Original Article

Null genotypes of Glutathione S-transferase M1 and T1 and risk of oral cancer: A meta-analysis

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Abstract

Background: Glutathione S-transferase M1 (GSTM1) and Glutathione S-transferase T1 (GSTT1) null genotypes have been considered risk factors for many cancers. Numerous studies have been conducted to evaluate the association of null genotype of GSTM1 and GSTT1 with increased susceptibility to oral cancers, and these have produced inconsistent and inconclusive results. In the present study, the possible association of oral cancer(OC) with GSTM1 and GSTT1 null genotypes was explored by a meta analysis.

Materials and Methods: A meta-analysis was conducted on published original studies retrieved from the literature using a bibliographic search from two electronic databases: MEDLINE (National library of medicine, USA) and EMBASE. The pooled odds ratio and presence of publication bias in those studies were evaluated.

Results: A total of 49 studies concerning oral cancer (OC) were identified for GSTM1 null genotype. Similarly, 36 studies were identified for GSTT1 null genotype. The pooled OR was 1.551(95% confidence interval [CI]: 1.355–1.774) for the GSTM1 null genotype, while for GSTT1 null genotype, the pooled OR was 1.377 (95% CI: 1.155–1.642). No evidence of publication bias was detected among the included studies.

Conclusion: The results suggest that the Glutathione S-transferase M1 and Glutathione S-transferase T1 null genotypes significantly enhances the risk of developing oral cancer by a substantial percentage.

Keywords: GSTM1 null genotype, GSTT1 null genotype, head-and-neck neoplasm, meta-analysis, oral cancers, oral cavity polymorphisms

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INTRODUCTION

Oral cancer (OC) is the most common cancer in the world, accounting for 2.0% of all cancers. In India, oral cancer is the leading cause of cancer death amongst men.[1] Eastern and Western Europe and Australia/New Zealand also show high incidence rates, and the etiology of oral cancer has been linked to tobacco smoking, HPV infection, and ultraviolet radiation.[2,3,4,5,6] However, oral cancer, is confined only to a fraction of individuals who are subjected to these etiological factors, demonstrating that cancer susceptibility varies from

individual to individual. Therefore, variations in genetic host factors that contribute to carcinogenic pathways may have a role in the development of oral cancer susceptibility. The implication is that a genetic deficit in the enzymes that metabolise tobacco carcinogens is likely to have a role in a person's susceptibility to oral cancer.[6] Further more, animal studies that relate the association of carcinogens produced due to tobacco smoking with OCs, have bestowed the precise mechanism of oral carcinogenesis on abilities of metabolites formed as a resultant of tobacco smoking, to bind onto DNA forming DNA adducts.[7] Failure to repair such DNA adducts prior to DNA replication may induce mutation in oncogenes or tumor suppressor genes that can lead to malignant transformation of the cell and thereby initiating carcinogenesis.[8]

Further, these chemical carcinogens are eliminated by the action of xenobiotic metabolizing enzymes, that have molecular and cellular mechanisms for detoxifying and excreting harmful substances, and are distinguished into phase I and phase II enzymes. Phase I xenobiotic enzymes are involved in oxidation, reduction and hydroxylation, rendering hydrophobic substances into more hydrophilic in nature, which can easily be excretable,[9] examples of these enzymes include cytochrome P450s (CYPs), cyclooxygenases and aldo keto-reductases. Phase II xenobiotic enzymes are involved in introduction of polar groups moieties and thereby assisting in excretion.[9] Examples of phase II enzymes include uridine diphosphate glucuronosyltransferases, N, O-acetyltransferases, sulfotransferases and glutathione S-transferases (GSTs).

Glutathione-S-transferases (GSTs) a phase II enzymes, are known to protect cells from oxidative stress. Polymorphism of GSTM1 and GSTT1 has been extensively explored in various malignancies. It is hypothesized that any deleted variants of the GSTM1 and GSTT1 genes result in loss of functional activity, often reported as a factor influencing the individual susceptibility to cancer encouraging the concept of gene–environment interactions to Oral cancer risk.[10-12] However, this concept has been addressed by focal studies, but the results have been inconsistent and obscure.[10-13] Further, a previous meta-analysis has rested GSTM1 null genotype and risk of OC to be associated with only in Asian population,[14] yet another recent study has a related association of GSTM1 null polymorphisms with increased risk of OC.[15] In addition to this, a recent meta-analysis on association of null genotype GSTT1[15] with OC suggests an increased risk of development of OC. However, this comes with limited literature due to criteria for selection of studies. Therefore, whether null genotypes of GSTM1 or GSTT1 is a risk factor for OC remains obscured. Hence, in the

present study, an evidence-based quantitative meta-analysis was conducted to address this controversy.

