Comparison of Biochemical Markers (Glutathione S Transferase and Glutathione Reductase) in Patients With Habit of Tobacco Consumption and Cancer of Oral Cavity

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Abstract :

Introduction : Glutathione plays an important role in phase-II detoxification of carcinogens. In this study, a comparison of serum Glutathione S Transferase(GST) and Glutathione Reductase (GR) levels and their co-relation was done among four groups of individuals which included oral cancer patients, patients suffering from oral premalignant conditions, healthy volunteers who were habitual tobacco addicts and non tobacco users. **Material and Methods :** 100 subjects were divided into 4 groups of 25 each consisting of, Group 1:(OC) Patients with histologically proven squamous cell carcinoma of oral cavity with a history of habitual tobacco use. Group 2: (OPC) Patients with premalignant conditions of the oral cavity with history of habitual tobacco use. Group 3 : (TNOC) Healthy volunteers with habits of tobacco use but no oral pathology. Group 4 : (CONTROL) Healthy volunteers with no oral pathology and no history of tobacco use. Both GST and GR activity in the serum were analyzed spectrophotometrically. **Results :** GR activity was significantly higher in group 1, 2, 3 than group 4. Serum GST activity was higher in group 3 (TNOC) as compared to group 4(C). Group 2(OPC) had a statistically significant lower mean GST level. Mean GST was lower in Group1&2 than in Group 3&4 patients, while there was no significant change in GR levels. No statistical significance was found in mean GST or GR values in various stages of the disease or with duration of tobacco. **Conclusion :** GST and GR are potent biochemical markers and assessment of these markers allows us to identify subjects with high risk of developing cancer and targeting them for intensive tobacco cessation intervention.

Key words: Glutathione Reductase, Glutathione S Transferase, Oral cancer.

Introduction :

Oral cancer is an insidious devastating malignancy and is one of the five leading sites of cancer in India. [1] Overall, an estimated 12.7 million new cancer cases were detected and 7.6 million cancer deaths were reported in 2008 in the world .[2] For both genders combined, cancer of the mouth and pharynx ranks sixth in the world. The highest rates in the world for oral cancer are found in France, the Indian subcontinent, Brazil, and central & eastern Europe. In high-prevalence areas, cases occur prior to the age of thirty five due to heavy abuse of various forms of tobacco. [3] In spite of significant advances in surgery and radiation therapy, the five-year survival has remained at about 52% for the past few decades [4]. Among the oral tumors, 90% of them are squamous cell carcinomas (SCC), which arise from the mucosal lining. [5]

Taken together, the effects of tobacco use, heavy alcohol consumption, and poor diet probably explain over 90 percent of cases of head and neck cancer. More than 300 carcinogens have been identified in tobacco smoke or in its water-soluble components that reach into saliva. [6]

Metabolism of carcinogens usually involves oxygenation by p450 enzymes in cytochromes, and then conjugation, in which the enzyme glutathione S transferase (GST) is involved. Polymorphisms of the p450 and GST genes are currently under active study in the search for genetic markers of susceptibility to head and neck cancer, and indeed to tobacco-related cancers at many other body sites. Not all results are consistent, however. [7] Recently GST has attracted interest in the field of diagnosis and monitoring of malignancy. [8] Glutathione, an antioxidant plays an important role in

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phase-II detoxification of carcinogens. The levels of glutathione are maintained by glutathione-depleting as well as replenishing enzymes such as glutathione-s-transferase (GST) and glutathione reductase (GR), respectively.[9]

In this study a comparison of serum GST and GR levels and their co relation was done among four groups of individuals which included oral cancer patients, patients suffering from oral premalignant conditions, healthy volunteers who were habitual tobacco addicts and non tobacco users.

Aims and Objectives :

Analysis and clinicopathological correleation of plasma Glutathione S Transferase (GST) and plasma Glutathione Reductase (GR) levels among patients of oral cancers and pre cancerous patients, habitual tobacco users and non tobacco users.

