RESEARCH COMMUNICATION

Glutathione S-transferase M1 and T1 Status and the Risk of Laryngeal Cancer: a Meta-analysis

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Abstract

Background: Polymorphic variations in GSTM1 and GSTT1 have been implicated as risk factors for various cancers. A number of studies conducted to assess their association with susceptibility to laryngeal carcinomas have yielded inconsistent and inconclusive results. In the present study, the possible association of laryngeal cancer risk with GSTM1 and GSTT1 null genotypes was explored by a meta analysis. Method: A meta-analysis was carried out on case-control studies collected from the literature. The pooled odds ratio (OR) and presence of publication bias in those studies were evaluated. Results: A total of 20 studies concerning laryngeal cancer were identified. The results showed that the pooled OR was 1.22 (95% CI 1.03-1.43) for the GSTM1 polymorphism while for GSTT1 polymorphism, the pooled OR was 1.23 (95% CI 0.96-1.58). No evidence of publication bias was detected among the included studies. Conclusion: The results suggest that the GSTM1 deficiency significantly increases susceptibility to laryngeal cancer whereas GSTT1 null genotype might not be a risk factor.

Keywords: Laryngeal cancer - GSTM1 - GSTT1 - meta-analysis - polymorphism - risk.

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Introduction

Carcinoma of the larynx is one of the most commonly occurring carcinomas of the upper respiratory tract (Jaskula-Sztul et al., 1998). The incidence and mortality of laryngeal carcinoma worldwide are 1,50,677 and 81,892, in number, respectively (Globocan, 2008). The use of tobacco and alcohol are considered as important environmental etiological factors that increase the risk of developing laryngeal cancer (Gajecka et al., 2005). Despite many individuals exposed to these environmental factors through tobacco and alcohol consumption, laryngeal cancer develops only in a small group of exposed people, suggesting the existence of inter-individual cancer susceptibility (Tripathy and Roy, 2006). It is hypothesised that part of the susceptibility to laryngeal cancer may be determined by the inter-individual difference in the genetic host factors that contribute to carcinogenic mechanisms. Therefore it is logical to suspect that the genetic defect in the enzymes processing tobacco carcinogens may play a role in determining the individual susceptibility to laryngeal cancer.

Various types of chemicals have the potential to induce cancer in a variety of tissues of various hosts, including man. The work of Millers and their colleagues has defined the role of metabolism in carcinogen activation, which indicated commonality between carcinogens in being chemically reactive towards DNA by forming DNA adduct (Miller and Miller, 1966; 1971; Dipple et al., 1968). The formation of such adducts lay down the strategies in molecular epidemiology and bio monitoring (Dipple, 1995).

Most chemical carcinogens undergo metabolic activation in the cells to form reactive intermediates that react with DNA (Hayes et al., 2005). This could be result of natural mechanisms that cells employ to spare themselves of toxins, generally by making them more water soluble. Metabolism of xenobiotic (foreign) compounds is carried out in the cell by broad spectrum oxidative enzymes (Phase I metabolism) that introduce polar groups (e.g. hydroxyl groups) into chemical molecules and render the molecules suitable substrates for conjugation (Phase II metabolism) with one of a variety of hydrophilic groups. The resulting conjugate is substantially more water soluble than the parent compound and thus more readily excreted (Phillips, 2007).

Glutathione S-transferases are enzymes involved in phase II xenobiotics metabolism and also provide protection against oxidative stress (Hayes et al., 2005). The null genotype of GSTM1 (in which glutathione S-transferase μ1 is absent) results in an inability to detoxify tobacco-related carcinogens potentially increasing the risk for smoking-related cancer such as lung and bladder cancer. GSTM1 genotypes are important for laryngeal cancer susceptibility, because the GST enzymes are detected in the squamous mucosa of the head and neck with site specificity with laryngeal mucosa expressing the highest concentration of GST- μ isoform compared with

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other GSTs (Hashibe et al., 2003).

The GSTT1 (glutathione S-transferase θ1) is known to be expressed in adult liver and also in red blood cells (Hayes et al., 2005). Since its expression in red blood cells is considered to play a more prominent role than GST- μ in detoxifying carcinogens, there exist studies which indicate the potential role of GSTT1 related to squamous cell carcinoma of the head and neck region including laryngeal cancers. Though a number of studies have focussed on GSTM1 and GSTT1 genetic variation with respect to laryngeal cancer, they have yielded conflicting results. Whether GSTM1 or GSTT1 polymorphism is a risk factor for laryngeal cancer remains largely uncertain. In the present study, an evidence based quantitative meta-analysis was conducted to address this controversy.

