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# Genetic Polymorphism at GSTM1 Gene Locus in Patients with Potentially Malignant Disorders

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## ABSTRACT

**Background**: Oral cancer in Asian population is commonly attributed to tobacco usage and the nitroso compounds within. Certain host and environmental factors play an active role in development of carcinoma. One such host factor is genetic constitution of the individual. The xenobiotic compounds which enter our body, undergo various metabolic reactions before they become active carcinogens. These reactions are mediated by the phase I and phase II enzymes. Glutathione S Transferase (GST) is one of the phase II enzyme or the detoxifying enzyme. Absence of synthesis of GST can be one of the etiologic factor in development of malignancy.

**Objective:** To study GSTM1 null genotype and its role in genetic susceptibility to carcinogenesis in patients with leukoplakia and OSMF comparing with healthy controls.

**Materials and Method:** 50 patients with precancerous lesions and 50 controls were included in the study. Tissue biopsy and peripheral venous blood was withdrawn for DNA extraction. These samples were then exposed to PCR to check for GSTM1 gene deletion.

**Result:** Higher frequency of GSTM1 null genotype was noted in patients with OSMF i.e. 37.5%. However no difference was found in the frequency of GSTM1 null genotype when patients with precancerous lesions were compared with healthy controls having tobacco related habits.

**Conclusion:** GSTM1 Null genotype may be a high penetrance risk factor in patients with premalignant lesions and conditions and should be evaluated for the potential cause in malignant transformation of such lesions.

**Keywords:** GSTM1 gene, leukoplakia, oral submucous fibrosis, polymorphism, potentially malignant disorders.

## INTRODUCTION

Cancer is considered as the most devastating and debilitating disease in India and other Asian countries and is a major cause of mortality. Oral cancer is the sixth most common cancer in the world and has particularly high incidence in south east Asian countries as stated by Johnson 1991; Parkin et al 1997[1,2]. Oral cancer, quite often is preceded by appearance of premalignant lesion namely leukoplakia and oral submucous fibrosis. The incidence of such premalignant lesion and condition, caused due to the intake of tobacco, is reported to be high among Indian population.

Ng et al (1993)stated that although there is overwhelming evidence of the etiological association between tobacco and oral cancer,

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majority of tobacco habitués do not suffer from cancer[3]. This can be hypothesized as the interaction between environmental agents and host susceptibility factors. Spitz (1999) suggested that genetically determined factors can significantly alter the effects of environmental carcinogens and that genetic variability might come into play at any phase of multistage carcinogenic process[4].

The environment gene interaction in carcinogenesis is well reflected by phase I and phase II enzymes that are involved in the metabolism of carcinogens. All carcinogens are lipophilic and have a tendency to be converted into water soluble hydrophilic compound and can be easily removed from the body through the excretory system. This conversion or detoxification of carcinogen is achieved by the addition of one atom of oxygen to the carcinogenic compound brought about by the superfamily of cytochrome P450 phase of enzymes. This process of detoxification also leads to formation of reactive intermediates. GST's (Glutathione S transferases) are group of phase II enzymes that are primarily involved in detoxifying carcinogenic metabolites and neutralize the harmful effects of reactive intermediates[5].

Seidegard et al (1988) put forth that in the (Glutathione S Transferaseμ) GSTμ class, GSTM1 and GSTM3 gene which lie on human chromosome 1p13 are known to be polymorphic[6]. "Polymorphism" is defined as less frequent phenotypes that occur in less than 1% of population and are caused by gene mutation, frame shift mutation or gene deletions resulting in most cases, but not always in enzymes with reduced or no activity.

Thus GSTM1 null genotype has decreased capability in detoxifying carcinogens. This hypothesized role of GSTM1 in carcinogenesis suggests that deleterious somatic changes may occur at high frequency in GSTM1 null homozygotes than in GSTM1 positive individuals.

Studies have shown that loss of GSTM1 gene is associated with increased risk of lung bladder and breast cancer[7,8,9]. Data also suggests that null genotype of GSTM1 are risk factors for developing oral leukoplakia and consequently oral cancer in habituated betel quid and tobacco chewers in Indian ethnicity[10]. Thus a study was undertaken to determine the GSTM1 polymorphism in patients with leukoplakia and oral submucous fibrosis and its comparison with patients having long standing habit but no evidence of oral lesion i.e. healthy controls.

