Association of GSTT1 and GSTM1 Null Polymorphisms with Premalignant Laryngeal Lesions in South Indian Population

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Abstract- The objective of this study is to identify association of GSTM1 and GSTT1 null gene polymorphisms with risk of cancer in defined populations in South India with histologically confirmed pre-malignant laryngeal lesions. Different parameters were taken into consideration and a demographic risk profile was defined as reported from a hospital-based cancer registry in Hyderabad, Andhra Pradesh, India. 23 histologically confirmed pre-malignant laryngeal cancer tissue and blood samples were collected and analyzed for GSTM1 and GSTT1 null polymorphisms by PCR method. GSTT1 null polymorphism was found to be more associated with the risk of malignancy than GSTM1 null polymorphism in the study population.

Index Terms- GSTM1, GSTT1, pre-malignant laryngeal lesions, South India.

I. INTRODUCTION

Cancer is an uncontrolled proliferation of cells that can in principle arise from every tissue. Cancer currently accounts for one-fifth of the total mortality in high-income countries and is the second leading cause of death after cardiovascular diseases. Majority of neoplasms (90%) are head and neck squamous cell carcinomas (HNSCC) that develop in the squamous layer of the mucosal lining, in upper aerodigestive tract, corresponding to 25% of cancer of larynx.[1]

Laryngeal cancer is the second most common respiratory cancer after lung cancer. Larynx cancer is the seventeenth most common cancer in men in the UK, but is uncommon in women. In the larynx, epithelial changes are frequently noted[26]. It is one of the worst recurrence rates for any malignancy, is known to be influenced by several environmental factors. Its incidence is increasing over time in much of the world and this increase is generally accepted to be related to changes in tobacco and alcohol consumption. It is a relatively common cancer in men, but rarer in women. Most laryngeal cancers are squamous cell carcinomas, reflecting their origin from the squamous cells which form the majority of the laryngeal epithelium.

Familial clusterings of laryngeal cancer have been reported but no systematic evaluation of the clinical feature of the disease or an in-depth analysis of familial forms of the disease has been made. Studies revealed a distinct clinical pattern of disease in familial cases of laryngeal cancer, which may provide a valuable basis for the identification of genetic determinants of this malignancy. [2]

The incidence of laryngeal cancer in males around the world is at its highest in Spain accounting to 18.0 %, followed by USA (17.3 %), Australia (9.8 %), India (9.7 %) with China (0.8 %) and Gambia, Western Africa (0.2 %) recording the least cases.The incidence in India is highest in Nagpur, Maharashtra recording to 7.6 % of the laryngeal cancer cases, followed by Pune (6.7 %), Delhi (6.5 %) and Kolkata (5.7 %) with the least in Kerala accounting to 3.3 %. The incidence rate in Andhra Pradesh is 3.73 %. (**CANCER ATLAS INDIA.org, A** Project of the **National Cancer Registry Programme,** *(Indian Council of Medical Research)* Supported by the **World Health Organization**). According to the Department of Biostatistics and Cancer Registry in Kidwai Memorial Institute of Oncology, Bangalore, Karnataka, 4.6 % males, 0.4 % females and a total of 2.3 % represent tobacco related laryngeal cancer in India.

A pre-malignant lesion is a lesion that, while not cancerous, has strong potential for becoming cancerous. It is an apparently benign, morphologically altered tissue which has a greater than normal risk of containing a microscopic focus of cancer at biopsy or of transforming into a malignancy after diagnosis. Precancerous condition can be defined as a disease or patient habit which does not necessarily alter the clinical appearance of local tissue but is known to have a greater than normal risk of pre-cancer or cancer development[3].

Precancerous laryngeal lesions are pathologic conditions of the epithelium, which can undergo malignant degeneration in a higher rate than other epithelial lesions[4]. The diagnosis and management of premalignant lesions of the larynx has not progressed much in the last decade.

A wide spectrum of lesions ranging from dysplasia to in situ carcinoma has to be considered when dealing with laryngeal precancerous conditions. Early vocal cord cancer has a very high cure rate when treated[5]. Laryngeal premalignant lesions range from simple hyperplasia or keratosis to carcinoma in situ (CIS). According to WHO, it is characterized into hyperplasia, keratosis, mild, moderate or severe dysplasia, and CIS. No universally accepted histopathologic classification system. A male predominance is noted in premalignant laryngeal lesions. A positive correlation between age and degree of dysplasia shows that as the degree of dysplasia increases, there is an increase in malignancy risk.

Leukoplakia and erythroplakia are two clinical lesions widely considered to be premalignant[6]. Leukoplakia, exophytic keratosis, and polypoid thickening are early signs which may arouse the suspicion of malignancy[4].

