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Prolidase, Paraoxonase-1, Arylesterase Activity In Oral Squamous Cell Carcinoma

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ABSTRACT

In many types of cancer, reactive oxygen and nitrogen products have been detected at high levels. Arylesterase, and paraoxonase1 are esterase enzymes that have strong antioxidant characteristics. The prolidase enzyme is a rate-limiting metalloenzyme which also plays a role in collagen turnover, and is dependent on its NO-activity. In this study, we aimed to investigate the possible relationship between serum paraoxonase1, arilesterase and prolidase enzyme activity in patients with squamous cell carcinoma of the oral cavity and to investigate the etiology and mechanism of oral cavity cancer.

The study included 24 patients with oral cavity cancer, and 22 healthy age- and sex- matched individuals. Arylesterase, paraoxonase1 and prolidase activities were measured using spectrophotometry.

Paraoxonase1 activity was 11.6±2.32 nmol/l in the patient group and 29.46±6.18 nmol/l in the controls. Arylesterase activity was 32.2±14.57 nmol/l in the patient group and 80.71±7.23 nmol/l in the controls. Prolidase activity was 39.51±3.02 nmol/l in the patient group and 19.53±1.13 nmol/l in the controls. The mean paraoxonase1 and arylesterase levels in the patient group were statistically lower than the control group and the mean prolidase levels were high (p = 0.0001).

In our study, arylesterase and paraoxonase1 enzyme activity was low in patients with oral cavity cancer. The prolidase activity was higher in the same group. As a result, paraoxonase1, arylesterase and prolidase enzyme activities play an important role in the etiopathogenesis of oral cavity cancers. In addition, more research should be done on both clinical and molecular levels of oral cavity cancer.

Key Words: Oral cavity cancer, prolidase, paraoxonase 1, arylesterase, antioxidant

Introduction

Currently, oral cavity cancers are quite common among the diagnosed cancers. Each year, more than 300.000 new oral cavity cancer cases are diagnosed worldwide (1). The ratio of oral cavity cancers (not including lip cancers) is 14.1% in all head and neck cancers. The majority of the individuals with diagnosed oral cavity cancer are in the 6th decade and approximately 60% are men. Squamous cell carcinoma (SCC) accounts for about 86.3% of malignant tumors of the oral cavity. Despite recently developed treatment modalities and surgical techniques, the five-year survival rate is generally poor, especially in men and elderly patients (65 years and older) (2,3).

Smoking and alcohol consumption is considered as a substantial factor in the etiology of oral cavity tumors. Important factors in etiology include dietary habits, profession, human papillomavirus infection, socioeconomic levels, ultraviolet exposure, chronic irritation and genetic predisposition (1,2).

The mechanism that reveals carcinogenesis may include oxidative stress and free radical formation. Rising oxidative stress has been shown to damage molecules such as DNA and is associated with carcinogenesis. Oxidative processes are associated with the production of reactive oxygen molecules that cause cancer in humans and be associated with detoxification. In the development of oral cancers, lipid peroxidation, cellular level of total antioxidants and oxidation of unsaturated fatty acids in free radicals have been identified as risk factors (1,4). Oxidative stress is a balance between toxic reactive products (reactive oxygen products and reactive nitrogen products) and studies have shown that

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antioxidative defense mechanisms and carcinogenesis correlate with increased oxidative stress (5,6,7). Studies also have demonstrated that free radical production and increased oxidative stress can damage biological molecules such as DNA and play a role in carcinogenesis in many different cancer types (1,7).

The biological mechanisms of the human body have many endogenous free radical scavenging systems such as paraoxonase-1 and arylesterase (1). Serum esterases such as serum paraoxonase 1 (PON-1) and arylesterase (ARE), which destroy carcinogenic liposoluble radicals, belong to the endogenous free radical antioxidant system (ROS) family present in the human body (1,5). Antioxidants and antioxidant enzymes are very important in the antioxidative mechanism (5,8).