## MATERIAL AND METHOD

The study comprised of 50 patients with premalignant lesion/condition i.e. leukoplakia and oral submucous fibrosis and 50 patients who had tobacco related habit but no lesion and these acted as controls, visiting the OPD of Department of Oral Diagnosis and Radiology, Government Dental College and Hospital, Mumbai. Patients with premalignant intraoral lesions were randomly selected from the OPD, a thorough case history of the patients was obtained and the lesions were evaluated clinically. The patients were then informed about the study and a written consent was obtained. Incisional biopsy of the lesion was done followed by withdrawal of peripheral venous blood from the patient. The tissue was used to confirm the clinical diagnosis of the lesion. DNA extraction from the blood using phenol chloroform technique was further carried out. The extracted DNA was then subjected to polymerase chain reaction(PCR), and the results were analysed.

PCR is a highly specialized research tool with many uses in medicinal laboratories in genetic, microbiological virological analysis. It basically involves reiterate amplification of a small part of DNA to many folds. PCR is used to amplify a short well defined part of DNA. This can be single gene or just part of a gene. It is possible to amplify specific DNA sequences (50bp – 1.5kb) of impure intact or fragmented DNA in few hours.

The PCR reaction is carried out in a thermocycler(The PCR reaction in our study were carried out at ACTREC Kharghar). This is the machine that heats and cools the reaction tube within it to the precise temperature required for each step of the reaction to prevent evaporation of the reaction mix. Heated lid is placed on top of the reaction tubes or a layer of oil is put on the surface of the reaction mix. PCR assay utilizes a pair of primers [M12S (sense) – 5' CTG CCC TAC TGA TTG ATG 3', M1AS (anti sense) – 5' CTG GAT AGc AGA TCA TGC 3', M2AS (antisense) - 5' GAC TCA CTE TGA GCA TAG CAC 3'. I unit of recombinant Taq DNA

Table I: Age wise distribution of patient

| Age group | Leukoplakia | OSMF | Total |
|---|---|---|---|
| 10-20 yrs | 0 | 03 | 03 |
| 21-30 yrs | 05 | 08 | 13 |
| 31-40 yrs | 08 | 05 | 13 |
| 41-50 yrs | 09 | 0 | 09 |
| 51-60 yrs | 05 | 01 | 06 |
| 61-70 yrs | 04 | 0 | 04 |
| 71 yrs and above | 02 | 0 | 02 |
| | N=33 | N=17 | N=50 |

Table II: Distribution of patient according to gender

| Sex | Leukoplakia | OSMF | Total | Percentage |
|---|---|---|---|---|
| Male | 30 | 15 | 45 | 90 |
| Female | 03 | 02 | 05 | 10 |

Table III: Frequency of GSTM1 null genotype in leukoplakia patients

| | No of patients | GSTM1(-) % |
|---|---|---|
| Males | 30 | 8(26.66) |
| Females | 03 | 0 |
| Homogeneous | 8 | 3(37.5) |
| Non homogeneous | 25 | 5(20) |
| Multiple tobacco habits | 12 | 4(33.33) |

Table IV: Frequency of GSTM1 null genotype in SMF patients.

| | No of patients | GSTM1(-)% |
|---|---|---|
| Male | 15 | 6(40) |
| Female | 02 | 0(0) |
| Total no of patients | 17 | 6(37.5) |
| Multiple tobacco habit | 05 | 3(60) |

Table V: Frequency of GSTM1 null genotype in control patients

| | No of patients | GSTM1(-)% |
|---|---|---|
| Males | 45 | 13(28.9) |
| Females | 05 | 1(20) |
| Chewers | 28 | 7(25) |
| Smokers | 15 | 6(40) |
| Multiple tobacco habits | 05 | 1(20) |

polymerase], that are selected from the opposite strands of the gene which is specific to a particular organism. A sample analysed by PCR using these primers yields an amplification product, the size of which is determined by distance of the two primers from each other. The amplified product is then run on a 0.2% polyacrylamide gel, used to perform electrophoresis. After the electrophoresis the gel is observed under ultraviolet light to check for the results.