MATERIALS AND METHODS

Selection of studies
Two investigators independently searched two electronic databases, MEDLINE (National Library of Medicine, USA) and EMBASE, for studies pertaining to the deficiency of enzymes GSTM1 and GSTT1 and the risk of oral cancer, covering all papers published up to October 2021. The search was conducted using the combination of the following search GSTM1, GSTT1, oral cancers, mouth neoplasm, glutathione, null genotype. Additional articles were also manually retrieved via the references cited in these publications and review articles.

The following criteria were used for the selection of articles for the meta-analysis: (1) Only studies which explicitly describe the association of oral squamous cell carcinoma with GSTM1/GSTT1 null genotypes; (2) The sources of cases and controls, as well as the histopathological diagnosis of oral squamous cell carcinoma, should be mentioned; (3) Individuals should have been genotyped solely through the use of polymerase chain reaction technique; (4) The sample size, odds ratios (ORs), and 95% CIs, as well as any other information that can be used to deduce the results should have been stated. Accordingly, the exclusion criteria used were: (1) design and the definition of the experiments were obviously different from those of the selected papers; (2) the sample size, source of cases and controls and other essential information was not presented and (3) reviews and literature that is repeated.

Extraction of data
Data from the selected articles were extracted and entered into MedCalc, version 20.018. The extraction was performed by two investigators independently. For conflicting evaluations, an agreement was reached following a discussion. For each study, the author, year of publication, country where the study was carried out, number, race and gender of patients and controls, control source (hospital based or population based) and matching of cases and controls were rigorously tabulated.

Statistical analysis
The OR of GSTM1 and GSTT1 polymorphisms in OCs for each study was recalculated and their corresponding 95% CIs were recorded. Presumption was made that all the studies considered are estimating different effect sizes (alternative hypothesis [HA]), and therefore, null hypothesis (H0) was that all studies are estimating the

same effect size. To determine whether study heterogeneity exists or not, Q statistics was used, and the I^2 statistic was used to quantify the percentage of variation across studies that is attributable to heterogeneity rather than chance.[16,17] If, *P* value was ≤ 0.05, indicated evidence against the null hypothesis and null hypothesis was rejected and HA was accepted and ORs were pooled according to the random effect model by DerSimonian and Laird method,[18] otherwise fixed-effect model by inverse variance method was used.[9] To identify publication bias, Begg rank correlation and Egger regression test were used.[19]

RESULTS

A total of 51 studies associating GSTM1 null genotype with respect to oral squamous cell carcinoma were identified. After a careful review, two irrelevant studies were excluded based on the inclusion and exclusion criteria. The data Pertaining to one study as that of Park JY[20] et al., for computing purpose in this meta-analysis, was divided into two as the study was performed in two different populations (African Americans and Caucasians) with a larger sample size and both the data were published in a single literature, accounting for two different populations. A database was established according to the extracted information from each published literature as indicated in table 1. A total of 36 studies were listed for GSTT1 null genotype after excluding 1 study based on the inclusion and exclusion criteria, as indicated in Table 2.

Out of 49 studies included in the meta-analysis of GSTM1, 33 studies came from Asian countries, 6 from European countries and 10 from American countries. The source of controls in all the studies of GSTM1 was predominantly hospital based followed by population based and combination of hospital and population based constituting 27 studies, 17 studies and 5 studies, respectively. The controls were matched with case's sex, age, geographical location, ethnicity and race in 22 studies, 20 studies, 7 studies, 9 studies and 4 studies, respectively. Socioeconomic status of cases was matched with controls in 4 studies, and in 1 study, hospital distribution was matched with controls. In 2 studies, control matching with cases was done with habits. In GSTT1 null genotype, there were 26 studies from Asian countries, 3 from European countries and 7 from American countries. Source of controls from 20 studies were hospital based, 13 studies were population based and 3 studies were mixed by hospital and population based. The controls were matched with case's sex, age, geographical location, ethnicity and race in 15 studies, 19 studies, 7 studies, 8 studies and 2 studies, respectively. Socioeconomic status of cases was matched with controls in 3 studies, and

in 1 study, hospital distribution was matched with controls. In 4 studies, control matching with cases was done with habits. In 14 studies of GSTM1 and 10 studies of GSTT1, matching concerning case and control was not mentioned.

