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GSTM1 polymorphism and oral leukoplakia

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BACKGROUND: Molecular epidemiological studies have now provided evidence that an individual susceptibility to cancer is mediated by genetic and environmental factors. Genetic polymorphisms have been described for enzymes involved in the metabolism of tobacco carcinogens and cancer risk is determined by the degree of expression and/or activity of enzymes involved in carcinogen activation or deactivation. The objective of this study was to investigate the *GSTM1* null polymorphism and the risk for oral leukoplakia in individuals with tobacco-smoking habit in a Brazilian population.

METHODS: A total of 52 tobacco-smoking patients with oral leukoplakia and 52 tobacco-smoking controls were recruited in a Brazilian population. The *GSTM1* genotypes were studied by polymerase chain reaction-based methods.

RESULTS: The frequency of the *GSTM1* null genotype in the group with oral leukoplakia (57.7%) was statistically different from the controls (34.6%; OR: 2.57, 95% CI: 1.16–5.69, $P < 0.05$). The stratification of the samples according to the level of dysplasia showed increased prevalence of *GSTM1* null genotype on lesions with moderate/severe histological dysplasia (68.2%) compared with the control group (31.9%). This difference was statistically significant (OR: 4.59, 95% CI: 1.29–16.33, $P < 0.05$).

CONCLUSION: In conclusion, the *GSTM1* null genotype may increase the risk for oral leukoplakia development.
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Keywords: *GSTM1*; oral leukoplakia; polymorphism

Introduction

Oral leukoplakia is defined as 'a chronic white mucosal macule which cannot be characterized clinically or pathologically as any other disease' (1, 2). The development of oral leukoplakia is strongly associated with exogenous exposure to carcinogens, mainly smoking, chewing tobacco and betel nut. The use of tobacco is the most important, and has been present in 80% of the cases (3, 4). Oral leukoplakia shows predilection for man (70%), generally up to 40 years old (5) and may occur as a single or multiple lesion (6). Histologically, the leukoplakia may show mild, moderate or severe dysplasia (6, 7). Although the presence of nodular, verrucous or erythroplastic, or severe dysplasia may relate to increased risk for malignant transformation, these markers are not useful in individual cases. The annual transformation rate of oral leukoplakia seems not to exceed 1% (8).

Molecular epidemiological studies have now provided evidence that an individual susceptibility to cancer is mediated by genetic and environmental factors (9). Numerous carcinogenic components present in the tobacco smoking require metabolic activation by phase I enzymes (e.g. cytochrome P450 oxidases-like CYP1A1 and CYP2E1) or deactivation by phase II enzymes (e.g. glutathione S-transferase, GST). GSTs are a supergene family coding for five multigene enzyme groups referred to as μ or M, θ or T, π, α and κ, which conjugate glutathione to genotoxic eletrophiles such as polycyclic aromatic hydrocarbons, epoxybutanes, ethylene oxide and halomethanes. GST-conjugated compounds are generally rendered hydrophilic and non-toxic, and are easily excreted (10, 11).

Genetic polymorphisms have been described for enzymes involved in the metabolism of tobacco carcinogens and cancer risk is determined by the degree of expression and/or activity of enzymes involved in carcinogen activation or deactivation (11). The *GSTM1* null polymorphism results in a loss of expression, resulting in decreased ability to detoxify GST-μ-conjugated carcinogens (12). Considering that tobacco is the main aetiological factor in oral cancer and that oral leukoplakia is the most prevalent potentially malignant lesion, together with the fact that *GSTM1* polymorphism increases the risk for oral squamous cell carcinoma (13), the purpose of this study was to investigate the *GSTM1* null polymorphism and the risk for oral

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leukoplakia in individuals with tobacco-smoking habit in a Brazilian population.