GSTM1 positive samples show a band at 270 base pairs and the band at160 base pairs which correspond to M2 gene which acts an internal control [Fig 1]. Lane L2 shows the marker DNA. L3, L4, L8 show absence of the gene thus indicating a null genotype.

## RESULTS

The present study on GSTM1 polymorphism in patients with leukoplakia and SMF included 100 patients of which 33 were leukoplakia and 17 were SMF and 50 were control patients. The youngest patient in our group was 12 yrs and oldest was 77 yrs. Most of our patients having leukoplakia and SMF were in the age range of 31-50 yrs (51.51%) (Table I). This study comprised of 45 males and 5 females with lesions, thus giving us a male:female ratio of 9:1(Table II).

Of the 50 patients, 28 (56%) had more than one habit. Chewing tobacco with lime was the most predominant habit with 18 out of 50(36%) patients exhibiting the same. Of the 33 patients having leukoplakia, 25(75.75%) were diagnosed of having homogeneous leukoplakiahistopathologically and 8(24.24%) were found to have non homogeneous leukoplakia.

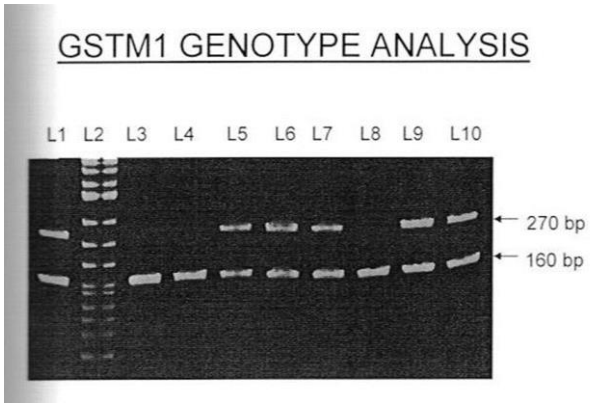


Fig 1: GSTM1 genotype analysis on PCR.

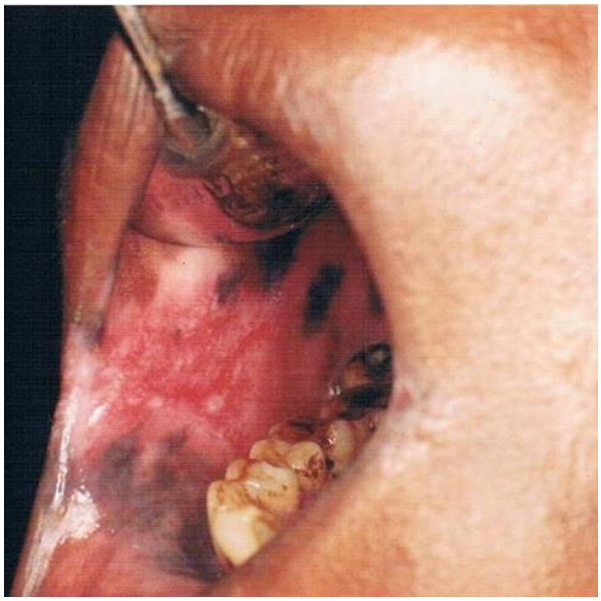

Fig2: Speckled Leukoplakia involving right buccal mucosa.

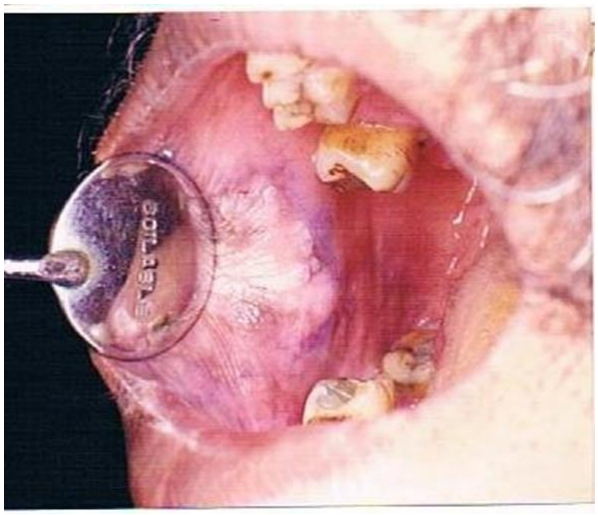

Fig 3: Homogeneous Leukoplakia involving right buccal mucosa.