Population frequencies
A total of 7049 cases and 10,308 controls from 49 included case–control studies for GSTM1 null genotype were analyzed, out of which 3677 cases and 4200 controls showed the null genotype constituting 52.1% among the cases and 40.7% among the controls. Whereas, in GSTT1, 5169 cases and 7307 controls from 36 included case–control studies were analyzed, of which 1759 cases and 1849 controls showed the null genotype accounting for 34.02% among the cases and 25.3% among the controls.

Meta-analysis
Heterogeneity when tested for all the 50 studies of GSTM1 null genotype gave Chi-square-based Q-value of 267.6496 with 50 degrees of freedom (df), I^2 of 81.32% and $P = 0.0001$, indicating heterogeneity across the studies. Therefore, a random-effect model was used and the overall OR for GSTM1 null genotype was 1.551 with 95% CI of 1.355–1.774 as indicated in figure 1. On the basis of these findings, it is likely that GSTM1 null status significantly increases the susceptibility to OC.

Likewise, heterogeneity test for all the 36 studies of GSTT1 null genotype yielded Chi-square-based Q-value of 183.8086 with 36 degrees of freedom (df), I^2 of 80.41% and $P = 0.0001$, indicating heterogeneity across the studies. Hence, a random-effect model was used for GSTT1 null genotype also, which showed an overall OR of 1.377 with 95% CI of 1.155–1.642 as indicated in figure 2. The data implied that GSTT1 null genotype was significantly associated with OC risk.

For the diagnosis of publication bias, both Egger's test and Begg's test, when applied, showed no evidence of publication bias as *P* was 0.2454 and 0.2844 in GSTM1 and GSTT1 null genotype, respectively. Hence, none of the studies were excluded.

DISCUSSION

The synthesis, species-specific expression and distribution of GST are considered an evolutionary adaptive mechanism against endogenous and exogenous toxic metabolites.[21,22] GST consists of three major families of proteins,[23] of which cytosolic GSTs (cGSTs) are extensively explored[9] and constitute a larger class divided into cGST alpha (A, α), mu (M, μ), Pi (pi, π), omega (ω), theta (T, θ),

