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OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(4):84-7

Research Article Doi: 10.17546/msd.547201

Comparison of serum glutathione peroxidase levels in healthy controls and patients with oral cavity malignancy

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Abstract

Objective: In this study, we investigated the glutathione peroxidase enzyme activity changes in patients with oral cavity malignancies.

Material and Methods: Twenty-five patients with oral cavity malignancy and 24 healthy individuals were included in the study. There was no statistically significant difference between the groups in terms of age, gender and smoking-alcohol consumption habits.

Results: Glutathione peroxidase activity was found to be significantly lower in the blood of patients with oral cavity malignancies than the control group (2,238±0,039, p: 0,0001).

Conclusion: The level of glutathione peroxidase in the blood can be used as an important biomarker for the prognosis and to guide to the treatment approaches for the patients with oral cavity malignancy.

Keywords: Oral cavity malignancy, glutathione peroxidase, antioxidant enzyme

Introduction

Oral cavity malignancies are among the most common malignancies in the world and constitute approximately 8% of all malignancies (1). They are the most common subgroup of cancer in the head and neck region (except for non-melanoma skin cancer) (2). Oral squamous cell carcinoma is one of the most prevalent malignancies in the head and neck region and ranks sixth among all tumors worldwide (3). Despite advances in surgical techniques and treatment methods, the 5-year survival rate is quite low (1). Although the exact etiology is not clearly known, many factors such as smoking, alcohol consumption, tobacco chewing, poor oral hygiene and chronic irritation may play a role in the etiology (4). The primary approach for the treatment of oral cavity tumors includes surgical intervention. Adjuvant radiotherapy (RT) is used in the presence of risk factors after the primary surgery and in advanced stage disease; RT is used for definitive treatment when surgery is not possible in early stage disease. Chemotherapy (CT) may be added to the adjuvant RT in high risk cases (2).

Carcinogenesis is generally divided into three main stages: initiation, promotion, and progression. Reactive oxygen products and free radicals which may leads to hyper- and hypo- methylations of DNA are considered to play a role especially in the initiation and promotion stages (5).

Enzymes such as superoxide dismutase, catalase and glutathione peroxidase are involved in the protective mechanisms against free radicals. The production of reactive oxygen species (ROS) is an essential element of aerobic cellular metabolism. The imbalance between ROS production and antioxidant mechanism efficiency results in oxidative stress, leading to many diseases such as oral cavity malignancies (6).

Glutathione peroxidase (GPx) is a selenocysteine-dependent enzyme. GPx in cells is the most important hydrogen peroxide (H2O2) scavenging enzyme. This antioxidant enzyme catalyzes the reduction of H2O2 and leads to the oxidation of glutathione (GSH) which can be reduced by GSH reductase using NADPH (7, 8).

In this study, we compared the changes in serum glutathione peroxidase activity between the control group of healthy individuals and patients with oral cavity cancers.

Received 31-03-2018 **Accepted** 29-04-2019 **Available Online** 30-04-2019 **Published** 30-04-2019
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http://dx.doi.org/10.17546/msd.547201

Material and Methods

In this study, the patients were selected from the patients who admitted to ENT clinic of Bagcilar Training and Research Hospital with a diagnosis of oral cavity squamous cell carcinoma. There was no other malignancy in the patient group except for oral cavity squamous cell carcinoma. Forty-nine people were included in the study. The patient group consisted of 25 (6 female, 19 male), the control group 24 individuals (8 females, 16 males, healthy individuals recruited from ENT outpatient clinic). Subjects aged between 28-85 years were included in the study. Smoking and alcohol habits, histopathological diagnosis of the patients were recorded from the patient follow-up data. A case report form was prepared for each individual. After informed consent, 5 ml venous blood samples were collected from each patient and each control. The blood was allowed to clot for 15 minutes and then centrifuged at 5.000 rpm for 10 minutes. Separated sera were stored at -80°C until analyzed.

Estimation of cytosolic GPx activity in hemolysate was based on the Paglia and Valentine method using hydrogen peroxide and the rate of disappearance of NADPH at 37°C and recorded spectrophotometrically (340 nm). The activity of the enzyme was presented as U/gHb.

Statistical Evaluation: In this study, statistical analysis was performed using NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program.

In addition to descriptive statistical methods (mean, standard deviation), independent t-test was used for comparison of paired groups and chi-square test was used for the comparison of qualitative data. The results were evaluated at $p<0.05$ level.

Results

Twenty-five patients (19 males and 6 females) with oral cavity malignancy and 24 healthy controls (16 males, 6 females) were included in the study (table 1). The mean age was 55.8±13.4 years in the patient group and 54.5±11.91 years in the control group. No statistically significant difference was found in age and sex distributions between the control and patient groups (p= 0.722) (table2). No statistically significant difference was found between the control patient groups in terms of smoking distribution (p= 0.913) (table 2). There was no statistically significant difference between the control and patient groups in terms of alcohol consumption (p= 0.684) (table 2).

The glutathione peroxidase activity was 0.238±0.039 U/gHb in the patient group and 0.825±0.152 U/gHb in the control group. Glutathione peroxidase was significantly lower in the study group than the control group (p= 0.0001) (table 2).

