

RESEARCH ARTICLE

Meta-analysis of GSTM1 and GSTT1 Polymorphisms and Risk of Nasopharyngeal Cancer

Archana Krishna Murthy^{1*}, Vinod Kumar², KP Suresh³

Abstract

Background: Studies of associations between genetic polymorphism of glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) with risk of nasopharyngeal cancer (NPC) have generated conflicting results. Thus, a meta-analysis was performed to clarify the effects of GSTM1 and GSTT1 polymorphisms on the risk of developing NPC. **Materials and Methods:** A literature search in two electronic databases namely PubMed and EMBASE up to December 2012 was conducted and eligible papers were finally selected based on the inclusion and exclusion criteria. The pooled odds ratio (OR) and presence of heterogeneity and publication bias in those studies were evaluated. **Results:** A total of 9 studies concerning nasopharyngeal cancer were evaluated. Analyses of all relevant studies showed increased NPC risk to be significantly associated with the null genotypes of GSTM1 (OR=1.43, 95% CI 1.24-1.66) and GSTT1 (OR=1.28, 95% CI=1.09-1.51). In addition, evidence of publication bias was detected among the studies on GSTM1 polymorphism. **Conclusions:** This meta-analysis demonstrated the GSTM1 and GSTT1 null genotypes are associated with an increased risk of NPC.

Keywords: Nasopharyngeal cancer - GSTM1 - GSTT1 - meta-analysis - polymorphism - risk

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Introduction

Nasopharyngeal carcinoma (NPC) is considered as one of the rarer cancer forms globally, the incidence of NPC has been quoted as 84,000 cases diagnosed annually, with an age standardized rate (ASR) of 1.2 per 100,000 for both sexes (Ferlay et al., 2008). NPC represents 24th most frequently diagnosed cancer form globally and 22nd within the developing countries (Jemal et al., 2011). However, the distribution of this cancer is highly skewed, the highest incidence rates seen in China and Southeast Asian region (Cheng et al., 2003). The NPC is most prevalent in Chinese and Malaya population and is a leading cause of death among Cantonese in Southern China (Guo et al., 2003).

The aetiology of NPC is majorly attributed to three risk factors namely infection with Epstein-Barr virus (EBV), genetic predisposition, and environmental pollutants like cigarette smoking, formaldehyde vapours, occupational exposure to products of combustion, and cotton dust (ICMR bulletin, 2003). Besides, Chinese foods such as salt-cured fish and smoke-dried meat which while cooking aerosolize carcinogenic nitrosamines that are subsequently inhaled may also pose a risk of developing NPC (Guo et al., 2003). Despite many individuals being exposed to these risk factors, only a minority of them develop NPC. This evidence suggests that the inter-individual differences in susceptibility can be attributed to the individual's

effectiveness in the detoxification of these chemicals which in turn is ascribed to genetic differences.

Glutathione S-transferases constitute a super-family of ubiquitous, multifunctional enzymes, which play a key role in cellular detoxification (Ye et al., 2004). GSTM1 and GSTT1 are known to be highly polymorphic. This genetic variation may change an individual's susceptibility to carcinogens and toxins. Homozygous deletions of these genes, referred to as GSTM1 null and GSTT1 null, respectively, result in lack of enzyme activity and therefore have been associated with increased risk for a number of cancers including NPC. Though a number of studies have focussed on GSTM1 and GSTT1 genetic variation with respect to NPC, they have yielded contradictory results. Hence, an evidence based quantitative meta-analysis was conducted to address this controversy. In addition, the risks of developing NPC in relation to GSTM1 and GSTT1 null genotypes were also analysed.

Materials and Methods

Selection of studies

Studies with information on GSTM1 and GSTT1 deficiency and the risk of nasopharyngeal cancer were identified by bibliographic search in two electronic databases; MEDLINE and EMBASE, covering all papers published up to December 2012. The search strategy

¹Department of Public Health Dentistry, MR Ambedkar Dental College, Bangalore, ²Department of Oral and Maxillofacial Pathology, Geetam Dental College, Vishakapatam ³Animal Disease Monitoring and Surveillance, Bangalore, India *For correspondence: archanakm20@gmail.com

used was conducted using the combination of following search terms ‘GSTM1, GSTT1, nasopharyngeal cancers, polymorphisms, head and neck, neoplasm, carcinoma, glutathione’. A manual review of the references cited in the selected articles was conducted to retrieve additional 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 nasopharyngeal cancer with GSTM1 / GSTT1 polymorphisms; 2) Case-control studies; 3) The nasopharyngeal 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 studies where patients were overlapped.

