Original Article

GSTM1 AND GSTT1 GENES ANALYSIS IN HEAD AND NECK CANCER PATIENTS

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ABSTRACT

Objective. To establish the clinical and demographic profile and identify risk factors among patients with head and neck cancer, relating them to the polymorphism of GSTT1 and GSTM1.

Methods. One hundred patients with head and neck cancer and 100 control group individuals without history of neoplasm were analyzed. The molecular analysis was made by multiplex polymerase chain reaction. For statistical analysis, data were tabulated and compared by the Fisher's exact test, the Chi-square test and multiple logistic regression were also used.

Results. There was prevalence of smokers (OR = 5.32, CI 95% CI = 2.04-13.86 p = 0.0006), alcohol drinkers (OR = 5.04, CI 95% = 2.19-11.59 p = 0.0001) in head and neck cancer patients. The GSTT1 null genotype was found in 47% of the patient and 41% of the control group (OR = 0.67; CI 95%= 0.34-1.35; p = 0.2648). Likewise, the GSTM1 null genotype was found in 66% of the patient and 75% of the control group (OR = 2.25; CI 95%= 1.05 - 4.84; p = 0.0368). The combined GSTT1 and GSTM1 gene null genotype shown association between GSTM1*0/GSTT1 and occurrence of head and neck carcinoma (OR = 7.64; CI 95%= 1.72-34.04; p = 0.0076). Analysis of clinical-pathological features showed association between GSTT1 null genotype and larynx, the inverse relation between this genotype and pharynx.

Conclusion. In our study it was possible to establish an association between the nullity of GSTM1 genotype and that of combined GSTT1/GSTM1*O ([]/[-] genotypes and head and neck cancer.

Key words: Head and neck neoplasias. Glutathione transferase. Tobacco. Alcoholic drinks.

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Introduction

Head and neck cancer (HNC) is a term that designates malignant neoplasias occurring in upper aerodigestive tract, that is, deriving from the oral cavity epithelium, pharynx, larynx, whose incidence of occurrence correspond, respectively, to 40%, 15% and 25%.[1-3]. The most common histological type is the squamous cells or spinocellular carcinoma occurring in 90% of the patients.[3,4] This type of cancer is the fifth more common type in the world and is associated with low life expectancy rates and high morbidity rates when diagnosed in advanced stages.[3]

In Brazil, according to the Instituto Nacional do Câncer (National Institute of Cancer - INCA), the estimation for oral cavity cancer for the year 2010 is 13,250 new cases for male sex and 4,880 in women, in a total amount of 18,130 new cases.[5].

Various factors are related with the development of head and neck cancer, but smoking and alcohol drinking are considered independent risk factors.[2,3,6,7] When associated, they have a synergistic effect, increasing the risk of developing cancer in this anatomical region in even 15 times.[3,8,9] There is a higher incidence among male individuals, but an increase in its occurrence among women is evidenced due to the increase in alcohol and tobacco consumption.[3]

Tobacco is constituted by carcinogenic agents, with

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N-nitrosamines and aromatic amines, which, interacting with genetic material, may result in the formation of DNA adducts, which favor cellular mutations and reactive hyperplasia in the mucosa of upper aerodigestive tract. Alcohol is not genotoxic, but it might act in synergy with tobacco, suppressing the removal of nitrosamines in inhibiting the various isoforms of the cytochrome P450 superfamily (*CYP*s), enzymes involved in the cellular detoxification process, increasing adducts formation.[9-11].

Two groups of enzymes are involved in the process of biometabolization of chemical compounds of the tobacco and alcohol: the enzymes of oxidative metabolism (Phase I) and conjugating enzymes (Phase II). Phase I oxidative enzymes, mainly the families pertaining to the cytochrome *P-450* superfamily (*CYP*s), convert many compounds into highly reactive metabolites. On the other hand, Phase II enzymes act deactivating Phase I products, making metabolites hydrophilic and liable to excretion as a result of their conjugation with the endogenous substrate (glutathione, sulphate, glucose, acetate) by means of the action of glutathione S-transferases (*GST*s), UDP-glucuronyl transferases and N-acetyltransferases.(*NAT*s) [12-17].

The glutathione S-transferases (*GST*s) are an important family of enzymes involved in the biosynthesis and metabolism of many substances, including detoxification of exogenous chemical carcinogens, such as aromatic polycyclic hydrocarbons present in the tobacco. They comprise four classes of genes (α, μ, π, and θ) and each class, on their turn, include various genes.[18] Polymorphisms in genes that codify the *GSTM1* and *GSTT1* may alter their expression or function and result in activation or detoxification of carcinogenic compounds.[12-16].

