Original Article

Evaluation of glutathione peroxidase in the blood and tumor tissue of oral squamous cell carcinoma patients

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Abstract

Aims and Objectives: The lowered antioxidant capacity and the oxidant–antioxidant imbalance have been considered to play a role in multistage carcinogenesis. The deleterious effects produced by reactive oxygen species depend on the imbalance between oxidant and antioxidant status in the body, so this study is aimed to evaluate the levels of antioxidant enzyme, glutathione peroxidase (GPx), in the blood and tumor tissues of oral squamous cell carcinoma (OSCC) patients in comparison with healthy controls.

Materials and Methods: The study comprised of 38 participants divided into two groups. Group 1 comprised of 20 patients with OSCC and Group 2 comprised of age- and sex-matched 18 healthy individuals free of any habits and systemic illness. The levels of GPx were estimated in the blood and tissue samples in both groups by Paglia and Valentine method using a Commercial Biochemical assay kit (RANDOX), by ultraviolet-visible spectrophotometer.

Results: The GPx levels were elevated in the whole blood and the tissue samples of OSCC cases as compared to the control group. It was also found that the GPx levels were increased in the tumor tissue with respect to the histopathological grading of the OSCC cases.

Conclusion: Detection of antioxidant status may be useful to choose correct radiotherapy or chemotherapy, to monitor the effectiveness of the therapeutic strategy and to determine tumor resistance to therapy. Hence, the evaluation of GPx enzyme level can be used as a prognostic marker in patients with OSCC.

Keywords: Antioxidants, glutathione peroxidase, oral squamous cell carcinoma, oxidative stress

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INTRODUCTION

Oral cancer is a major form of cancer worldwide and is one of the most common malignancies in India accounting for 30%–40% of all cancers.[1] Tobacco is the primary etiological factor and other factors include alcohol, genetic predisposition and a diet lacking micronutrients.[2]

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Antioxidants have an important role in the prevention of cancers at various stages. The oxidant–antioxidant imbalance has been considered to play a role in multistage carcinogenesis.[3] Antioxidants are the first line of defense against free radical damage and are essential for maintaining optimum health and well-being. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are the three major enzymatic antioxidant

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defense systems responsible for scavenging free radicals and nascent oxygen.[4]

Since the deleterious effects produced by reactive oxygen species (ROS) depend on the imbalance between oxidant and antioxidant status in the body, this study was aimed to evaluate the levels of antioxidant enzyme, GPx, which forms the main antioxidant defense system. GPx helps to remove reactive species once formed. It functions to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.[5] As this scavenging antioxidant is concentrated more in the cytosol and cell membrane,[6] the whole blood was used for the analysis of GPx.

Tobacco and other tobacco-related products have direct effect on the oral mucosal tissue and cause malignant transformation. Therefore, tumor tissue was also evaluated to see if there are any changes in the lesional tissue for the levels of GPx as compared to the normal healthy tissue.

MATERIALS AND METHODS

Study population

This was a case–control study which included a total of 38 participants. They were divided into two groups. Group 1 comprised of 20 patients with oral squamous cell carcinoma (OSCC) diagnosed histopathologically and not received any prior treatment. Group 2 comprised of age- and sex-matched 18 healthy individuals free of any habits and any systemic illness. Institutional ethical clearance was obtained for the study and informed consent was obtained from all the patients prior to the study. Group 1 (OSCC) patients were divided into well (Grade I)-, moderate (Grade II)- and poorly (Grade III)-differentiated carcinoma, whereas clinically, they were categorized into Stages I/II/III/IV on the basis of the tumor, node and metastasis staging system.

Collection of samples

Blood and tissue samples were obtained from both the groups. Under all aseptic precautions, incisional biopsies were taken from the representative sites in OSCC cases and for control group, healthy tissue was obtained during minor surgical procedures such as operculectomy, removal of impacted third molars, implant cases and gingivectomy in orthodontic cases. The biopsied tissue was washed with normal saline 2–3 times to remove the red blood cells and then stored in normal saline at −80°C till the analysis. Under all aseptic conditions, 2 ml of venous blood was collected from antecubital fossa of each patient from both the groups in heparin bulb and was stored at −80°C till the analysis.