Table 1: Summary of studies on GSTM1 null genotype in oral cancer

Name	Year	Country	Source of Controls	Control Matching	Case n/N	Controls n/N	OR	95% CI
M Deakin[46]	1996	England	Hospital	None	22/40	316/577	1.01	0.530-1.922
Park[48]	1997	USA	Hospital, Healthy Population	Age, Sex, Race	55/109	58/109	0.9	0.526-1.524
Hung[47]	1997	Taiwan	Healthy Population	Age, Ethnicity	24/41	71/123	1.03	0.505-2.118
Matthias C[49]	1998	Germany	Hospital	None	71/122	95/178	1.22	0.764-1.936
Jourenkova Mironova[50]	1999	France	Hospital	Age, Sex, Hospital Distribution	30/67	90/172	0.74	0.419-1.302
Katoh[51]	1999	Japan	Hospital	None	54/92	68/147	1.65	0.975-2.795
K Tanimoto[52]	1999	Japan	Hospital	Age, Sex	43/100	40/100	1.13	0.645-1.987
M Sato[11]	2000	Japan	Healthy Population	Age, Sex	92/142	64/142	2.24	1.391-3.614
JY Park[20]	2000	USA	Hospital	Age, Sex, Race	20/63	21/133	2.48	1.224-5.026
JY Park[20]	2000	USA	Hospital	Age, Sex, Race	51/101	104/213	1.07	0.666-1.717
Nomura T[53]	2000	Japan	Hospital	None	77/114	15/33	2.5	1.134-5.500
Sreelekha[54]	2001	India	Healthy Population	Age	48/98	20/60	1.92	0.985-3.741
Kietthubthew[55]	2001	Thailand	Healthy Population	Age, Sex, Residence, Habits	30/53	16/53	3.02	1.356-6.709
M Hahn[56]	2002	Germany	Healthy Population	Ethnicity	56/94	49/92	1.29	0.723-2.312
Buch[57]	2002	India	Healthy Population	Region of Origin	146/297	111/450	2.95	2.160-4.036
S Gronau[58]	2003	Germany	Hospital	Smoking, Alcohol	41/73	66/129	1.22	0.687-2.178
Sikdar[12]	2004	India	Hospital	Ethnicity	84/256	85/259	1	0.692-1.444
Xie[13]	2004	USA	Healthy Population	Region of Origin	49/132	65/143	0.71	0.437-1.148
Drumond[59]	2004	Brazil	Hospital	Socio Economic Status, Age, Sex	46/70	40/82	2.01	1.044-3.880
Majumder[60]	2005	India	Hospital	None	104/310	117/348	1	0.721-1.378
Chung Ji Liu[61]	2005	Taiwan	Hospital	Sex, Smoking, Areca Chewing	66/114	55/100	1.13	0.654-1.934
Huang[79]	2006	China	Hospital, Healthy Population	None	54/87	39/87	2.01	1.100-3.688
Sharma A[80]	2006	India	Healthy Population	None	21/40	29/87	2.21	1.030-4.746
T Sugimura[68]	2006	Japan	Hospital	None	59/122	126/241	0.86	0.553-1.322
Biselli[67]	2006	Brazil	Not Mentioned	Age, Sex, Race, Alcohol, Ethnicity	13/26	29/60	1.07	0.426-2.684
Gattas[81]	2006	Brazil	Hospital	Age, Sex	24/38	39/102	2.77	1.281-5.985
In Ho Cha[82]	2007	Korea	Healthy Population	None	37/72	123/209	0.74	0.432-1.266
Ananthraman[83]	2007	India	Healthy Population	Age, Sex, Habit	198/451	269/727	1.33	1.049-1.693
Hatagima[84]	2008	Brazil	Hospital	Age, Sex, Race	95/231	93/212	0.89	0.613-1.303
L Varela Lema[25]	2008	Spain	Hospital	None	27/53	62/130	1.14	0.601-2.158
Losi-Guembarovski[85]	2008	Brazil	Hospital, Healthy Population	Age, Region	36/91	42/81	0.61	0.332-1.113
Bathi[86]	2009	India	Healthy Population	Age, Sex, Socio Economic Status	16/30	36/60	0.76	0.315-1.844
R Amtha[69]	2009	Indonesia	Hospital	Age, Sex	49/81	90/162	1.23	0.712-2.108
Chen[87]	2010	Taiwan	Healthy Population	Age, Sex, Geographic Location, Ethnicity	87/164	152/274	0.91	0.615-1.337
Yadav[70]	2010	India	Healthy Population	Age, Sex, Ethnicity	66/136	120/270	1.18	0.780-1.782
Sharma R[88]	2010	India	Hospital	Region of Origin	35/73	53/201	2.57	1.475-4.485
Cordero[89]	2010	Santiago	Healthy Population	None	24/48	24/124	4.17	2.028-8.561
Masood[90]	2011	Pakistan	Hospital	Age, Sex	35/228	12/150	2.09	1.045-4.163
Lourenco[91]	2011	Brazil	Hospital	Sex, Ethnic Origin	10/29	66/142	0.61	0.263-1.395
Ruwali[92]	2011	India	Healthy Population	Ethnicity	84/170	160/500	2.08	1.456-2.959
Shukla D[93]	2012	India	Hospital	Age, Sex, Tobacco	51/150	27/150	2.35	1.373-4.012
Zhang[94]	2012	China	Healthy Population	None	415/600	265/600	2.84	2.238-3.593
Mondal[95]	2013	India	Hospital, Healthy Population	Age, Sex	59/124	37/140	2.53	1.510-4.229
Singh RD[96]	2014	India	Hospital	Region of Origin, Socio Economic Status	53/122	42/127	1.56	0.929-2.601
Tanwar R[39]	2015	India	Hospital	None	70/80	10/100	63	24.844-159.759
Maurya SS[40]	2015	India	Hospital, Healthy Population	Ethnicity, Geographical Area, Socio Economic Status, Sex	131/300	234/750	1.71	1.298-2.252
Zakiullah[41]	2015	Pakistan	Healthy Population	Age, Ethnicity	159/200	86/151	2.93	1.831-4.693
Dong TT[42]	2016	China	Hospital	Age, Place of Origin, Gender	519/750	331/750	2.84	2.302-3.515
Rao KA[43]	2017	India	Hospital	None	12/15	11/15	1.46	0.264-8.010
Sarvani S[44]	2019	Iran	Hospital	Sex, Age, Ethnicity	29/50	27/63	1.84	0.869-3.903
Overall OR (Random -effect model)							1.551	1.355-1.774

n=Number of cases positive for GSTM1 null genotype, *N*=Total number of cases, OR=ODDS Ratio, CI=Class Interval

delta (δ), sigma (σ) and zeta (ζ)[21,22,24] and the GSTs have a critical role in cell growth, oxidative stress, as well as in disease progression and prevention. GST, demonstrate polymorphisms in humans, which may be a prime cause for interindividual variations in responses to xenobiotic.[21]

Likewise, in most of the cancers, genetic mechanism also influence the initation and progression of oral cancer. Further, OC is also found to be associated with environmental factors such as tobacco usage. Hence, it is important to evaluate the gene–environment