ARE and PON-1 are serum esterases and are strong antioxidant enzymes that have similar antioxidant enzymes but act differently (5,8). Prolidase is an enzyme that contains a group of metalloproteinases that accelerate protein catabolism during oxidative and nutritional stress conditions (such as cancer progression) (9). Physiologically, PON-1 is transported in association with high-density lipoproteins in serum and protects low-density lipoproteins against oxidation (5,10). Like PON-1 and arylesterase is also encoded by a similar gene and has similar active sites (6). It has been shown that degradation of the activity of both enzymes is associated with severe morbid conditions such as cerebrovascular diseases (5).

Prolidase is a metalloenzyme responsible for the hydrolysis of imido peptides containing proline. Prolidase is necessary for the preservation, reconstitution, and degradation of collagen structures in humans. Dysregulation of prolidase activity disrupts collagen metabolism (9). Prolidase activity induced by matrix metalloproteinases through tumor cell-mediated cytokines leads to collagen degradation and increased serum proline level. As a consequence, there have been recent discussions regarding the upregulation of hypoxia-inducible factor-1 (HIF-1) activity, which has stress-induced gene expression and angiogenic signaling, by increased prolidase activity. Under normal conditions, HIF-1 is inactivated. Activated HIF-1 by this mechanism may play a role in increasing angiogenesis and tumor spread. It has also been shown that prolidase activity is increased in the presence of nitric oxide (NO-), an important oxidative stress product, however, prolidase expression remains unchanged. NO- is thought to regulate prolidase activity by phosphorylating the serine/threonine regions associated with the prolidase enzyme via cGMP kinase (9).

It was shown in many studies that serum PON-1and ARE activity are associated with various types of cancers. Therefore, serum PON-1 and ARE activity are likely to be important in SCC carcinogenesis due to their antioxidant effects (1,6).

Fluctuations in prolidase activity can be detected during the development of some cancers and diseases. Fluctuations in prolidase activity can indicate both the presence of a disease state and the severity of the pathology. Literature studies have reported that prolidase activity is increased in some cancer types (9,11,12,13,14). This suggests that the increase in prolidase activity in cancers occurs due to enhanced collagen degradation and turnover, which is manifested in tumor metastases and spread (9,15).

Today, the actions of these enzymes on cancer development and their levels in cancer patients are being investigated in many studies. In this study, we purposed to investigate PON-1, ARE and prolidase enzyme activities and their roles in carcinogenesis (contribution, effect) in patients with oral cavity cancer.

Material and Method

This non-randomized, prospective clinical study was approved by the ethics committee of clinical research at Health Sciences University, Istanbul Bagcılar Research and Education Hospital (2018.09.2.01.078.r1.090).

Patients in this study were recruited from those who had oral cavity squamous cell carcinoma (OSCC) diagnosis and had been consulted to the otorhinolaryngology clinic. Individuals in the study group did not have any malignancy other than SCC of the oral cavity. Forty-six people were included in the study. The study group consisted of 24 (5 female, 19 male) and the control group consisted of 22 (4 female, 18 male, healthy, voluntarily, selected from the ear, nose and throat outpatient clinic). Included in the study were individuals aged between 34 and 84. Cigarette smoking and alcohol consumption habits of the patients and histopathologic diagnoses were recorded from patient follow-up data. A case report form was prepared for each individual. After obtaining informed consent forms from the patients, 5 ml of venous blood was taken from patients and controls. The blood was allowed to clot for 15 minutes and then was centrifuged for 10 minutes at 5,000 rpm. Serums were stored at -80 ° C.

