Brazilian Dental Science

unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.4322/bds.2022.e2956

Influence of nicotine dependence, current smoking status and gender on *CYP1A1, CYP1B1* and *CYP2A6* expression in oral squamous cell carcinoma

Influência da dependência nicotínica, parâmetros de consumo de tabaco e gênero na expressão de CYP1A1, CYP1B1 e CYP2A6 em carcinoma de células escamosas oral

Adriana Ávila ALMEIDA[1], Celso Muller BANDEIRA[1,2], Mônica Ghislaine Oliveira ALVES[1,3], Maria Beatriz Nogueira PASCOAL[4,5], José Francisco Salles CHAGAS[4], Morun Bernardino NETO[6], Patrícia Pimentel de BARROS[1], Fábio Daumas NUNES[7], Celina Faig Lima CARTA[1], Janete Dias ALMEIDA[1]

1 - Departamento de Biociências e Diagnóstico Bucal, Instituto de Ciência e Tecnologia de São José dos Campos, Universidade Estadual Paulista, São José dos Campos, SP, Brasil.
2 - Faculdade de Ciências Médicas de São José dos Campos – Humanitas, São José dos Campos, SP, Brasil.
3 - Faculdade de Medicina da Universidade Anhembi/ Morumbi, São José dos Campos, SP, Brasil.
4 - Departamento de Cirurgia de Cabeça e Pescoço, Faculdade São Leopoldo Mandic, Campinas, SP, Brasil.
5 - Departamento de Cirurgia de Cabeça e Pescoço do Hospital Municipal Dr. Mário Gatti, Campinas, SP, Brasil.
6 - Departamento de Ciências Básicas e Meio Ambiente, Universidade de São Paulo, São Paulo, SP, Brasil.
7 - Departamento de Patologia Oral da Faculdade de Odontologia da Universidade de São Paulo, São Paulo, SP, Brasil.

ABSTRACT

Objective: Tobacco smoke is composed of cancer-causing chemicals referred to as carcinogens. These carcinogens are metabolized by the enzymes of the cytochrome P450 (CYP) family. Our objective was to evaluate the correlation of tobacco consumption parameters with *CYP1A1, CYP1B1* and *CYP2A6* expression using qRT-PCR in samples of oral squamous cell carcinoma (OSCC). **Material and Methods:** The sample was divided into 2 groups: Cancer (36 subjects) and non-Cancer (12 subjects). The smokers' participants (36) were evaluated regarding their Nicotine dependence (ND) was assessed by the Fagerström test for cigarette dependence (FTCD). Questions regarding tobacco consumption like the number of cigarettes/day (CPD), duration of use, and pack-years were also evaluated. The Mann-Whitney and Spearman correlation tests were used at a significance level of 5%. **Results**: 48 participants were included, 32 men (66.7%), 36 smokers (75%) and 27 smokers with OSCC (56.3%). Samples of OSCC expressed more *CYP1A1*, *CYP1B1*, and *CYP2A6*. Especially, the *CYP1B1* gene was significantly expressed in OSCC samples, regardless gender or tobacco use. No women expressed *CYP2A6*, as well as, non-smokers did not express the *CYP1A1* and *CYP2A6* genes. *CYP1A1* gene was higher among men ($P = 0.021$). **Conclusion**: Lack of exposure to tobacco may justify the absence of *CYP1A1* and *CYP2A6* expression in non-smokers. The *CYP1B1* gene was significantly expressed in the cancer presence despite gender or tobacco use. The assessment of ND and quantification of tobacco consumption are important instruments in monitoring smokers with benign oral lesions and, especially, in the presence of cancer.

KEYWORDS

CYP1A1; CYP1B1; CYP2A6; Carcinoma; Squamous cell; Tobacco use disorder.

RESUMO

Objetivo: A fumaça do tabaco é composta de substâncias químicas cancerígenas conhecidas como carcinógenos. Esses carcinógenos são metabolizados pelas enzimas da família do citocromo P450 (CYP). Nosso objetivo foi avaliar a correlação dos parâmetros do consumo de tabaco com a expressão de CYP1A1, CYP1B1 e CYP2A6 por qRT-PCR em amostras de carcinoma de células escamosas bucal (CCEB). **Material e Métodos:** A amostra foi dividida em 2 grupos: Câncer (36 indivíduos) e sem Câncer (12 indivíduos). Os participantes fumantes (36) foram

avaliados quanto à dependência nicotínica (DN) pelo teste de Fagerström para dependência de cigarro (TFDC). Questões relacionadas ao consumo de tabaco como número de cigarros / dia (CPD), tempo de uso e anos-maço também foram avaliadas. Os testes de correlação de Mann-Whitney e Spearman foram utilizados com nível de significância de 5%. **Resultados:** foram incluídos 48 participantes, 32 homens (66,7%), 36 fumantes (75%) e 27 fumantes com CCEB (56,3%). Amostras de CCEB expressaram mais CYP1A1, CYP1B1 e CYP2A6. Especialmente, o gene CYP1B1 foi significativamente expresso em amostras de CCEB, apesar do sexo ou uso de tabaco. Nenhuma mulher expressou CYP2A6, assim como, não fumantes não expressaram os genes CYP1A1 e CYP2A6. O gene CYP1A1 foi maior entre os homens (P = 0,021). **Conclusão:** A falta de exposição pode justificar a ausência da expressão dos genes CYP1A1 e CYP2A6 entre não fumantes. O gene CYP1B1 foi significativamente expresso na presença de câncer, independentemente do sexo ou do uso de tabaco. A avaliação da DN e a quantificação do consumo de tabaco são importantes instrumentos no acompanhamento de fumantes com lesões bucais benignas e, principalmente, na presença de câncer.