The *GSTM1* gene (1p13.1chromosome) is polymorphic and 20% to 50% of the individuals did not express the enzyme due to a genic deletion in homozygosis (*GSTM1**0) [19]; this frequency is higher in Caucasoids and Asians than in Africans.[20] The *GSTT1* gene (22q11.2 chromosome) is equally polymorphic, presenting null genotype by deletion (*GSTT1**0) and consequent complete loss of enzymatic activity. The frequency of individuals who do not express the enzyme is higher in Asians (60%) and Africans (40%) than in Caucasoids (20%).[21, 22]

The polymorphisms of the GSTM1 and GSTT1 genes involved may result in differences in the enzymatic activity, possibly favoring mechanisms 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 the null genotype of *GSTM1*,[7, 21, 23-25] while others do not.[26-30] The same occurs with the null genotype of *GSTT1*, showing an association with the disease[7,23,25] or not.[26,27,30-32] This way, the development of head and neck cancer is the result of the interaction between environmental factors and genetic heritage.[24,33]

Therefore, the objectives of this work were to establish the clinical, sociodemographic profile and identify risk factors (smoking and alcohol drinking) of head and neck cancer patients.

Methods

This study was approved by the Ethics Committee of the teaching institution SISNEP-0976.0.140.000-05. Participants were included after the obtention of the Informed Consent Form and submitted to a standardized questionnaire, information is maintained under secrecy.

In this case-control study individuals who did not present a family history of cancer and did not possess a personal background of cancer were evaluated. Thus, 100 patients were evaluated in out-patient follow up in the Otorhinolaryngology and Head and Neck Surgery service in a university hospital in the North-West of the São Paulo State, who received the histopathological diagnosis of head and neck spinocellular carcinoma, regardless of clinical staging. All the samples of peripheral blood were collected before the beginning of the treatment. The control group includes 100 individuals from other services offered by the same university hospital.

Varieties analyzed were age, gender, and exposition to risk factors (smoking and alcohol). Individuals who consumed more than 100 cigarettes (commercial or handmade) for the whole life were classified as smokers, and the patients who drank more than four alcoholic drinks (distilled or fermented) per week during six months or more, according to Ahrendt et al., 2000.[34]

Clinical data referring to the primary site of the tumor and clinical staging were classified according to the *Union Internationale Contrele Cancer* (UICC), 2002 and the *American Joint Commitee for Cancer* (AJCC), 1997.[35]

The genomic DNA was obtained through peripheral blood and the amplification by PCR performed, according to Miller et al.[36] The product of PCR was submitted to electrophoresis in 1.5% agarose gel, colored with ethidium bromide, in which *GSTT1* is observed as a fragment of 480 base pairs (bp), *GSTM1* with 219 bp and the *CYP1A1* with 312 pb. A sequence of the exon 7 of the *CYP1A1* gene was used as internal control of amplification.

Demographic data and those pertaining to genotypic distribution of the polymorphisms were tabled and compared by the Fisher's exact test and the Chi square test. The model of logistic regression was used to determine the effect of the dependent variables in the head and neck cancer and to group the clinical-pathological characteristics as dependent variables. The results presented in *odds ratio* (OR) and 95% confidence interval (IC - 95%). The level of significance was established in 5% (p=0.05).

Results

Data pertaining to the sociodemographic profile (gender and identity) and exposition to risk factors (smoking and alcohol drinking habits) were analyzed. In this study male individuals were dominant in the group of patients, with mean age of 58.46 years; the control group is represented by 68% of male individuals and mean age of 55.32 years. Age median is equal to 56 years (Table 1).

The two main habits associated to HNC, smoking and alcohol drinking, were both expressively more frequent in the group of individuals with neoplasia (Table 1). The frequency

of genetic polymorphisms are equally presented in Table 1.

Data of the multivariate analysis model (Table 1) show that smoking [OR: 5.32; IC 95% (2.04-13.86); p=0.0006], and drinking [OR: 5.04; IC 95% (2.19-11.59); p=0.0001] and the null genotype *GSTM1* [*GSTM1*0*] [OR: 2.25; IC 95% (1.05-4.84); p=0.0368] are predicting factors for head and neck cancer.

Table 2 presents the results of the combined genotypes analysis, in which *GSTT1/GSTM1**0 ([ ] / [-]) presents an increase for the risk of head and neck cancer [OR= 7.64; IC 95% (1.72-34.04); p= 0.0076].

