Int J Acad Med Pharm, 2020; 2(2); 72-75
International Journal of Academic Medicine and Pharmacy
journal homepage: www.academicmed.org

Comparison of Total Serum Sulfhydryl and Glutathione S Transferase Activities in Patients with Oral Cavity Malignancies and Healthy Control Individuals

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Article info

Received: 13.04.2020
Received in revised form: 27.04.2020
Accepted: 04.05.2020
Available online: 05.06.2020

Keywords

Glutathione S Transferase
Oral cavity malignancy
Total Serum Sulfhydryl level

Abstract

Research Article

Serum glutathione S transferase is one of the enzymatic antioxidants that play an important role in cell protection against oxidant stress. Serum sulfhydryl level is among the non-enzymatic antioxidant products that play a crucial role in scavenging of elevated reactive oxygen species produced as a result of deterioration in oxidative stress homeostasis. We were examined the changes in these two mechanisms of activity enzyme some antioxidant (Serum glutathione S transferase and serum total sulfhydryl) in patients with oral cavity malignancy. Forty-five people were included in the study. The patient group consisted of 20 (5 female, 15 male), control group 25 (6 female, 19 male, healthy individuals who were recruited from ear-nose-throat outpatient clinic). Serum glutathione S transferase activity and total sulfhydryl level were measured by spectrophotometric method. The mean value of glutathione S transferase (0.005±0.002 nmol/l) in the study group was found to be significantly lower than the control group (0.046±0.007 nmol/l) (p=0.0001). The mean value of total sulfhydryl in the study group (0.344±0.350 mmol/l) was significantly lower than the control group (2.002±0.271 mmol/l) (p=0.0001). Glutathione S transferase activity and total sulfhydryl level that play a role in defending against the reactive oxygen species are among the important and promising parameters which can be used in the follow-up of cancer patients, in the selection of treatment modalities and in cancer research. As a result, glutathione S transferase and total sulfhydryl may be a marker in oral cavity cancers.

INTRODUCTION

Oral cavity malignancies are among the most common malignancies worldwide and constitute approximately 8% of all malignancies[1]. Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in the head and neck region and ranks sixth among all tumors worldwide[2]. Despite advances in surgical techniques and treatment methods, the 5-year survival rate is quite low[1]. Although the exact etiology is not known, many factors such as smoking, alcohol, tobacco chewing, poor oral hygiene and chronic irritation may play a role in the etiology[3].

Reactive oxygen species (ROS) such as superoxide and hydrogen peroxide play a role in many diseases, including cancer. ROS play an important role in the onset and progression phases of carcinogenesis process. Glutathione S transferase is one of the critical enzymes involved in the destruction of reactive oxygen species[3]. Sulfhydryl is a non-enzymatic antioxidant that plays a role in this mechanism[4].

In this study, we compared the changes in serum glutathione S transferase activity and total serum sulfhydryl levels in the control group of healthy individuals and patients with oral cavity cancer.

MATERIAL and METHODS

This non-randomized prospective clinical laboratory study was approved by the Clinical Research Ethics Committee of our hospital. (2018.12.1.01.094.r3.114). Patients and control subjects gave written informed consent.

In this study, the patients were selected from the patients who had received a diagnosis of oral cavity squamous cell carcinoma and who were admitted to our hospital ear nose and throat (ENT) clinic. There was no other malignancy except for oral cavity squamous cell carcinoma in the patient group. Forty-five people were included in the study. The patient group consisted of 20 (5 female, 15 male) and control group 25 (6 female, 19 male, healthy individuals recruited from ENT outpatient clinic). The study included subjects aged between 28 and 76 years. Smoking and alcohol habits, along with

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http://dx.doi.org/10.29228/jamp.42882

histopathological diagnoses of the patients were recorded from the patient follow-up data. A case report form was prepared for each individual. After obtaining informed consent, 5 ml venous blood samples were collected from each patient and control subject. The blood was allowed to clot for 15 minutes and then centrifuged at 5000 rpm for 10 minutes. Separated sera were stored at -80 °C until analysis.