Table 1: Types of oral cavity malignancy in the patient group

Pathology	Studied Group	
	n	%
SCC of the Floor of Mouth	4	16,00%
Lower Lip SCC	4	16,00%
Buccal Mucosal SCC	3	12,00%
SCC of the tongue	10	40,00%
Gingival SCC	1	4,00%
Hard Palate SCC	2	8,00%
Soft Palate SCC	1	4,00%

Table 2: Statistical data in patient and control groups

		Control Group n:24		Study Group n:25		P
Age		54,5±11,91		55,8±13,4		0,722*
Sex	**Male**	16	66,67%	19	76,00%	0,470+
	Female	8	33,33%	6	24,00%	
Smoking	**Yes**	9	37,50%	9	36,00%	0,913+
	No	15	62,50%	16	64,00%	
Alcohol Consumption	**Yes**	14	58,33%	16	64,00%	0,684+
	No	10	41,67%	9	36,00%	
Glutation Peroxidase		0,825±0,152		0,238±0,039		0,0001*

*** Independent t test + Chi-Square test**

http://dx.doi.org/10.17546/msd.547201

Discussion

It is believed that cancer cells exposes to much higher amount of ROS than normal cells and actively regulate multiple antioxidant systems to avoid the harmful effects of oxidative stress. Theoretically, overexpression of antioxidants may have an anticancerogenic effect in certain cancers by limiting the oxidants, but it can also lead to the survival of transformed cells by limiting apoptotic mechanisms in some cancers. In conclusion, the role of antioxidants in tumor formation and prognosis has been controversial for several years (7, 9). Therefore, we compared the levels of glutathione peroxidase in patients with oral cavity malignancy in the early stage with the levels in healthy control subjects and we examined the clinical implications of this in the literature.

Lee et al. found that glutathione peroxidase expression was higher in tissue samples of patients with oral squamous cell carcinoma and reported that this finding could be used as a useful biomarker in the survival and follow-up of recurrence in patients with Oral squamous cell carcinoma. (7). Banerjee et al. also found that mitochondrial glutathione peroxidase levels in tissue samples of patients with oral SCC were higher than healthy tissue samples. However, as the tumor stage increased, the enzyme level began to decrease. Therefore, they have recommended this enzyme activity assay as a biomarker that can be used in the follow-up of tumor progression and in selecting treatment modalities (10). In our study, serum glutathione peroxidase activity was significantly lower in patients with oral SCC than in healthy control subjects at the initial diagnosis stage independent of the tumor stage. This suggests that the enzyme activity can be used as a biomarker to assist in the diagnosis of malignancy and prognosis.

In a study conducted by Fu et al. in 2016, glutathione peroxidase levels were found to be higher in tissue samples of patients with oral SCC compared to the patients with verrucous carcinoma. Therefore, they suggested that in contrast to verrucous carcinoma, distant metastasis and local spread were more frequent in cases with oral SCC (11). Furthermore, in many cases of pathology, distinction between verrucous carcinoma and early stage oral SCC may be difficult. The determination of enzyme activity appears to be a useful biomarker in the diagnosis of more aggressive oral SCC cases and in preventing the selection of over-treatment modalities in verrucous carcinoma. Fu et al. found that in 2010, glutathione peroxidase levels were higher in patients with buccal mucosal SCC. This enzyme activity has been found to be higher in patients who had a better survival (5).

Malinowska et al. suggested that increasing the activity of glutathione peroxidase by using copper (II) complex can be used to create an anticancer treatment protocol (6). In the light of these results, it can be interpreted that, determination of the enzyme activity initially and the activity enhancing preparations added to the treatment protocol by selecting the appropriate patient population may be effective in improving patient survival.

Balasenthil et al. have observed that glutathione peroxidase activity increases in oral carcinoma tissue samples that they have experimentally formed in hamsters compared to normal tissues. This was interpreted as the increase in this enzyme activity might play a role in carcinogenesis (12). These studies are very helpful in selecting treatment modality and provide a better understanding of carcinogenesis.

Among the study groups of Gurudath et al., which included oral squamous fibrosis, oral leukoplakia and oral cancer patients, oral cancer group showed the lowest GPx levels (8). Low glutathione peroxidase levels indicate that many cancer cells cannot detoxify hydrogen peroxide. (13) These results are consistent with our study.

Significant evidence indicates that antioxidant enzymes prevent both initiation and promotion of cancerogenesis. In the literature, the low activity of these enzymes has been interpreted as playing a key role in the progression of the lesion (8).

Conclusion

As a result, antioxidant enzyme levels are an interesting research topic that has a potential role in cancerogenesis and is the basis of the cellular antioxidant defense mechanism. Therefore, glutathione peroxidase may be a potential biochemical marker to assess the disease process. This study evaluates the change in enzyme level in healthy patients. This antioxidant enzyme may also serve as a guide for therapeutic targets and prognosis in patients suffering from such a disease. We believe that the role of antioxidant mechanisms in cancerogenesis, diagnosis and treatment methods and prevention of cancerogenesis in the future is an area that should be considered and investigated. However, further detailed biochemical and histopathological studies are needed to determine the actual role of biochemical parameters in various clinical stages.

All procedures performed in studies involving 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 form: Informed consent was obtained from all individual participants included in the study.

Acknowledgement: Approval of Bagcilar Training and Research Hospital Clinical Research Ethics Committee was obtained for this non-randomized prospective clinical laboratory study (2019.03.1.01.020).

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **ŞB, HD, AK** were contributed to planning the research, patient examination, ŞB, AK examination of biochemical parameters **ŞB** preparation of the article, and revisions.

Ethical issues: All Authors declare, originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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