Factors contributing to malignant transformation of laryngeal pre-neoplastic lesions remain largely unknown. Potential etiologic factors may be related to a genetically controlled sensitivity to environmental carcinogens.[7]

Studies show it takes an average of 3.9 years between the first laryngeal biopsy and the diagnosis of invasive carcinoma. About 5-18% of epithelial dysplasias become malignant[8, 9, 10, 11].

With the completion of the first draft of the human genome and the availability of cheaper and quicker technique genotyping technologies, there is a rapidly increasing interest in identifying genes and genetic polymorphisms that predispose people to increased risk of cancer.[12, 13]

Polycyclic aromatic hydrocarbons (PAHs), present in tobacco smoke or metabolites of alcohol, are precursors of chemicals that increase the risk of cancer. 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.

Most PAHs first require activation by Phase I enzymes, such as cytochrome P4501A1 (CYP1A1), to become an ultimate carcinogen. These activated forms may be subjected to detoxification by Phase II enzymes, especially glutathione *S*-transferases (GSTs).[14]

The glutathione *S*-transferases (GSTs) are a family of enzymes known to play an important role in the detoxification of several carcinogens found in tobacco smoke. GSTs are dimeric proteins that catalyze conjugation reactions between glutathione and tobacco smoke substrates, such as aromatic heterocyclic radicals and epoxides. Conjugation facilitates excretion and thus constitutes a detoxification step. In addition to their role in phase II detoxification, GSTs also modulate the induction of other enzymes and proteins important for cellular functions, such as DNA repair[15]. This class of enzymes is therefore important for maintaining cellular genomic integrity and, as a result, may play an important role in cancer susceptibility. In humans, based on their primary structures, GSTs are divided into seven families/classes: α (alpha), μ (mu), π (pi), τ (theta), ϕ (sigma), ω (omega), and ζ (zeta).[16,17,18] Deletion of these genes results in a lack of enzyme activity and a reduction in the elimination of carcinogenic substances.[19] Two loci in particular, GSTM1 and GSTT1, may be of relevance for susceptibility to squamous cell carcinoma of the head and neck (SCCHN).[20]

The role of the GST enzymes in detoxification mechanisms of the carcinogenic compounds has led to the hypothesis that if the individual's genotype at GST locus encodes a deficient GST enzyme it may result in increased risk of cancer.[46] GSTM1 and GSTT1 can both detoxify carcinogenic polycyclic aromatic hydrocarbons, such as benzo[a]pyrene[21] 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[15,22]. 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[23].

The GSTM1 gene located on chromosome 1p13.3[35,36] codes for cytosolic GST μ class enzyme, is known to be highly polymorphic and has a deletion polymorphism that when homozygote (GSTM1 null) results in the complete absence of functional gene product [35,37]. GSTT1 locus has been mapped on chromosome 22q11.2[22].

Some studies showed that polymorphisms in CYP1A1 and GSTM1 may increase the risk of cancer.[24, 25] By contrast, other studies showed no significant association between the null genotypes of these genes with the risk of cancer.[26-34]
The relationship between genetic polymorphism of the above genes and pre-malignant laryngeal lesions was not previously investigated in South India. Therefore, the aim of this study was to investigate the genetic polymorphisms in GSTM1 and GSTT1 and its association with pre-malignant laryngeal cancer risk in a specific South Indian population.

II. MATERIALS AND METHODS

A. Sample collection

23 cases of clinically confirmed premalignant lesions of the larynx were taken for study. The patients had histologically confirmed pre-malignant laryngeal lesions, who reported during the period from 2010-2011 in one hospital-based cancer registry of Govt. ENT Hospital, Hyderabad, Andhra Pradesh, India.

The age range for patients with premalignant laryngeal lesions was 18 - 48 years and 7 - 43 in years in females and in controls, 25-50 years in males and 18-38 years in females (Table 1).

Table 1: Distribution based on age and gender in clinically confirmed cases with pre-malignant laryngeal lesions (n = 23)

Age Group	No. of males (n = 13)	No. of females (n = 10)	**P* value
0 – 10 years	2	3	< 0.001*
11 - 20 years	2	1	
21 – 30 years	6	4	
31 – 40 years	2	-	
41 – 50 years	1	2	

**P* value obtained by Chi-square test

Three retrospective tissue samples and 20 prospective blood samples were obtained from Pathology lab of the Govt. ENT Hospital, Hyderabad, AP, India. The tissue samples were of 2 females (7 years and 21-30 years old) and 1 male (21-30 years old). Fresh blood samples from 23 healthy individuals, who had no risk factor habits, were used as controls.

