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Original research

# *GSTM1* and *GSTT1* null polymorphism and antioxidant levels in oral submucous fibrosis, leukoplakia and oral cancer patients among a South Indian Population

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ABSTRACT

*Objective:* We investigated the null polymorphism in *GSTM1* and *GSTT1* genes and the antioxidant levels in oral submucous fibrosis (OSMF), leukoplakia and oral cancer patients along with healthy controls in a South Indian cohort.
*Methods:* Genotyping was done using multiplex PCR and the antioxidant levels were estimated using biochemical methods Association between genotypes and different diseased states was determined by odds ratio with 95% confidence interval (CI) and chi-square analysis and for antioxidant levels student's *t*-test was used.
*Results:* The relative risk for *GSTM1* and *GSTT1* gene polymorphisms was statistically insignificant for the 3 patient groups *vs.* controls. Comparing the frequency of the null genotypes between the groups of patients only *GSTM1* polymorphism revealed a significant difference between OSMF & oral cancer subjects ($p = 0.02$). Further, analysis of the antioxidant parameters shows ceruloplasmin levels to be significantly elevated between patient groups and controls and among OSMF *vs.* cancer patients ($p < 0.05$). Similarly, malondialdehyde and glutathione levels were found to be significantly elevated among cancer and both cancer and leukoplakia subjects respectively in comparison with controls ($p < 0.05$). Analysis between the patient groups revealed glutathione levels to be significantly elevated between OSMF *vs.* cancer patients and cancer *vs.* leukoplakia patients ($p < 0.05$).
*Conclusion:* In conclusion, this study finds *GSTM1* null genotype and antioxidants (ceruloplasmin and glutathione levels) to be significantly higher in oral cancers than in precancerous lesions, and suggesting that they might be associated with the malignant transformation of the oral precancers.


## 1. Introduction

Oral precancerous lesions (PCL) such as oral submucous fibrosis (OSMF) and leukoplakia are early indicators of damage to the oral mucosa with a transformation rate of 2–12% to form malignancies [1]. In India, oral cancer is the most common malignant neoplasms accounting for 20–30% of various types of cancers [2]. Tobacco consumption in both smoke and smokeless form is an established etiological factor in the development of cancers of the oral cavity. Tobacco smoke consists of nearly 60 carcinogenic compounds among which polycyclic aromatic hydrocarbons (PAHs), nitrosamines, aldehydes and ketones form the major carcinogens while the consumption of smokeless tobacco occurs with the concomitant use of several additives which can alter cancer risk [3].

Most of these carcinogens are lipophilic and tend to convert into water-soluble hydrophilic compounds that are easily removed through the excretory system. Detoxification of these carcinogens is achieved through phase-II enzymes of which glutathione *S*-transferases (GST) are an important constituent [4]. These enzymes are a ubiquitous family of multifunctional proteins which comprise

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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of four classes namely α, μ, π, and θ [5]. GST μ (GSTM1-GSTM5) and GST θ (GSTT1-GSTT2) have 5 and 2 members respectively [6]. Sequence variation or polymorphisms in these genes can alter the expression, function and/or activity of this enzyme and in turn cause cancer risk [7].

Although all tobacco smokers and betel quid chewers do not necessarily develop cancer or precancerous condition, the possibility of genetic susceptibility or predisposition plays an important role in the development of these states [4]. Progression of the pathological process has been closely related to cell injury and one of the important causes of membrane damage is by free radicals. Excess free-radical formation leads to cancer, atherosclerosis, ageing and is also known to be involved in neoplastic transformation and disease progression [8,9]. To defend damage caused by these reactive oxygen species (ROS) the human body has

been endowed with several endogenous antioxidant systems. These systems can be divided into enzymatic and nonenzymatic groups which are closely linked to each other [10].