Materials and Methods

Selection of studies

Studies with information on GSTM1 and GSTT1 deficiency and the risk of laryngeal cancer were identified using two electronic databases; Medline (National Library of Medicine, USA) and EMBASE, covering all papers published up to December 2010. The search was conducted using the combination of following search terms 'GSTM1, GSTT1, laryngeal cancers, polymorphisms, head and neck, neoplasm, carcinoma, glutathione'. Additional articles were also manually retrieved via the references cited in these publications and review articles. When several articles were identified for the same population, only the most updated source was referred.

The following criteria were used for the selection of articles for the meta-analysis:1) Articles explicitly describing studies in the association of laryngeal cancer with GSTM1 / GSTT1 polymorphisms; 2) Case-control studies; 3) The laryngeal cancer diagnoses and the sources of cases and controls should be stated and the studies in which individuals were genotyped by PCR technique only; 4) The size of the sample, odds ratios (ORs) and their 95% confidence intervals (CIs) or the information that can help deduce the results should also be stated; 5) Those publications that gave data to allow the calculation of such outcomes were also selected.

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; 3) Reviews and literature that is repeated.

Extraction of data

Data from the selected articles were extracted and entered into SAS 9.2 database. The extraction was performed by 2 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), tumour site, and matching of cases and controls were rigorously tabulated.

Statistical analysis:

The study-specific crude odds ratio of GSTM1 and GSTT1 polymorphisms and laryngeal cancers were recalculated for each study along with their corresponding 95% confidence intervals. Each study was treated as a separate stratum. To take into account the possibility of heterogeneity across the studies, a Chi-square based Q statistic test was performed. If the result of the heterogeneity test was $p > 0.05$ indicating the absence of heterogeneity, ORs were pooled according to fixed – effect model by Inverse Variance method, otherwise, the random effect model by DerSimonian and Laird Method was used. (Cooper HM and Hedges LV, 1994). To identify publication bias, Begg Rank Correlation and Egger Regression test were used (Begg CB and Mazumdar M, 1994 and Egger M et al., 1997).

Results

A total of 34 studies regarding GSTM1or GSTT1with respect to laryngeal cancer were identified. After a careful review, irrelevant 21 papers were excluded based on the inclusion and exclusion criteria. Then, 7 papers were included from the reference lists of the selected papers. A database was established according to the extracted information from each article and has been listed in tables 1 and 2.

Of the 20 studies included in the meta-analysis of GSTM1 and GSTT1, 3 were carried out in Asian countries, 5 in American countries and 12 in Europe. Hospital patients were used as controls in 6 studies whereas general population were controls in 8 studies and 6 did not mention

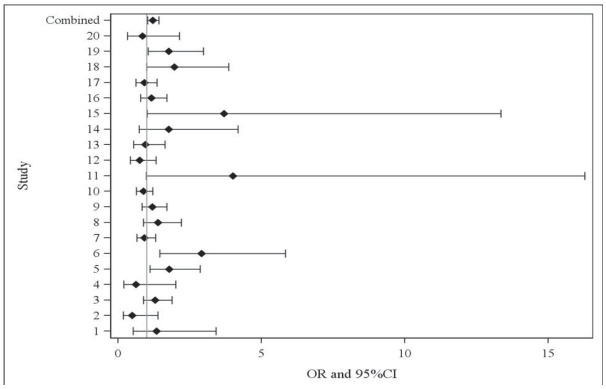

Figure 1. Forest Plot of Odds Ratio of GSTM1 Deficiency and Risk of Developing Laryngeal Cancer

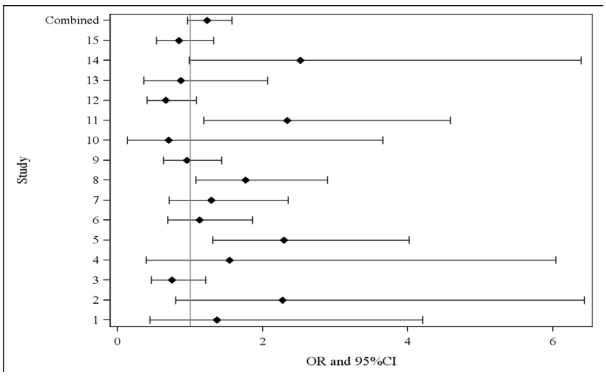

Figure 2. Forest Plot of Odds Ratio of GSTT1 Deficiency and Risk of Developing Laryngeal Cancer

