RESEARCH COMMUNICATION

GSTM1, GSTT1 and CYP1A1 Polymorphisms and Risk of Oral Cancer: a Case-control Study in Jakarta, Indonesia

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Abstract

Purpose: to investigate genetic polymorphisms in GSTM1, GSTT1 and CYP1A1 and the association with the risk of oral cancer in the Jakarta population. Method: A total of 81 cases and 162 controls matched for age and sex were selected from 5 hospitals in Jakarta. Sociodemographic data using questionnaires were obtained and peripheral blood samples were collected with informed consent for PCR-RFLP assay. Conditional logistic regression analysis was performed to obtain the association between the risk of oral cancer and GSTM1, GSTT1 and CYP1A1 polymorphisms. Results: GSTM1 and GSTT1 null were slightly overrepresented among cases (60.5% and 45.7% respectively) compared to controls (55.6% and 41.4% respectively), but no statistically significant differences were observed. In contrast, the distribution of CYP1A1 polymorphism was higher among controls compared to cases (52.5 % versus 42.4 %). The odds ratio of null GSTM1 and GSTT1 genotypes was slightly higher compared to wild type genotypes (OR 1.19, 95% CI 0.70-2.02 and OR 1.19, 95% CI 0.72-2.05 respectively). Furthermore, the presence of CYP1A1 polymorphism did not increase the risk of oral cancer (OR 0.70, 95% 0.39-1.25). Conclusion: Genetic polymorphisms of GSTM1, GSTT1 and CYP1A1 may not be risk factors for oral cancer in the Jakarta population.

Key Words: Oral cancer risk - metabolizing enzyme polymorphisms - Jakarta, Indonesia

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Introduction

Oral cancer is one of the sixth most common cancers worldwide (Stewart et al., 2003a). Tobacco exposure and alcohol drinking have been established as major risk factors of oral cancer in most of countries in the world (Stewart et al., 2003b). In addition, betel quid chewing is also associated with the disease in some countries, such as India and Taiwan (Znaor et al., 2003; Jacob et al., 2004; Yang et al., 2005). Despite the risk of tobacco exposure, alcohol drinking and quid chewing, the majority of the patients who smoke or chew tobacco do not get cancer suggesting that factors that influence carcinogen-exposed individuals in developing malignancies may thus involve a combination of exposure and genetic polymorphisms in genes that may modulate their capacity in metabolizing the carcinogens mentioned above (Rebbeck, 1997; Nair et al., 1999; Sreelekha et. al., 2001).

Most carcinogens are not biologically active when they enter the body. They need to be converted into biologically active forms before they can interact with host DNA to cause mutations. Numerous carcinogens present in tobacco are metabolized by various enzymes, namely, metabolic activation by phase I (Cythocrome P450/CYP) and detoxification by phase II enzymes (glutathione S-transferase/GSTs) (Lazarus et al., 2000). Phase I (CYP) monooxygenases metabolism involves an initial oxidation of most endogenous chemicals (e.g. hormones and fatty acids) and exogenous chemicals (e.g. polyaromatic hydrocarbons/PAHs, aromatic amines and mycotoxins).

Two polymorphic sites, a T to C transition, 1194 bp downstream of exon 7, generating a new MspI cleavage site and the closely linked exon 7, A to G transition (isoleucine-valine, ile:val) polymorphism, are associated with an increase in CYP1A1 enzymatic activity towards benzo[a]pyrene (Park et al., 1997) and higher inducibility or enchanced catalytic activity of the valine-type CYP1A1 enzyme (Hayashi et al., 1991). This metabolic activation step is followed by phase II metabolism, which frequently involves detoxifying carcinogenic metabolites which is catalyzed by gluthathione S-transferases (GSTs). This enzyme also plays an important role in determining an individual's ability to metabolize various carcinogens particularly benzo[a]pyrene. In humans, based on their primary structures, GSTs are divided into seven families/ classes: α (alpha), μ (mu), π (pi), τ (tetha), ϕ (sigma), ω

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(omega), and ζ (zeta) (Mannervik et al., 1985; Mannervik et al., 1992; Meyer et al, 1991). Deletion of these genes results in a lack of enzyme activity and a reduction in the elimination of carcinogenic substances. Two of the enzymes, GSTM1 and GSTT1 have been extensively studied in relation to several known carcinogens (Nasca, 2001).