Therefore, an overall balance between production and removal of ROS is important in various cancers including oral cancer [11]. Reactive free radicals damage cells by the initiation of lipid peroxidation of the polyunsaturated fatty acids which has direct effect on the membrane structure and also influences membrane fluidity, crosslinking, structure and function. Lipid peroxidation generates a carbonyl compound called malondialdehyde (MDA) whose levels indicate oxidative damage to the cells [12]. Glycoprotein ceruloplasmin belongs to a group of acute phase reactants and is a copper containing protein of plasma whose levels are also known to increase during oxidative stress [13]. Similarly glutathione directly scavenges free radicals and sometimes acts as a substrate for glutathione peroxidases and glutathione S-transferases during the detoxification of ROS [14].

A number of studies have reported on the association between *GSTM1* and *GSTT1* null genotypes with PCL, oral cancers and with various antioxidant levels [15–20]. However, there has been inconsistency between results involving different ethnicities and population groups. Therefore, the present study was carried out with a preliminary aim to detect the frequency of *GSTM1* and *GSTT1* null polymorphisms and antioxidant levels (MDA, ceruloplasmin and glutathione) in rendering susceptibility to oral cancers from precancerous lesions (OSMF and leukoplakia) among a South Indian population from Telangana.

## 2. Materials and method

### 2.1. Subjects

This hospital based study was conducted in the Department of Oral Medicine and Radiology, Govt. Dental College and Hospital, Afzalgunj, Hyderabad and Institute of Genetics and Hospital for Genetic Diseases, Begumpet, Hyderabad. The study was conducted on 75 patients consisting 25 cases each of OSMF, leukoplakia and oral cancer patients diagnosed clinically and histopathologically along with 25 healthy control subjects. The study was approved by the ethical committee of the study hospitals and blood samples from the study subjects were collected only after obtaining the written informed consent. Subjects with systemic diseases like diabetes mellitus, hypertension, cardiovascular diseases, and other diseases were excluded from the study. The inclusion criterion for the controls was absence of a prior history of cancer or any other oral lesions. All subjects included in the study were above the age group of 18 years and all the samples were collected only after obtaining the written informed consent followed by a structured questionnaire.

### 2.2. DNA isolation and genotyping

Two ml of blood was collected in EDTA vaccutainers and genomic DNA was extracted from blood samples using standard phenol-chloroform method. The evaluation for *GSTT1* and *GSTM1* was done using PCR technique. The primers used for the amplification of the *GSTT1* gene are forward-5′ TTC CTT ACT GGT CCT CAC ATCTC 3′ and reverse-5′ TCA CCG GAT CAT GGC CAG CA 3′, for *GSTM1* gene forward-5′ GAA CTC CCT GAA AAG CTA AAGC 3′ and reverse-5′ GTT GGG CTC AAA TAT ACG GTG 3′, for internal control albumin forward-5′ GCC CTC TGC TAA CAA GTC CTA 3′ and reverse-5′ GCC CTA AAA AGA TCG CCA ATC 3′. The amplified product for *GSTT1* is 480 bp, for *GSTM1* is 215 bp and the internal control albumin was detected at 350 bp. Separation of amplified PCR products was done on 2.0% agarose gel.

### 2.3. Biochemical analysis

Three ml of blood was collected in clot activators and serum was separated by centrifuging at 3000 rpm for 15 min into fresh eppendorf tubes while plasma was separated from 2 ml of blood collected in EDTA tubes. Estimation of MDA, the major lipid peroxidation product was done from plasma by thiobarbituric acid reaction assay (TBARS) [21]. Ceruloplasmin was estimated from serum using the copper-oxidase method while the glutathione level was estimated by following the procedure of glutathione peroxidase method [22,23].

### 2.4. Statistical analysis

Frequency distribution of wild and null genotypes of patients and controls was compared. Relative risk was estimated by calculating odds ratio (OR) at 95% confidence interval (CI). A Student's *t*-test was applied to assess the difference in the levels of antioxidants. $P < 0.05$ was considered as significant. The *p*-values were adjusted by Bonferroni corrections for mean antioxidant levels among controls and patients and ANOVA was performed for determining the relationship between MDA, ceruloplasmin and glutathione levels with GSTM1 genotypes in patients and controls. All the calculations were done in SPSS software (version 17.0).