Table 1. Summary of Case-control Studies on GSTM1 Genotype and Laryngeal Cancer

SL. No.*	Author (Year)	Country	Control Source	Matching of Conterols	Cases (n/N)	%GSTM1 Deficiency	Cntrols (n/N)	%GSTM1 Deficiency	OR (95% CI)
8	Jahnke V (1996)	Germany	Not available	None	151/269	56.2	112/216	52	1.19 (0.83-1.70)
15	Coutelle C (1997)	France	Hospital	None	14/18	77.8	18/37	48.6	3.69 (1.02-13.35)
8	Jourenkova N (1998)	France	Hospital	Age, sex and hospital	78/129	60.5	90/172	52.3	1.39 (0.88-2.21)
13	Jaskula-Sztul R (1998)	Poland	Population healthy	None	29/171	49.1	32/180	57.7	0.94 (0.54-1.64)
16	Matthias (1998)	Germany	Hospital	None	151/265	57	95/178	53.4	1.16 (0.79-1.70)
12	Morita S (1999)	Japan	Hospital	None	30/69	43.5	83/164	50.6	0.75 (0.43-1.32)
18	Hong (2000)	Korea	Population healthy	None	56/82	68.3	33/63	52.4	1.96 (0.99-3.86)
11	Hanna (2001)	USA	Hospital	Age, sex and smoking	16/20	80	10/20	50	4.0 (0.98-16.3)
17	To- Figueras (2002)	Spain	Population healthy	None	96/204	47.1	100/203	49.3	0.92 (0.62-1.35)
6	Gronau S (2003)	Germany	Population healthy	Age, sex , alcohol and smoking	39/53	73.6	68/139	48.9	2.91 (1.45-5.83)
7	Risch A (2003)	Germany	Population healthy	Age and sex	127/245	51.8	135/251	53.8	0.93 (0.65-1.32)
20	Bardakci (2003)	Turkey	Not available	None	17/36	47.2	18/35	54.3	0.85 (0.33-2.15)
14	Unal M (2004)	Turkey	Not available	None	19/42	45	15/47	32	1.76 (0.74-4.18)
19	Li (2004)	China	Not available	None	50/89	56.1	69/164	42.1	1.77 (1.05-2.97)
10	Gajecka M (2005)	Poland	Population healthy	None	140/292	48	164/321	51.1	0.88 (0.64-1.21)
1	Gattas (2006)	Brazil	Hospital	None	10/22	45.4	39/102	38.2	1.35 (0.53-3.41)
2	Biselli (2006)	Brazil	Not available	Age, sex, race and alcohol	7/22	31.8	29/60	48.3	0.50 (0.18-1.40)
3	Peters ES (2006)	USA	Population healthy	Age, sex and residence	81/135	60	404/753	53.7	1.30 (0.89-1.88)
4	Goloni-Bertollo (2006)	Brazil	Not available	Age, sex and ethnicity	6/16	37.5	22/45	48.9	0.63 (0.20-2.02)
5	Acar H (2006)	Turkey	Population healthy	Geographic region	57/110	51.8	74/197	37.6	1.79 (1.12-2.87)