Materials and methods

Subjects and sample collection

Fifty-two smokers patients (mean age = 47.9 years; range: 25–87) with oral leukoplakia and 52 healthy smokers volunteers (mean age = 48.6 years; range: 29–81) were included in the study. The criteria for the diagnosis of oral leukoplakia were previously reported (2). All subjects were selected at the Dental Clinics of the School of Dentistry and were of the same geographical region as well as socio-economic status. The sex and age of both groups were matched. All subjects had smoked at least 10 cigarettes per day over a period of 20 years. There were 31 males (59.6%) and 21 (40.4%) females in the patient's group. Ethnicity was not established as the hazards of judging Brazilians by colour, race and geographical origin (14). The lesions located at the floor of the mouth, tongue and soft palate were grouped together as high-risk site group, while all the oral sites were included in the group of low-risk site. The grade of epithelial dysplasia was established as described elsewhere (15). The histopathological features of epithelial dysplasia considered were loss of polarity of the basal cells, presence of more than one layer of cells having a basaloid appearance, increased nuclear-cytoplasmic ration, drop-shaped rete process, irregular epithelial stratification, increased number of mitotic figures, presence of mitotic figures in the superficial half of the epithelium, cellular pleomorphism, nuclear hyperchromatism, enlarged nucleoli, reduction of cellular cohesion and diskeratosis (6). Local ethical committee approved the study protocol, and informed consent was obtained from all patients.

Oral swabs were taken from each subject. The procedure was performed on the contralateral intact mucosa of the patients with oral leukoplakia. The swabs from the control subjects were collected from their labial mucosa. The swabs were performed with sterile plastic tips and placed immediately in Eppendorf microtubes contain 500 µl of Krebs buffer (NaCl 20%, KCl 2%, $CaCl_2{\cdot}2H_2O$ 2%, $MgSO_4$, KH_2PO_4, $C_6H_{12}O_6$). The pellet obtained after 10 min of centrifugation at 17 900 *g* was stored at −20°C until processing. The DNA extraction was carried out as described by Boom et al. (16).

Polymerase chain reaction

The *GSTM1* genotypes were studied by polymerase chain reaction as described previously (17). The amplification was performed in a final volume of 25 µl containing 5 µl of genomic DNA, 0.75 mM $MgCl_2$, 40 mM KCl, 10 mM Tris-HCl (pH 8.4), 0.1% Triton X-100, 11.4 µl H_2O, 2.5 µl dNTPs and 2.5 U *Taq* DNA polymerase. Samples were subjected to 5 min at 94°C, followed by 35 cycles of amplification at 95°C for 30 s, 64°C for 1 min and 72°C for 1 min. The run was terminated by a 7 min elongation step at 72°C. A negative control reaction without DNA and samples with known *GSTM1* genotype were always used. The reaction produced a 220 bp product. Amplification of the β-globin gene was used as an internal control. All samples were amplified using a DNA thermal cycler (Programmable Thermal Controller, PTC). The product was analysed in a 6.5% polyacrylamide gel electrophoresis followed by silver stain.

Statistical analysis

Statistical analysis was performed by chi-square and Fisher's exact tests; and significance was set at *P*-value of < 0.05.

Results

The frequencies of the *GSTM1* genotypes between cases and controls are shown in Table 1. Individual homozygous for the wild-type *GSTM1* (+/+) and heterozygous (+/0) were grouped together. The frequency of the *GSTM1* null genotype in the group with oral leukoplakia (57.7%) was statistically different from the controls [34.6%; odds ratio (OR): 2.57, 95% CI: 1.16–5.69, $P < 0.05$]. When the groups were stratified according to the gender and site of occurrence, no statistical difference was observed. However, the stratification of the samples according to the level of dysplasia showed increased prevalence of *GSTM1* null genotype on lesions with moderate/severe histological

Table 1 Glutathione S-transferase (*GSTM1*) genotypes in oral leukoplakia patients and controls

