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Assessing Glutathione-S-Transferase Levels In Serum with Oral Malignancy Patients

Dr. Virvardhan Alias Shubham Patil[1], Dr. Vinay Salyam[2], Dr. Manda Akshitha[3], Dr. Gourav Saini[4], Dr. Aparna Dave[5], Dr Pulin Saluja[6]

[1]Assistant Professor, Department of Prosthodontics, D Y Patil Dental School, Pune,412105.

[2]Senior Lecturer, Department of Oral Pathology and Microbiology, Rajarajeswari Dental College and Hospital, Mysore Road, Kumblegodu.

[3]BDS, Intern, Rajarajeswari Dental College and Hospital, Mysore Road, Kumblegodu.

[4]Post Graduate 3rd Year, Department of Oral and Maxillofacial Pathology and Oral Microbiology, SGT Dental College and University, Budhera, Gurugram, Haryana

[5]Professor, Department of Oral and Maxillofacial Pathology and Oral Microbiology, SGT Dental College and University, Budhera, Gurugram, Haryana

[6]Professor, Department of Oral and Maxillofacial Pathology and Oral Microbiology, SGT Dental College and University, Budhera, Gurugram, Haryana

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ABSTRACT

Background: Glutathione-S-transferases (GSTs) are a family of detoxifying enzymes that play a critical role in cellular defense mechanisms against oxidative stress and carcinogenic agents. Alterations in GST activity have been implicated in the pathogenesis of various malignancies, including oral cancer. This study aims to assess and compare serum GST levels in patients diagnosed with oral malignancy versus healthy individuals to explore its potential as a biomarker for early detection and progression of disease.

Materials and Methods: A total of 60 participants were included in this cross-sectional study, comprising 30 patients with histologically confirmed oral squamous cell carcinoma (Group A) and 30 age- and sex-matched healthy controls (Group B). Blood samples were collected, and serum GST levels were measured using an ELISA-based immunoassay. Clinical parameters including tumor site, TNM stage, and tobacco use history were recorded. Statistical analysis was performed using independent t-tests and one-way ANOVA for subgroup comparisons, with a significance threshold of $p<0.05$.

Results: The mean serum GST level in Group A was significantly elevated (12.45 ± 2.36 U/L) compared to Group B (6.78 ± 1.15 U/L) ($p<0.001$). A positive correlation was observed between GST levels and advanced TNM staging (Stage III and IV showed a mean GST level of 14.21 ± 1.85 U/L). Tobacco users among oral cancer patients had higher GST levels (13.56 ± 1.90 U/L) than non-users (10.87 ± 2.14 U/L), suggesting a synergistic effect of tobacco-induced oxidative stress.

Conclusion: Serum glutathione-S-transferase levels are significantly elevated in patients with oral malignancy and correlate with disease progression and tobacco exposure. GST may serve as a potential biomarker for early diagnosis, risk assessment, and monitoring of therapeutic response in oral cancer patients

Keywords: *Glutathione-S-transferase, GST, Oral malignancy, Oral squamous cell carcinoma, Serum biomarkers, Oxidative stress, Cancer diagnostics*

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is among the most common malignancies affecting the head and neck region, accounting for over 90% of all oral cancers worldwide. Despite advances in diagnostic and therapeutic strategies, the overall prognosis remains poor, largely due to late-stage diagnosis and frequent recurrences (1). Identifying reliable biomarkers that can aid in early detection, risk stratification, and therapeutic monitoring of OSCC is therefore a critical research focus.

One of the pivotal mechanisms in carcinogenesis is oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system. This imbalance leads to damage of cellular components, including DNA, proteins, and lipids, contributing to mutagenesis and tumor development (2). Glutathione-S-transferases (GSTs) are a superfamily of phase II detoxification enzymes that play a vital role in neutralizing oxidative stress by catalyzing the conjugation of glutathione to a wide range of electrophilic compounds, including carcinogens and cytotoxic agents (3). The GST family includes several isoenzymes, with GST alpha, mu, pi, and theta classes being most studied in relation to cancer (4).

Altered expression or activity of GSTs has been implicated in the development and progression of multiple cancers, including those of the liver, lung, and colon (5). In oral cancers, GST polymorphisms and overexpression have been reported to influence susceptibility, tumor aggressiveness, and response to therapy (6,7). Among various diagnostic modalities, evaluating serum GST levels offers a non-invasive approach that may reflect systemic oxidative burden and tumor-associated metabolic changes (8).