Procedure for the estimation of glutathione peroxidase levels

The levels of GPx were estimated in the blood and tissue samples in both groups, i.e., cases and controls by Paglia and Valentine method using a Commercial Biochemical assay kit (RANSEL; Glutathione Peroxidase kit by RANDOX), by ultraviolet (UV)–visible spectrophotometer.

Method to evaluate glutathione peroxidase levels in the whole blood

0.05-ml heparinized whole blood diluted with 1-ml diluting agent (R3) was incubated for 5 min and then 1 ml of hemoglobin reagent was added to it. Diluted blood sample (20 μl), reagent R1 (glutathione, glutathione reductase [GR], nicotinamide adenine dinucleotide phosphate [NADPH], 1000 μl) and reagent R2 (cumene hydroperoxide, 40 μl) were pipetted into a test tube, mixed well and readings were taken at the initial absorbance of sample and reagent blank after 1 min and timer was started simultaneously. Further readings were again taken after 1 and 2 min. Reagent blank value was subtracted from that of the sample.

Method to evaluate glutathione peroxidase levels in the tissue

Tissues were weighed individually and accordingly the phosphate-buffered saline, pH 7.4, was added, and the tissue was homogenated and centrifuged at 2500 rpm for 10 min. And, the supernatant was used for the assessment of GPx.

Biochemical analysis

The levels of GPx were estimated by Paglia and Valentine method,[5] which is based on the principle that GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroxide. In the presence of GR and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm is measured at 37°C on UV spectrophotometer, and data are expressed as U/dl in whole blood and U/g of protein concentration in the tissue samples.

Statistical analysis

The findings were expressed as mean ± standard deviation. Statistical analysis was performed using unpaired *t*-test. $P < 0.05$ was considered statistically significant and $P < 0.01$ as statistically highly significant.

RESULTS AND OBSERVATIONS

The results obtained of the assessed GPx in OSCC patients and healthy controls are shown in Table 1 for the whole

blood and tissue samples, respectively. Comparison of the levels of GPx among the OSCC group and control group showed a statistically highly significant difference for mean GPx levels in the whole blood ($P < 0.01$, $t = 6.86$) and in tissue proteins ($P < 0.001$, $t = 16.24$).

The mean GPx levels within different clinical stages of OSCC were compared. From the statistical analysis, no significant difference was found either in the blood ($P > 0.05$, $t = 0.48$) or in the tumor tissue ($P > 0.05$, $t = 0.26$) of OSCC patients in different clinical stages [Table 2].

Table 3 depicts the comparison of mean GPx levels in the whole blood and tissue within histopathological grades of OSCC. Statistically no significant difference was found in the mean GPx levels in the blood ($P > 0.05$, $t = 0.12$) between all the three histopathological grades of OSCC cases. The results in the tumor tissue showed that the mean GPx levels increased with the increasing grade of the disease. From the statistical analysis, it was observed that the difference of mean GPx levels between Grade I and Grade II ($P < 0.01$, $t = 7.18$) and Grade II and Grade III ($P < 0.01$, $t = 9.01$) OSCC cases was statistically highly significant.

Table 1: Comparison of glutathione peroxidase levels in the blood and the tissue between oral squamous cell carcinoma cases and controls

Study groups	Whole blood (U/dl)	Tissue (U/g of protein)
Group 1: OSCC (*n*=20)	1924.89±421.5	15.96±7.67
Group 2: Control (*n*=18)	1038.51±348.1	7.96±2.09
t	6.86	16.24
Significance	HS	HS

HS: Highly significant, *n*: Number of patients, OSCC: Oral squamous cell carcinoma

Table 2: Comparison of glutathione peroxidase levels in the blood and tissue within different clinical stages of oral squamous cell carcinoma cases

Clinical stages	Whole blood (U/dl)	Tissue (U/g of protein)
Stage III (*n*=12)	1714.02±1001.2	16.19±7.68
Stage IV (*n*=08)	1503.15±920.3	16.43±8.2
t	0.48	0.26
Significance	NS	NS