Table 2: Summary of studies on GSTT1 null genotype in oral cancer

Name	Year	Country	Source of controls	Control Matching	Case n/N	Controls n/N	OR	95% CI
M Deakin[46]	1996	England	Hospital	None	04/34	94/509	0.59	0.203-1.711
Hung[47]	1997	Taiwan	Healthy Population	Age, Ethnicity	24/41	65/123	1.26	0.616-2.575
Jourenkova Mironova[50]	1999	France	Hospital	Age, Sex, Hospital Distribution	15/67	27/172	1.55	0.765-3.139
Katoh[51]	1999	Japan	Hospital	None	44/92	75/147	0.88	0.522-1.482
Sreelekha[54]	2001	India	Healthy Population	Age	18/98	05/60	2.48	0.867-7.063
Kietthubthew[55]	2001	Thailand	Healthy Population	Age, Sex, Residence, Habits	18/53	25/53	0.58	0.263-1.261
Buch[57]	2002	India	Healthy Population	Region of Origin	54/297	55/450	1.6	1.061-2.400
S Gronau[58]	2003	Germany	Hospital	Smoking, Alcohol	11/73	19/136	1.09	0.489-2.411
Xie[13]	2004	USA	Healthy Population	Region of Origin	39/132	42/143	1.01	0.600-1.694
Sikdar[12]	2004	India	Hospital	Ethnicity	42/256	32/259	1.39	0.847-2.287
Drumond[66]	2005	Brazil	Healthy Population	Socio Economic Status, Age, Sex	73/87	34/81	7.21	3.500-14.843
Majumder[60]	2005	India	Hospital	None	54/310	54/348	1.15	0.760-1.735
Chung Ji LiU[61]	2005	Taiwan	Hospital	Sex, Smoking, Areca Chewing	51/114	37/100	1.38	0.796-2.386
Huang[79]	2006	China	Hospital, Healthy Population	None	47/87	42/87	1.26	0.694-2.284
T Sugimura[68]	2006	Japan	Hospital	None	46/122	105/241	0.78	0.502-1.225
Sharma A[80]	2006	India	Healthy Population	None	17/40	13/87	4.21	1.780-9.947
Gattas[81]	2006	Brazil	Hospital	None	10/38	18/102	1.67	0.689-4.032
Biselli[67]	2006	Brazil	Not Mentioned	Age, Sex, Race, Alcohol	06/26	14/60	0.99	0.331-2.935
Ananthraman[83]	2007	India	Healthy Population	Age, Sex, Habit	45/451	114/727	0.60	0.413-0.860
Hatagima[84]	2008	Brazil	Hospital	Age, Sex, Race	49/231	48/212	0.92	0.586-1.443
Losi-Guembarovski[85]	2008	Brazil	Hospital, Healthy Population	Age, Ethnicity, Region	30/91	23/81	1.24	0.647-2.379
Bathi[86]	2009	India	Healthy Population	Age, Sex, Socio Economic Status	20/30	45/60	0.67	0.256-1.738
R Amtha[69]	2009	Indonesia	Hospital	Age, Sex	37/81	67/162	1.19	0.697-2.041
Chen[87]	2010	Taiwan	Healthy Population	Age, Sex, Geographic Location, Ethnicity	79/164	149/274	0.78	0.529-1.149
Yadav[70]	2010	India	Healthy Population	Age, Sex, Ethnicity	42/136	85/270	0.97	0.623-1.518
Masood[90]	2011	Pakistan	Hospital	Age, Sex	57/228	28/150	1.45	0.873-2.415
Lourenco[91]	2011	Brazil	Hospital	Sex, Ethnic Origin	7/29	27/142	1.36	0.525-3.498
Ruwali[92]	2011	India	Healthy Population	Ethnicity	48/170	103/500	1.52	1.018-2.258
Guo[65]	2012	China	Hospital	None	208/300	133/300	2.84	2.031-3.968
Mondal[95]	2013	India	Hospital, Healthy Population	Age, Sex	51/124	32/140	2.36	1.384-4.016
Singh RD[96]	2014	India	Hospital	Region of Origin, Socio Economic Status	28/122	17/127	1.93	0.994-3.739
Zakiullah[41]	2015	Pakistan	Healthy Population	Age, Ethnicity	95/200	35/151	3	1.876-4.793
Dong TT[42]	2016	China	Hospital	Age, Place of Origin, Gender	355/750	152/750	3.54	2.813-4.444
Sarah D Mello[97]	2016	India	Hospital	None	22/30	23/25	0.24	0.0456-1.253
Rao KA[43]	2017	India	Hospital	None	01/15	2/15	0.46	0.0375-5.750
Sarvani S[44]	2019	Iran	Hospital	Sex, Age, Ethnicity	12/50	10/63	1.67	0.656-4.272
Overall OR (Random -effect model)							1.377	1.155-1.642