Physical examination was carried out and information from patients on their age, occupation, nature of job, illness, lifestyle,

habits such as smoking, alcohol consumption and chewing tobacco (Table 2), exposure to chemicals or radiation, family history, surgical history, etc. were recorded using a standard questionnaire. Volunteers recruited for the study were informed about the study and their consent was taken.

Table 2: Distribution of cases based on risk factor habits

Parameters	Patient group (n = 23)	Control (n = 23)	*P value
Smoking	1	0	< 0.001*
Alcohol	0	0	
Chewing Habits	2	0	
Smoking & Alcohol	4	0	
Chewing & Alcohol	1	0	
No habits	15 (65 %)	23 (100 %)	

*P value obtained by Chi-square test

B. DNA extraction

The 3 retrospective formalin-fixed, paraffin embedded tissue samples of 4 µ - 1 mm thickness was deparaffinized using xylene, followed by ethanol wash and tissue lysis using Proteinase-K. The resulting cell lysate was heated at 95°C for 8 minutes to inactivate the Proteinase-K. Standardised phenol-chloroform method was used to extract DNA from the tissue samples.[39-43]

Similarly 5 millilitre blood samples were collected from 20 patients clinically diagnosed with pre-malignant laryngeal lesions and from 23 healthy individuals. They were collected into EDTA tubes and stored at -20°C until DNA extraction was performed using standard protocols. DNA was extracted from anti-coagulated blood (EDTA) samples by Proteinase-K digestion and phenol-chloroform extraction.

C. GSTM1 and GSTT1 polymorphism

The protocol for analysing null genotypes was considered from G. Tirumala Vani et al.,[45] Regina Grazuleviciene et.al.,[46] Rozati et al.,[35] M. Singh et al.[47] and standardised in the working lab.

The primers used for analysing GSTM1 gene: 5'-GAACTCCCTGAAAAGCTAAAGC-3' and 5'-GTTGGGCTCAAATATACGGTGG-3'; GSTT1 gene: 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCATGGCCAGCA-3'; β-globin gene: 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'.

Multiplex-PCR was performed in a 50 µl reaction mixture containing 5 µl of 10X PCR buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl), 1.5 mM MgCl2, 200 µM dNTPs, 25 pM each of GSTM1 primers, GSTT1 primers and β-globin primers, 5 U Taq DNA Polymerase and 50 ng of genomic DNA. The mixture was made upto 50 µl by adding auto-claved double distilled water. PCR was carried out for 35 cycles in a DNA thermal cycler using a thermal profile of initial denaturation at 95°C for 2 mins, denaturation at 95°C for 40s, annealing at 62°C for 40s and primer extension at 72°C for 40s and final extension at 72°C for 8 mins.

D. Analysis of PCR products

After PCR amplification, The PCR products were then separated on a 2% agarose gel at 150 V for 1.5 h and stained with 1 µg ml^{-1} ethidium bromide at 25°C for 10 min. The gel was carefully taken out after electrophoresis and placed in UV gel doc for analysis of the amplified products and gel documentation. Three bands of final PCR products were observed on the gel viz., GSTM1 gene-215 bp, GSTT1-480 bp and β-globin (internal control)-268 bp. The 215 bp band and 480 bp band were not observed in samples with GSTM1 and GSTT1 null genotypes respectively (Illustrative Fig.1).

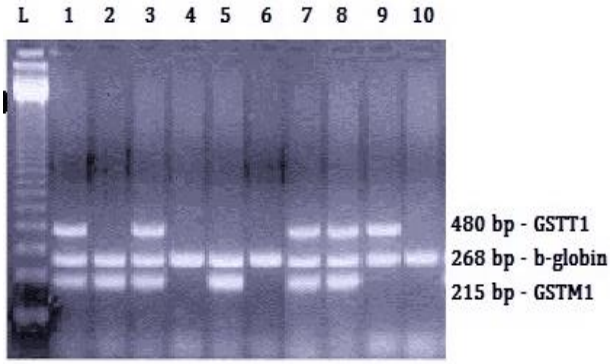

Fig.1 PCR products from the co-amplification of GSTM1 (215 bp), GSTT1 (480 bp) and the internal control, β-globin (268 bp). Lane 9 did not have only 215 bp band, representing GSTM1 null genotype. Lanes 2,5 did not have only 480 bp band, representing GSTT1 null genotype. Lanes 4,6,10 did not have 215 and 480 bands representing both GSTM1/GSTT1 null genotypes. L= 1 Kb ladder. (Illustrative figure)

E. Statistical analysis

The data were analyzed using SPSS version 19 (SPSS Inc., Chicago, IL, USA). Significance (*P*) values and Correlation values were determined by Pearson Chi-square test to assess the association of gender with age groups, habits and type of premalignancy. *P* value of <0.05 was considered to be statistically significant. Any *P* value more than 0.05 was considered non-significant in this particular study population.