Extraction of data

Data from the selected articles were extracted and entered into STATA, version 10.1 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 null polymorphisms and nasopharyngeal cancers were recalculated for each study along with their corresponding 95% confidence intervals. 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 Mantel-Haenszel method, otherwise, the random effect model by DerSimonian and Laird Method was used (Cooper and Hedges, 1994). To identify publication bias, Egger Regression test was used (Egger et al., 1997).

Results

A total of 14 studies regarding GSTM1 and GSTT1 were identified. Based on the inclusion and exclusion criteria, 5 studies were excluded and finally 9 studies pertaining to GSTM1 and 5 studies regarding GSTT1 were selected. A database was established according to the extracted information from each article and has been listed in Tables 1 and 2.

Of the included 9 studies, 7 were carried out in Asian countries, 1 in America and 1 in Europe. General population was used as source of controls in 3 studies whereas hospital patients were controls in 2 studies and 4 did not mention the source of controls. In 3 studies, the controls were age and sex-matched with cases and in 2 studies, controls were matched with cases according to the geographical location. In the other 4 studies, matching was not mentioned.

Population frequencies

For GSTM1 polymorphism, the data from the 9 included case-control studies showed 1294 cases and 1967 controls, of which 747 cases and 956 controls had the null genotype. The frequencies of GSTM1 deficiencies ranged from 51.1-64.1% among the cases and 33-55.6% among the controls.

For GSTT1 polymorphism, total study subjects were

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

SL. No.	Author (Year)	Country	Control Source	Matching of controls	Cases (n/N)	%GSTM1 deficiency	Controls (n/N)	%GSTM1 deficiency	OR (95%CI)
1	Nazar-Stewart (1999)	USA	Population healthy	Geographical area, age and sex	45/83	54.2	63/142	44.4	1.48 (0.86, 2.56)
2	Da (2002)	China	Not available	None	48/80	60	36/80	45	1.83 (0.98, 3.43)
3	Cheng (2003)	Taiwan	Population healthy	Age, sex and residence	173/314	55.1	169/337	50.1	1.22 (0.90, 1.66)
4	Deng (2004)	China	Not available	None	56/91	61.5	64/135	47.4	1.77 (1.03, 3.05)
5	Liao (2005)	China	Not available	None	50/80	62.5	32/72	44.4	2.08 (1.09, 3.99)
6	Tiwawech (2005)	Thailand	Hospital	Age	50/78	64.1	74/145	51	1.71 (0.97, 3.02)
7	Bendjemana (2006)	France	Not available	None	24/45	51.1	33/100	33	2.32 (1.13, 4.76)
8	Guo (2008)	China	Population healthy	Geographic region	204/341	59.8	328/590	55.6	1.19 (0.91, 1.56)
9	Jiang (2011)	China	Hospital	Age and sex	97/182	53.3	157/366	42.9	1.52 (1.06, 2.17)

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

SL. No.	Author (Year)	Country	Control Source	Matching of controls	Cases (n/N)	%GSTT1 deficiency	Controls (n/N)	%GSTT1 deficiency	OR (95%CI)
1	Cheng (2003)	Taiwan	Population healthy	Age, sex and residence	160/316	50.6	174/336	51.8	0.96 (0.7, 1.3)
2	Deng (2004)	China	Not available	None	54/91	59.3	55/135	40.7	2.12 (1.24, 3.65)
3	Bendjemana (2006)	France	Not available	None	9/45	20	16/100	15.5	1.31 (0.53, 3.25)
4	Guo (2008)	China	Population healthy	Geographic region	164/338	48.5	269/585	46	1.11 (0.85, 1.45)
5	Jiang (2011)	China	Hospital	Age and sex	120/182	65.9	180/366	49.2	2.00 (1.38, 2.89)

