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Research Article

Metabolic Gene Polymorphisms and Antioxidant Influence Risk of Pre Oral Cancer and Oral Cancer

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Abstract

Many polymorphic genes that code enzymes involved in carcinogen biotransformation have been reported to be associated with development of cancer. GSTT1, GSTM1 genes that code phase II enzyme belongs to the glutathione S-transferases (GSTs) family and seems relevant for the susceptibility to oral diseases as it detoxify carcinogenic tobacco smoke to its excretal form. The aim of our study was to determine the association of GSTT1, GSTM1non null/null polymorphisms with the plasma level of GR, GSH and GST and susceptibility in pre oral cancer and oral cancer in North Indian population. The study group consisted of 230 patients of precancerous oral lesions (Leukoplakia 70, Oral Sub mucous fibrosis 90, Lichen Planus 70), 72 oral cancer and 300 cancer free healthy controls subjects. Genotyping was done by multiplex PCR method and antioxidant/antioxidant enzymes like reduced glutathione (GSH), glutathione reductase (GR) and glutathione S-transferase (GST) were estimated in blood plasma. Genotype frequency was calculated by Chi-square test. GST, GR and GSH activity were estimated spectrophotometrically. Null genotype of GSTT1 and GSTM1 polymorphisms increased the risk of oral diseases (OR=6.00, OR=2.62).The null genotype of GSTM1 polymorphism also increased the risk of OSMF & leukoplakia (OR=3.85, OR=3.82). Smokers, tobacco chewers and individual with more than one oral habit with null genotype of GSTT1 were at increased risk of pre oral cancer lesions (OR=12.15, OR=4.10, OR=23.95).

Keywords: oral cancer; pre oral cancer; genetic polymorphism; pcr; gstt1; gstm1

Introduction

Oral cancer is the eighth most common cancer in the world and it causesmore than4,260,000 cases per annum among which ~128,000 passes annually (Hussain 2016). In India, frequency of oral cancer is 12.8 per 100,000 in males and 7.5 per 100,000 in females (Parul 2018). The frequency is high in rural areas in comparison to urban (Reddy 2004). It is complex disease related to genetic as well as environmental factors (Nigam 2016). Main etiological factors of oral cancer are smoking and alcohol consumptions while chewing tobacco or consumption of smokeless tobacco is particularly the main causalkey of oral cancer among Indian population (IARC 2012). In many patients occurrence of oral cancer is preluded with the development of precancerous oral lesions (Napier 2008; Rajaraman 2015). Pre oral cancer like lichen planus, leukoplakia and oral sub-mucous fibrosis have capacity to get converted into malignancy under the influence of several environmental factors/carcinogens [Rajaraman 2015; Radoi 2013; Guha 2013). However, genetic polymorphisms which may alter the metabolization of carcinogens (Hiyama 2008; Liao 2014)may influence the risk of having oral precancerous lesions and oral cancer (Scully 2000).

GSTT1 (Glutathione S-Transferase Theta 1) gene is present on chromosome number 22q11 and synthesize a phase II metabolic enzyme. Active metabolites of tobacco carcinogens get detoxified by this Phase II enzyme (Saikrishna 2013). Polymorphism in *GSTT1* gene results in complete deletion of *GSTT1* gene results in absence of its enzymatic activity and is known as null genotype. The null genotype has a low capacity to detoxify hence leads to the formation of DNA adducts and DNA damage which may eventually promote carcinogenesis. In

Caucasians and Asians, Null genotype of *GSTT1*has been found to influence the risk of breast(Kimi 2016), lung (Sharma 2015), prostate (Wang 2016) and bladder cancer (Yu 2017).

GSTM1 (Glutathione S -Transferase Mu 1) is a protein coding gene presented on chromosome number 1p13. It is highly polymorphic in nature. It codes for the mu class of enzymes which conjugates glutathione to electrophilic compound such as therapeutic drugs, carcinogens, environmental toxins and oxidative stress products and help in their detoxification. Null mutations of *GSTM1* have been linked with an increased risk of several cancers as it is associated with compromised detoxification of carcinogens and environmental toxins. Null genotype of *GSTM1*has been reported to be involved in the development of breast cancer (Kimi 2016) and lung cancer (Sharma 2015).