Serum total sulfhydryl level (TSH) was measured by spectrophotometric method. 100 mL of the sample was mixed with 1.500 mL of potassium phosphate buffer (pH=7.4, 0.1 M). Immediately after mixing with 400 mL of DTNB solution (2 mM), it was incubated for 5 minutes at 37 °C. The absorbance values of the samples were determined against the reagent blank at 412 nm using Shimadzu UV-1601 spectrophotometer. The extinction coefficient was determined by using e_{max}=13600 M-1 cm-1 and the results were given in mmol/L[5].

Serum glutathione S transferase activity (GST) was also measured by spectrophotometric method. The GST was pre-incubated at 37° C for 10 min in 1ml of incubation mixture containing 850 µl of 0.1 M phosphate buffer at pH: 6.5 and CDNB reagent (20 mM). The reaction was initiated by adding 50 µl of 20 mM GSH and 50 µl serums. The reaction was carried out at 1-minute intervals, measuring absorption at 340 nm for 5 minutes. Simultaneously, deionized water was used for serum. Then O.D change/min was calculated. The GST was determined using the molar extinction coefficient [9.6 mM-l cm-l] of the GST[6].

Statistical evaluation

In this study, statistical analysis was performed by NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package software 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 patients (15 male, 5 female) with oral cavity malignancy and 25 healthy controls (19 male, 6 female) were included in the study (table 1). The mean age was 53.55±12.64 years in the patient group and 54.68±11.11 years in the control group. No statistically significant difference was found between age and sex distributions of the control and the patient groups (p=0.751) (table2). No statistically significant difference was found between the control and the patient groups in terms of smoking (p=0.540) (table 2). There was no statistically significant difference between the control and the patient groups in terms of alcohol use (p=1) (table 2).

Glutathione S transferase activity was 0.005±0.002 nmol/l in the patient group and 0.046±0.007 nmol/l in the control group. The mean value of glutathione S transferase in the study group was significantly lower than the control group (p=0.0001) (table 2).

The total sulfhydryl level was 0.344±0.350 mmol/l in the patient group and 2.002±0.271 mmol/l in the control group. The mean value of total Sulfhydryl in the study group was significantly lower than the control group (p=0.0001) (table 2).

Table 1: Oral cavity malignancy types, study group

Pathology	Study Group	
Floor of mouth SCC	4	%20.00
Lower lip SCC	4	%20.00
Buccal Mucosa SCC	2	%10.00
Tongue SCC	6	%30.00
Gingival SCC	1	%5.00
Hard palate SCC	2	%10.00
Soft palate SCC	1	%5.00

Table 2: Statistical data of the patient and the control groups

		Control Group		Study Group		p
Age		54.68±11.11		53.55±12.64		0.751*
Gender	Male	19	%76.00	15	%75.00	
	Female	6	%24.00	5	%25.00	0.938+
Smoking	Absent	11	%44.00	7	%35.00	
	Present	14	%56.00	13	%65.00	0.540+
Alcohol use	Absent	15	%60.00	12	%60.00	
	Present	10	%40.00	8	%40.00	1+
Total Sulfhydryl		2.002±0.271		0.344±0.350		0.0001*
Glutathione S Transferase		0.046±0.007		0.005±0.002		0.0001*

*Independent t test +Chi Square test

DISCUSSION

Reactive oxygen species (ROS) are produced in response to normal cellular processes in the body. Low concentrations of ROS are required in many sub-cellular events in the body, while high concentrations may have harmful effects. Under normal physiological conditions, the cells may compensate for the detrimental effects of reactive oxygen species with antioxidant defense system consisting of free radical scavengers, including non-enzymatic antioxidants such as

superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase and thiols along with various other substances (i.e. nutrients such as vitamin E, vitamin C, b-carotene and flavonoids). Antioxidant defense systems works together to alleviate the oxidative stress caused by the augmented production of free radicals. Any change in one of these systems may disrupt this balance, cause cellular damage and result in malignant transformation[1,7,8].