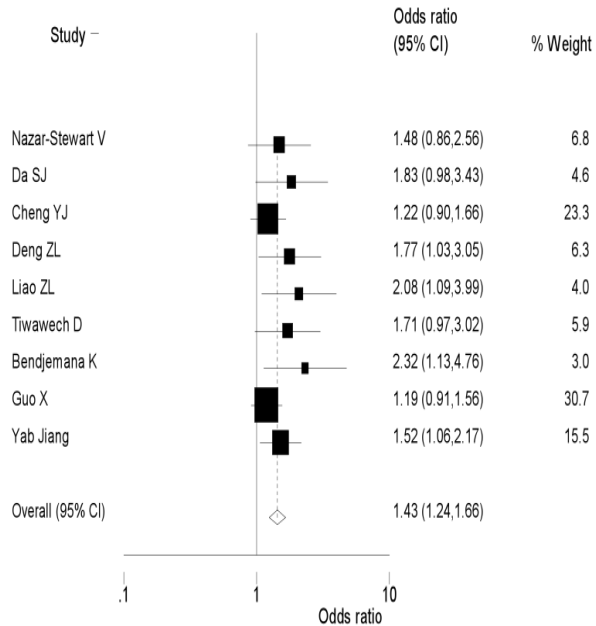


Figure 1. Forest Plot of *GSTM1* Deficiency and Risk of Developing Nasopharyngeal Cancer

972 cases and 1522 controls of 48.9% and 40.5% of cases and controls respectively had null genotype.

Test of heterogeneity

The analysis of heterogeneity for all the 9 studies of *GSTM1* gave the Chi square Q value of 7.57 with 8 degree of freedom (df) and $p=0.477$ indicating lack of heterogeneity and hence the fixed effect model was used. Similarly, the association of *GSTT1* null genotype and NPC risk, the Chi square Q value was 23.6 with 4 df and $p=0.100$ also suggesting the absence of heterogeneity. Therefore the fixed effect model was used for the analysis.

Meta-analysis

The overall OR for *GSTM1* null genotype from the included 9 case-control studies was 1.43 (95%CI 1.24-1.66) and the test for overall effect Z value was 4.95 ($p<0.05$) using the fixed effect model (Figure 1). The results indicate that *GSTM1* null status significantly increases the susceptibility to NPC.

With regard to *GSTT1* null genotype, the overall OR for the 5 studies was 1.28 (95%CI 1.09-1.51) and the Z value was 2.95 ($p<0.05$) using fixed effect model (Figure 2). The data implied that *GSTT1* null genotype also has significant association to NPC.

Publication bias

For the diagnosis of publication bias, the Egger's test, when applied, showed an evidence of publication bias ($p<0.05$) for *GSTM1* polymorphism. However, for *GSTT1* polymorphism, p value of Egger's test was more than 0.05, ($p=0.415$) Thus, the results above suggested that publication bias was not evident in this meta-analysis.

Discussion

In the present meta-analysis, risk of development of

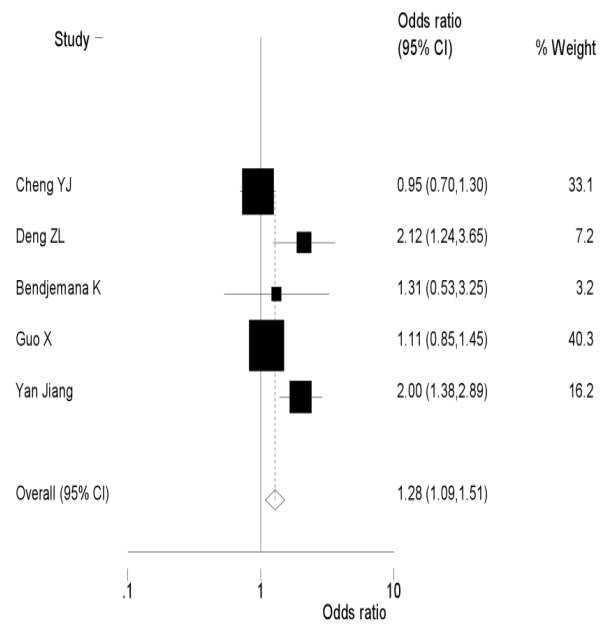


Figure 2. Forest Plot of *GSTT1* Deficiency and Risk of Developing Nasopharyngeal Cancer

nasopharyngeal cancer in individuals with *GSTM1* null and *GSTT1* null genotype were tabulated and analyzed statistically. The outcome of this analysis showed *GSTM1* null status significantly increases the susceptibility to NPC which was also true with the *GSTT1* null status demonstrating significant association with NPC development.