*Sl, Nos. corresponding to the numbers in the forest plot

Table 2. Summary of Case-control Studies on GSTT1 Genotype and Laryngeal Cancer

SL. No.*	Author (Year)	Country	Control Source	Matching of Conterols	Cases (n/N)	%GSTM1 Deficiency	Cntrols (n/N)	%GSTM1 Deficiency	OR (95% CI)
8	Jahnke V (1996)	Germany	Not available	None	56/269	20.9	28/216	12.9	1.77 (1.08-2.89)
7	Jourenkova N (1998)	France	Hospital	Age, sex and hospital	25/129	19.4	27/172	15.7	1.29 (0.71-2.35)
13	Jaskula-Sztul R (1998)	Poland	Population-healthy	None	10/171	17.5	12/180	21.7	0.87 (0.37-2.07)
15	Matthias (1998)	Germany	Hospital	None	51/263	19.4	45/203	22.2	0.85 (0.54-1.33)
11	Hong (2000)	Korea	Population healthy	None	47/82	57.3	23/63	36.5	2.34 (1.19-4.58)
10	Hanna E (2001)	USA	Hospital	Age, sex and smoking	3/20	15	4/20	20	0.71 (0.14-3.66)
12	To-Figueras (2002)	Spain	Population healthy	None	35/204	17.2	48/203	23.6	0.67 (0.41-1.09)
6	Risch A (2003)	Germany	Population healthy	Age and sex	38/245	15.5	35/251	13.9	1.13 (0.69-1.86)
14	Unal M (2004)	Turkey	Not available	None	17/42	40	1047	21	2.52 (0.99-6.39)
9	Gajecka M (2005)	Poland	Population healthy	None	54/290	18.6	61/316	19.3	0.96 (0.64-1.44)
1	Gattas (2006)	Brazil	Hospital	None	5/22	22.7	18/102	17.6	1.37 (0.45-4.21)
2	Biselli (2006)	Brazil	Not available	Age, sex, race and alcohol	9/22	40.9	14/60	23.3	2.28 (0.81-6.43)
4	Goloni-Bertollo (2006)	Brazil	Not available	Age, sex and ethnicity	4/16	25	8/45	17.8	1.54 (0.39-6.04)
5	Acar H (2006)	Turkey	Population healthy	Geographic region	33/110	30	31/197	15.7	2.30 (1.31-4.02)
3	Peters ES (2008)	USA	Population healthy	Age, sex and residence	23/135	17	162/753	21.5	0.75 (0.46-1.21)

*Sl, Nos. corresponding to the numbers in the forest plot

the source of controls. In 7 studies, the controls were age and sex-matched with cases and in 1 study, controls were matched with cases according to the geographical location. In the rest 12 studies, matching was not mentioned.

Population frequencies

For GSTM1 polymorphism, the data obtained from the 20 studies included in the meta-analysis included 2289 cases and 3347 controls. The frequencies of GSTM1 deficiencies ranged from 31.8% to 80% among the cases and 32% to 57.7% among the controls. For the study by Peters ES the required data was obtained backwards from the OR given (Peters, 2006).

For GSTT1 polymorphism, total study subjects were 4848, of which 2020 were cases and 2828 were controls. The number of cases in the studies included in the meta-analysis for GSTT1 deletion varied from 20 to 269 patients. The frequency of the GSTT1 null in the control

group ranged from 12.9% to 36.5%, with considerable variation depending on where the study was carried out.

Meta-analysis:

The analysis of heterogeneity for all the 20 studies gave the Chi square value of 46.7216 with 19 degree of freedom (df) and p = 0.0004 using the random effect model for the GSTM1 polymorphism. The overall OR for GSTM1 was 1.22 (95% CI 1.03 – 1.43). Hence, it is likely that GSTM1 null status significantly increases the susceptibility to laryngeal cancer.

Likewise, for the association between GSTT1 null genotype and laryngeal cancer risk, the Chi square value for the heterogeneity of the 15 studies was 51.8618 with 14 df and p = 0.00 in random effect model. The overall meta-OR was 1.23 (95% CI 0.96 – 1.58). The data implied that GSTT1 null genotype has no significant association to laryngeal cancer risk.

For the diagnosis of publication bias, both Egger's test and Begg's test, when applied, showed no evidence of publication bias ($p < 0.05$) and hence none of the studies were excluded.

Discussion

A large number of researchers have elucidated the possible association between GSTM1 and GSTT1 deficiency and the risk of laryngeal cancer. However, many studies have produced inconsistent conclusions. These potential misleading views prompted our meta-analysis, which to our best of knowledge is the first such study carried out to derive an estimate of risk associated with GSTM1 and GSTT1 null status with susceptibility to laryngeal cancer.

The data from 21 studies was used in the meta-analysis with over 2289 cases and 3347 controls. Based upon these data, the results of the analysis suggest that GSTM1 is significantly associated with a modest increased risk of laryngeal cancer whereas GSTT1, though positively associated is not significantly associated. It was also observed that 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.

Epidemiological studies have provided evidence that individual susceptibility to cancer is mediated by both genetic and environmental factors (Varela-Lema et al., 2008). Given that the exposure to carcinogens is a major risk toward cancer development, the hypothesis that modulation of carcinogen metabolism either by activation or detoxification is under genetic control is a plausible mechanism for explaining inter individual susceptibility. There exist three major families of proteins exhibiting glutathione transferase activity: cytosolic GST, mitochondrial GST and microsomal GST (Ladner JE et al., 2004) The cytosolic GSTs constitute a large class of GST and are extensively studied as they demonstrate polymorphisms in humans and may be prime cause inter-individual variations in responses to xenobiotics (Hayes et al., 2005). Null mutations of GSTM1 and GSTT1, the phase II enzymes have shown to abolish enzyme activities resulting in increased susceptibilities to environmental toxins and carcinogens and therefore have been linked with an increase in risk of cancer.