GSTM1 and GSTT1 can both detoxify carcinogenic polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (Pemble et al., 1994) and the absence of the GSTM1 and GSTT1 genes has been reported to increase the risk of several common cancers, particularly those caused by cigarettes smoking including cancers of the mouth, lung, bladder and breast (Rebbeck, 1997; Hayes et al., 1995). Although GSTM and GSTT are considered as low-penetrance genes they may still contribute significantly to the number of cancer cases in the general population because of their high prevalence (Zheng, 2001).

The loss of GSTM1 enzymatic activity due to the homozygous null genotype has been reported to occur in about 50% of Caucasians (such as white populations of Europe and north America) and Asians (Rebbeck, 1997; Hayes et al., 1995; Zheng et al, 1993). Previous studies on the relationship between this polymorphism and oral cancer showed varying results. Some studies showed that polymorphisms in CYP1A1 and GSTM1 may increase the risk of oral cancer (Wogan et al., 2004; Tanimoto et al., 1999). By contrast, other studies showed no significant association between the null genotypes of these genes with the risk of oral cancer (Sharma et al., 2006; Hung et al., 1997; Sreelekha et al., 2001; Cha et al., 2007; Park et al., 1997; Park et al., 2000; Olshan et al., 2000; Deakin et al., 1996 and Worrall et al., 1998). The geographical and ethnic variations in the distribution of genotype frequencies of both GSTs and CYP alleles maybe partly responsible for the discrepant observations. The relationship between genetic polymorphism of the above genes and oral cancer was not previously investigated in Indonesia. Therefore, the aim of this study was to investigate the genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and its association with oral cancer risk in an Indonesian population.

Materials and Methods

Subjects

A hospital based, case-control study of oral cancer was conducted in 5 hospitals in Jakarta, the capital city of Indonesia in the period between January 2005 to April 2006. Cases were individuals with histologically confirmed oral squamous cell carcinoma (OSCC) who seeked treatment in these selected centers. Eighty one eligible cases aged 23-74 years (mean age 47.4 years ± 12.4) were included. One hundred and sixty two hospital-based control individuals matched for age and sex within 5 years were selected. Controls were selected among non cancer patients who attended the same centers for minor aliments. Two control patients were matched to each case (1:2). Non-Indonesian citizens and those who had cancer, cardiovascular disease and are currently undergoing treatment were excluded from this study. Both cases and controls were interviewed using structured questionnaires developed for Indonesians to collect information on sociodemographic and risk habits (smoking, alcohol and betel quid chewing). A signed informed consent was obtained from all participants and the study was approved by Trisakti University (as the main centre) and University of Malaya, Kuala Lumpur. Five milliliters of intra-venous blood was drawn from each case and control subjects and placed in EDTA tubes. Genomic DNA was extracted from peripheral blood from case and control subjects using QIAamp DNA mini kit (Qiagen, USA) following manufacturer's instructions. Subsequently, DNA concentration was measured using the Nanodrop spectrophotometer.

Genotyping assay for GSTM1 and GSTT1

The homozygous null polymorphisms GSTM1 and GSTT1 were determined using multiplex PCR, slightly modified from Nair et al (1999), for simultaneous amplification of both genes. The co-amplification of an albumin gene fragment served as an internal positive control to indicate a successful amplification reaction. PCR was performed in a 50 μl reaction containing 20 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTPs, GSTM1 primers at 3 μg/ml each, GSTT1 primers at 2 μg/ml each, albumin primers at 1 μg/ml each, and 50–100 ng of genomic DNA. The reaction was preheated at 99.9°C for 2 minutes before Taq DNA-Polymerase (2.5 U; Go Taq, Promega, USA) was added. PCR was performed in the GeneAmp PCR system 9700 (Applied Biosystems, USA). After an initial denaturation at 95°C for 5 minutes, amplification was carried out for 40 cycles at 94°C for 1 minute, 64°C for 1 minute and 72°C for 1 minute, followed by final elongation at 72°C for 7 minutes. The sequences for the primer pairs were as previously reported (Nair et al., 1999). The PCR products were electrophoresed in a 2% agarose gel for analysis.