III. RESULTS

800 cases of head and neck squamous cell carcinoma was considered for an epidemiological study out of which, a total of 23 cases of clinically confirmed pre-malignant laryngeal lesions were reported during the study period of 2010-2011 in one hospital-based cancer registry of Govt. ENT Hospital, Hyderabad, Andhra Pradesh. Total no. of males diagnosed with

pre-malignant lesions of the larynx was 13 and total no. of females was 10 (Table 1). Maximum patients were in the age group of 21-30 years. 15 out of 23 patients i.e., 65% of patients did not indulge in any kind of risk factor habits (Table 2). 4 of 23 (17%) patients had a combined habit of smoking and alcohol consumption, which was the highest risk factor habit recorded in the study population.

Three different types of pre-malignant laryngeal lesions were observed in the study population viz., Recurrent multiple papilloma of larynx, Juvenile laryngeal papillomatosis and Laryngeal papillomatosis.

11 cases of recurrent multiple papilloma of larynx, 7 cases of laryngeal papillomatosis and 5 cases of juvenile laryngeal papillomatosis. 7 males and 4 females had recurrent multiple papilloma of larynx, 4 males and 3 females had laryngeal papillomatosis, 2 males and 3 females had juvenile laryngeal papillomatosis (Table 3 & 4). *P* value had a significant value of less than 0.001.

Table 3: Distribution of cases based on gender and type of pre-malignant lesions (n = 23)

Pre-malignant laryngeal lesions	Total patients (23) *n* (%)	⊥Males (13) *n* (%)	±Females (10) *n* (%)	*P value
Recurrent multiple papilloma of larynx	11 (47.8)	7 (53.8)	4 (40)	< 0.001*
Juvenile laryngeal papillomatosis	5 (21.7)	2 (15.3)	3 (30)	
Laryngeal papillomatosis	7 (30.4)	4 (30.7)	3 (30)	

**P* value obtained by Chi-square test. ⊥Percentage of all male cancer sites. ±Percentage of all female cancer sites.

Table 4: Distribution based on type of premalignant laryngeal lesions, gender and age group (n = 23)

Type of lesion	0 – 10 years		11 – 20 years		21-30 years		31- 40 years		41-50 years	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Recurrent multiple papilloma of larynx	-	-	1	1	4	2	2	-	-	1
Juvenile laryngeal papillomatosis	2	3	-	-	-	-	-	-	-	-
Laryngeal papillomatosis	-	-	1	-	2	2	-	-	1	1

Table 5 represents the results of GSTM1 gene polymorphism. The frequency of GSTM1 null genotype was 56.5% in study patients as against 39.1% in controls. Similarly, the frequency of GSTM1 wild type or non-null genotype in study patients was 43.4% as against 60.8% in controls.

Table 5: Distribution of GSTM1 polymorphisms in controls and patient groups

Group	GSTM1			
	Wild-type	%	Null-type	%
Controls (n = 23)	14	60.8	9	39.1
Test samples (n = 23)	10	43.4	13	56.5

GSTT1 null genotype was 52.1% in study patients as against 26% in controls. Similarly, GSTT1 wild-type genotype was 47.8% in test samples as to 73.9% in controls [Table 6].

Table 6: Distribution of GSTT1 polymorphisms in controls and patient groups

Group	GSTT1			
	Wild-type	%	Null-type	%
Controls (n = 23)	17	73.9	6	26
Test samples (n = 23)	11	47.8	12	52.1

A combination of smoking and alcohol habits, followed by chewing habits had a significant P value of <0.005 in the patient group. Whereas in controls, none of the risk factor habits were of any significant value [Table 2].

Table 7: Statistical significances of GSTM1 and GSTT1 polymorphisms

Genes	*P* values	OR	95% CI		df
			Lower bound	Upper bound	
GSTM1	0.197	4.667	0.702	31.036	1
GSTT1	0.015	0.889	0.138	5.723	1

P values – calculated by Pearson chi-square test; OR – odds ratio; 95% CI – 95% confidence intervals; df – degrees of freedom

Statistical significances of GSTM1 and GSTT1 polymorphisms have been described in Table 7. Odds ratio was 4.667 for developing GSTM1 null polymorphism and 0.889 for GSTT1 null polymorphism. 95% CI values for GSTM1, lower bound - 0.702 and upper bound - 31.036. Relative risk for GSTM1 null polymorphism is 1.815 and 0.389 positive. Relative risk for GSTT1 null polymorphism is 0.944 and 1.063 for positive. 95% CI values for GSTT1, lower bound – 0.138 and upper bound- 5.723. Degrees of freedom was 1 in both cases. Asymptotic *t*-value for GSTM1 was 1.678 and 0.119 for GSTT1.