In relation to the localization of the neoplasia, primary anatomical sites occurred with the frequency of 35% in the oral cavity, 26% in the pharynx, 36% localized in the larynx, and in 3% it was possible to identify the primary site of the tumor.

The analysis of the clinical parameters and polymorphisms showed association between the *GSTT1* null genotype with its occurrence in the larynx [OR= 5.33; IC 95% (1.99-14.36); p=0.0009] and inverse relation in the pharynx [OR= 0.32; IC 95% (0.12-0.89); p=0.0282]. For the other anatomic sites studied, besides the tumor extension (parameter T), lymphonodal commitment (parameter N) and occurrence of metastases (parameter M), no associations could be identified.

Table 1 - Percentage distribution of demographic data and those of the genes involved in head and neck cancer patients and individuals with no history of neoplasia

Variables	Patients %	Control %	OR (IC 95%)	p
Age				
Median				
< 56 years	63	62	Reference	
≥ 56 years	37	38	4 (1,97-8,13)	0,0001
Gender				
Male	87	68	Reference	
Female	13	32	0,90 (0,35-2,37)	0,8406
Smoking				
No	08	48	Reference	
Yes	92	52	5,32 (2,04-13,86)	0,0006
Alcohol drinking	18	56	Reference	
No	82	44	5,04 (2,19-11,59)	0,0001
Yes				
GSTT1 Gene				
Positive	53	59	Reference	
Negative	47	41	0,67 (0,34-1,35)	0,2648
GSTM1 Gene				
Positive	34	25	Reference	
Negative	66	75	2,25 (1,05-4,84)	0,0368

Table 2 - Frequency of combined genotypes in head and neck cancer patients and in the control group

Genotypes	Patients	Control	OR (IC)	p
GSTM1 [+]/GSTT1 [+]	28	35	Reference	
GSTM1 [-]/GSTT1 [-]	14	19	1,52 (0,42-5,58)	0,5214
GSTM1 [+]/GSTT1 [-]	38	40	1,03 (0,46-2,34)	0,9396
GSTM1 [-]/GSTT1 [+]	20	6	7,64 (1,72-34,04	0,0076

Discussion

This study confirms the fact that the development of head and neck cancer is related to some habits, as smoking and alcohol drinking. Epidemiological studies show that the exposition to tobacco and alcohol are the strongest risk factors for the disease.[18,37]

In this study we have evidenced an increased frequency of the *GSTM1* null genotype in patients with head and neck cancer, when compared to a group of individuals with no history of neoplasia. Results of meta-analysis demonstrate the association of the *GSTM1* null genotype and the increase of risk for the development of head and neck cancer.[38-41]

In a multivariate logistic analysis, we have evidenced that the *GSTT1/GSTM1**0 ([ ]/ [-]) combined genotype provides a greater susceptibility for the development of spinocellular carcinoma of head and neck, similar results to those found by other authors. [7,23,42,43]

Various studies could not correlate the null *GSTT1* genotype with the head and neck cancer,[26,27,43] which was a result found in our study. Similarly, there was no association between this type of neoplasia and the null combined genotypes null *GSTT1* and null *GSTM1*, as observed by Singh et al.[7] On the other hand, a Chinese study has shown a correlation between the double null genotype *GSTT1 and GSTM1* with nasopharynx cancer among men.[44]

With respect to the relation between genes deletion and specific anatomic sites, Duarte et al.[25] found an association between the *GSTM1* null genotype and the increase in risk of development of leukoplasia in the oral cavity. On the other hand, in our study we have identified a relation between the occurrence of larynx carcinoma with *GSTT1* null genotype and, on the contrary, a protective effect of this genotype in the pharynx. These data are due to embryological, histological, and molecular differences between anatomic regions, resulting from then the peculiar biologic behavior of this type of tumor in the various anatomic localizations. [45,46]

Histopathological parameters with tumor extension, invasion of lymphonodes and metastases were evaluated, but they remain controversial in the literature, because there is a relation in some studies, such as Mathias et al.[47] who revealed the association between *GSTT1* null genotype with the occurrence of T3/T4 tumors and the absence of lymphonodes; while Losi-Guembarovski et al.[32] did not find a statistically significant association, which was what occurred in our study.

Conclusion

In our study we were able to establish, by means of multivariate logistic analysis, the association of male gender, advanced age, smoking and alcohol habits with the occurrence of head and neck cancer, as well as the *GSTM1 genotype's nullity;* there was a higher occurrence in oral cavity and

association between the occurrence of this tumor in the larynx with the null genotype.

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