Genotyping Assay for CYP1A1

Isoleucine/valine polymorphism of CYP1A1 was determined using PCR-RFLP, modified from a method done by Sreelekha and colleagues (2001). PCR was performed in a 50 μL reaction volume containing 20 mM Tris-HCl pH 9.0, 50 mM KCl, 25 mM MgCl2, 200 μM dNTPs, and 100-200 ng of genomic DNA. The reaction was performed at 94°C for two minutes before 0.5 μl of Taq DNA-Polymerase (5U; Promega, USA) was added. PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystem, USA). After an initial denaturation at 94°C for 5 minutes, amplification was carried out for 30 cycles at 94°C for one minute, 68°C for one minute and 72°C for one minute, followed by a final elongation at 72°C for ten minutes. The sequences for the primer pairs were as previously described by Sreelekha and colleagues (2001). Following PCR, the CYP1A1 fragments were digested with Nco1 as previously described (Sreelekha et al., 2001). The digested DNA fragments were electrophoresed on a 4% agarose gel, stained with ethidium bromide and visualized using the Chemimager (Alpha Innotech, USA) to determine the

Table 1. Characteristic of Cases and Controls

	Controls (162)	Cases (81)
Age group		
22-34	24 (14.8)	16 (19.8)
35-49	69 (42.6)	30 (37.0)
>49	69 (42.6)	35 (43.2)
Gender		
Male	100 (61.7)	50 (61.7)
Female	62 (38.3)	31 (38.3)
Marital status		
Married	134 (82.7)	73 (90.1)
Single	15 (9.3)	5 (6.2)
Divorced/Widowed	13 (8.0)	3 (3.1)
Risk Habits		
No habit	34 (42.0)	79 (48.8)
Smoking	30 (37.0)	65 (40.1)
Smoking & alcohol	10 (12.3)	14 (8.6)
Smoking & betel quid	3 (3.7)	0 (0.0)
Smoking, alcohol, betel quid	1 (1.2)	1 (0.6)

genotype.

Statistical analyses

Stratified analysis using Chi square test was used to calculate significance in the frequency distribution of the genes. From this data, the odds ratio was calculated using conditional logistic regression with STATA 8 version to determine the risk of oral cancer.

Results

Forty-eight out of 81 cases of oral cancer had tongue carcinomas (59.3%, ICD-10 C01-C02). This was followed by the gum (19.8%, ICD-10 C03), buccal mucosa (11.1%, ICD-10 C06, C06.1-2, C06.8) and lip (4.9 %, ICD 10-C00). The occurrence of oral cancer at the other sites were less that 5%. Forty six (56.8%) patients were diagnosed with moderately differentiated OSCC. Well differentiated OSCC accounted for 17 cases (21%) with the remaining 18 (22.2%) being poorly differentiated OSCC. The data from this study shows that 58% of cases had risk habits in comparison to 51.9% of controls (see Table 1). The most common habit practiced among the cases was smoking (54.2%), either as a single habit or in combination with alcohol consumption and betel quid chewing. Alcohol drinking and betel quid chewing alone were only practiced in a small number of subjects (1.2% and 2.5%, respectively).

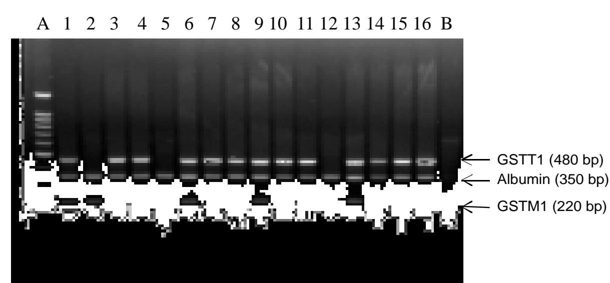

Figure 1. A Representative Image of Multiplex PCR Analysis of GST Polymorphisms. Lane A is molecular weight marker. Lane B is negative control. Lanes 1-16 are samples. Albumin as internal positive controls are shown at 350 bp in all PCR reaction. GSTM1 wild type was shown at 220bp in lanes 1,2,6,9,13 whereas GSTT1 wild type was shown at 480bp in lanes 1,3,4,6,7,8,9,10, 11,13,14,15,16. The GSTM1 deletion (null) was shown in lanes 3,4,5,7,8,10,11,12,14, 15,16 and GSTT1 null is shown in lanes 2,5,12

