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Serotonin Transporter Gene Polymorphisms: A Case-Control Study

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Considerable evidence indicates that serotonergic mechanisms, particularly the serotonin transporter, are involved in alcoholism and tobacco use and are influenced by polymorphism of the promoter region of 5HTT (5-HTTLPR). As alcohol and tobacco consumption have been implicated in the pathogenesis of oral cancer, the purpose of this study was to investigate 5-HTTLPR polymorphism in patients with oral squamous cell carcinoma (OSCC) compared with a control group in a sample of Brazilian patients. One hundred and three patients affected by OSCC and 103 volunteers without OSCC were genotyped for 5-HTTLPR. Both groups were matched for age, sex and tobacco use. The chi-squared test was used for statistical analysis (α=0.05). There was no statistically significant difference in 5-HTTLPR genotypes between case and control group (p= 0.408). In conclusion, the present investigation demonstrated that serotonin transporter polymorphisms are not implicated in the OSSC development.

Key Words: oral squamous cell carcinoma, 5-HTTLPR, polymorphism.

INTRODUCTION

The gene for serotonin transporter (*SLC6A4*) is located on chromosome 17q12 and consists of a promoter and 14 exons spanning 31 Kb (1,2). A polymorphic region of *SLC6A4*, identified by a 44 bp insertion-deletion in the promoter region (5-HTTLPR), is known. The two alleles, termed long (L) and short (S), exhibits different phenotype (1). *In vitro* studies have demonstrated that the short allele (S) has been associated with reduced transcriptional efficiency that causes a reduced serotonin expression and uptake of the neurotransmitter (1). Some studies have suggested a possible involvement of the 5-HTTLPR genotype with psychiatric disorders (3), suicidal behavior (4), depression (5), anxiety (6), alcoholism (7), smoking (8) and recurrent aphthous ulceration (9).

Oral squamous cell carcinoma (OSCC) is the most common cancer in the oral cavity. This tumor usually affects more men than women, usually in the middle to later decades of life. In Brazil, an incidence of 14,120 new cases of OSCC was estimated in 2010, with a ratio of 10.64 cases for 100,000 male inhabitants and of 3.76 for female inhabitants (10). Although tobacco smoking and alcohol consumption are the two most strongly implicated risk factors for development of OSCC, studies have tried to elucidate the biological behavior of this disease (11-15).

The fact that 5-HTTLPR genotype is associated with alcohol consumption (7,16,17) and tobacco use (8,18), led us to investigate a possible association between functional genetic 5-HTTLPR polymorphism with alcohol and tobacco consumption in a sample of Brazilian patients affected by OSCC.

PATIENTS AND METHODS

Subjects, Sample Collection and DNA Extraction

One hundred and three consecutive subjects (mean

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age= 57.2 ± 10.4 years old; range 36-82 years old) with OSCC and 103 age, sex and tobacco use matched control subjects (mean age= 57.3 ± 10.4 years old; range 36-82 years old) were included in this study. There were 77 (74.8%) males and 26 (25.2%) females in the case group. In the OSCC group 92 (89.3%) patients were smokers and 75 (72.8%) were drinkers. In the control group, 90 (87.4%) were smokers while 63 (61.2%) were drinkers. The cases were recruited from the Oral Diagnosis Clinic at the Federal University of Minas Gerais - UFMG, Brazil. Both the case and control groups were of the same geographic area and had identical socioeconomic status. No evaluation of the psychological status of patients was performed. Ethnicity was not established as the hazards of judging Brazilians by color, race and geographical origin was recently demonstrated (19). The tobacco and alcohol consumption were calculated as follows: 20 manufactured cigarettes = 4 hand-rolled, black tobacco cigarettes = 4 cigars = 5 pipefulls with regular pipe tobacco; ethanol concentration in beer = 5%, wine = 10%, hard liquor and 'cachaça' (Brazilian sugar cane alcoholic beverage) = 50% (20). Former-smokers were analyzed together with current smokers. The same criterion was used to analyze the alcoholic beverages consumption. The time of tobacco and ethanol consumption was informed by the patient in the interview. The time of tobacco consumption was used to by dichotomize the sample in two groups, on the basis of the median: less than 34 years and more than 34 years. While the median of time of alcohol consumption was 20 years and the time of alcohol consumption was calculated in two groups: less than 20 years and more than 20 years.

Oral mucosa swabs were taken once from the subjects on the buccal mucosa. The swabs were performed with sterile plastic tips, placed immediately in Eppendorf microtubes containing 500 µL of Krebs buffer, and the pellet obtained after 10 min of centrifugation at 9300 g was stored at -20ºC until processing. The study protocol was approved by the institutional Ethics Committee (ETIC104/05) and informed consent was obtained from all patients. DNA extraction was carried out as described by Boom et al. (21) and modified as below.

The insertion/deletion in the *5HTT* gene-linked polymorphic region (5-HTTLPR) was assessed only by polymerase chain reaction (PCR) amplification. The sequences of PCR primers were 5'-CCGCTCTGAATGCCAGCACCTAAC-3` and 5'-AGAGGGACTGAGCTGGACAACCAC-3`. PCR was carried out in a 50 µL mixture containing 6 µL of DNA solution (~ 80 ng), 1 unit/reaction of Taq DNA polymerase (Phoneutria Biotecnologia, Belo Horizonte, MG, Brazil), Special PCR buffer (Phoneutria Biotecnologia), deoxynucleoside triphosphates (0.1 mM/reaction of each dNTP) (Amersham Biosciences Piscataway, NJ, USA), and primers (20 pmol/reaction). This solution was subjected to 2 min at 94ºC, followed by 40 cycles of 30 s at 94 ºC, 30 s at 68 ºC and 45 s at 72 ºC. The run was terminated by final elongation at 72ºC for 5 min. Lid temperature was 103ºC. All samples were amplified using a DNA thermal cycler (Programmable Thermal Controller, PTC, Hamburg, Germany). Allele sizes were determined by comparison of bands with size standards after electrophoresis in a 6.5% polyacrylamide gel and silver staining. Amplification of the 5-HTTLPR gave two alleles differing by 44 bp (L with 522 bp and S with 478 bp) (7).