NS: Not significant

Table 3: Comparison of glutathione peroxidase levels in the blood and tissue between histological grades of oral squamous cell carcinoma cases

Histopathological grades	Whole blood (U/dl)	Tissue (U/g of protein)
I (*n*=13)	1916.09±546.98	12.75±3.08
II (*n*=4)	1948.63±509.65	16.89±11.11
III (*n*=3)	1931.39±665.7	28.65±2.71
t	0.12	7.18
Significance	NS	HS

NS: Not significant, HS: Highly significant

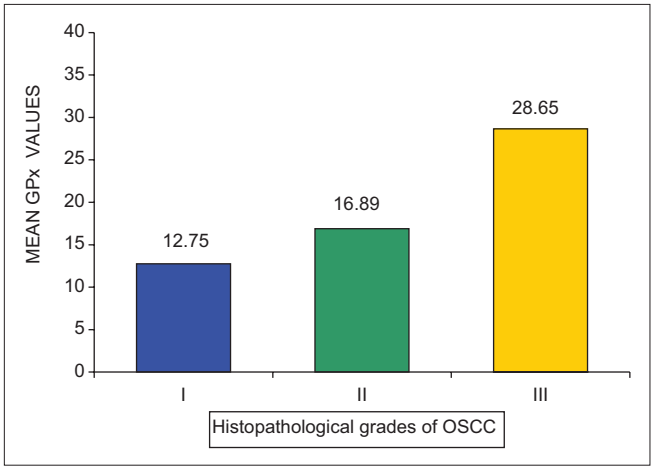
As compared to the blood, the GPx levels were increased in the tumor tissue with respect to the histopathological grading of the OSCC cases.

DISCUSSION

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins and carbohydrates for energy; however, it does not come without a cost. Oxygen is a highly reactive atom that is capable of becoming a part of potentially damaging molecules (ROS), commonly called free radicals. Free radicals are capable of attacking healthy cells of the body, causing them to lose their structure and function and may transform normal cells into malignant one. Cell damage caused by free radicals appears to be a major contributing factor for carcinogenesis.[7]

The human body has developed several enzymatic and nonenzymatic antioxidants that scavenge the free radicals and inhibit the neoplastic process. Any changes in one of these systems may break this equilibrium and cause cellular damages and ultimately malignant transformation.[2] GPxs are a family of selenium-dependent enzyme with at least four isoenzymes identified to date. It protects cells against oxidative damage by reducing hydrogen peroxide and a wide range of organic peroxides.[8]

Epidemiological studies have shown a casual relationship between the incidence of oral cancer and tobacco consumption.[9] In India, chewing tobacco and betel quid, usually mixed with other toxins such as slaked lime, is a common custom that causes oral cancer, which is responsible for 50%–90% of the cases.[10] In the present study, all the OSCC patients had the habit of chewing tobacco; few patients particularly between the ages



Graph 1: Comparison of glutathione peroxidase levels in the tumor tissue with histopathological grades of oral squamous cell carcinoma

of 30 and 45 years also had other chewing habits such as gutkha, betel quid and areca nut along with tobacco habit.

The cell of origin of OSCC is the oral keratinocyte, in which DNA mutation can be spontaneous, but mutagens increase the mutation rate.[11] Chewing of tobacco results in a local exposure of oral mucosa to tobacco-specific nitrosamines. In our study, we evaluated the GPx levels in tumor tissue, as the localized tissue abuse is rampant in the form of tobacco-related habits. Blood is an indicator of systemic condition. Hence, tissue and blood were studied so as to understand localized as well as systemic changes associated with OSCC.

In the present study, the mean GPx level in the whole blood was 1924.89 ± 421.5U/dl in the OSCC group and 1038.51 ± 348.10U/dl in the control group. The mean GPx level was significantly higher ($P < 0.01$) in the OSCC group compared to that in control group.