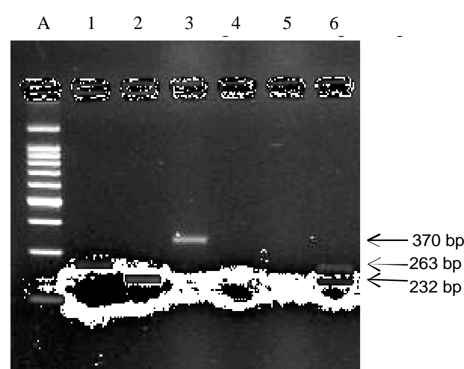

Figure 2. Other samples of RFLP for CYP1A1. Lane A contains DNA molecular weight markers. Lane 3 showed uncut CYP1A1 product (370 bp), lane 1 showed polymorphism bands at 263 bp (valine/valine). Lane 2 single bands at 232 bp for wild type allele of CYP1A1 and lane 6 showed polymorphism, consists of double bands at 263 bp and 232 bp (isoleucine/valine)

Representative muliplex PCR and RFPL findings are illustrated in Figures 1 and 2. The frequency in the distribution of genetic polymorphism of GSTM1, GSTT1, and CYP1A1 among cases and controls is displayed in Table 2. In this study, GSTM1 and GSTT1 null was

Table 2. Crude Odds Ratios (OR) of GSTs and CYP1A1 and Oral Cancer

Genetic polymorphism		Controls (162)	Cases (81)	Crude OR	95% CI	P value
GSTM1	Wild type	72 (44.4)	32 (39.5)	1.00	Reference	-
	Null	90 (55.6)	49 (60.5)	1.19	0.70-2.02	0.527
GSTT1	Wild type	95 (58.6)	44 (54.3)	1.00	Reference	-
	Null	67 (41.4)	37 (45.7)	1.19	0.72-2.05	0.463
CYP1A1	Wild type	77 (47.5)	45 (55.6)	1.00	Reference	-
	Polymorphism	85 (52.5)	36 (44.4)	0.70	0.39-1.25	0.226
Combinations 2 genes						
GSTM/GSTT both wild type		41 (25.3)	17 (21.0)	1.00		
GSTM/GSTT either1 expressed		85 (52.5)	42 (51.9)	1.19	0.59-2.40	0.630
GSTM/GSTT both null		36 (22.2)	22 (27.2)	1.14	0.68-3.00	0.342
Combination 3 genes						
GTSM/GSTT/ CYP1A1 wild type		21 (13.0)	7 (8.6)	1.00		
GSTM/GSTT either null and CYP1A1		65 (40.1)	34 (42.0)	1.74	0.63-4.84	0.287
GSTM/GSTT null and CYP1A1 wild type		51 (31.5)	32 (39.5)	2.06	0.76-5.60	0.158
All 3 gene polymorphisms		25 (15.4)	8 (9.9)	1.05	0.34-3.28	0.934

slightly overrepresented among cases (60.5% and 45.7% respectively) compared to controls (55.6% and 41.4%), but no statistically significant differences were observed between cases and controls. In contrast, the distribution of CYP1A1 polymorphism (Ile/Val and Val/Val) was higher among controls compared to cases (52.5 % versus 42.4 %). The ORs and 95% CI associated with GSTM1, GSTT1 or CYP1A1 polymorphism genotypes and oral cancer were calculated and presented in Table 2. The null GSTM1 and GSTT1 genotypes increased the risk of oral cancer marginally (OR 1.19, 95% CI 0.70-2.02 and OR 1.19, 95% CI 0.72-2.05 respectively). However, polymorphism in CYP1A1 did not increase the risk of oral cancer (OR 0.70, 95% 0.39-1.25). When evaluated in combination, polymorphisms in 2 or more genes appeared to increase the risk of oral cancer; however this was not statistically significant.

Discussion

Molecular epidemiological studies have shown varying evidence that individual susceptibility to cancer is mediated by both genetic (gene polymorphisms) and environmental factors (smoking, alcohol consumption and quid chewing). The inherited differences in the effectiveness of detoxification or activation of carcinogens play a crucial role in host susceptibility. However, polymorphism of GSTM1, GSTT1 and CYP1A1 genes and their effects on oral cancer studied in many countries world wide for the last thirty years have displayed varying results. Meta-analysis data on ethnic differences in the baseline frequencies of GSTM1 null among control subjects have reported variations between 24 to 57.7% in Asians, 46.6 to 53.8% in Europeans and 15.9 to 57.8% in Americans (Ye et al., 2004).