Material and Methods :

This prospective study was conducted at the department of ENT, Head and Neck Surgery at a tertiary care hospital in the Anand district of Gujarat, India. A total of 100 subjects were recruited for the study. Subjects were divided into four groups of 25 individuals each in the following manner.

Group 1: (OC) Patients who have developed histologically proven squamous cell carcinoma of oral cavity with a history of habitual tobacco use.

Group 2: (OPC) Patients with premalignant conditions of the oral cavity with a history of habitual tobacco use.

Group 3: (TNOC) Healthy volunteers with habits of tobacco use but no oral pathology.

Group 4: (CONTROL) Healthy volunteers with no oral pathology and no history of tobacco use.

The use of tobacco included smoking of cigarettes, bidis, cigar, chillum and hukka, tobacco use also included chewing and ingestion of ghutka, mava and khaini. The duration of tobacco abuse was recorded in each case.

Those with malignancy other than oral cavity were excluded from the study. Blood sample was collected between 9:00 am to 11:00 am, with prior consent of the patients to participate in the study. 4ml of venous blood was collected from each individual under aseptic precautions. These samples were centrifuged at 3000 rpm for 10 minutes. Separated sera were stored at -20° C until analysis.

Clinical staging of malignancy in group 1 and group 2 was done according to American Joint Committee on Cancer (AJCC) (1997) criteria.[10] Detailed clinical evaluation was done in all subjects including healthy volunteers. Both GST and GR activity in the serum were analyzed spectrophotometrically using a Beckman DU 640 scanning spectrometer. Blood samples were analyzed and values were expressed as mean ± SE (standard error). The enzyme activity was expressed as ηmol product formed /minute/mg of protein.

Statistical Analysis: All data were analyzed using SPSS statistical software version 10. Students' 't' test was performed to compare GST and GR activities in cancerous and non cancerous patients. Differences in enzyme activities were considered to be significant when $p<0.05$.

Results :

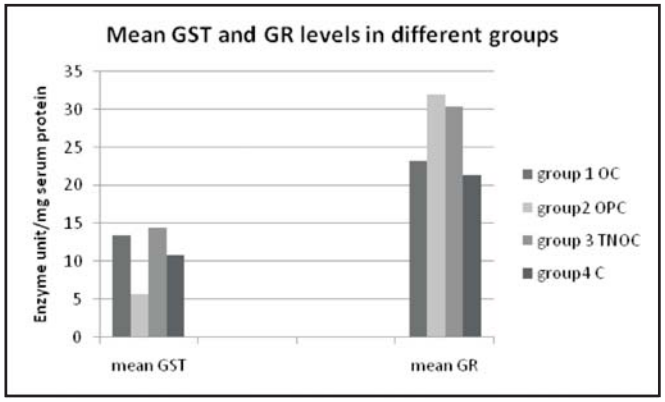
Out of the total 100 subjects recruited for the study, 84 were males and 16 were females. Age of the patients ranged from 19 to 70 years. Group 1 patients were histologically diagnosed cases of squamous cell carcinoma of the oral cavity; out of which 10 cases were early stages and 15 cases were advanced stages. Staging was based on AJCC criteria and TNM classification and for statistical purpose, stage 1 &2 were considered early and stage 3 & 4 were considered as advanced. Group 2 consisted of 25 patients suffering from precancerous lesions of the oral cavity that included oral sub mucous fibrosis (OSMF) and oral

leukoplakia (OL). In group 2, 14 patients were OSMF and 11 were OL. Tobacco associated free radical and oxidative stress related response in terms of antioxidant enzyme activities (GST/GR) were studied in all four groups. In group 1(OC), serum estimation of GST/GR was done prior to start of treatment. The serum GST and GR activities among all four groups are shown in Table 1 and and a comparison of the same is shown in Figure 1.

