RESEARCH ARTICLE

DETERMINATION of SOME ANTIOXIDANT ACTIVITIES and OXIDATIVE STRESS LEVES in PATIENTS with LIP and ORAL CAVITY CANCER

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ABSTRACT

Squamous cell carcinomas constitute the majority of oral cavity tumors. Poor oral hygiene, smoking, chronic irritation and alcohol consumption are counted as etiological factors. In study, it was aimed to determine oxidative stress levels and some antioxidant activities such as superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) in cancer of the lip and oral cavity. Malondialdehyde (MDA) levels, SOD, GSH and CAT activities were measured using the spectrophotometric method. The results showed that SOD, CAT and GSH levels decreased significantly in the patient group ($p < 0.05$), while MDA levels increased significantly ($p < 0.05$). Lack of antioxidants can increase the development of oral and lip cancers.

Keywords: *Lip and oral cavity cancers, SOD, GSH, CAT, MDA*

1. INTRODUCTION

Cancer is a state of excessive proliferation of cells. There are factors that predispose to the formation of cancer. Many factors such as environmental and family family history can be counted among the causes of cancer. Apart from free radicals, reactive oxygen nitrogen species (RONS) also participate in the initiation of carcinogenesis. Free radicals are formed from nitrogen, sulfur and oxygen molecules inside the cell [1]. ROS consists of oxygen molecules such as $O_2 \bullet^-$, $\bullet OH$, $1O2$), $O3$, $RO2$ and H_2O_2. ROS and Reactive Nitrogen Species (RNS) are two important agents of DNA damage [1].

Oral cavity cancer with an incidence of more than 300,000 cases per year; It is defined as cancers of the hard and soft palate, lips, cheeks, tongue, floor of the mouth, sinuses and pharynx. Oral cavity cancer, whose prognosis is improved with early diagnosis, has a survival rate of 75% at 5 years for stage 1 [2]. Oral cavity cancer is more common in men than women in many countries. The increased risk with aging can lead to death in those aged 50 and above. Although oral cavity cancer is seen in all parts of the world, it is more in the south of Asia,especially in Pakistan, Sri Lanka, Taiwan, India, France in Europe, Slovenia, Slovakia, Hungary and the Latin region of America. It has been reported that the incidence of lip cancer is higher in Caucasians than in blacks in Australia and Canada. [3]. Excessive alcohol, tobacco consumption and exposure to ultraviolet sun rays are among the causes of oral cavity cancer. On the other hand, another risk factor for oral cavity cancer has been reported to be human papillomavirus [4].

ROS can control many cellular tasks. Lipid peroxidation is a chain reaction controlled by free radicals and causes the oxidation of unsaturated lipids. MDA can accelerate the increase of cell damage. It is a highly reactive substance [5,6,7].

In this study, we aimed to determine oxidative stress levels and some antioxidant activities such as GSH, CAT and SOD in cancer of the lip and oral cavity.

2. MATERIALS and METHODS

2.1. Materials
This study consisted of a total of 50 individuals: 25 were made up of patients diagnosed with lip and oral cavity cancer and 25 were from the healthy control group. Patient and healthy control groups were selected from clinic Urology Van Yuzuncu Yil University and Istanbul Bagcılar Training and Research Hospital. The study started with the decision of the Ethics Committee dated 31.05.2016 and numbered 2016/13
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2.2. Methods
For measurements, blood was taken from sick and healthy individuals (4cc). Blood samples were separated from plasma by centrifugation at 4000 rpm for 5 minutes in the biochemistry laboratory, and the obtained serums were preserved until processed (at 20°C).

2.2.1. Determination of SOD activity
SOD was measured or analyzed with the system prepared by "Marklund et al" [8].

2.2.2. Determination of CAT activity
CAT activity was measured according to the method developed by Aebi [9].

2.2.3. Determination of level (GSH
GSH was performed using the Beutler method [10].

2.2.4. Determination of level MDA
MDA level was measured according to the method developed by Gutteridge [11].

2.2.5. Statistical analysis

Mean±standard deviation were used as descriptive statistics. In two-group comparisons in terms of continuous variables; The t test was used when the normal distribution was provided, and the Mann-Whitney U statistics were used when it was not. In addition, The cutoff value that separates the patient was determined by performing the ROC curve analysis. For the level of significance, $p < 0.05$ was taken and SPSS (ver: 13) statistical package program was preferred for analysis.