Serum PON activity was measured by the method described in previous studies (7). ARE activity was measured by targeting the levels of phenylacetate hydrolysis. Tris-HCl buffer containing CaCl2 and

Table 1. Statistical data of patients and control groups

		Control Group n:22		Patient Group n:24		p
Age		53.59±11.05		55.42±13.54		0.621*
Sex	Male	18	81.82%	19	79.17%	
	Female	4	18.18%	5	20.83%	0.821+
Tobacco	No	11	50.00%	8	33.33%	
	Yes	11	50.00%	16	66.67%	0.251+
Alcohol	No	15	68.18%	15	62.50%	
	Yes	7	31.82%	9	37.50%	0.686+
Arylesterase (kU/L)		80.71±7.23		32.2±14.57		0.0001*
Paraoxonase 1 (U/L)		29.46±6.18		11.6±2.32		0.0001*
Prolidase (U/L)		19.53±1.13		39.51±3.02		0.0001*

*Independent t test +Chi square test

phenylacetate, 50 µl of serum at pH 8.0 was added and then, the activity of arylesterase activity was measured at 270 nm (7) . Mean measurement activity of prolidase was done according to the method specified by Kitchener and Grunden (9).

Statistical Analysis: NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program was applied and analyzes were done. Descriptive statistical methods and independent t-test were used to compare data and the chi-square test was used to compare qualitative data. Statistical significance was evaluated as $p < 0.05$.

Results

The study included 23 patients (19 men, 5 women) and 22 healthy controls (18 men, 4 women) with oral cavity malignancy (Table 1). The mean age was 55.42 ± 13.54 in the study group and 53.59 ± 11.05 in the control group. When the control and study groups were compared, there was no statistically significant difference between the groups in terms of age and gender distribution, tobacco smoking($p=0.251$) and alcohol consumption ($p = 0.668$) (Table1). The PON-1 activity was 11.6 ± 2.32 nmol / l in the study group and 29.46 ± 6.18 nmol / l in the control group. When statistical calculations were evaluated in the context of PON-1 levels, the levels in the study group were found to be significantly lower than those in the control group ($p = 0.0001$) (Table 1). The ARE activity was measured at 32.2 ± 14.57 nmol / l in the study group and 80.71 ± 7.23 nmol / l in the control group. The mean ARE levels in the study group were statistically significantly lower than the control group ($p = 0.0001$) (table 1). Prolidase activity was 39.51 ± 3.02 nmol / l in the study group and 19.53 ± 1.13 nmol / l in the control group. The mean prolidase levels in the study group were statistically significantly higher than the control group ($p = 0.0001$) (Table 1).

Discussion

Oxidative stress refers to an imbalance between the ability of a biological system to easily detoxify toxic reactive intermediates or to repair the resulting damage by producing ROS (5). It also reflects the impairment of ROS homeostasis due to either an increase in ROS or a decrease in ROS scavenging capacity (3). Toxic reactive intermediates can be separated into two groups as reactive oxygen products (H_2O_2, O_2; O^{-2} etc.) and reactive nitrogen products (NO^-, $ONOO^-$ etc.) (5). ROS occurs as a result of routine cellular activities in the body. While these products are required at low concentrations in some cellular events, they can cause harmful effects at high levels (3). ROS at high concentrations can disrupt cellular lipids, proteins, and DNA, which affect enzyme activity and membrane function; therefore, oxidative stress is considered a cause of tumor formation (5). Oxidative stress can be caused by various causes such as interaction with carcinogens, environmental influences, and genetic interactions, and has been shown to increase the pathogenesis of many diseases, including cancers (3). Some biological causes and mechanisms can be the source of the production of ROS in cells. Some of these factors include increased metabolic activity, mitochondrial dysfunction, peroxisomal activity, altered activity of many enzymes, oncogene activity in a cancer cell, receptor signaling (5,16,17). At the same time, cancer tissue has also been infiltrated by activated macrophages that increase ROS and cytokine production in large quantities and enhance inflammatory response (5, 18).