Table 1: Mean GST and GR levels in all groups

Groups	Mean GST	Mean GR
Group 1 OC	13.5	23.3
Group 2 OPC	5.7	32
Group 3 TNOC	14.5	30.4
Group 4 C	10.9	21-4

Figure 1: The comparison of mean GST and GR levels among Group 1, 2, 3 and 4

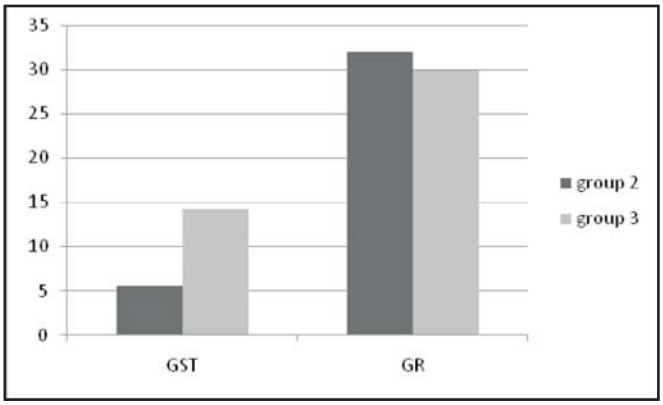


Serum GST activity was higher in group 3 (TNOC) as compared to group 4(C). Group 2(OPC) had a statistically significant lower mean GST level. Group 1 also had a low GST level which was however statistically not significant. GR activity was significantly higher in group 1, 2& 3 than group 4. GST activities was higher in group 3 as compared to group 4 as shown in table 2 .(Table 2 and Figure 2).

Table 2 : Comparison of mean GST and GR among group 2 and group 3

Enzyme	Group	n	Mean	SD	Std. Error of Mean
GST	2	25	5.7272	3.8622	0.7727
	3	25	14.416	4.2128	0.8426
GR	2	25	32.1460	11.2011	2.2402
	3	25	30.4996	10.4739	2.0948

Figure 2: Comparison of mean GST and GR among group 2 and group 3

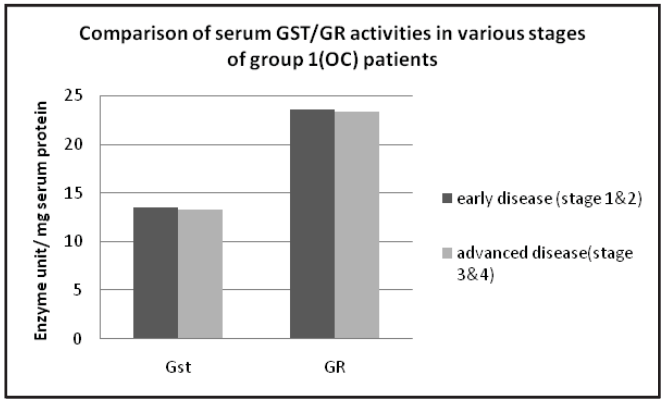


Comparison of GST and GR activity was also done among patients with no oral cavity lesions (Group 3&4) and patients with cancerous or precancerous lesions of the oral cavity (Group 1 & 2). Mean GST was found to be lower in cancerous and precancerous patients while there was no significant change in serum GR levels as shown in Table 3.

Table 3: Comparison of GST and GR in patients of cancerous and precancerous lesions of the oral cavity (group 1 & 2) against healthy volunteers (group 3 & 4).

Enzyme	Group	n	Mean	SD	Std.Error of Mean
GST	1 & 2	50	9.5652	5.8373	.8255
	3 & 4	50	12.7045	3.7667	.5327
GR	1 & 2	50	27.7778	9.4918	1.3423
	3 & 4	50	25.9968	8.8609	1.2531

Figure 3 : Comparison of mean GST / GR activity in early and advanced stages of Group 1 (OC) patients.