		GSTM1 genotype				
	n	*0/0 (%)*	*+/0 or +/+ (%)*	χ^2	*P-value*[a]	*OR (95% CI)*
Control	52	18 (34.6)	34 (65.4)			
Oral leukoplakia	52	30 (57.7)	22 (42.3)	5.57	< 0.05	2.57 (1.16–5.69)
Grade of displasia						
Absent/mild	30	15 (50.0)	15 (50.0)	–	n.s.	–
Moderate/severe	22	15 (68.2)	7 (31.8)	5.81	< 0.05	4.59 (1.29–16.33)
Location						
High risk	9	5 (55.6)	4 (44.4)	–	n.s.	–
Low risk	43	25 (58.1)	18 (41.9)	–	n.s.	–

[a]Vs. control.
[a]n.s., not significant; 0/0, *GSTM1* null; +/0, *GSTM1* heterozygous; +/+, *GSTM1* wild-type homozygous; CI, confidence interval; OR, odds ratio.

dysplasia (68.2%) compared with the control group (31.9%). This difference was statistically significant (OR: 4.59, 95% CI: 1.29–16.33, $P < 0.05$).

Discussion

Epidemiological studies have demonstrated a significant influence of tobacco and alcohol consumption on the risk for oral cancer. Oral leukoplakia is the most common potentially malignant lesion of the oral mucosa. Although tobacco use is related to oral leukoplakia, alcohol consumption has not a significant association with it (18). Because exposure to chemical carcinogens acts as an important mechanism of oral cancer development, the investigation of genetic polymorphisms of drug-metabolizing enzymes and cancer susceptibility is of significant interest.

GSTM enzymes may offer protection against DNA damage induced by free radicals and metabolites of polycyclic aromatic hydrocarbons (19). The lack of the *GSTM1* activity is a result of a homozygous deletion (null genotype) of the *GSTM1* gene. The null genotype results in a loss of expression, resulting in decreased ability to detoxify GST-μ-conjugated carcinogens (11). The *GSTM1* null genotypes (0/0) polymorphism has been linked to increased susceptibility to oral squamous cell carcinoma development (13, 17, 20, 21). In addition, this polymorphism seems to be a risk factor for developing multiple primary neoplasms in the upper aerodigestive tract (22). In the present study, a positive association between the *GSTM1* null genotype and oral leukoplakia in Brazilian subjects was observed. This polymorphism was also demonstrated to be a risk factor for developing oral leukoplakia in ethnic Indian betel quid/tobacco chewers (23).

In our study, the frequencies of female or male patients in the case group who were null for the *GSTM1* genotype was not statistically different from the female or male control group respectively. No statistical difference was also observed when the samples were stratified by the location of the lesion. These results may be due to the low number of subjects after segregation. The presence of the GSTM null genotype increased the risk factor to lesions showing histological moderate/severe dysplasia. We may speculate that the lack of *GSTM1* activity would make the oral tissues more susceptible to the action of tobacco carcinogens and to the development of a high-grade level of dysplasia. Longitudinal studies evaluating the impact of this polymorphism on malignant transformation of oral leukoplakia would be of interest and could be an interesting tool for targeting patients with oral leukoplakia at particular risk for future cancerous lesion.

In conclusion, the present study shows a positive association between the presence of *GSTM1* null genotype and the development of oral leukoplakia. Indeed, this association varies in accordance with the histological grade of dysplasia.