Most of the studies[12-15] showed the increased levels of GPx in the serum/plasma of blood. However, in the present study, the GPx level was evaluated in the whole blood as the cell membrane of blood cells contains the maximum amount of GPx concentration as compared to the blood serum or plasma.

The increased levels of GPx in the whole blood, observed in the present study, might be due to higher magnitude of oxidative stress since all our patients were in advanced clinical stages (III and IV) having a large tumor burden, and there might be leakage of GPx from the tissue to the blood to control the oxidative stress due to increased tumor burden.

Nagini *et al*.[12] measured thiobarbituric acid-reactive substances, reduced glutathione, GPx and SOD in the blood of OSCC patients. Lipid peroxidation product, CAT and SOD levels significantly decreased, whereas reduced glutathione and GPx levels elevated. It was hypothesized that a decrease in peroxidizable substrates in cancers is associated with an increase in antioxidant capacities, conferring a selective growth advantage to cancer cells.

Surapaneni and Vishnu[16] observed a significant increase in erythrocyte malondialdehyde, SOD and GPx levels in OSCC patients compared to the normal. These results are in line with our findings. GPx, an oxidative stress-inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes. The rise in the activity of GPx could be due to its induction to counter the effect of increased oxidative stress.

OSCC is a localized lesion due to tissue abuse habits, and the continuous exposure of the oral mucosal tissue to these chemical carcinogens will lead to increased oxidative stress and ultimately increased cell proliferation and tumor growth. In general, the defense mechanism of body will reflect by producing the increased levels of antioxidants (as in the present study) in the blood to combat the increased levels of free radicals generated by the growing tumor cells.

The mean GPx level in the tumor tissue was 15.96 ± 7.67U/g of protein in the OSCC group and 7.96 ± 2.09 U/g of protein in the control group. The mean GPx level was highly significant ($P < 0.001$) in the OSCC group compared to that in control group. These results were in accordance with those of studies by Gokul *et al*.,[17] Nagini *et al*., Hristozov *et al*.,[13] Fiaschi *et al*.,[14] Rasheed *et al*.,[15] Subapriya *et al*.[18] and Srivastava *et al*.,[19] in the OSCC patients.

These results are in agreement with the studies done on tumor tissue GPx levels in human colorectal cancer, lung cancer, hepatocellular carcinoma,[20] breast cancer,[21] cervix cancer[22] and laryngeal cancer[23] and also in the studies by Baskaran *et al*.[24] in albino rats. Balasenthil *et al*.[25] compared the antioxidant changes in the tumor tissue of hamster and human oral mucosa and found increased levels of GPx in the tumor tissue.

In our study, we observed no significant correlation between clinical stages of the disease and levels of GPx in whole blood or in the tumor tissue.

In the present study, we found a significant positive correlation between GPx levels with increase in the grades of OSCC, in the tumor tissue only but not in the blood levels of GPx. The increase in the mean GPx level in tumor tissue was observed with advancing histological grade of OSCC. It was 12.75 ± 3.08 U/g of protein in Grade I OSCC cases and 16.89 ± 11.11U/g of protein in Grade II and 28.65 ± 2.71U/g of protein in Grade III OSCC cases [Graph 1]. These findings are in agreement with the recent studies by Srivastava *et al*.[19] who found a positive correlation between GPx levels with increase in the grades of OSCC, in the tumor tissue as in our study.

Inci *et al*.[23] have suggested that the detection of antioxidant status may be useful to choose correct radiotherapy or chemotherapy, to monitor the effectiveness of the therapeutic strategy and to determine the tumor resistance to therapy. According to the results obtained from our study and from the supportive literature studies, we can conclude that evaluation of GPx enzyme level can be

used as a biomarker of oxidative stress to determine the progression of various stages of cancer.

CONCLUSION

From the present study, it is evident that antioxidant status and oxidative damages in the cell structure are related to tumor process, indicating augmentation of oxidative stress in OSCC. Further elaborative studies with a larger sample size could ratify the value of glutathione peroxidase as a prognostic marker of oxidative stress to determine the progression of various stages of cancer.

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Conflicts of interest

There are no conflicts of interest.

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