The risk of cancer is also enhanced by free radicals which are controlled naturally by antioxidant. GSH is the important intracellular antioxidant which protects organisms against oxidative stress and toxicity caused by deleterious hydrogen peroxide (Beevi 2004; Kumar 2012). It also plays major role against oral cancer by detoxifying the carcinogens and protecting lipid peroxidation by maintaining immune functions, regulating lymphocytic and mitogenic responses (Richie 2008). (GST) Glutathione S-transferase is a family of intracellular protein which detoxifies activated carcinogen metabolites by catalysis of their reaction with GSH(Coles 2001). Glutathione reeducates (GR) is another enzyme which is involved in the metabolism of xenobiotics and formation of the correct disulfide bonds of numerous protein.

The present study is aimed to determine the role of *GSTM1*, *GSTT1* polymorphisms with the development of oral cancer and pre oral cancer. Along with the association of these polymorphisms with the plasma level of GR, GSH and GST levels was also explored.

Materials and methods:

Study Subjects

This study was evaluated on 602 subjects including 302 patients with previously treated and pathologically confirmed oral pre cancer and cancer who were registered at department of Oral Pathology & Microbiology, King George's Medical University and 300 healthy controls. Institutional Ethics Committee of the King George's Medical University, Lucknowhas been approved this study. An informed written consent was obtained from all subjects. Venous blood samples were collected in EDTA tubes and stored at −80 °C, till DNA extraction. DNA was extracted from blood samples by salting out method (Suguna 2014). Plasma was separated from the blood by centrifugation and was used for biochemical estimation.

Genotyping by PCR

Genotyping for GSTT1, GSTM1polymorphisms were done by polymerase chain reaction (PCR) method. PCR products were generated by using 10 ng of genomic DNA in 10 ul volume reactions containing 20 mMTris--HCl, 50 mMKCl, 2.0 mM MgCl2, 0.11 mM each dNTP, 0.3 mM each primer and 0.3 U Platinum Taq DNA polymerase (Invitrogen, Paisley, UK). The PCR products were stained with ethdium bromide and visualize on a 2% agarose gel. The genotype results were regularly checked and compared with known genotypes as controls using direct DNA sequencing of the amplified fragments (Figure 1,2).

Biochemical Assay

Reduced Glutathione (GSH) assay.

The concentration of reduced glutathione was estimated in blood plasma. 100µL plasma was taken and the plasma proteins were precipitated with the addition of equal volume of 5% trichloroacetic acid (TCA). 0.1 mlof supernatant was taken and to this 0.9mL of 0.2M phosphate buffer (pH 7.5) was added to make the volume 1ml. To this 2ml of freshly prepared DTNB was added and incubated for 15 mins. The intensity of yellow color formed was spectrophotometrically read at 412nm against blank. GSH concentration was calculated by using the molar extinction coefficient of DTNB ($14150 M^{-1}cm^{-1}$)(Moron 1979)'.

Glutathione reductase (GR) assay.

GR activity in the plasma samples was measured by preparing a 3 ml assay mixture containing 0.2mmol/L freshly prepared nicotinamide adenine dinucleotide phosphate (NaDPH), 0.5mmol/Loxidized glutathione (GSSG), 0.1mol/L phosphate buffer and 0.1ml of plasma sample. Decrease in absorbance at 340nm was measured for 3 minutes.GR activity was calculated using the molar extinction coefficient of NADPH (=$6.2 Mm^{-1}cm^{-1}$). One unit of GR is defined as the amount of enzyme required to catalyze the reduction of 1 µmol/L of GSSG per minute at pH 7.5. One molecule of NADPH is oxidized per molecule of GSSG reduced. Therefore, the oxidation of NADPH (measured by loss of A340 nm) directly correlates with GSSG reduction (Sheokand 2008).

Glutathione S-transferase (GST) assay.

Plasma GST activity in the study subjects were estimated spectrophotometrically with reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) as substrates. The assay mixture consisted one ml of assay cocktail containing 980µlPBS pH 6.5, 10µl of 100 mM CDNB and 10 µl of 100 mM GSH.An increase in absorbance was measuredat 340nm for 3 minute. GST activity was calculated using extinction coefficient of 9.6nm-1 cm-1 and was expressed as n molar of CDNB conjugation/min (ref protocol). One unit of GST activity is the amount of enzyme which produces 1 µmol of GS-DNB conjugate/min under the conditions of the assay (Seyyedi 2005).