Excessive amounts of released ROS not only affect the redox balance of the cell, but also damage other cellular mechanisms, including DNA, cellular proteins and lipids. Oxidative stress reflects the deterioration in ROS homeostasis due to either elevated ROS or reduced ROS scavenging capacity. This imbalance can be caused by a variety of causes, such as exposure to carcinogens, environmental factors or genetic changes, and it has been shown to increase in the pathogenesis of many diseases, including cancer. Cancer is a multi-stage process involving initial, development, and progression phases, all of which can be triggered by ROS and thus facilitate tumor growth. Oxidative stress is potentially harmful to cells. Thus, in the case of malignant neoplasms, intrinsic oxidative stress in cancer cells may have dramatic consequences, such as cancer cell proliferation, genetic instability promotion, and changes in cellular sensitivity to anticancer agents[1,7,8]. Oxidative stress, an important etiological factor in carcinogenesis, has been studied in patients suffering from various cancers, including OSCC.

Glutathione S transferase is one of the most important enzymes involved in the ROS scavenging system. The level of this enzyme may be an important marker to detect the body's ROS scavenging capacity. In their study, Prabhu et al. found that this enzyme activity was reduced in patients with oral cavity cancer compared to the control group[3]. In another study by Prabhu and colleagues conducted in patients with cervical carcinoma, no significant difference was found in enzyme activity between the patients and healthy controls[9]. In our study, we found that glutathione S transferase enzyme activity was lower in the patient group. In the literature, we found very few studies related to glutathione S transferase in patients with malignancy. Glutathione S transferase is one of the antioxidants that can be studied in the future for the detection of malignancy, progression follow-up and determining treatment options.

Serum total sulfhydryl level reflects the total reductive thiol level in the serum. Free sulfhydryl groups are modifiers of events regulated by redox. In many studies, the role of low-molecular anti-oxidants such as ascorbate and glutathione has been highly valued, however a key player in the first line of defense of chemistry associated with free radicals is nitric oxide, which, together with ROS, determines the cellular redox tone. The NO/ROS balance depends on the thiol redox state (i.e. how much it is reduced against oxidized thiols), and this is related to the circulating pool of free thiols in the blood. Plasma thiol groups disrupt the peroxidative chain and allow repair of oxidatively damaged molecules.

In their study, Gupta et al [8]. observed a significant depletion of the plasma thiols and concluded that this reflected the increased pro-oxidant environment of the cells and correlated with increased lipid peroxides in the circulation of cancer patients[8]. In our study, the total sulfhydryl level of the patient group was significantly lower than the control group ($p<0.0001$). In their study, Frenay colleagues reported that measurement of serum total sulfhydryl level may be an important initial parameter for monitoring the risk profile in renal transplant patients[10]. Kolanjiappan et al. found that the majority of sulfhydryl groups in oral tissues of cancer patients consisted of reduced glutathione, and that their levels were quite high[11]. They commented that this could lead to increased cell proliferation and excessive tumor cell expression in malignant tumors[11]. In our study, the reduced level of total serum sulfhydryl groups in patients with a diagnosis of oral cavity malignancy led us to consider the idea that this may be used as a monitoring parameter for disease progression and tumor spread during the follow-up period in advanced stages.

The most important limiting factor of our study is the scarcity of the patient population. However, we think that our findings are important because of the low number of studies conducted in the literature and ours is the first study performed in patients with oral cavity malignancy. In addition, the correlation between antioxidant enzyme activity and tumor progression, tumor progression and treatment should be investigated with further studies. We think that it is necessary to investigate this subject in larger study populations.

In conclusion, we believe that low glutathione s transferase activity and total serum sulfhydryl levels in patients with oral cavity malignancy might be signs of impaired ROS homeostasis and these parameters can be used for follow-up of cancer progression. In addition, GST, and TSH may play an

important role in the etiopathogenesis of oral cavity cancers. As a result, GST and TSH may be a marker in oral cavity cancers. Further studies are needed to establish their role in determining and planning of treatment options.

Conflicts of Interest

The authors declare that they have no conflict of interests.

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