IV. CONCLUSIONS

A pre-malignant lesion is a lesion that, while not cancerous, has strong potential for becoming cancerous. It is important to identify the right type of the pre-malignant lesion in order to have an insight into the stage of pre-malignancy and specific treatment plan. The study population reported 3 types of pre-malignant conditions i.e., Recurrent multiple papilloma of larynx, Juvenile laryngeal papillomatosis and Laryngeal papillomatosis.

Papillomas are benign epithelial tumor growths similar to warts on the skin[47]. Malignant degeneration to squamous cell carcinoma can occur, but is very rare. It is a relatively uncommon disease in adults while approximately 70% of this disease showing juvenile onset (at 10 years of age or less).[48,49] Based on clinical parameters, the papillomas were divided into juvenile multiple papillomas, adult multiple papillomas and adult solitary papillomas[50]. Laryngeal papillomatosis, also known as recurrent respiratory papillomatosis or juvenile multiple papilloma of the larynx, is a rare disease characterized by the development of tumors in the larynx. These tumors can reoccur frequently, may require repetitive surgery, and may interfere with breathing.

Glutathione S-transferases are a family of dimeric enzymes that play an important role in the detoxification of several carcinogens found in tobacco smoke, significant role in cellular protection against toxic foreign chemicals and oxidative stress and other cellular functions, such as DNA repair.

Normal and malignant squamous cells of the larynx have been shown to express the GST-mu isoform in the highest concentration compared with the other GST enzymes.[20,51-54] Persons who do not have the ability to produce the GSTM1 enzyme potentially accumulate more DNA adducts through their inefficiency at excreting activated carcinogens.

GSTT1 (GST-theta isoform) is expressed not only in the adult liver but also in human erythrocytes and, as a result, is believed to play a more global role than GSTM1 in detoxification of carcinogens in the body[55]. Unlike the GSTM1 enzyme, however, GSTT1 has detoxification and activation roles.[20,15,55] The presence of GSTT1 enzyme within red blood cells may allow red cells to act as a detoxification sink among those who are able to synthesize the enzyme. Interestingly, if the capacity for removal of detoxification products from the circulation is exceeded among those with GSTT1 functionality, the risk of carcinogenesis may be increased compared with risk among those who have no function of the enzyme.[20] Persons with homozygous deletions of the GSTM1 and GSTT1 locus have no enzymatic functional activity of the respective enzymes.

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 tobacco chewing). The inherited differences in the effectiveness of detoxification or activation of carcinogens play a crucial role in host susceptibility. However, polymorphism of GSTM1 and GSTT1 genes and their effects on cancer studied in many countries worldwide for the last thirty years have displayed varying results.

The polymorphisms of the GSTM1 and GSTT1 genes involved may result in differences in the enzymatic activity, possibly favoring mechansims that increase the susceptibility to cancer. Studies relating these polymorphisms of deletion with the occurrence of head and neck carcinoma diverge between themselves, some demonstrate the association of these neoplasias with null genotype of GSTM1 or GSTT1, while others do not.[38,46,56-65]

In our study, we did not find evidence for a strong interaction between risk factor habits, metabolizing enzyme polymorphisms, and the risk of malignancy. 65% of patients did not indulge in any type of risk factor habits and yet had a pre-malignant condition. There was no clear pattern of increasing risk in the presence of risk factors habits for at-risk genotypes of GSTM1 and GSTT1.

Our study evidenced an increased frequency of the GSTT1 and GSTM1 null genotype in patients with clinically confirmed pre-malignant laryngeal lesions, when compared to control group of individuals. The wild-type genotypes were in higher frequency in control cases for both gene groups. Our results conclude that since the null-genotypes were more in test samples than the wild-type genotypes, it may be associated with an increase in risk for the development of malignancy. However, it cannot be confirmed as one of the reasons though. The statistical results show that GSTT1 null polymorphism are of significant value ($P = 0.015$) whereas, GSTM1 null polymorphisms are not ($P < 0.05$). These data are confined to a specific and small population and hence cannot be used to reflect the same for general population.