Statistical analysis

The distributions of genotype were checked for the Hardy–Weinberg equilibrium (HWE). Comparison between case and control were assessed by the chi-square test with 1 degree of freedom. Odds ratio (OR) was calculated to measure the odds of having diseases with the relative frequency of different genotypes of the cases and controls. Odds ratio and p values were calculated by the software Epi-Info programme (http://wwwn.cdc.gov/epiinfo/). Biochemical values were expressed as the mean ± standard deviation (SD). Biochemical data obtained were subjected to statistical analysis using SPSS version 17 software.Student's t test was used to compare the data among the groups and the differences were considered statistically significant if p values were 0.05 or less.

Results:

Demographics of the study population

The demographic profile including age, sex, habitual risk factor, tumor staging and environmental risk factors which may contribute to the development of oral cancer and pre oral cancer disease, are shown in Table 1. This study involved 302 oral cancer patients, including 203 (67%) male and 99 (33%) female. Calculated mean age of subject was 46.67. The mean age of controls [219(73%) males and 81(27%) females] was 38.02. Tobacco, smoking chewing and alcohol consumption have been observed to be significantly associated with the development of oral diseases (p value <0.0001). Types of oral diseases included in this study are also showed in Table 1, which includes 23.33% leukoplakia, 30% OSMF, 23.33% lichen planus, 23.33% malignancy. Clinical parameters of oral cancer patient including tumor stage, tumor T status, lymph node involvement, metastasis and cell differentiated grade are shown in Table 1.

Demographic Character	Cases n=302 (%)	Control n=300 (%)	p- value
Male	203 (67)	219 (73)	Ref
Female	99 (33)	81 (27)	0.144
Age distribution			
21– 40	89 (29)	160 (53)	Ref
41- 90	213 (71)	140 (47)	**<0.0001***
Mean age	46.67	38.02	
Median age	47	37.5	
Habitual risk			
Alcohol consumption			
Yes	41 (14)	11 (04)	Ref
No	261 (86)	289 (96)	**<0.0001***
Smoking			
Yes	141 (46)	78 (26)	Ref
No	161 (54)	222 (74)	**<0.0001***
tobacco chewing			
Yes	134 (44)	73 (25)	Ref
No	168 (56)	227 (75)	<0.0001*
Type of oral diseases			
Leukoplakia	70 (23.33)	-	-
O.S.M.F	90 (30.00)	-	-
Lichen planus	70 (23.33)	-	-
Malignancy	72 (23.33)	-	-
Clinical features of oral cancer subjects			-
Tumor Stage I II III IV	 8 (11) 11 (15) 18 (25) 35 (49)	-	-
Tumor T Status T1+T2 T3+T4	 10(13) 62 (87)	-	-
Lymph Node N0 N1+N2	 25 (34) 47 (66)	-	-
Metastasis M0 M1	 54 (75) 18 (25)	-	-
Cell differentiated grade Grade 1 >Grade 1	 31 (45) 39 (55)	-	-

***=significant value**

Table 1: Demographic parameters of patients and controls and their association with risk of Oral pre cancer & cancer

Individual & Combined genotypes of GSTT1 and GSTM1 polymorphisms and the risk of oral diseases

The frequency distribution of different genotypes of GSTT1 and GSTM1 polymorphisms among the cases of oral disease and control are presented in Table 2. GSTT1 and GSTM1 null genotypes showed significant association with the risk of oral cancer and oral pre cancer (p= 0.0001and 0.0001, respectively). Our combined genotype analysis showed that individuals who lacks both the GSTT1 and GSTM1 genes (null/null genotype) were at 7 times higher risk for the development of oral disease compared to individual who possess both the genes (non-null/non null genotype) (p= 0.0001,OR=7.27; 95% CI 3.26-16.25).