In the present study, we found that GTSM1 was slightly overrepresented in cases as compared to controls (60.5% vs. 55.5%). Among controls, the prevalence of GSTM1 null was within the range that was reported for the Asian, European and American population although it appears to be in the upper range. However, this study found that GSTM1 null genotype did not increase the risk of oral cancer in the Jakarta population (OR 1.19, 95% CI 0.70-2.02) in contrast to results from a pooled analysis presented by Hashibe and colleagues (2003) using data from Asian, European and American countries (OR 1.53, 95% CI 1.19-1.97). The risk of oral cancer remained statistically insignificant after adjusting for the influence of the main risk factor in this study which is smoking. This finding is supported by many other studies in several countries including India (Sharma, 2006; Sreelekha et al., 2001), Taiwan (Hung, et al., 1997), Korea (Cha, 2007), United States of America (Olshan et al., 2000; Park et al., 1997; Park et al., 2000), and in the United Kingdom (Deakin et al., 1996; Worral et al., 1998). In contrast, there are studies that reported an association of genetic polymorphism in GSTM1 with oral cancer development, in their respective populations. Such studies have been reported for the Japanese population where the null GSTM1 genotype increased the risk of oral cancer by 2.2 times (Sato et al., 1999; Katoh et al.,1999; Kihara et al., 1997), whilst among the Thais, GSTM1 null conferred a 2.6 times increase in risk (Kiethewbew et al., 2001). Interestingly, Nair and colleagues also reported a significant higher risk of oral leukoplakias and precancerous lesions amongst individuals with GSTM1 null genotype (Nair et al., 1999).

The prevalence of GSTT1 null genotype amongst controls in this study is within the range reported for Asians (41.4%) by Hashibe and colleagues (2003). However no significant difference was observed between cases and controls ($\chi 2$=0.4118, P = 0.521). Previous studies reported ORs of 1.13 (95% CI 0.97-1.32) in whites, 0.88 (95% CI 0.49-1.57) in African-Americans and 1.19 (95% CI 0.87-1.63) in Asians based on pooled analysis. Again, similar results for pooled ORs was observed in this study suggesting that the genetic polymorphism in GSTT1 does not increase the risk of oral cancer in the Jakarta population (OR 1.19, 95% CI 0.72-2.05). This result is also supported by eight out of fourteen studies in a meta-analysis performed by Geisler and Olshan, (2001) which reported a weak association between GSTT1 null genotype and oral cancer in different ethnic groups (Buch et al., 2002; Deakin et al., 1996; Kietthubthew et al., 2001; Sreelekha et al., 2001) and results from pooled analysis done by Hashibe et al (2003).

In contrast to the numerous studies available for GST, relatively fewer studies examined the role of CYP1A1 polymorphisms in relation to oral cancer development. Most of the research in the CYP gene was done on pharynx, larynx and lung cancers. In this study, even though the prevalence of CYP1A1 polymorphism was found to be slightly higher in controls than cases, the difference was not statistically significant (P = 0.226). The result is consistent with a study done for Caucasians suffering from head and neck cancers (Hahn et al., 2002; Oude Ophius et al., 1998) and a meta-analysis done by Hashibe and colleagues (2003). However, studies in the Japanese and Indian populations have indicated that polymorphisms in CYP1A1 enhanced the risk of oral cancer (Sato et al., 1999; Sreelekha et al., 2001; Morita et al., 1999). Conflicting data on the association between polymorphism of GSTM1, GSTT1 and CYP1A1 and oral cancer may arise from many sources.

As enumerated by Marques and colleagues (2006), the problems can be encountered from differences in the distribution of polymorphic genotype among ethnic and geographical groups worldwide, as well as the methodological issues. Failure to properly sample the cases and controls for hospital based study can lead to bias of the study population if the controls do not reflect the exposure and/or genotype distributions of the source population. Matching is often utilized to increase the efficiency of statistical adjustment of confounding factors. The selection bias and residual confounding may be introduced when matching factors are not accounted for in analysis (Geisler et al., 2001). Besides that, the distinct role of enzymes in different tissues and cancers is another possibility yielding a different feature of genotypes. Thus, the ethnic differences in allelic frequency of GSTM1 and GSTT1 polymorphism and the differences in environment and lifestyle risk factors may also lead to discrepancy.

When considering the latter, the types of tobacco used in cigarettes in Jakarta population (which comprised mainly of kretek) may have significantly different mixtures from other cigarettes found in other countries and thus may have different mechanisms in influencing the interaction with drug metabolizing enzymes in the body particularly when study groups were from different ethnic populations who were exposed to different carcinogens (Sharma et al., 2006). In conclusion, the results of our study suggest that genetic polymorphisms of GSTM1, GSTT1 and CYP1A1 does not increase the risk of oral cancer in the Jakarta population.

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