The relationship between habitual risk factors and prevalence of premalignancy risk was not statistically significant with GST polymorphism in the study group. The distinct role of enzymes in different tissues and cancers yields different features of genotypes. Thus, there might be an instance of divergence

between the ethnic differences in allelic frequency of GSTM1 and GSTT1 polymorphism and the differences in environmental and lifestyle risk factors.

Detecting malignancy at an earlier stage, upon finding any early symptoms i.e., pre-malignant stage is very important in diagnosing and treating a disorder. Delay by people in getting medical attention and treatment at the right time, reduces the possibility of identifying disorders in pre-malignant stage. It is necessary to carry out more studies on a larger population to access further probable patterns leading to malignancies. Genetic analysis and counselling of at-risk population can be provided thereby ensuring early diagnosis and management.

REFERENCES

[1] General Introduction Head and neck squamous cell carcinoma

[2] Marisa Gariglio, Barbara Azzimonti, Laura Hertel, et al. Demonstration of Multiple HPV Types in Laryngeal Premalignant Lesions Using Polymerase Chain Reaction and Immunohistochemistry, Journal of Medical Virology 59:110-116 (1999).

[3] http://www.maxillofacialcenter.com/BondBook/mucosa/erythroplakia.html

[4] FL. ANGHELINA et.al, Clinical, morphological and immunohistochemical aspects in laryngeal premalignant lesions, Romanian Journal of Morphology and Embryology 2006, 47(2):169–174.

[5] Patrick Bradley, oncologynews.biz, Volume 2 Issue 5 • February/March 2008, Page 5.

[6] oralcancerfoundation.org, Premalignant Lesions, Chapter IV.

[7] Gallo, O; Franchi, A; Chiarelli, I; Porfirio, B; Grande, A; Simonetti, L; Bocciolini, C; Fini-Storchi, O (ACTA OTO-LARYNGOLOGICA. SUPPLEMENTUM Vol. 527, 1997) ((PMID): 9197477)

[8] Axell T, Pindborg JJ, Smith CJ, van der Waal I., Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21 1994. J Oral Pathol Med 1996; 25:49-54.

[9] Silverman S Jr, Gorsky M, Lozada F., Oral leukoplakia and malignant transformation: a follow-up study of 257 patients. Cancer 1984; 53:563-8.

[10] Kaugars GE, Burns JC, Gunsolley JC. Epithelial dysplasia of the oral cavity and lips. Cancer 1988; 62:2166-70.

[11] Lumerman H, Freedman P, Kerpel S., Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol 1995;79:321-9.

[12] Zondervan K, Cardon L, Kennedy S Development of a Web site for the genetic epidemiology of en¬dometriosis. Fertil Steril. 2002; 78: 777-781.

[13] Kennedy S. Genetics of endometriosis: a review of the positional cloning approaches. Semin Reprod Med. 2003; 21: 111-118.

[14] Lazarus P, Park PY (2000). Metabolizing enzyme genotype and risk for upper aerodigestive tract cancer. Oral Oncol, 36, 421-31.

[15] Hayes J, Pulford D. The glutathione S-transferase supergene family: regulation of GST* and the contribution of the isoenzymes to cancer chemoprevention and drug resistance. Crit Rev Biochem Mol Biol 1995;30:445–600.

[16] Mannervik B, Alin P, Guthenberg C, et al (1985). Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. Proc Natl Acad Sci USA, 82, 7202-6.

[17] Mannervik B, Awatshi YC, Board PG, Hayes JD, Di Ilio C (1992). Nomenclature for human glutathione S-transferases. Biochem J, 282, 305-8.

[18] Meyer DJ, Coles B, Pemble SE, et al (1991). Theta new classes of glutathione S-transferases purified from rat and man. Biochem J, 274, 409-14.

[19] Rahmi Amtha et al, GSTM1, GSTT1 and CYP1A1 polymorphisms and Risk of Oral Cancer: a Case-control Study in Jakarta, Indonesia, Asian Pacific Journal of Cancer Prevention, Vol 10, 2009, 21-26.

[20] Stacy A. Geisler, Andrew F. Olshan, GSTM1, GSTT1, and the Risk of Squamous Cell Carcinoma of the Head and Neck: A Mini-HuGE Review, Am J Epidemiol Vol. 154, No. 2, 2001, 95-105.

[21] Pemble S, Schroeder KR, Spencer SR, et al (1994). Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J, 15, 271-6.

[22] Rebbeck TR (1997). Molecular epidemiology of human gluthation S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomark Prev, 6, 733-43.

[23] Zheng W. Epidemiological studies of genetics factors for cancer. In Philip C Nasca and Harris Pastides. (eds). 'Fundamentals of Cancer Epidemiology'. Maryland, Aspen, p. 2001, 103-12.