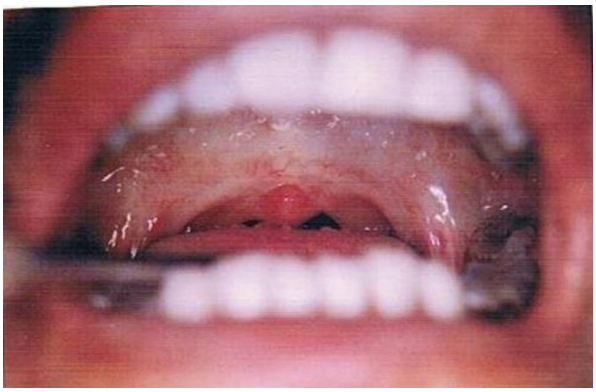

Fig 4: OSMF involving the Soft Palate.

As shown in the table (II) of the 33 leukoplakia patients, 30 were males and three were females. Of the 30 male patients 8(26.66%) patients showed a null genotype. .

The frequency of GSTM1 null genotype was found to be high (37.5%) in patients with non homogeneousleukoplakia [Fig 2] in comparison to 20% in patients with homogeneous leukoplakia [Fig 3] (Table III). In Patients with oral submucous fibrosis [Fig 4] out of the 17 patients15 were male and 2 were females and GSTM1 null genotype was found in 6(37.5%) out of the17 patients. Thus frequency of GSTM1 null genotype was found to be higher in SMF patients, i. e. 37.5% in 17 patients (Table IV) as compared to 28% in controls(Table V).

Out of the 50 patients 17 patients had multiple tobacco habit. Of these 7(41.17%) were found to be GSTM1 null positive. Among the 50 control patients, 45 were males and 5 were females. Out of the 45 male patients 13(28.9%) patients were found with GSTM1 null genotype and 1 female patient(20%) out of the 5 had a null genotype. Out of the 14 controls with GSTM1 null genotype 7(25%) patients had a tobacco chewing habit, 6 (40%) patients had a tobacco smoking habit and 1(20%) patient had a tobacco smoking and chewing habit.

When the data was analysed statistically using chi square test with the following formula: Chi square test = $\sum (0\text{-}E)^2/E$

Where O = Obsereved frequency

E = Expected frequency

The chi square value was 9.67 and probability(p) was 0.28 which is greater than 0.05. the higher probability value indicates non significant results.

## DISCUSSION

Glutathione S transferase are detoxifying enzymes expressed in different organs in the body. Levels of these enzymes determine susceptibility of an individual to toxic chemicals. The genes coding these enzymes are important in detoxifying of tobacco carcinogens. There are five classes of GST enzymes. Of these the GSTμ gene which lies on human chromosomes, 1p13 is polymorphic. Some individuals show deletion of an entire structural gene sequence. Two functional alleles GSTM1a and GSTM1b have been identified and a non-functional allele GSTM1o has been identified[11,12,13].

In some individuals both the alleles for GSTM1 locus are intact and their genotype for GSTM1 is homozygous (+/+).In some individuals one of the allele is intact and their genotype will beheterozygous (+/-). Some individuals will have deletions in both the alleles and their genotype will be null (-/-)[14].

In the present study the frequency of GSTM1 null genotype was found to be high (37.5%) in patients with non homogeneousleukoplakia This is in accordance with the study carried out by Nair et al(1999)[10]. They have also found a high percentage of GSTM1 null genotype in non homogeneousleukoplakia i.e. 89.7%, in contrast to our study. This can be attributed to the fact that, non homogeneousleukoplakia have a higher rate of malignant transformation in comparison to homogeneous leukoplakia. Thus GSTM1 null genotype and a non-homogeneous leukoplakia may be considered to be at a high risk in development of carcinoma.