## 3. Results

The demographic features of the study subjects have been summarised in Table 1. The mean age of OSMF, leukoplakia and cancer patients was calculated to be 31.2, 42 and 49 years respectively, while that for controls it was found to be 45.9 years. Analysis of the smoking status in the study population involving its frequency and duration revealed a statistical significance among OSMF, leukoplakia and cancer patients with respect to controls ($p < 0.0001$). Similarly, the betel chewing frequency also revealed a statistical significance in all the 3 patient groups *vs.* controls ($p < 0.0001$) however, its duration revealed a statistical significance only among leukoplakia ($p = 0.0004$) and cancer ($p = 0.0018$) patients. The wild and null genotypes of *GSTM1* and *GSTT1* genes have been summarised in Table 2. The *GSTM1* null genotype was present in 44% of controls, 68% of oral cancer patients, 52% of oral leukoplakia and in 36% of OSMF patients. Similarly the *GSTT1* null genotype was present in 20% of controls, 36% of oral cancer patients 24% of oral leukoplakia and in 28% of OSMF patients. The relative risk at 95% CI estimated for *GSTM1* gene for oral cancer patients was 2.7 (0.8–8.5), for oral leukoplakia 1.3 (0.4–4.1) and for OSMF 0.7 (0.2–2.2) which was statistically insignificant. Similarly relative risk at 95% CI estimated for *GSTT1* gene for oral cancer patients was 2.2 (0.6–8.0), for oral leukoplakia was 1.2 (0.3–4.8) and for OSMF it was 1.5 (0.4–5.7) which was statistically insignificant (Table 2).

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**Table 1**
Demographic features of study population.

| | Controls | OSMF | p-value | Leukoplakia | p-value | Cancer | p-value |
|---|---|---|---|---|---|---|---|
| Mean Age | 45.9 | 31.2 | | 42 | | 49 | |
| Male:Female | 20:5 | 22:3 | | 21:4 | | 16:9 | |
| Smokers | 14 | 17 | | 15 | | 16 | |
| Frequency (mean ± S.D) | 10.7 ± 1.2 | 16.9 ± 2.3 | <0.0001 | 18.2 ± 1.8 | <0.0001 | 22.8 ± 4.6 | <0.0001 |
| Duration (mean ± S.D) | 11.6 ± 0.8 | 15.9 ± 3.0 | <0.0001 | 16.8 ± 2.3 | <0.0001 | 18.5 ± 1.7 | <0.0001 |
| Betel quid chewers | 9 | 25 | | 23 | | 25 | |
| Frequency (mean ± S.D) | 5.8 ± 2.7 | 14.9 ± 1.8 | <0.0001 | 13.7 ± 0.7 | <0.0001 | 11.2 ± 4.2 | <0.0001 |
| Duration (mean ± S.D) | 7.1 ± 1.5 | 7.9 ± 3.1 | =0.25 | 9.2 ± 2.3 | =0.0004 | 9.5 ± 3.3 | =0.0018 |

**Table 2**
Distribution of wild and null genotype for GSTM1 and GSTT1 gene among different group of patients and controls.

| Groups (GSTM1) | Sample (n) | Wild (+/+ or +/−) n (%) | Null (−/−) n (%) | Relative risk | p-value | 95% CI |
|---|---|---|---|---|---|---|
| Control | 25 | 14 (56%) | 11 (44%) | 1 | | |
| Cancer | 25 | 8 (32%) | 17 (68%) | 2.7 | 0.08 | 0.8–8.5 |
| Leukoplakia | 25 | 12 (48%) | 13 (52%) | 1.3 | 0.57 | 0.4–4.1 |
| OSMF | 25 | 16 (64%) | 9 (36%) | 0.7 | 0.56 | 0.2–2.2 |
| Groups (GSTT1) | Sample (n) | Wild (+/+ or +/−) n (%) | Null (−/−) n (%) | Relative risk | p-value | 95% CI |
| Control | 25 | 20 (80%) | 5 (20%) | 1 | | |
| Cancer | 25 | 16 (64%) | 9 (36%) | 2.2 | 0.20 | 0.6–8.0 |
| Leukoplakia | 25 | 19 (76%) | 6 (24%) | 1.2 | 0.73 | 0.3–4.8 |
| OSMF | 25 | 18 (72%) | 7 (28%) | 1.5 | 0.50 | 0.4–5.7 |