[24] Wogan GN, Hecht SS, Felton JS, Conney AH, Loeb LA. Environmental and chemical carcinogenesis. Semin Cancer Biol, 2004, 14, 473-86.

[25] Tanimoto K, Hayashi S, Yoshiga K, Ichikawa T. Polymorphisms of the CYP 1A1 and GSTM1 gene involved in oral squamous cell carcinoma in association with a cigarette dose. Oral Oncol, 1999, 35, 191-6.

[26] Sharma A, Mishra A, Das BC, Sardana S, Sharma JK. Genetic polymorphism at GSTM1 and GSTT1 gene loci and susceptibility to oral cancer. Neoplasma, 2006, 53, 309-15.

[27] Hung HC, Chuang J, Chien YC, et al. Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. Cancer Epidemiol Biomarkers Prev, 1997, 6, 901-5.

[28] Sreelekha TT, Ramadas K, Pandey M, et al. Genetic polymorphism of CYP1A1, GSTM1 and GSTT1 genes in Indian oral cancer. Oral Oncol, 2001, 37, 593-8.

[29] Cha In-Ho, Park JY, Chung WY, et al. Polymorphisms of CYP1A1 and GSTM1 genes and susceptibility to oral cancer. Yonsei Med J, 2007, 48, 233-9.

[30] Park JY, Muscat JE, Ren Q, et al. CYP1A1 and GSTM1 polymorphisms and oral cancer risk. Cancer Epidemiol Biomark Prev, 1997, 6, 791-7.

[31] Park JY, Muscat JE, Kaur T, et al. Comparison of GSTM polymorphisms and risk of oral cancer between African-American and Caucasians. Pharmacogenetics, 2000, 10, 123-31.

[32] Olshan AF, Weissler MC, Watson MA, Bell DA. GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev, 2000, 9, 185-91.

[33] Deakin M, Elder J, Hendrickse C, et al. Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. Carcinogenesis, 1996, 17, 881-4.

[34] Worrall S, Corrigan M, High A, et al. Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome P450 CYP2D6. Pharmacogenetics, 1998, 8, 433–9.

[35] Roya Rozati et.al., The CYP1A1 and GSTM1 Genetic Polymorphisms and Susceptibility to Endometriosis in Women from South India, International Journal of Fertility and Sterility, Vol 2, No 3, Nov-Dec 2008, 105-112.

[36] Pearson WR, Vorachek WR, Xu SJ, Berger R, Hart I, et al. Identification of class-mu glutathione transferase genes GSTM1-GSTM5 on human chro¬mosome 1p13.3 Am J Hum Genet. 1993, 53, 220-233.

[37] Seidegard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA. 1988, 85, 7293-7297.

[38] Zheng T, Holford TR, Zahm SH, Owens PH, Boyle P, Zhang Y, et al. Cigarrete smoking, glutathione-S-transferase M1 and T1 genetc polymorfisms and breast cancer risk (United States). Cancer Causes Control. 2002;13:637-45.

[39] Cawkwell L, Quirke. Direct multiplex amplification of DNA from formalin fixed, paraffin wax embedded tissue section, J Clin Pathol: Mol Pathol 53, 51-52, 2000.

[40] Chan P K S, Chan D P C, To K-F, et al. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. Clin Pathol 54, 401-403, 2001.

[41] Jung K, Ha Y, Kim S-H, et al. Development of Polymerase Chain Reaction and comparison with In situ Hybridization for the detection of Haemophilus parasuis in formalin-fixed, Paraffin embedded tissues, J.vet. Med. Sci. 66(7), 841-845, 2004.

[42] Keohavong P, Gao W-M, Zheng K-C, et al. Detection of K-ras and p53 mutations in sputum samples of lung cancer patients using laser capture microdissection microscope and mutation analysis, Analytical Biochemistry 324, 92-99, 2004.

[43] Shi S-R, Cote R J, Wu L, et al. DNA extraction from archival formalin-fixed, Paraffin-embedded tissue sections based on the antigen retrieval principle: Heating under the influence of pH, The journal of Histochemistry & Cytochemistry 50, 1005-1011, 2002.

[44] G. Tirumala Vani et.al., Role of glutathione S-transferase Mu-1 (GSTM1) polymorphism in oligospermic infertile males, Andrologia, 42, 213-217.

[45] Regina Grazuleviciene et.al., Maternal Smoking, GSTM1 and GSTT1 Polymorphism and Susceptibility to Adverse Pregnancy Outcomes, Int. J. Environ. Res. Public Health 2009, 6, 1282-1297.

[46] M. Singh et al., Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer, Mutation Research, 638, 2008, 184–194.