GSTT1 Genotypes	Cases n (%)	Controls n (%)	p- value	Odds Ratio	95% CI
Non-null	180 (60)	270 (90)	Ref	1	1
Null	120 (40)	30 (10)	**0.0001***	**6.00**	**3.85-9.33**
GSTM1 Genotypes					
Non-null	221 (74)	264 (88)	Ref	1	1
Null	79 (26)	36 (12)	**0.0001***	**2.62**	**1.70-4.04**
Combined Genotype					
Non-null/Non-null	133 (44)	242 (81)	Ref	1	1
Non-null/Null	47 (16)	28 (09)	**0.0001***	**3.05**	**1.82-5.10**
Null/Non-null	88 (29)	22 (07)	**0.0001***	**7.27**	**4.35-12.15**
Null/Null	32 (11)	08 (03)	**0.0001***	**7.27**	**3.26-16.25**

*** significant association**

Table 2: Distribution of different genotypes GSTT1, GSTM1polymorphisms among cases of oral diseases (pre cancer and cancer) and healthy controls

Genotypes of GSTT1 and GSTM1 polymorphisms and risk of different pre oral cancer and oral cancer

The frequency of different genotypes of GSTT1, GSTM1 polymorphisms among patients of different pre oral cancer diseases, oral cancer and healthy control subjects are showed in Table 3.Compared to the non-null genotypes of GSTM1, GSTT1 polymorphisms the risk of developing OSMF was significantly higher with null genotypes (OR=14.82; 95%CI 8.39-26.19 and OR=3.85; 95% CI 2.20-6.72, respectively)and are listed in Table 3.Null genotype of GSTT1 polymorphism was significantly increased the risk for the development of lichen planus, leukoplakia, and malignancy (p value=0.0009, OR=3.11, 95% CI=1.61-6.00; p value=0.0001, OR=7.15, 95%CI=3.91-13.08; p value=0.01, OR=2.45, 95% CI=1.23-4.86).Individual who possess the GSTT1 gene but lacks GSTM1 gene (i.e having GSTT1 non-null/GSTM1null genotype) were at increased risk of OSMF and leukoplakia (OR=6.82, and 5.40 respectively). Similarly risk of OSMF, Lichen planus and leukoplakia were higher among the subjects lacking the GSST1 gene but possess the GSTM1 gene (i.esubjects with GSTT1 null/GSTM1non null genotype)(OR= 23.25, 4.12 and 10.08 respectively). With reference to non-null/ non-null genotypes for GSTT1 and GSTM1 genes risk of developing OSMF and Leukoplakia lesion were significantly higher with the null/null genotype (OR= 25.47and 4.34 respectively).

Genotypes	**OSMF n (%)**	**p-value&OR (95% CI)**	**LichenPlanus n (%)**	**p-value&OR (95% CI)**	**Leukoplakia n (%)**	**p-value&OR (95% CI)**	**Malignancy n (%)**	**p-value&OR (95% CI)**	**Control n (%)**
GSTT1 Genotypes									
Non-null	34 (38)	Ref	52 (75)	Ref	39 (56)	Ref	55 (79)	Ref	270 (90)
Null	56 (62)	**0.0001* 14.82 (8.39-26.19)**	18 (25)	**0.0009* 3.11 (1.61-6.00)**	31 (44)	**0.0001* 7.15 (3.91-13.08)**	15 (21)	**0.01* 2.45 (1.23-4.86)**	30 (10)
GSTM1 Genotypes									
Genotypes	**OSMF n (%)**	**p-value&OR (95% CI)**	**Lichen Planus n (%)**	**p-value&OR (95% CI)**	**Leukoplakia n (%)**	**p-value&OR (95% CI)**	**Malignancy n(%)**	**p-valueOR & (95% CI)**	**Controls n (%)**
Non-null	59 (66)	Ref	55 (78)	Ref	46 (66)	Ref	61 (87)	Ref	264 (88)
Null	31 (34)	**0.0001* 3.85 (2.20-6.72)**	15 (22)	0.06 2.00	24 (34)	**0.0001* 3.82**	09 (13)	0.84 1.08 (0.49-2.36)	36 (12)

				(1.02-3.90)		**(2.09-7.00)**			
Combined Genotype									
Non-null/ Non-null	19 (21)	Ref	40 (57)	Ref	24 (34)	Ref	50 (71)	Ref	242 (81)
Non-null/Null	15 (17)	**0.0001* 6.82 (3.12-14.91)**	12 (17)	0.02 2.59 (1.21-5.51)	15 (21)	**0.0001* 5.40 (2.54-11.48)**	05 (07)	0.96 0.86 (0.31-2.34)	28 (09)
Null/Non-null	40 (44)	**0.0001* 23.15 (11.50-46.59)**	15 (21)	**0.0002* 4.12 (1.97-8.61)**	22 (32)	**0.0001* 10.08 (4.88-2.08)**	11 (16)	0.04 2.42 (1.10-5.30)	22 (07)
Null/Null	16 (18)	**0.0001* 25.47 (9.66-67.12)**	03 (05)	0.44 2.26 (0.57-8.91)	09 (13)	**0.0001* 4.34 (4.00-32.12)**	04 (06)	0.29 2.42 (0.70-8.35)	08 (03)