**Table 3**
Comparing frequency of GSTM1 polymorphism between different patient groups.

| GROUP | Number of patients | GSTM1 | | p-value |
|---|---|---|---|---|
| | | Wild n (%) | Null n (%) | |
| CANCER | 25 | 8 (32) | 17 (68) | 0.24 |
| LEUKOPLAKIA | 25 | 12 (48) | 13 (52) | |
| CANCER | 25 | 8 (32) | 17 (68) | 0.02 |
| OSMF | 25 | 16 (64) | 9 (36) | |
| LEUKOPLAKIA | 25 | 12 (48) | 13 (52) | 0.25 |
| OSMF | 25 | 16 (64) | 9 (36) | |

**Table 4**
Comparing frequency of GSTT1 polymorphism between different patient groups.

| GROUP | Number of patients | GSTT1 | | p-value |
|---|---|---|---|---|
| | | Wild n (%) | Null n (%) | |
| CANCER | 25 | 16 (64) | 9 (36) | 0.35 |
| LEUKOPLAKIA | 25 | 19 (76) | 6 (24) | |
| CANCER | 25 | 16 (64) | 9 (36) | 0.54 |
| OSMF | 25 | 18 (72) | 7 (28) | |
| LEUKOPLAKIA | 25 | 19 (76) | 6 (24) | 0.74 |
| OSMF | 25 | 18 (72) | 7 (28) | |

Analysis of the null genotypes between the 3 patient groups for *GSTM1* gene revealed a significant difference between OSMF & cancer patients (p = 0.02). However, there was no significant difference between OSMF & leukoplakia (p = 0.25); leukoplakia & cancer (p = 0.24) patients (Table 3). For *GSTT1* null gene polymorphism there was statistically no significant difference found between any of the patient groups (p > 0.05) (Table 4).

Biochemical analysis of antioxidant ceruloplasmin levels significantly increased in patients as compared to controls (p < 0.05), the mean values for controls was 32.87 ± 3.69 mg/dl, for OSMF patients it was 36.47 ± 4.55 mg/dl, among leukoplakia subjects it was 38.39 ± 5.01 mg/dl and for oral cancer patients it was 40.49 ± 2.17 mg/dl, adjusted p value after bonferroni correction was found to be significant (p' = 0.002) (Table 5). However, its mean levels when studied between the patient groups reveal significance only between OSMF and cancer patients (p = 0.0002) (Table 6). For antioxidant MDA the mean value for controls, OSMF, leukoplakia and oral cancer patients was calculated to be 2.97 ± 1.09 μm/ml, 3.82 ± 2.55 μm/ml, 3.93 ± 2.48 μm/ml and 4.34 ± 1.69 μm/ml respectively. The MDA levels were significantly increased in cancer patients as compared to controls (p = 0.001) but there was no significant difference in leukoplakia & OSMF patients when compared with controls (p = 0.08 and p = 0.13), adjusted p value after bonferroni correction was found to be non-significant (p' = 0.195) (Table 5). Further, the MDA levels between the patient groups revealed no significant difference (p > 0.05) (Table 6). For antioxidant glutathione the mean levels among controls, OSMF, leukoplakia and oral cancer subjects was reported to be 4.46 ± 0.39 μg, 4.56 ± 0.68 μg, 4.80 ± 0.60 μg and 5.55 ± 0.91 μg respectively. Significant difference was found between the mean glutathione levels of oral cancer patients with controls (p = 0.000) and leukoplakia patients with controls (p = 0.02). However, there was no significant difference in OSMF patients when compared with controls (p = 0.53), adjusted p value after bonferroni correction was found to be non-significant (p' = 0.454) (Table 5). Significant difference in glutathione levels was also seen between two patient groups i.e OSMF *vs.* cancer (0.00006) and cancer *vs.* leukoplakia patients (p = 0.001) (Table 6).