n=Number of cases positive for GSTT1 null genotype, *N*=Total number of cases, OR=ODDS Ratio, CI=Class Interval

interactions and also gene–gene interactions. However, only environmental risk factors are well established in the development of oral squamous cell carcinoma, whereas collaborative role of environmental factors and genetic polymorphisms has exhibited diversity with inconsistent results.[15] Considering the fact that the development of cancer is attributed to carcinogenic exposure, then the genetic mechanism governing the mechanics of carcinogen metabolism may be a probable mechanism to elucidate interindividual susceptibility.

However epidemiological studies have indicated the combined role of genetic and environmental factors in individual susceptibility to cancer.[25] Over the years, it has been postulated that a reduced detoxification by phase II enzymes was directly proportional to susceptibility to cancers,[26] which is emphasized by toxicology studies that associates increased sister chromatid exchange (SCE) in GSTM1 null genotype and baseline frequency of SCE in GSTT1 null genotype in tobacco smokers.[27,28] Further, studies have correlated upregulation of GST with tumor stage and differentiation with GSTs.[28,29] Hence, it is logical to believe that deprived status of these enzymes can contribute toward tumorigenesis and survival depending on the initiative stage or propagative stage of carcinogenesis respectively.

A large number of studies have contemplated the association of GSTM1 and GSTT1 deficiency and the risk of OC. Studies pertaining to GSTM1 and GSTT1 null genotypes have demonstrated complete abolished enzyme activities of GSTM1 and GSTT1, respectively. This may enhance the exposure of individuals to environmental toxins and carcinogens and may be
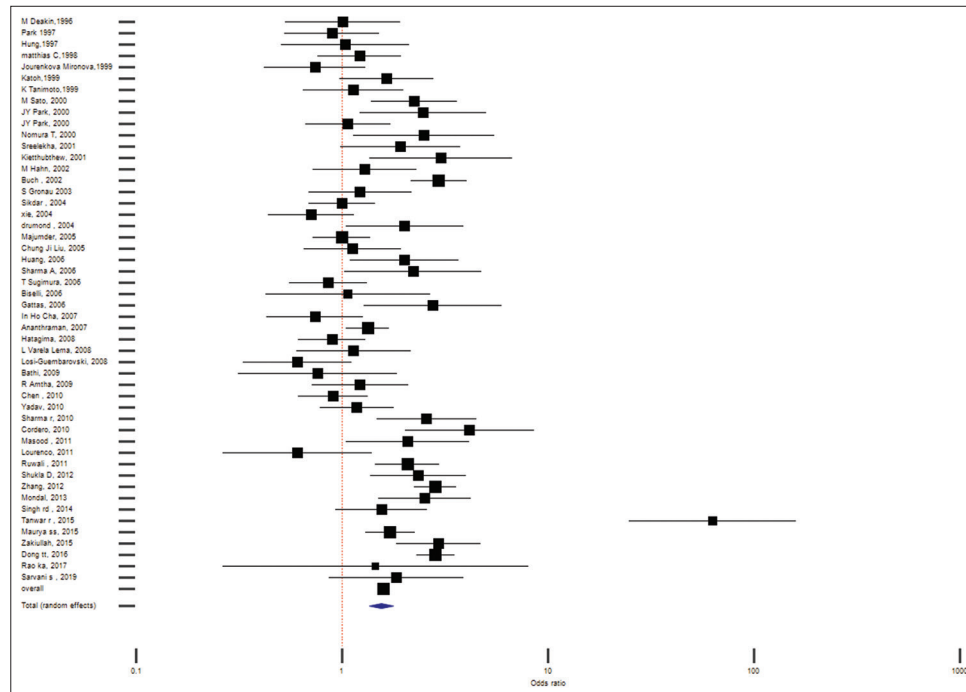
Figure 1: Forest Plot of Odds Ratio of GSTM1 Deficiency and Risk of Developing Oral Cancer

responsible for an Individuals increased susceptibility to cancer[30]. However, these studies have produced inconsistent conclusions. Previous meta-analyses suggest that GSTM1 deficiency might modestly increase the risk of head-and-neck cancer.[31-33] In contrast to these, a number of meta-analyses indicated no marked association of GSTM1 null mutations with hepatocellular cancer,[34] gastric cancers,[35] brain tumors,[36] nasopharyngeal cancer[37] and prostate cancer.[38]