[47] Abramson, A.L., Steinberg, B.M. and Winkler, B.M. Laryngeal papillomatosis: clinical histopathologic and molecular studies, Laryngoscope 1987; 97: 678-685.

[48] Takashi Hirano et.al, Endoscopic Diagnosis and Treatment of a Case of Respiratory Papillomatosis, Diagnostic and Therapeutic Endoscopy, 1997, Vol. 3, pp. 183-187.

[49] Weiss, M.D. and Kashima, H.K. Tracheal involvement in laryngeal papillomatosis. Laryngoscope 1983,93, 45-48.

[50] Henning lindeberg, Laryngeal papillomas: histomorphometric evaluation of multiple and solitary lesions, Clin Otolaryngol Allied Sci. 1991 Jun,16(3),257-60.

[51] Janot F, Massaad L, Ribrag V, et al. Principal xenobiotic-metabolizing enzyme systems in human head and neck squamous cell carcinoma. Carcinogenesis 1993,14,1279–83.

[52] Bongers V, Snow GB, Braakhuis BJ. The role of glutathione S-transferases in head and neck squamous cell carcinogenesis. Eur J Cancer B Oral Oncol 1995,31B, 349–54.

[53] Mulder T, Manni J, Roelofs H, et al. Glutathione S-transferases and glutathione in human head and neck cancer. Carcinogenesis 1995,16,619-24.

[54] Mulder T, Manni J, Roelofs H, et al. Glutathione peroxidases in human head and neck cancer. Acta Otolaryngol 1995, 115,331-3.

[55] Landi S. Mammalian class theta GST and differential susceptibility to carcinogens: a review. Mutat Res 2000, 463, 247–83.

[56] Soya SS, Vinod T, Reddy KS, Gopalakrishnan S, Adithan C. Polymorfisms of glutathione-S-transferase genes (GSTM1, GSTT1, GSTP1) and upper aerodigestive tract cancer risk among smokers, tobacco chewers and alcoholics in a Indian population. Eur J Cancer. 2007,43,2698-706.

[57] 24. Suzen HS, Guvenc G, Turanli M, Comert E, Duydu Y, Elhan A. The role of GSTM1 and GSTT1 polymorphisms in head and neck cancer risk. Oncol Res. 2007,16,423-9.

[58] Duarte EC, Ribeiro DC, Gomez MV, Ramos-Jorge ML, Gomez RS. Genetic polymorphisms of carcinogen metabolizing enzymes are associated with oral leukoplakia development and p53 overexpression. Anticancer Res. 2008,28,1101-6.

[59] Biselli JM, Leal RCAC, Ruiz MT, Goloni-Bertollo EM, Maníglia JV, Rossit ARB,et al. Polimorfismos GSTT1 e GSTM1 em indivíduos tabagistas com carcinoma espinocelular de cabeça e pescoço. Rev Bras Otorinolaringol. 2006,72,654-8.

[60] Goloni-Bertollo EM, Biselli JM, Correa LCL, Maniglia JV, Rossit ARB, Ruiz MT. et al. Avaliação da influência da nulidade dos genótipos GSTT1 e GSTM1 na carcinogênese em cabeça e pescoço. Rev Assoc Med Bras. 2006,52,365-8.

[61] Rossini A, Rapozo DCM, Soares Lima SC, Guimarães DP, Ferreira MA, Teixeira R, et al. Polymorfisms of GSTP1 and GSTT1, but not of CYP2A6, CYP2E1 or GSTM1, modify the risk for esophageal cancer in a western population. Carcinogenesis. 2007,28,2537-42.

[62] Reszka E, Czekaj P, Adamska J, Wasowicz W. Relevance of glutathione S-transferase M1 and cytochrome P450 1A1 genetic polymorphisms to the development of head and neck cancers. Clin Chem Lab Med. 2008,46,1090-6.

[63] Amtha R, Ching CS, Zain R, Razak IA, Basuki B, Roeslan BO, Gautama W, Purwanto D. GSTM1, GSTT1 and CYP1A1 polymorphisms and risk of oral cancer: a case-control study in Jakarta, Indonesia. Asian Pac J Cancer Prev. 2009,10,21-6.

[64] Buch SC, Notani PN, Bhisey RA. Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population. Carcinogenesis. 2002,23,803-7.

[65] Losi-Guembarovski R, Cólus IM, De Menezes RP, Poliseli F, Chaves VN, et al. Lack of association among polymorphic xenobiotic-metabolizing enzyme genotypes and the occurrence and progression of oral carcinoma in a Brazilian population. Anticancer Res. 2008, 28, 1023-8.

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