Studying the relationship between *GSTM1* genotypes with antioxidant levels revealed a significant association only for ceruloplasmin levels with *GSTM1* genotypes among leukoplakia subjects (p = 0.013). No significant association was observed for *GSTM1* genotypes with ceruloplasmin, MDA and glutathione levels among OSMF, cancer and control subjects (p > 0.05) (Table 7).

## 4. Discussion

Tobacco consumption is a known risk feature for oral cancer and preneoplastic states and other etiological factors such as alcohol consumption, dietary habits, viral infection and genetic factors can contribute to early transformation of PCL to oral cancers. Previous studies indicate three fourths of all oral and pharyngeal cancers

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**Table 5**
Mean Ceruloplasmin, MDA and Glutathione levels among controls and patients.

| Ceruloplasmin levels | Number of patients | Mean (mg/dl) | SD | Difference from CONTROL MEAN ± SD | p-value |
|---|---|---|---|---|---|
| Control | 25 | 32.87 | 3.69 | | |
| Cancer | 25 | 40.49 | 2.17 | 7.65 ± 1.52 | 0.000 |
| Leukoplakia | 25 | 38.39 | 5.01 | 5.55 ± 1.32 | 0.000 |
| OSMF | 25 | 36.47 | 4.55 | 3.63 ± 0.86 | 0.004 |
| | | | | | p' = 0.002. |
| **MDA levels** | **Number of patients** | **Mean(μm/ml)** | **SD** | **Difference from CONTROL MEAN ± SD** | **p-value** |
| Control | 25 | 2.97 | 1.09 | | |
| Cancer | 25 | 4.34 | 1.69 | 1.37 ± 0.60 | 0.001 |
| Leukoplakia | 25 | 3.93 | 2.48 | 0.96 ± 1.39 | 0.08 |
| OSMF | 25 | 3.82 | 2.55 | 0.85 ± 1.46 | 0.13 |
| | | | | | p' = 0.195. |
| **Glutathione levels** | **Number of patients** | **Mean (μg)** | **SD** | **Difference from CONTROL MEAN ± SD** | **p-value** |
| Control | 25 | 4.46 | 0.39 | | |
| Cancer | 25 | 5.55 | 0.91 | 1.09 ± 0.52 | 0.000 |
| Leukoplakia | 25 | 4.80 | 0.60 | 0.34 ± 0.21 | 0.02 |
| OSMF | 25 | 4.56 | 0.68 | 0.10 ± 0.29 | 0.53 |
| | | | | | p' = 0.454. |

p' = p-value after Bonferroni adjustment.

**Table 6**
Comparison of mean values for the three antioxidant levels between the patient groups.

| Ceruloplasmin | | |
|---|---|---|
| **Groups** | **t-value** | **p-value** |
| OSMF *vs.* Leukoplakia | −1.418 | 0.16 |
| OSMF *vs.* Cancer | −3.987 | 0.0002 |
| Cancer *vs.* Leukoplakia | 1.923 | 0.06 |
| **MDA** | | |
| **Groups** | **t-value** | **p-value** |
| OSMF *vs.* Leukoplakia | −0.154 | 0.87 |
| OSMF *vs.* Cancer | −0.849 | 0.40 |
| Cancer *vs.* Leukoplakia | 0.683 | 0.50 |
| **Glutathione** | | |
| **Groups** | **t-value** | **p-value** |
| OSMF *vs.* Leukoplakia | −1.323 | 0.19 |
| OSMF *vs.* Cancer | −4.357 | 0.00006 |
| Cancer *vs.* Leukoplakia | 3.440 | 0.001 |

**Table 7**
Relationship of Mean ± SD values of ceruloplasmin, MDA and glutathione levels with GSTM1 genotypes among patient groups.