Cancer is primarily a multi-stage process involving initiation, development, and progression, in which ROS plays important roles (3, 19, 20). Prolidase is the enzyme family that contains a group of proteases that catalyze the cleavage of proteins into smaller peptides

and amino acids, and they are mainly involved in the cellular proline turnover. The largest proline repository in the human body is collagen tissue and its activity is increased in the case of nutritional stress (such as cancer) and tumor spread where collagen destruction is present (9). Oxidative stress, an important factor in the etiology of carcinogenesis, has been studied in patients with malignancy. Among these studies, the number of those performed in the patient group with malignancy of the oral cavity is rather small (1,3). We examined the PON-1 and ARE enzyme actions involved in the ROS scavenging system as well as prolidase enzyme activity levels that play a role in collagen degradation and proline turnover as a marker of tumor spread. With the anticipation that cigarette smoking and alcohol consumption along with age and sex might have an enhancing effect on oxidative stress mechanisms, we made sure there was no statistically significant difference between the groups concerning these parameters in our study.

In a study group of 57 patients with oral cavity malignancy, Metin et al. found PON-1 activity as 21.9±5 unit/l and in 59 control group as 120.4±2 unit/l (1). Similar to this study, we found PON-1 activity was 11.6±2.32 nmol/l in the patient group and 29.46±6.18 nmol/l in the control group. These values were statistically significant in both studies ($p < 0.0001$). It was also seen that the results of a study conducted by Malik and his colleagues were also compatible with our study (3).

Previous studies in the literature reported that PON-1 levels were significantly lower in patients with malignancy than in the healthy control group (4,5,6,7,21). Malik et al. have suggested that the results they have found may be crucial for examining the carcinogenesis process and assessing the effect of oxidative stress on this process (3). We can conclude that the results of our study are by findings from the literature.

ARE, as mentioned above, is an enzyme involved in the ROS scavenging system like PON-1. In this study, ARE enzyme activity was 32.2±14.57 nmol/l in the patient group. ARE enzyme activity was statistically significantly lower in the control group ($p<0.0001$). These results are consistent with the study of Malik et al. These authors have concluded that ARE levels can be used as an important marker in evaluating the carcinogenesis process like PON-1enzyme (3). Afşar et al. found that ARE and PON-1 levels were higher in colorectal cancer patients than in healthy controls (7). However, in a study conducted by Bulbuller et al., PON-1 levels were low, similar to the results of our study which was done on the colorectal cancer patient group (22). The same findings for PON-1 was reported by a study of Eroğlu et al. on patients with prostate carcinoma (23). In a study by Michalac et al. on a group of patients with ovarian cancer, results for ARE enzyme activity were similar to those of our study (5). However, our study has not been consistent with some studies in the literature. This can be interpreted as the role of oxidative stress may be different in various types of cancer.

In previous studies, prolidase enzyme activity is increased in nutritional stress. Among the reasons for this may be the use of collagen in the body as an energy source. It is thought that the increase in the level of prolidase is due to collagen degradation and the depletion of the proline pool, which is manifested as tumor metastases and spread (9). In our study, the prolidase level was significantly higher in the patient group (39.51±3.02 nmol/l) than the control group ($p<0.0001$). Our results are consistent with many studies in the literature (11,12,13,14,15,24). However, these studies do not contain a group of patients with oral cavity malignancies. In this case, we believe that higher levels of prolidase activity may be used to determine the stage of the disease, to monitor the extent of the disease and to choose treatment modalities in patients with early diagnosis of oral cavity malignancy. High prolidase levels might play a role as an important marker as well. The higher levels of prolidase activity may be used in the process of diagnosis and management of the patients with oral cavity malignancy.

We purposed to research the role of PON-1, ARE and prolidase in carcinogenesis and to provide preliminary data that should be further validated in other populations to fully understand the mechanism leading to activity changes and its effect on OSCC. However, in this study scarce number of cases, economic reasons and consideration of the environmental factors that could affect the enzyme activities prevented the diversification of study groups.

In conclusion, this study is the first preliminary study in the literatüre on oral cavity cancer fort he included enzyme activities. PON-1, ARE and prolidase enzyme activities may play an important role in the etiopathogenesis of oral cavity cancers to contribute the course of diagnosis and management. Further research concerning enzyme activity and oral cavity cancers will provide more precise data.

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