Statistical Analysis

Statistical significance of differences between case and control group distributions for alleles and genotypes were determined using chi-squared with Yates's correction. A significance p value ≤ 0.05 was used.

The evaluation of the Hardy-Weinberg equilibrium was performed by comparing observed and expected frequencies of heterozygotes and homozygotes. All statistical analyses were performed using BioStat 3.0 software (Optical Digital Optical Technology, Belém, PA, Brazil).

Table 1. Distribution of the genotypes in the groups.

Group	Genotypes			Total n (%)	p value
	l/s n (%)	l/l n (%)	s/s n (%)		
Cases	53 (51.5)	31 (30.1)	19 (18.4)	103 (100.0)	0.408
Controls	44 (42.7)	34 (33.0)	25 (24.3)	103 (100.0)	

Pearson's chi-square: the p values are result of the comparison with the control.

RESULTS

The genotype frequency of 5-HTTLPR polymorphism is shown in Table 1. The frequency of the genotype in the group of OSCC and control was not statistically different (p=0.408). The observed distribution of 5-HTTLPR genotype in the case group (LL:LS:SS; 31:53:19) was not statistically different from those (30:51:21) expected from the Hardy-Weinberg equilibrium equation (p= 0.66). No statistically significant differences (p>0.05) were identified between genotype and allele distributions amongst cases and controls in relation to smokers and non-smokers, alcohol consumers and non-consumers (data not shown), and duration of such habits (Tables 2 and 3).

DISCUSSION

Tobacco smoking and alcohol consumption are the two most strongly implicated risk factors for development of OSCC (13,20). Smoking is a complex phenotype that may be influenced by serotonergic mechanisms. Previous studies have demonstrated association between 5-HTTLPR polymorphism with smoking (8). In one study the L/L + L/S genotypes was associated with smoking (22), but this evidence was not confirmed by others (23). A putative role of the 5-HTTLPR polymorphism for alcoholism has also been demonstrated in the literature (24,25).

Patients with OSCC have the profile of smokers and heavy drinkers (13,20). The control group was matched with the OSCC group and therefore also showed a profile of smoking and alcohol consumption. In two groups there was no statistically significant difference between 5-HTTLPR genotypes. The results suggest that smoking and drinking habits of OSCC patients are not determined by a change in the 5-HTTLPR polymorphism.

The involvement of 5-HTTLPR polymorphism with smoking and alcoholism is still controversial and suggests that other factors may be involved in determining such behavior.

In the present study association between 5-HTTLPR polymorphism and the risk behavior for OSCC was not observed. Therefore, it could be suggested that serotonin transport imbalance may not be important to the disease development. In addition, the use of tobacco or alcohol was not related to the serotonin transporter gene polymorphism, which indicates that these habits were not influenced by this genotype.

In conclusion, based on the obtained results, the present investigation demonstrated that serotonin transporter polymorphisms are not implicated in the OSSC development.

Table 2. Frequency of genotypes in drinkers.

Group	Genotype	Time of alcohol use		p value
		<20 years N (%)	>20 years N (%)	
Cases	l/s	22 (51.2)	31 (51.7)	0.490
	l/l	11 (25.6)	20 (33.3)	
	s/s	10 (23.3)	9 (15.0)	
	Total	43 (100.0)	60 (100.0)	
Controls	l/s	29 (42.6)	15 (42.9)	0.698
	l/l	24 (35.3)	10 (28.6)	
	s/s	15 (22.1)	10 (28.6)	
	Total	68 (100.8)	35 (100.0)	

Pearson's chi-square: the p values are result to the comparison with the control.

Table 3. Frequency of genotypes in smokers.

Group	Genotype	Time of tobacco use		p value
		<34 years N (%)	>34 years N (%)	
Cases	l/s	21 (42.9)	32 (59.3)	0.097
	l/l	15 (30.6)	16 (29.6)	
	s/s	13 (26.5)	6 (11.1)	
	Total	49 (100.0)	54 (100.0)	
Controls	l/s	23 (39.7)	21 (46.7)	0.703
	l/l	21 (36.2)	13 (28.9)	
	s/s	14 (24.1)	11 (24.4)	
	total	58 (100.0)	45 (100.0)	

Pearson's chi-square: the p values are result to the comparison with the control.

RESUMO

Consideráveis evidências indicam que mecanismos serotoninérgicos, particularmente o transportador de serotonina, estão envolvidos no alcoolismo e no uso de fumo e são influenciados pelo polimorfismo da região promotora do 5HTT (5-HTTLPR). Como o consumo de álcool e fumo está implicado na patogênese do câncer, o objetivo deste estudo foi investigar o polimorfismo 5-HTTLPR em pacientes com carcinoma bucal de células escamosas (CBCE) comparado com um grupo controle em uma amostra de pacientes brasileiros. Cento e três pacientes afetados por CBCE e 103 voluntários sem história de CBCE foram genotipados para 5-HTTLPR. Ambos os grupos foram pareados pela idade, gênero e uso de fumo. O teste do qui-quadrado foi usado para análise estatística. Não houve diferença estatística entre os genótipos dos grupos caso e controle (p= 0,408). Concluindo, a presente investigação demonstrou que os polimorfismos do transportador de serotonina não estão implicados no desenvolvimento do CBCE.

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