| OSMF | Genotypes | N = 25 | Mean | Std. deviation | p-value |
|---|---|---|---|---|---|
| Ceruloplasmin | Wild | 16 | 35.8 | 3.7 | |
| | Null | 9 | 37.7 | 5.7 | |
| | | | | | =0.321 |
| MDA | Wild | 16 | 3.3 | 1.4 | |
| | Null | 9 | 4.7 | 3.8 | |
| | | | | | =0.194 |
| Glutathione | Wild | 16 | 4.6 | 0.8 | |
| | Null | 9 | 4.5 | 0.5 | |
| | | | | | =0.738 |
| **Leukoplakia** | **Genotypes** | **N = 25** | **Mean** | **Std. deviation** | **p-value** |
| Ceruloplasmin | Wild | 12 | 40.9 | 2.5 | |
| | Null | 13 | 36.1 | 5.7 | |
| | | | | | =0.013 |
| MDA | Wild | 12 | 3.4 | 1.4 | |
| | Null | 13 | 4.4 | 3.1 | |
| | | | | | =0.317 |
| Glutathione | Wild | 12 | 4.6 | 0.4 | |
| | Null | 13 | 4.9 | 0.7 | |
| | | | | | =0.206 |
| **Oral cancer** | **Genotypes** | **N = 25** | **Mean** | **Std. deviation** | **p-value** |
| Ceruloplasmin | Wild | 8 | 40.3 | 2.4 | |
| | Null | 17 | 40.6 | 2.1 | |
| | | | | | =0.753 |
| MDA | Wild | 8 | 4.4 | 1.3 | |
| | Null | 17 | 4.3 | 1.8 | |
| | | | | | =0.890 |
| Glutathione | Wild | 8 | 5.4 | 0.7 | |
| | Null | 17 | 5.6 | 0.9 | |
| | | | | | =0.586 |
| **Controls** | **Genotypes** | **N = 25** | **Mean** | **Std. deviation** | **p-value** |
| Ceruloplasmin | Wild | 14 | 32.2 | 2.3 | |
| | Null | 11 | 33.7 | 4.9 | |
| | | | | | =0.320 |
| MDA | Wild | 14 | 3.0 | 1.3 | |
| | Null | 11 | 2.9 | 0.7 | |
| | | | | | =0.820 |
| Glutathione | Wild | 14 | 4.5 | 0.3 | |
| | Null | 11 | 4.4 | 0.5 | |
| | | | | | =0.540 |

to be caused by heavy smoking and consumption of alcohol. GST's play an important role in xenobiotic metabolism because this phase II enzyme catalyze the conjugation of glutathione with tobacco smoke substrates, resulting in increased water solubility and renal excretion [24]. The GST family detoxifies carcinogens, reactive oxygen species and lipid peroxidation products, yielding excretable hydrophilic metabolites and lack of these enzymes may potentially increase susceptibility to various cancers because of a decreased ability to detoxify carcinogens [25,26].

Therefore, in this study we examined the null gene polymorphism of *GSTM1* and *GSTT1* genes that have been widely studied in susceptibility to precancerous stages (OSMF, leukoplakia) and oral cancers. The earlier studies have mostly evaluated these genes as separate studies involving OSMF, leukoplakia or oral cancer subjects. Hence, our present study explores the genetic susceptibility of *GSTM1*, *GSTT1* genes along with antioxidants ceruloplasmin, MDA and glutathione levels in a composite retrospective case-control study population.

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Our evaluations of the 2 null gene polymorphisms indicate the relative risk to be highest among oral cancer subjects for both the genes although with no statistical significance. However, a significant statistical association was found for *GSTM1* gene when examined between cancer patients and OSMF patients ($p = 0.02$). Different studies on diverse population groups show a variation in the frequency of the *GSTM1* and *GSTT1* null genotype [27]. Presence of null genotypes results in lack of elimination of toxic carcinogens from the body leading to their accumulation and DNA adduct formation. The *GSTT1* null genotype is also known to play a role in leukoplakia and oral cancer. Null genotype in *GSTT1* gene in the present report was higher in cancer followed by OSMF, leukoplakia and controls.