In a meta-analysis, pertaining to OC, Zhao *et al.*[14] have suggested that the effect of GSTM1 null polymorphisms on the risk of OC may differ by ethnicity, and that asian population with tobacco habits are at higher risk of developing OC. A meta-analysis, as that of Zhao *et al.,*[14] is limited with literature up to 2013 and therefore does not contain the published data of recent years, as that of Tanwar *et al.*[39] Maurya *et al.,*[40] Zakiullah *et al.,*[41] Dong *et al.,*[42] Rao *et al.*[43] and Saravani *et al.*[44] Further, studies as that of Maurya *et al.,*[40] Zakiullah *et al.*[41] and Dong *et al.*[42] consists of larger sample size which are adequately powered studies which can influence[45] the meta-analysis results. Yet, another study, as that of Lopes *et al.*,[15] indicates the association of GSTM1 null polymorphisms with increased risk of OC but the meta-analytic study of Lopes *et al.*[15] has left out initial studies as that of Deakin *et al.*[46] Hung *et al.,*[47] Park *et al.,*[48] Matthias *et al.,*[49] Jourenkova-Mironova *et al.,*[50] Katoh *et al.,*[51] Tanimoto *et al.,*[52] Sato *et al.,*[11] Park *et al.,*[20] Nomura *et al.,*[53] Sreelekha *et al.,*[54] Kietthubthew

et al.,[55] Hahn *et al.,*[56] Buch *et al.*[57] Gronau *et al.,*[58] Sikdar *et al.*,[12] Xie *et al.*[13] Drumond *et al.,*[59] Majumder *et al.*[60] and Liu *et al.*[61] towards the association of GSTM1 null genotype with OC.

In addition to this, null genotype of GSTT1 has been suggested to increase the risk of numerous cancers such as lung cancer,[62] colorectal cancer,[63] gastric cancer,[64] leukemia,[32] head-and-neck cancer[30] and OC.[65,66] In contrast, few studies did not find an association of GSTT1 null genotypes with a greater risk of OC.[67-70] Very recent meta-analyses, as that of Lopes *et al.,*[15] have suggested that GSTT1 null polymorphisms may increase the risk of development of OC. Meta-analysis of Lopes *et al.*[15] has left out studies as that of M Deakin *et al.,*[45] Hung *et al.,*[46] Jourenkova-Mironova *et al.,*[50] Katoh *et al.,*[51] Sreelekha *et al.,*[54] Kietthubthew *et al.,*[55] Buch *et al.,*[57] Gronau *et al.,*[58] Sikdar *et al.,*[12] Xie *et al.,*[13] Drumond *et al.,*[66] Majumder *et al.*[60] and Liu *et al.*[61] Out of these nonincluded studies, three studies are of adequately powered studies, two studies as that of Buch *et al.*[57] and Majumder *et al.*[60] negate the association of GSTT1 null genotype with OC and one study as that of Sikdar *et al.*[12] only relates GSTT1 polymorphism among heavy chewers with OCs. Thus, these studies may influence the meta-analysis results. These ambiguous views created the necessity of meta-analysis to extract an estimate of risk associated with GSTM1 and GSTT1 null status with susceptibility to OC.
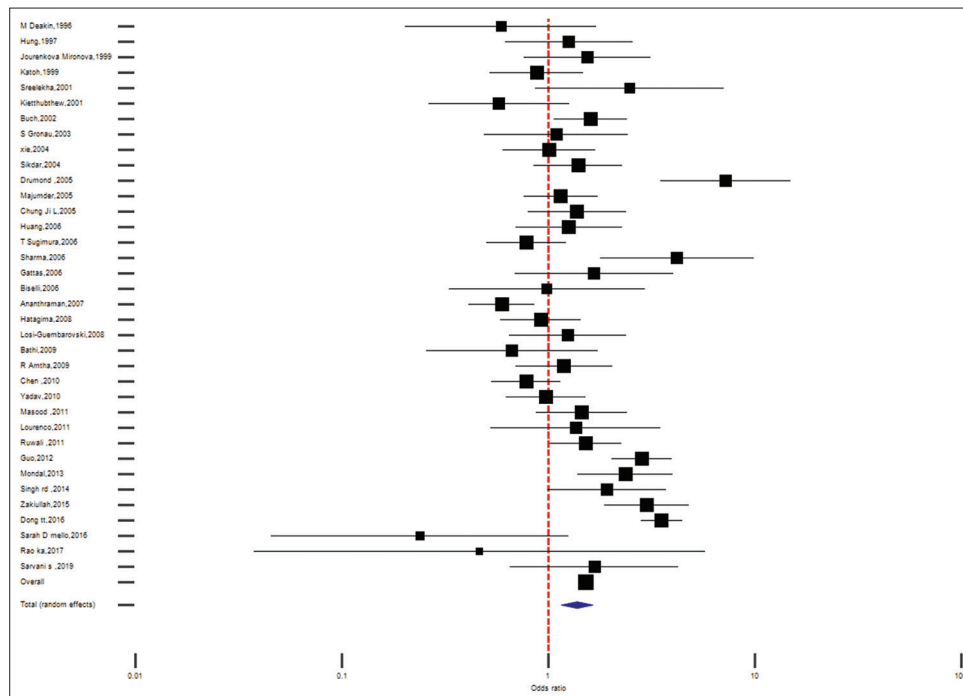
Figure 2: Forest Plot of Odds Ratio of GSTT1 Deficiency and Risk of Developing Oral Cancer