Analysis of serum GST & GR activity was also done separately in group 1(OC) patients and comparison was done among early and advanced stage of the disease. No statistical significance was found in mean GST & GR values in various stages of the disease as shown in table 4 and figure 2. Serum GST activities did not have any statistical co-relation with duration of tobacco consumption.

Table 4 : Comparison of mean GST/GR activity in early and advanced stages of Group 1 (OC) patients

Enzyme	Stage	n	Mean	Std Deviation	Std Error of Mean
GST	Early	10	13.5500	5.0796	1.6063
GST	Advanced	15	13.3053	4.9463	1.2771
GR	Early	10	23.5510	4.3284	1.368
GR	Advanced	15	23.3153	4.4756	1.1556

Discussion :

Living organisms are continuously exposed to non-nutritional foreign chemical species. These xenobiotics may interact deleteriously with an organism, causing toxic and sometimes carcinogenic effects. Cells possess an impressive array of enzymes capable of bio-transforming a wide range of different chemical structures and functionalities. The GSTs comprise a complex and widespread enzyme super family that has been subdivided further into an ever-increasing number of classes based on a variety of criteria. [11]

Proteins of the GST family (glutathione S-transferases) are a group of phase II detoxification enzymes involved in detoxifying a wide range of hazardous substances, such as reactive oxygen species (ROS) or xenobiotics. Thus, their role among other things, is the protection of DNA against oxidative damage, which may lead to mutations, and in consequence, favour carcinogenesis. [12, 13] Many studies are available concerning the polymorphisms of GST genes as factors modulating the risk of contracting cancer, including gastrointestinal cancer. A meta-analysis of studies conducted in recent years on the Chinese population showed a relationship between GSTM1 & GSTT1 null genotypes and an increased risk of development of hepatocellular carcinoma (HCC). [14]

Our study showed a higher mean GST activity in group 1&3 compared to group 4. The higher mean GST is probably due to the transcriptional activation of this enzyme through antioxidative responsive element and xenobiotic responsive element as an adaptive response to oxidative stress. The low level of GST among group 2 is probably due to the null genotype that does not show GST induction. Our study is consistent with the studies of Patel et.al which also reported a higher GST and GR activity in patients of untreated cancer as compared to healthy people who were non tobacco users. No significant differences in GSH content, GST enzyme activity or isoenzyme composition were however found by Mulder et.al amongst patients of oral and laryngeal carcinoma and in normal individuals. [15] Comparison of mean GST and GR was also done among cancerous and precancerous conditions of the oral cavity (group 1&2) as against healthy volunteers irrespective of their smoking habits (group 3 & 4). Mean serum GST activity was found to be decreased in cancer patients while GR levels did not show significant difference. (Table 3) It could be hypothesized that GST being glutathione depleting enzyme utilizes GSH as a substrate which is depleted in cancer patients because of excessive free radical scavenging mechanism. GST being dependent on GSH becomes non functional as soon as GSH is

depleted thus rendering cancer patients relatively unprotected against oxyradicals. Similar results were reported by Patel et al. [9]

Analysis of serum GST and GR activity was also done separately in group 1(OC) patients and comparison was done among early and advanced stage of the disease. No statistical significance was found in mean GST and GR values in various stages of the disease as shown in table 4. Similar results were reported by Patel et.al in which no correlation was found between stage of head and neck cancer and GST or GR activity. However, a study carried out by Prabhu et.al showed a significant increase in serum total glutathione-s-transferase levels in patients with stage IV oral cancer as compared to stage II and stage III oral cancer. This shows that alterations in serum total Glutathione-s-transferase levels may have a role in cancer progression.[16]

Conclusion :

GST and GR are potent biochemical markers and assessment of these markers allows us to identify subjects with high risk of developing cancer and targeting them for intensive tobacco cessation intervention. Patients with precancerous changes and low GST can undergo regular follow up to detect early invasive carcinoma.

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