***= significant value**

Table 3: Distribution of different genotypes GSTT1,GSTM1 polymorphisms among the subjects of oralsubmucous fibrosis, Lichenplanus, Leukoplakia, oral cancer & controls

GSTT1, GSTM1 gene polymorphisms in relation to different habit of patients with oral cancer and pre oral cancer

Distribution of different genotypes for GSTT1 and GSTM1 null/non-null polymorphisms among the tobacco chewers, alcohol consumers and smokers are detailed in Table 4. The null genotype of GSTT1 was found to increase the risk for pre oral cancer and that was irrespective of individuals oral habits (Table 4). Whereas, the risk of oral cancer was increased by the GSTT1 Null genotype only in the individuals who had more than 1oral habit (OR=9.11).The null genotype of GSTM1 was found to increase the risk of pre oral cancer among the smokers and individuals with more than 1 oral habits (OR=26.75 and 7.22 respectively). No interaction of GSTM1 genotype with the oral habits were observed in risk modulation of oral cancer.

Genotypes	**Pre-oral cancer n (%)**	**p-value**	**Odd ratio (95% CI)**	**Oral cancer n (%)**	**p- value**	**Odd ratio (95% CI)**	**Controls n (%)**
GSTT1 Genotypes							
No Habit							
Non-null	39 (17)	Ref	1	1 (01)	Ref	1	165 (55)
Null	40 (17)	**0.0001***	**8.90 (4.65-17.03)**	1 (01)	0.51	8.68 (0.52-144.66)	19 (07)
Smokers							
Non-null	38 (16)	Ref	1	1 (01)	Ref	1	44 (15)
Null	21 (09)	**0.0003***	**12.15 (2.67-55.27)**	00	-	-	2 (0.7)
Tobacco							
Non-null	21 (09)	Ref	1	19 (28)	Ref	1	30 (10)
Null	23 (10)	**0.007***	**4.10 (1.54-10.93)**	3 (04)	0.71	0.59 (0.13-2.51)	8 (02)

Alcohol							
Non-null	5 (02)	Ref	1	00	Ref	1	00
Null	4 (02)	-	-	1 (01)	-	-	00
>1 Habit							
Non-null	22 (10)	Ref	1	34 (49)	Ref	1	31 (10)
Null	17 (08)	**0.0003***	**23.95 (2.96-193.67)**	10 (15)	**0.03***	**9.11 (1.10-75.43)**	1 (0.3)
GSTM1 Genotypes							
No Habit							
Non-null	60 (26)	Ref	1	2 (03)	Ref	1	157 (52)
Null	19 (08)	0.09	1.84 (0.95-3.55)	1 (02)	0.93	2.90 (0.25-33.20)	27 (9)
Smokers							
Non-null	37 (16)	Ref	1	1 (02)	Ref	1	45 (15)
Null	22 (10)	**0.0001***	**26.75 (3.44-208.09)**	00	-	-	1 (01)
Tobacco							
Non-null	32 (14)	Ref	1	20 (28)	Ref	1	32 (10)
Null	12 (05)	0.32	2.00 (0.66-5.98)	01 (1)	0.40	0.26 (0.02-2.38)	06 (02)
Alcohol							
Non-null	04 (02)	Ref	1	01 (01)	Ref	1	00
Null	04 (02)	-	-	00	-	-	00
>1 Habit							

Non-null	27 (12)	Ref	1	37 (53)	Ref	1	30 (10)
Null	13 (05)	**0.01***	**7.22 (1.49-34.96)**	07 (10)	0.35	2.83 (0.54-14.68)	02 (01)

***significant association**

Table 4: Habitual riskof different genotypes GSTT1, GSTM1polymorphisms among oral pre cancer (Oral submucous fibrosis + Lichenplanus, +Leukoplakia), cancer and controls

Interaction between GSH, GR, GST with GSTT1, GSTM1 gene polymorphisms

The activity of GST and GR and plasma level of GSH were estimated and correlated with different genotypes of GSTT1 and GSTM1 polymorphisms. The plasma level GSH and the activity of GST and GR was similar between the different genotypes of GSTT1 and GSTM1 non null/null polymorphism (Table 5).