Nasopharyngeal cancer (NPC) is an aggressive tumour with a high potential for nodal and distant metastasis. This tumour is relatively rare in most areas of the world but common in Southeast Asia (Lin et al., 2002). The risk factors include infection with Epstein-Barr virus (EBV), genetic predisposition, and environmental pollutants. However development of such a tumour is still not clear, presently hypothesised to metabolic activation of carcinogenic compounds by Phase I enzymes such as cytochrome P4502E1 (CYP2E1) to yield carcinogens such as an epoxide form of benzo(a)pyrene and aflatoxin that can further interact with host DNA. The epoxide thus formed may be detoxified by phase II enzymes, particularly GSTs, resulting in cancer inhibition. Therefore, NPC susceptibility to carcinogens is dependent on the metabolic balance between phase I and phase II enzymes customized to individual which meant that persons who carried genotypes for high activity of phase I enzymes and low activity of phase II enzymes were at high risk of developing NPC (Hayashi et al., 1991; Kihara et al., 1995). Further, human papilloma virus (HPV16) infection has been found to reduce *GSTM1* enzyme activity and *GSTM1* mRNA levels in human cervical keratinocytes in culture (Chen and Nirunskisiri, 1999). Hence, the present meta-analysis found that individuals with *GSTM1* null and *GSTT1* null genotype showed significant increase in the susceptibility to NPC with pooled OR being 1.43 and 1.28, respectively.

Over the past decades, a large number of meta-analyses have been done to investigate the association between *GSTM1* and *GSTT1* polymorphisms and various

cancers. These include meta-analyses that suggest GSTM1 deficiency increases the risk of head and neck cancer (Hashibe et al., 2003; Ye et al., 2004; Tripathy and Roy, 2006), cervical cancers (Gao et al., 2011; Liu and Xu, 2012) 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), brain tumours (Sima et al., 2012), colorectal cancers (Zhao et al., 2012), ovarian cancers (Economopoulos et al., 2010), melanoma (Nie et al., 2011). In this study, the results indicate that null GSTM1 genotype might increase susceptibility to NPC which was in accordance with the evidence-based meta-analysis pertaining to GSTM1 and GSTT1 polymorphisms on nasopharyngeal cancer by Zhuo et al. (2009). 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), gastric cancer (Saadat, 2006), leukaemia (Ye and Song, 2005), cervical cancers (Gao et al., 2011), breast cancer (Chen et al., 2011), bladder cancer (Gong et al., 2012) and head and neck cancer (Hashibe et al., 2003) has been demonstrated. In this study, the results indicate that null GSTT1 genotype might increase susceptibility to NPC which was not in line with evidence-based meta-analysis of Zhuo et al. (2009) pertaining to GSTM1 and GSTT1 polymorphisms on nasopharyngeal cancer as the latter indicated no association.

In the present meta-analysis, no evidence of heterogeneity is observed across studies. However, there existed publication bias which was evident in Egger's test in relation to GSTM1 null genotype. In theory, publication bias could affect the results of the pooled analysis (Vogl et al., 2004). Publication bias can occur if studies with insignificant associations or null results are less likely to get published than studies with significant results, and subsequently the former will not be included in the pooled analysis. This would lead to biased results and hence the results must be interpreted with caution due to the fairly low power when applied to a few meta-analytically investigated studies. On the other hand, it is a known fact that published studies are generally of greater quality than the unpublished ones (Boccia et al., 2006). Hence, if high-quality scored studies are more likely to yield valid information than low-quality studies, we can conclude that, on the basis of the currently available data, an additional slight risk of nasopharyngeal cancer for GSTM1 null individuals may exist.

Even though pooling and analyzing individual data from original studies has several advantages; the results of the present meta-analysis should be interpreted in light of a few limitations. Firstly, the study was a meta-analysis of case control studies, some of which were hospital-based; thus, selection bias might exist. Secondly, the present study was based on published articles only; therefore, publication bias exists, as suggested from the Egger's test in relation to GSTM1 null genotype and nasopharyngeal cancer.

In conclusion, the meta-analysis suggests that GSTM1 null and GSTT1 null genotype may be associated with NPC susceptibility and they may be a potential risk

factor for NPC. However, future studies with larger study populations and more rigorous designs are needed to investigate the gene effects and the potential effect of environmental factors on nasopharyngeal cancer.

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