References

1. Kramer IRH, Lucas RB, Pindborg JJ, Sobin LH. WHO Collaborating Centre for oral precancerous lesions. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol* 1978; **46**: 518–39.
2. Axéll T, Pindborg JJ, Smith CJ, van der Waal I, an International Collaborative Group on Oral White Lesions. Oral white lesions with special reference to precancerous and tabacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18–21, 1994. *J Oral Pathol Med* 1996; **25**: 25–49.
3. Johnson NW, Warnakulasuriya S, Tavassoli M. Hereditary and environmental risk factors; clinical and laboratory risk matters for head and neck, especially oral, cancer and precancer. *Eur J Cancer* 1996; **5**: 5–17.
4. Neville BW, Day TA. Oral cancer and precancerous lesions. *CA Cancer J Clin* 2002; **52**: 195–215.
5. Mehta FS, Gupta PC, Pindborg JJ. Chewing and smoking habits in relation to precancerous and oral cancer. *Clin Oncol* 1981; **99**: 35–9.
6. van der Waal I, Axéll T. Oral leukoplakia: a proposal for uniform reporting. *Oral Oncol* 2002; **38**: 521–6.
7. Karabalut A, Reibel J, Therkildsen MH, Praetorius F, Nielsen HW, Dabelsteen E. Observer variability in the histologic assessment of oral premalignant lesions. *J Oral Pathol Med* 1995; **24**: 198–200.
8. Scheifele C, Reichart PA. Is there a natural limit of the transformation rate of oral leukoplakia? *Oral Oncol* 2003; **39**: 470–5.
9. Sikdar N, Mahmud A, Paul RR, Roy B. Polymorphism in CYP1A1 and CYP2E1 genes and susceptibility to leukoplakia in Indian tobacco users. *Cancer Lett* 2003; **195**: 33–42.
10. Perera FP, Weinstein IB. Molecular epidemiology: recent advances and future directions. *Carcinogenesis* 2000; **21**: 517–24.
11. Lazarus P, Park JY. Metabolizing enzyme genotype and risk for upper aerodigestive tract cancer. *Oral Oncol* 2000; **36**: 421–31.
12. Wiencke JK, Kelsey KT, Lamela RA, Toscano WA Jr. Human glutathione S-transferase deficiency as a marker of susceptibility to epoxide-induced cytogenetic damage. *Cancer Res* 1990; **50**: 1585–90.
13. Drummond SN, Noronha JCM, De Marco L, Gomez RS. GSTM-1 polymorphism and oral squamous cell carcinoma. *Oral Oncol* 2004; **40**: 52–5.
14. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci U S A* 2003; **100**: 177–82.
15. Paula AMB, Gomez RS. Immunolocalization of p53, glutathione S transferase π and CD57 antigens in oral leukoplakia. *Anticancer Res* 2001; **21**: 379–86.
16. Boom R, Sol CJA, Salimans MMM, Jausen CK, Wertheim-Van Dillen PME, Van Der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990; **28**: 495–503.
17. Sreelekha TT, Ramadas K, Pandey M, Thomas G, Nalinakumari KR, Pillai MR. Genetic polymorphism of CYP1A1, GSTM1 and GSTT1 genes in Indian oral cancer. *Oral Oncol* 2001; **37**: 593–8.
18. Harris CK, Warnakulasuriya KAAS, Cooper DJ, Petrs TJ, Gelbier S. Prevalence of oral mucosal lesions in a alcohol misusers in south London. *J Oral Pathol* 2004; **33**: 253–9.

19. Schneider J, Bernges U, Philipp M, Woitowitz HJ. *GSTM1*, *GSTT1*, and *GSTP1* polymorphism and lung cancer risk in relation to tobacco smoking. *Cancer Lett* 2004; **208**: 65–74.
20. Sato M, Sato T, Izumo T, Amagasa T. Genetic polymorphism of drug-metabolizing enzymes and susceptibility to oral cancer. *Carcinogenesis* 1999; **20**: 1927–31.
21. Nomura T, Noma H, shibahara T, Yokoyama A, Muramatusu T, Ohmori T. Aldehyde dehydrogenase 2 and glutathione S-transferase M1 polymorphism in relation to the risk for oral cancer in Japanese drinkers. *Oral Oncol* 2000; **36**: 42–6.
22. Jhavar S, Sarin R, Mulherkar R, Benner A, Agarwal JP. Dinshaw. Glutathione S-transferase M1 or T1 null genotype as a risk factor for developing multiple primary neoplasms in the upper aero-digestive tract, in Indian males using tobacco. *Oral Oncol* 2004; **40**: 84–91.
23. Nair UJ, Nair J, Mathew B, Bartsch H. Glutathione S-transferase M1 and T1 null genotypes as risk factors for oral leukoplakia in ethnic Indian betel quid/tobacco chewers. *Carcinogenesis* 1999; **20**: 742–8.

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