The presented meta-analysis from 50 published studies (The data Pertaining Park,[20] divided into two studies) suggests that null genotype of GSTM1 and null genotype of GSTT1 are significantly ($P = 0.0001$) associated with a modestly increased risk of OC, which may be attributed to expression of GST enzymes in the squamous mucosa of the head and neck[71-73] and secondly to activation of benzo[a] pyrene-7,8-dihydrodiol-9,10-oxide (benzo[a] pyrene) which later transforms into 7,8-diol-9,10-epoxide in tobacco-associated OC patients.[74,75] This 7,8-diol-9,10-epoxide is an identifiable substrate for the GSTM1 enzyme. GSTM1 null genotype individuals with adverse habits of tobacco accumulate more DNA adducts through their inefficiency at excreting activated carcinogens such as 7,8-diol-9,10-epoxide.[74,75] Further, if these DNA adducts, starts accumulating at locus of oncogenes or tumor suppressor genes, leads to somatic mutation and disruption of the cell cycle,which may lead to carcinogenesis.[76] Further, the ORs of null GSTM1 and null GSTT1 varied with geographic location. The prevalence of these genotypes in controls varied widely among and within regions. The data pertaining to GSTT1 null genotype meta-analysis from 36 studies showed that GSTT1 deficiency was associated with OC. This may be attributed to multifactorial role of the GSTT1, both activation and detoxification process, expression of GSTT1 in red blood cells resulting in more generalized detoxification process[9] and expression of GSTT1 in the squamous mucosa of the head and neck.[71-73] In the present

meta-analysis, GSTT1 null genotype was a significant risk factor for OC, in line with meta-analysis of esophageal cancers,[37] prostate cancer,[38] breast cancer[77] and OC.[15]

Within ethnic groups, categorization appears to be subjective and varied. There exists no unanimity on defining an "ethnic group." In consequence, ethnic groups, regardless how it is defined, will tend to evolve around social and political attitudes or developments.[78] Hence, basing ethnic identification upon an objective and rigid classification is nonreasonable. Down to the ground, in the meta-analysis, most of the studies relied on geographical location for ethnicity and failed to define a criterion for ethnicity-based recruitment of OC subjects and controls for the study. In the present meta-analysis, the pooled ORs for different ethnic groups was not conducted for two reasons: first, the stratification of studies based on ethnicity would have been vague and spurious, and second, due to the number of studies in each stratum defining particular ethnic group other than Asians would be very few or absent.

In the meta-analysis, the evidence of heterogeneity was observed across studies. The reasons for this might be methodological diversity, especially with arbitrary recruitment of cancer subjects and use of hospital based controls.[9] Nevertheless, from sensitivity analysis, it was found that studies that contributed toward heterogeneity did not demonstrate any significant alteration in the estimated

overall OR. Since only published studies were utilized in the meta-analysis, the combination of Egger's and Begg's test did not indicate the evidence of publication bias indicating results of the present study to be stable and credible.

CONCLUSION

In conclusion, the findings of this meta-analysis study suggest a significant role of GSTM1and GSTT1 null genotype in increasing the risk for OC; these findings have to be viewed with caution, as it contained substantial heterogeneity across studies. However, the estimated risk is tainted by heterogeneity across the studies and lack of exhaustive study designs. Further, studies with larger sample size exploring GSTM1and GSTT1 null genotypes among various demographic subgroups with optimum study design are needed to precisely conclude on risk of development of OC in individuals with GSTM1 or GSTT1 null genotype and habits of consumption of tobacco.

Conflicts of interest
There are no conflicts of interest.

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