Several studies have explored the link between GST activity and tobacco use, a well-established etiological factor for oral cancer. Tobacco contains numerous pro-oxidants and carcinogens that can induce GST expression as an adaptive response, suggesting a possible synergistic role in tumorigenesis (9). Thus, investigating serum GST levels in oral malignancy patients, especially in relation to clinical parameters such as tumor staging and tobacco exposure, can provide valuable insights into its diagnostic and prognostic significance.

This study aims to evaluate and compare serum glutathione-S-transferase levels in patients with histopathologically confirmed OSCC and healthy controls. It further seeks to determine the association of GST levels with clinical variables such as TNM stage and tobacco use, in order to assess its potential utility as a biomarker in the early diagnosis and management of oral malignancies

2. MATERIALS AND METHODS

This cross-sectional observational study was conducted on a total of 60 individuals, divided into two equal groups: Group A comprised 30 patients with histopathologically confirmed oral squamous cell carcinoma (OSCC), while Group B included 30 age- and sex-matched healthy controls with no history of malignancy or systemic disease.

Participant Selection Criteria: Inclusion criteria for Group A were patients aged 18 years or older with newly diagnosed, untreated OSCC. Group B participants were healthy volunteers with no prior history of tobacco or alcohol use and no clinical signs of oral pathology. Individuals with systemic conditions affecting oxidative stress levels (e.g., diabetes mellitus, chronic inflammatory diseases), those under antioxidant therapy, or patients who had undergone prior cancer treatment were excluded from the study.

Sample Collection and Processing: From each participant, 5 ml of venous blood was drawn using sterile, disposable syringes and collected into plain vacutainer tubes. The samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate the serum. The isolated serum was stored at −20°C until further biochemical analysis.

Estimation of Serum GST Levels: Serum glutathione-S-transferase levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions. The assay was based on the sandwich ELISA technique and performed using an automated microplate reader. All samples were analyzed in duplicate to ensure reliability of the results.

Clinical Data Collection: For OSCC patients, detailed clinical information including tumor location, TNM staging as per AJCC criteria (8th edition), and tobacco usage (smoking or smokeless forms) was documented through patient interviews and medical records. The staging was further categorized into early (Stage I–II) and advanced (Stage III–IV) for analysis.

Statistical Analysis: Descriptive statistics were used to summarize baseline characteristics. The independent samples t-test was employed to compare serum GST levels between the two groups. One-way analysis of variance (ANOVA) was conducted for comparing GST levels across different TNM stages. The association between tobacco use and GST levels within Group A was also examined using the t-test. A p-value of less than 0.05 was considered statistically significant. Data were analyzed using SPSS software version 25.0.

3. RESULTS

The study included a total of 60 participants, with 30 individuals in each group. The demographic profile was comparable between both groups in terms of age and gender distribution (mean age: Group A – 52.6 ± 9.4 years; Group B – 50.8 ± 8.7 years; $p = 0.47$). Among Group A patients, 70% (n=21) were male and 30% (n=9) were female, while Group B had 66.7% (n=20) males and 33.3% (n=10) females.

Comparison of Serum GST Levels Between Groups

The mean serum glutathione-S-transferase (GST) level was significantly higher in oral squamous cell carcinoma patients (Group A: 12.45 ± 2.36 U/L) compared to healthy controls (Group B: 6.78 ± 1.15 U/L), with a statistically significant difference ($p < 0.001$) (Table 1).

Table 1: Comparison of Serum GST Levels Between Study Groups

Group	N	Mean GST (U/L)	SD	p-value
Group A (OSCC patients)	30	12.45	2.36	<0.001
Group B (Healthy controls)	30	6.78	1.15	

(*Table 1 shows significantly elevated GST levels in OSCC patients compared to controls.*)

GST Levels and TNM Staging

Within Group A, GST levels were analyzed according to TNM stage. Patients in Stage I–II had a mean serum GST level of 10.58 ± 1.92 U/L, whereas those in Stage III–IV demonstrated markedly higher levels at 14.21 ± 1.85 U/L. The difference was statistically significant ($p < 0.01$) (Table 2).