Similarly, *GSTM1* null genotype was found to be more among cancer patients followed by leukoplakia patients, controls and OSMF patients; which is supported by findings from other studies [2,15,28]. Further, a recent study carried out by Tanwar et al. (2015) also reports *GSTM1* null polymorphisms to be significantly higher in cancer group as compared to subjects with habits and no oral lesions [29].

Recent meta-analysis reports the *GSTM1* null genotype to be a higher risk factor for oral cancer among Asians when compared with Caucasians [30–32]. Studies documenting role of *GSTM1* and *GSTT1* gene polymorphisms are inconsistent with some showing risks and few others reporting no risk. This disparity has been attributed to racial/ethnic differences and thus genotyping of *GSTM1* and *GSTT1* genes in different populations can be quite helpful in providing information about the differences in xenobiotic metabolism pathways due to variation within genes.

Enzymatic antioxidant levels in precancerous and cancerous stages also play a very vital role in maintenance of cellular antioxidant defence mechanism and therefore, can act as potential biochemical markers for evaluating the progression state of the disease. Antioxidants like ceruloplasmin increase during oxidative stress and especially during precancerous stages and in different kind of cancers [13,33]. Malondialdehyde levels on the other hand are generated by lipid peroxidation and are known to be involved in several diseases and inflammatory conditions. Another antioxidant called as glutathione a tripeptide, found ubiquitously in all cells protects cells against destructive effects of ROS. Serum levels of these antioxidants are found to be higher in patients with more oxidative stress as compared with healthy subjects. The increased level of antioxidants in patients can be attributed to the need of an innate defence mechanism in response to higher oxidative damage [34].

In the present study too we found higher levels of these antioxidants in cancer, leukoplakia and OSMF patients when compared with controls. Several other studies in oral cancer, leukoplakia and OSMF also support the same observation [13,35]. Comparison of mean values of the estimated antioxidant levels between patient group and controls reveals significant difference only between OSMF and cancer for ceruloplasmin levels. For MDA levels such a comparison did not show any significant difference between any patient groups. For glutathione significant difference was observed between OSMF and cancer and also in cancer and leukoplakia. This result is in partial agreement with a recently published report by Gurudath et al. (2012) that showed significant association of antioxidant levels with OSMF, leukoplakia and oral cancer [36]. Further, we also report that there is no relationship between *GSTM1* genotypes and in increase of antioxidants levels among patients.

Although the study suffers due to less sample size this preliminary study has formed the basis to understand cancer progression from precancerous lesions and suggests basic difference in genetic and antioxidant levels that can form the basis of prognostic marker study in the area of oral medicine. Further, it would also be interesting to evaluate the nutrigenomics status in such classified patients as it has been seen that the nutritional requirements are individual specific depending on genetic makeup of an individual (personalized diet) and influences ones response to pathological conditions. Such an interaction between diet and genetic makeup can tremendously influence the coping up of developing diseases especially so by increasing the immune defence and maintaining a balance between total oxidation status and total antioxidant response of the genome. Achievement of so with dietary habits, lifestyle modifications and one's own genetic system would thus prevent progression of diseased states into deadly cancers [37].

## 5. Conclusion

In conclusion, the present preliminary study in the population from Telangana (South India) reports *GSTM1* null genotype to be significantly found in oral cancers than OSMF and leukoplakia. This finding suggests that its deletion is associated with malignant transformation of the oral precancers. In addition to this, the study also shows evidence in support of the antioxidant levels to correlate well with the degree of oxidative damage and ceruloplasmin and glutathione levels to serve as potential biochemical markers in evaluating the disease progression between OSMF and oral cancer and also between cancer and leukoplakia. However, a longitudinal study with larger sample size and population from different ethinicities will provide more detailed information for these molecular and biochemical tests to qualify as essential prognostic markers.

## Conflicts of interest

None

## Ethical approval

Was obtained from necessary bodies

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