3. RESULTS

This study consisted of a total of 50 subjects, 25 of whom were healthy controls and 25 cancer of the lip and oral cavity. When Table 1 is examined for SOD, CAT and GSH enzyme activities, mean of the patient group was statistically lower than the average of the control group (Figure 1). When Table 1 was examined for MDA, the mean of the patient group was higher than the mean of the control group ($p < 0.05$) (Figure 2).

Table 1. Values of healthy individuals with cancer of the lip and oral cavity.

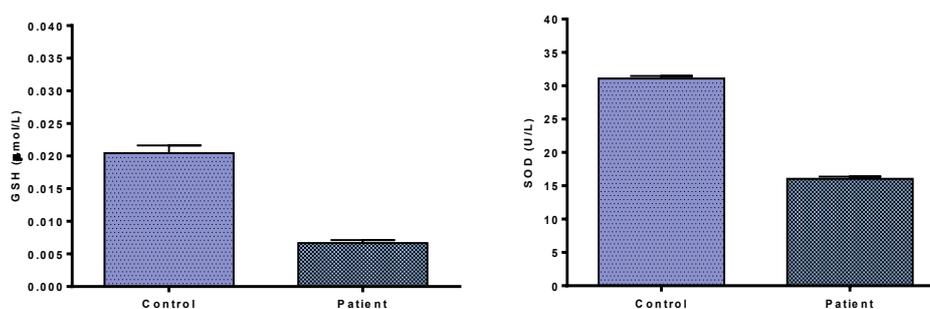| Parameters	Controls (n= 25) Mean±SD	Patients (n=25) Mean±SD	p
SOD (U/L)	31.0943 ± 1.8198	16.0459 ± 1.6496	0.001
GSH (µmol/L)	0.0204 ± 0.0011	0.0066 ± 0.0004	0.001
CAT (U/L)	0.2908 ± 0.0901	0.0733 ± 0.0219	0.001
MDA (µmol/L)	0.5011 ± 0.1473	1.3607 ± 0.2053	0.001

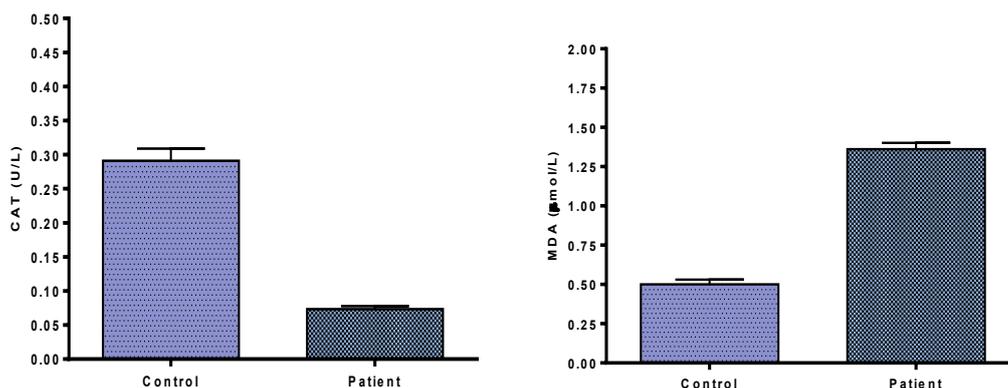**Figure 1**. SOD activity and GSH level for patient and control groups.

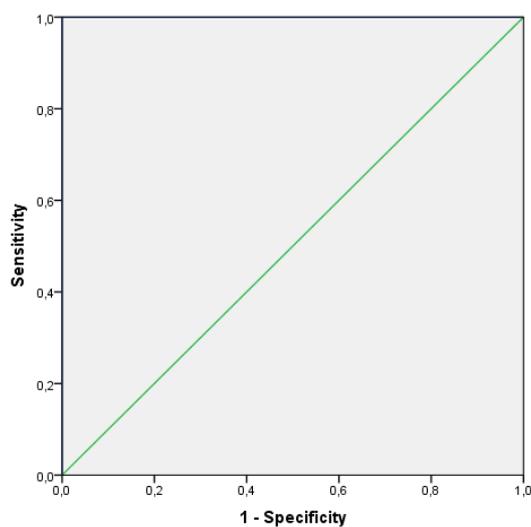**Figure 2**. CAT activity and MDA level for patient and control groups.

Figure 3. ROC Curve.