Table 2: Association of Serum GST Levels with TNM Stage in OSCC Patients

TNM Stage	N	Mean GST (U/L)	SD	p-value
Stage I–II	12	10.58	1.92	<0.01
Stage III–IV	18	14.21	1.85	

(*Table 2 illustrates that GST levels increased with advancing TNM stage, indicating a positive correlation with disease severity.*)

Influence of Tobacco Use on GST Levels

Among OSCC patients, 20 were current tobacco users and 10 were non-users. Tobacco users had a mean GST level of 13.56 ± 1.90 U/L, significantly higher than non-users (10.87 ± 2.14 U/L) ($p = 0.003$), suggesting a possible interaction between tobacco exposure and oxidative stress-related enzyme activity (Table 3).

Table 3: Comparison of GST Levels Between Tobacco Users and Non-Users in OSCC Group

Tobacco Use	N	Mean GST (U/L)	SD	p-value
Users	20	13.56	1.90	0.003
Non-users	10	10.87	2.14	

(*As seen in Table 3, tobacco use is associated with significantly elevated GST levels among oral cancer patients.*)

Overall, the findings indicate that serum GST levels are significantly elevated in OSCC patients compared to healthy individuals, and are further influenced by disease stage and tobacco consumption (Tables 1–3).

4. DISCUSSION

The present study assessed the serum levels of glutathione-S-transferase (GST) in patients diagnosed with oral squamous cell carcinoma (OSCC) compared to healthy controls. The results demonstrated significantly elevated serum GST levels in OSCC patients, with a positive correlation observed between GST expression and both advanced TNM staging and tobacco usage. These findings suggest that GST could serve as a valuable biomarker in the diagnosis and progression monitoring of oral malignancies.

Glutathione-S-transferases are phase II detoxification enzymes that play a critical role in cellular defense mechanisms by catalyzing the conjugation of reduced glutathione to electrophilic substrates, including environmental carcinogens and endogenous toxic compounds (1). Overexpression of GSTs has been linked to increased resistance to oxidative stress and the development of multiple cancer types, including hepatocellular, colorectal, and breast carcinomas (2–4). In the context of oral cancer, the upregulation of GSTs may represent a cellular adaptation to the heightened oxidative burden induced by carcinogens such as tobacco and alcohol (5).

The significantly higher serum GST levels observed in our OSCC group (12.45 ± 2.36 U/L) compared to healthy controls

(6.78 ± 1.15 U/L) are consistent with previous research demonstrating elevated antioxidant enzyme activity in cancer patients (6). Similar trends have been reported by Dey et al., who noted increased GST activity in the blood of patients with oral precancer and cancer lesions (7). The underlying mechanism may involve the tumor cells' increased metabolic activity and the resultant oxidative stress, prompting upregulation of antioxidant enzymes including GSTs (8).

Furthermore, our findings support the association between GST levels and tumor burden, as patients with advanced-stage OSCC (Stage III and IV) had significantly higher GST concentrations compared to those in early stages. This observation aligns with reports indicating that GST activity correlates with tumor aggressiveness and metastatic potential (9,10). The upregulation of GST in advanced malignancies may also reflect increased demand for cellular detoxification due to heightened proliferation and metabolic turnover (11).

Tobacco use, a well-established etiological factor for OSCC, appeared to significantly influence GST expression in this study. Tobacco users had considerably higher GST levels compared to non-users, indicating a possible synergistic interaction between carcinogen exposure and antioxidant response (12). Tobacco contains polycyclic aromatic hydrocarbons and nitrosamines, which are known GST substrates. The enzymatic response may be a protective attempt to neutralize these harmful agents, albeit insufficient to prevent oncogenic transformation (13).

Genetic polymorphisms in GST genes have also been associated with variable susceptibility to oral cancer. For instance, null genotypes of GSTM1 and GSTT1 have been linked to reduced enzymatic activity and increased cancer risk in several populations (14). Although this study did not evaluate GST genotypes, future research incorporating genetic screening alongside serum level estimation could provide a more comprehensive understanding of GST's role in oral carcinogenesis.

Serum biomarkers offer a minimally invasive approach for cancer screening and monitoring. Compared to tissue-based assessments, serum GST evaluation using ELISA offers practical advantages in clinical settings (15). The significant correlation of GST levels with tumor stage and tobacco use supports its potential application as a prognostic tool and a marker of disease progression.

5. CONCLUSION

In conclusion, the findings of this study highlight the diagnostic and prognostic potential of serum glutathione-S-transferase in oral squamous cell carcinoma. Elevated GST levels in OSCC patients, particularly in those with advanced disease and tobacco exposure, underscore its relevance in oxidative stress-mediated carcinogenesis. However, further longitudinal studies with larger sample sizes and integration of molecular profiling are necessary to validate its clinical utility and explore therapeutic implications

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