Previous meta analyses suggest that GSTM1 deficiency might modestly increase the risk of head and neck cancer (Hashibe et al., 2003; Tripathy and Roy, 2006; Ye and Song, 2005) and oral cancer (Zhuo et al., 2009). However, a number of meta-analyses indicated no marked association of GSTM1 null mutations with hepatocellular cancer (White et al., 2008), gastric cancers (La Torre et al., 2005), brain tumours (Lai et al., 2005), esopharyngeal cancer (Yang et al., 2005) and prostate cancer (Ntais et al., 2005). The data from present meta-analysis showed that GSTM1 deficiency was associated with laryngeal cancer. The plausible explanation can be that the metabolic action of GST enzymes including GSTM1 may differ from cancer site, the highest concentration of GSTM1 has been observed in laryngeal tissues relative to the other GSTs (Geisler and Olshan, 2001). However, another meta-analysis conducted in 2009 (Zhuo et al., 2009) failed to demonstrate a significant association of laryngeal cancer risk with GSTM1. This may be attributed to the non-inclusion of 6 studies: Jaskula-Sztul (1998), Hanna (2001), Gattas (2006), Biselli (2006), Peters (2006), Goloni-Bertollo (2006) in the previous meta-analysis which might have contributed significantly in the present study.

Likewise, null genotype of GSTT1 has been suggested to associate with risks of number of cancers. Marked association of GSTT1 deletion with lung cancer (Hosgood et al., 2007), colorectal cancer (Chen et al., 2005), brain tumour (Lai et al., 2005), gastric cancer (Saadat M et al., 2006), leukaemia (Ye and Song, 2005) and head and neck cancer (Hashibe M et al., 2003) has been demonstrated. However, in the present meta-analysis, GSTT1 deficiency was not a significant risk factor for laryngeal cancer, in line with meta-analysis of oesophageal cancers (Yang et al., 2005), prostate cancer (Ntais et al., 2005) and breast cancer (Vogl et al., 2004). Notably, these results should be interpreted with caution as the number of studies included was limited and the sample sizes in 6 out of 15 included studies were rather small.

Upon review, the design of some studies in evaluating GSTM1 and GSTT1 deficiency as risk for laryngeal cancer were unsatisfactory. To identify a relationship between the genotype and cancer risk, it is critical to examine large samples in the design of the case-control studies. As is evident in this study, about 50% of studies had samples less than 100 thus exaggerating the effect of null genotypes. Some of the case-control studies included used hospital based controls for comparison with cancer cases. Similarly, cases and controls were not matched in half of the studies. These findings suggest caution in the interpretation of such studies.

In the meta-analysis, the evidence of heterogeneity is observed across studies. The reasons for this might be uncontrolled, confounding and bias inherent in the study design, non-systematic and arbitrary acquisition of cancer samples and use of hospital based controls. Although evidence of heterogeneity exists, it was found through sensitivity analysis that studies that contribute to the heterogeneity do not significantly alter the estimate

of overall odds ratio.

Although only published studies were used 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.

It is a proven fact that variation in the geographic and ethnic factors between case and control individuals among studies may result in bias, which might confound the results of the meta-analysis. In the present meta-analysis, the pooled ORs for different ethnic groups was not conducted for 2 reasons, first, the stratification of studies based of ethnicity would have been ambiguous and spurious and second, if at all stratification was done, the number of studies in each stratum would be very few. Further, most of the included studies were on Caucasians and a few on Asians. Hence, studies concerning populations such as African and American are required.

As the case with most of the cancers, laryngeal cancer is also associated with environmental factors such as tobacco use and alcohol. Hence, it is important to evaluate the gene- environment interactions and also gene – gene interactions.

In conclusion, the findings of this meta-analysis study suggest a significant role of GSTM1 null genotype in increasing the risk for laryngeal cancer, whereas the relationship between GSTT1 deficiency and laryngeal cancer risk was not significant. However, the estimated risk is tarnished by small number of studies, presence of heterogeneity among the studies and less than optimal study designs. Further studies with larger sample size and scope for evaluating gene-environment and gene-gene interactions among various demographic subgroups are needed.

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