In the study, according to the results of ROC curve analysis, in separating the patient and control groups; The area under the curve was found to be 1.000±0.001 for MDA (Table 2). The cut-off value for MDA is seen as 0.86500 (Sensitivity 100%, Specificity 100%) (Figure 3).

Table 2: ROC analysis.

	Group	cut-off value	area under the curve	St. Error	Sensitivity	Specificity	p
MDA	Patient-	0.8650	1.000	0.001	1.000	1.000	0.001

(µmol/L)	Control

4. DISCUSSION and CONCLUSION

Oral cavity cancers, which are among the head and neck cancers, attract more attention due to their high incidence. There are complications and mortality resulting from the diagnosis of squamous cell carcinoma diagnosed, reaching 40% in the lip and mouth cavity, 25% in the larynx and 15% in the throat with a low incidence. Lesions mostly seen on the lower lip may originate from the skin and basal cell carcinomas may migrate to the lip [12].

Head and neck cancers (HNC) cover important organs of the body. It can be diagnosed very easily. It is a region that includes all parts of the mouth [13].

Cancer occurs with oxidative stress as a result of incompatibility between antioxidants and oxidants in the body. Oxidative stress causes some diseases. High ROS level creates lipid hydroperoxides (LHP) and lipid peroxidation (MDA). MDA causes damage to the structure of the cell. They are known to be mutagenic and carcinogenic [14,15]. In some of the studies, it was determined that lipid peroxidation decreased and antioxidant levels increased after radiotherapy in patients. However, in a related study, no difference was found between the levels of antioxidant enzymes and MDA in smokers and non-smokers with lip-mouth cancer groups and healthy non-smokers. It is known that the level of antioxidants and oxidants varies depending on the phase of the cancer during the enlargement of tumors [16]. MDA level was found to be higher in patients with oral cavity cancer than in the control group ($p < 0.05$). ROC curve analysis was performed in the study. According to this; The area under the curve for MDA was 1.000 ± 0.001 and the cut-off value were found as 0.8650 (sensitivity 100%, specificity 100%). In this case, the height of the MDA may be a marker in cancer lips and oral cavity.

In this study, we found that serum MDA levels increased and SOD, CAT and GSH levels decreased in patients with lip and oral cavity cancer. The increase in lipid peroxidation may increase the malignancy in the cell by increasing the radicals [17]. Antioxidant enzymes protect the cell against reactive oxygen radicals. GSH and CAT convert H_2O_2 to water, while SOD converts superoxide to H_2O_2 [17]. Selenium removes H_2O_2 from the environment with the help of GSH. Thus, cellular damage is prevented [18].

GSH, represented by a gene in humans, is a flavoenzyme and exposure to oxidative stress has also been shown to result in an increase in mRNA content. [19]. In a study in which GSH level was determined for oral cavity cancer risk, iron deficiency and low GSH level were associated with oxidative stress. [20]. In this study, the low GSH level of the patient group ($p < 0.05$) may be a marker for cancer lips and oral cavity.

CAT has a tremendous impact. It works well on metabolic systems. In a study examining the antioxidant levels in the serum of oral cavity cancer patients, it was determined that the CAT level was low [21]. In a study in which the role of oxidative stress and the activities of antioxidant enzymes were determined in patients, CAT level was found to be low [22]. In this study, CAT activity was also

lower than the control group (p < 0.05). In this case, low CAT activity may be a marker of cancer in the lips and oral cavity.

SOD constitutes the defense system, which is valuable in metabolic systems. Contributes to repair systems in metabolism [23]. SOD works effectively against cancers that may occur [6]. In a study evaluating oxidative damage in patients with oral submucous fibrosis, SOD level was decreased compared to healthy individuals [24]. In this study, low SOD activity (p < 0.05) of the patient group may be a marker of cancer in the lips and oral cavity.

In our study, it was concluded that activities antioxidant such as SOD, CAT and GSH decreased, while oxidative stress levels such as MDA increased in patients compared to controls. According to the ROC analysis, it can be said that the discrimination power of MDA is quite high and accordingly, it can be used as a diagnostic test to distinguish patients with lip and oral cavity cancer from the control group. Findings show that oxidative stress is associated with cancer of the lips and oral cavity, and increased oxidative stress can cause to rise of this disease. This study is a study showing the relationships between antioxidant enzymes and oxidative stress in cancer of the lip and oral cavity. In addition, more studies are needed in patients with lip and oral cavity cancer.

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