Discussion

GSTT1 and GSTM1 are Phase II metabolism pathway genes and are involved in detoxification of tobacco carcinogens by conjugation reaction. GSTM1 and GSTT1 absent/null genotype would low the capacity of the detoxification process. Thus, polymorphisms of GSTT1, GSTM1 genes may affect the susceptibility of different cancers.

In this study we observed that absent genotype of GSTT1 & GSTM1 polymorphisms increased the risk of oral diseases. Null genotype of GSTT1 polymorphism increased the risk of all kind of pre oral cancer & oral cancer and the null genotype of GSTM1 polymorphism increased the risk of OSMF and leukoplakia. Compared to presence of both the genes, absence of any one of them significantly increased the risk for oral diseases. The GSTT1 deletion genotype has been suggested by others to be associated with the risk of different cancers, including lung cancer in Asians (Raimondi 2006), gastric cancer (Saadat 2006), leukemia (Ye 2005), and hepatocellular carcinoma (White 2008). Polymorphisms in a GSTT1 and GSTM1 null genotype suggested to confer an increased risk for oral cancer by Sreelekha et al. (2008).

Genetic polymorphisms in GSTT1, GSTM1 and tobacco use to play key roles in development of different cancers (Choudhury 2014; 32. Talukdar 2013). In the present study genotypes of GSTT1 and GSTM1 were also found to interact with individuals oral habits like tobacco smoking and chewing in modulating the risk of both pre oral cancer and oral cancer. Similarly, absence of GSTM1 and GSTT1 genes showed to influence the risk of HNSCC risk among tobacco and alcohol consumers in the German population (Gronau 2003).

Antioxidants can reduce oxidative damage and inflammation, delay cancer progression, and prevent cancer recurrence by scavenging free radicals (Sarangarajan 2017). Different studies have successfully used vitamins or phytochemicals as anti-oxidative adjuvants for head and neck squamous cell carcinoma (Eastham 2018; Jain 2017). Anti-oxidative nutrients, which can also be obtained from a healthy diet that includes plenty of fresh vegetables and fruits, have been proposed to reduce the risk of head and neck cancer (Chang 2017). The use of tobacco may have suppressed the production of antioxidant enzymes, which was evident in pre oral and OSCC patients, who were tobacco users. The GSH/GSSG redox couple is commonly used in measuring oxidative stress inside the body. Previous studies have reported a strong correlation between decreased risks of oral cancer with increasing blood glutathione levels

(Richie 2008). In one Indian study Plasma glutathione level is consistently reduced in the advanced stages of oral cancer when compared to initial stages (Manoharam 2005). In glutathione redox cycle, GR, NAD(P)H-dependent enzymatic antioxidant, is the important enzyme and it efficiently maintains the reduced pool of GSH. Many studies reported that GRactivity getdecreased in oral cancer in response to oxidative stress (Fiaschi 2005). GSTT1 protein catalyses the conjugation of reduced glutathione to several electrophilic and hydrophobic compounds. Similarly GSTM1 synthesize a mu class of enzymes which conjugates reduced glutathione to electrophilic compound such as therapeutic drugs, carcinogens, environmental toxins and oxidative stress products and help in their detoxification. There fore presence or absence of these two enzyme may influence the risk of cancer by regulating the glutathione redox cycle. However, in our study we found plasma level GSH and the activity of GST and GR was similar between the different genotypes of GSTT1 and GSTM1 non null/null polymorphism in oral disease.

Conclusion:

The genetic polymorphism in GSTT1 and GSTM1 can be used as biomarkers of susceptibility for developing oral disease. However, we did not observe any correlation between the oxidative stress in patients and genotypes of GSTT1, GSTM1 polymorphism which may be due to small sample size or estimation of a few parameters and therefore, the findings only provide an index. Further studies are required to be conducted with large sample size to confirm the role of GSTT1, GSTM1 polymorphisms and oxidative stress in pre oral cancer and oral cancer patients before using these polymorphisms as susceptibility biomarker.

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Author contributions:

Kumud Nigam : writing original draft

Om prakash gupta: Analysis, Final editing & correction

Somali Sanyal: Visualisation, Resources, supervision

Shalini Gupta: Conceptualisation & study design

Saurabh Pratap Singh: Investigation and review

Reference:

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