophages has been demonstrated. Cluster designation (CD8) lymphocytes (T suppressor cells) predominate in the infiltrate, suggesting that immunosuppression is associated with progression of disease [15]. ROS are generated during irradiation by UV light, X-rays and gamma rays, they are the products of metal-catalyzed reactions, they are present as pollutants in the atmosphere, ROS are produced by neutrophils and macrophages during inflammation and they are the by-products of mitochondria-catalyzed electron transport reactions and other mechanisms [16]. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons usually confers a considerable degree of reactivity upon a free radical. The radicals derived from oxygen represent the most important class of such species generated in living systems [17]. Attraction of lymphocytes to a particular site would require cytokines [18]. Cytokines can stimulate production of ROS. The presence of apoptosis is a hallmark criterion in oral scc, and this indicates that ROS as essential mediators of apoptosis may play a crucial role in the disease process. This oxidative damage to the tissues may be a result of lipid peroxidation. Following such damage, a lack of antioxidant may result in incomplete elimination of ROS. Depletion of antioxidant is also known to enhance ROS generation and increase in ROS concentration [19]. Oxidative stress attributed to the formation of oxygen derived free radicals can cause cellular damage. Chain reaction of membrane lipid peroxidation could account from increased oxidative stress as consequence of free radical attacks [20]. Although the evidence that oxidative stress actually causes oral scc is not very strong, but there is sufficient evidence in other disease process that ROS increase risk of malignancy and antioxidant amount of saliva and plasma is lower in some premalignant diseases [3,11,13,26]. Lipid

peroxidation in turn increases ROS. Potent free radicals attack on the oral mucosa leading to various alterations in a wide spectrum from infection to lethal cancer has been suggested in the literature [21]. Thus, ROS may oxidize lipids, proteins and DNA leading to formation of oxidized products such as lipid hydro peroxides, protein carbonyls and 8-oxo-guanosine [22]. If these alterations occur to genes involved in normal homeostatic mechanisms that control proliferation and cell death, significant abnormalities are observed in the cell cycle, leading to the first cancer stage, initiation [23,24].

ROS such as superoxide radical, hydroxyl radical and hydrogen peroxides are frequently generated in the biological systems either by normal metabolic pathways or as a consequence of exposure to physical, chemical and biological agents. ROS attack bio membranes and lead to oxidative destruction of PUFAs by a chain reaction known as LPO.

ROS interfere with the structure and function of the cells, making them weak and defenseless. Overproduction of ROS within tissues can damage DNA and contribute to mutagenesis and carcinogenesis. ROS-mediated oxidative stress has been implicated in the pathogenesis of several diseases including cancer. Human body has, however, an array of sophisticated antioxidant defense mechanism to combat the deleterious effects of ROS-mediated oxidative damage [25]. Nagler et al. [8] in their experimental study showed the anticarcinogenic impact of saliva. They reported that saliva inhibits the production of ROS because it has antioxidant materials. The plasma antioxidant system elements are likely to be transported to areas where oxidative stress is localized, which may consider those antioxidant molecules or molecules may lead to synthesis of mRNA for antioxidant are likely to be transported from plasma to the body fluids in areas where ulcers occur [3]. Oxidative stress is caused by an imbalance between the production of reactive oxygen and the ability of the biological system to readily detoxify the reactive intermediates or easily repair the resulting damage. This usually results in the production of lipid peroxides [26]. It has been suggested that free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the inhaled cigarette smoke induce a gradually evolving process, initially expressed by dysplastic lesions of the mucosa, which are transformed into in situ carcinoma lesions and eventually result in full blown infiltrating and metastasizing oral SCC [17]. We believe that our finding indicates that there was a disturbance in the antioxidant defense mechanism leading to increased production of ROS. Our finding is in accordance with Beevi's study [11]. Beevi et al. [11] demonstrated a significant increase in lipid peroxidation products such as MDA and nitric oxide in oral scc patients. He concluded that enhanced lipid peroxidation with concomitant decrease in antioxidants is indicative of oxidative stress that provides evidence of the relationship between lipid peroxidation and oral cavity cancer. Ozben [12] emphasized that free radicals, particularly ROS have been proposed as common mediators for apoptosis and the mode of cell death depends on the severity of oxidative damage. In oral cancer, ROS increases and causes apoptosis. Sander et al. [26] reported high MDA levels in lichenplanus, leukoplakia, and cancer patients. Aghahosseini et al. [13] suggested that patients with OLP and SCC are more susceptible to an imbalance of antioxidant –oxidative stress status. In the human body, there is an antioxidant mechanism to maintain the balance of oxidation-reduction. The breakdown of this balance could lead to increased damage directly by ROS. Indeed, several diseases have been correlated to an imbalance of oxidation-reduction [13]. In our opinion, these data support our hypothesis that increased ROS and lipid peroxidation, and decrease of salivary and plasma antioxidant in oscc may enhance damage of cells and causes cancer.

Conclusion

Our results showed that salivary and plasma levels of total antioxidant status in oral scc patients were lower than those in healthy subjects.

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