It was found that all SMF patients had gutka chewing habit. Gutka contains betel nut, tobacco and lime, which generate reactive oxygen species(ROS) and 8 oxyguanine in DNA in vitro at the alkaline pH provided by the lime. ROS in part are responsible for the increased frequency of micronuclei observed in hamster cheek pouch upon exposure to betel quid ingredients and in exfoliated buccal mucosa cells of chewers. We also detected the formation of OH radicals in the oral cavity of subjects during chewing of betel nut, which may cause direct damage in the oral mucosa and promote growth of initiated oral epithelial cells. The contribution of GSTM1 super gene family to combat oxidative stress by conjugation pathway is well established and therefore absence of one or more GST enzymes could result in increased reactive oxygen species mediated damage[10]. Thus co-existence of OSMF and GSTM1 null genotype may further increase the risk of cancer development.

17 out of 50 patients had multiple tobacco habits, of these 41.17% were GSTM! Null positive. This might be an indicator to the high oxidative stress due to multiple tobacco related habits and hence a high percentage of deletions.

Thus looking at the overall data it was found that, on comparing the frequencies of GSTM1 null genotype between leukoplakia patients (26.66%) and controls (28%), there was no statistically significant difference. However, higher frequency of GSTM1 null genotype was found in patients with SMF (37.5%) as compared to controls. In the present study the frequency of GSTM1 null genotype in patients with precancerous lesion was 28%, which was found to be similar to that reported by Buch et al i.e. 24% and Dutta et al i.e. 26%[15,16].

Buch et al (2002) analysed 297 cancer patients and 450 controls. They have found GSTM1 null genotype as a risk factor for development of oral cancer among Indian tobacco habitués[15].

Sikdar N et al have found that increased lifetime exposure to tobacco smoking (> 11.5 pack years) increased the risk of leukoplakia in individuals with GSTM1 homozygous null genotype[17]

In several other studies carried out, by Deakin et al 1996, Lazarus et al 1998,Oude Ouphius et al 1998, Cheng et al 1999,Jowenkora et al 1999 and Olshal et al 2000Morita et al 1997, Park J Y et al 1997 and Tanimoto et al 1999on oral cancer they have reported no association between the GSTM1 null genotype and individual susceptibility to cancer at a specific site in the oral cavity[18,19,20,21,22,23,25,26,27,28]

Trizna et al and Hung et al reported an association between GSTM1 null genotype and an increased risk for head and neck cancer[24].

In a study carried out by Dutta S, Paul R.R and Roy B on 54 oral cancer patients and 60 control individuals, no association was seen between occurrence of oral cancer and GSTM1 null mutation, when the whole population was considered. But in patients below 50 years of age, a significant association was observed between GSTM1 null mutation and occurrence of oral cancer[16]

Katoh et al 1999, 2000, reported a significant correlation between GSTM1 null genotype and risk of oral cancer. Gronau S et al 2000 stated that GSTM1 deficiency predisposes to head and neck cancer especially to cancer of the larynx which is particularly exposed to tobacco smoke carcinogens. Shreelekha T.T et al 2001 stated in a study carried out on 98 oral cancer patients, that polymorphism at GSTM1 and GSTT1 null genotypes may confer an increased risk for oral cancer[29,30].

Few recent studies have also shown a positive association between the GSTM1 null genotype and head and neck cancer[31,32,33]

## CONCLUSION

We thus conclude that, higher frequency of GSTM1 null genotype was noted in patients with SMF i.e. 37.5% and in patients with non homogeneousleukoplakia i.e. 37.5%.Thus showing that GSTM1 Null genotype may be a high penetrance risk factor for developing oral premalignant lesion and consequently modulate the risk of developing cancer. However the study needs to be carried out with a larger sample size as presence of small sample size does not preclude its prevalence. Since our study was the first to analyse patients with SMF, and since we found an increased frequency of the null genotype in these patients, it might be considered a risk factor in patients with SMF.

This study needs to be carried out on a larger sample size and should have a follow up of the patients with tobacco habit but no oral lesions to know if they develop any such lesions. It will also be of interest to look at different polymorphic gene loci simultaneously which have shown to confer risk in oral cancer patients.

## CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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