PALAVRAS-CHAVE

CYP1A1; *CYP1B1*; *CYP2A6*; Carcinoma; Células escamosas; Transtorno por uso de tabaco.

INTRODUCTION

Smoking is the leading cause of preventable death worldwide, with devastating consequences for health, the environment, and the world economy, as well as being responsible for an increasing number of premature deaths, disability, and loss of life for active smokers, as well as passive smokers or ex-smokers [1]. At least twelve types of cancer are related to tobacco use, including head and neck cancer, e.g., oral, pharynx and larynx. Squamous cell carcinoma (SCC) is the histological type found in more than 90% of cases of oral cases [2,3].

The greater dependence on tobacco, combined with the physical and psychological discomfort caused by cancer treatment can intensify withdrawal symptoms, and decrease intention to quit. However, stop smoking decreases the chances of tumor recurrence, the manifestation of a second primary tumor, and treatment complications, and increases patient survival and quality of life [4–8]. Due to these characteristics, intensive and specialized support needs to be aligned with oncologic treatment, during and after, in intent to the abstinence. In order to achieve best outcomes, basic aspects of the smoking profile such as record the amount cigarettes, time of use and the degree of nicotine dependence, also allow to deduce the amount of tobacco carcinogens exposure, guiding the best strategy during the cessation process [4,9].

Tobacco carcinogens need to be activated in an oxidative phase called Phase I [10]. The main groups of carcinogens derived from tobacco are polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aromatic amines and all these components are metabolized by the enzymes of the cytochrome P450 family [10,11]. This enzyme family is responsible for 66% of the metabolism and bioactivation of carcinogens. Six enzymes, CYP *1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1,* and *CYP3A4* account for 77% of all metabolisms in this group [10].

CYP1A1 and *CYP1B1* enzymes are responsible for the metabolism of PAHs, nitrosamines, including those specific for tobacco, estrogen, and some chemotherapeutic agents [10]. The *CYP2A6* gene encodes an enzyme responsible for the metabolism of almost all nicotine, as well as nitrosamines and has become an important marker for studies of nicotine dependence [12].

The objective of this work was to evaluate the expression of Phase I genes *CYP1A1*, *CYP1B1* and *CYP2A6* in samples of oral squamous cell carcinoma and their correlation with tobacco consumption parameters.

MATERIAL AND METHODS

Sample selection

The present study was approved by the Ethics Committee (Protocol No. 1.033.312/2015). All participants signed the informed consent. It was a cross-sectional and ecological design study which included subjects older than 18 years, smokers, or non-smokers, attended consecutively at the Head and Neck Surgery Service of Hospital Municipal José Carvalho de Florence in the city of São José dos Campos/SP, Hospital and Maternity Celso Pierro of the

Pontifical Catholic University of Campinas/SP (PUC-Campinas) and Hospital Municipal Mario Gatti from Campinas/SP. All participants were diagnosed with oral squamous cell carcinoma (OSCC). Benign samples were collected at the clinic of Oral Diagnosis from the Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, Unesp from the gingiva, the floor of the mouth, tongue, palate, retromolar and buccal area.

The diagnosis followed standard pathological features findings. Patients with a history of any type of cancer treatment, whether surgery, radio or chemotherapy in any organ or system, as well as cases of lip cancer, were not included.

Nicotine dependence and smoking profile evaluation

The questions regarding tobacco use status included the age of initial regular use, number of cigarettes smoked per day (CPD), length of smoking (years) and pack-years,

The evaluation of nicotinic dependence was performed using the Fagerström Test for Cigarette Dependence (FTCD). This questionnaire consists of six questions scored according to the answers into one of five categories: very low (0 to 2 points), low (3 to 4 points), moderate (5 points), high (6 to 7 points) and very high (8 to 10 points). The cutoff for nicotine dependence (ND) was ≥ 4 points [13].

RNA extraction and analysis

The samples were stored in Allprotect tissue reagent solution (Qiagen, CA, USA) overnight in Eppendorff flask at 4 ° C, and after this period at -80 ° C.

Total RNA was extracted from all samples. It was used a Trizol kit (Ambion, Inc., Carlsbad, CA, USA) as recommended by the manufacturer. The concentration, purity, and quality of the RNA were verified using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Total extracted RNA (1 μg) was treated with DNase I (Turbo DNase Treatment and Removal Reagents - Ambion Inc., Carlsbad, CA, USA) and transcribed into complementary DNA (cDNA) using the SuperScript1 III First-Strand Synthesis SuperMix for qPCR Kit (InvitrogenTM, Carlsbad, CA, USA) according to the protocol recommended by the manufacturer. Initially, three reference genes were tested, ACTB (beta actin), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and TUBA1C (tubulin, alpha 1c), but at the end, the TUB1AC with greater compatibility by the sample was selected, as the efficiency of amplification (E) of the target and reference genes chosen should be approximately equal. The E can ideally vary between 90% and 100% (-3.6> slope> -3.1). This was determined using the equation: E = (10 –1/slope –1) Å~ 100.

Samples were evaluated by adding 1 microliter (μL) of RNA to measure absorbance at 260 (A260) and 280 (A280) nm. The quality of the RNA was analyzed by means of the A260/A280, and A260/A230 ratios, and concerning the A260/A280 ratio values obtained were between 1.8 and 2.0, and for A260/A230 values close to 1.2.

The sequences of the primers were confirmed on the NCBI / Gene Bank website, which was specific for the Homo sapiens species and homology. The primers chosen were *CYP1A1* (Forward - CTTCCGACACTCTTCCTTCG, Reverse - GGTTGATCTGCCACTGGTTT), *CYP1B1* (Forward - CAGGCAGAATTGGATCAGGT, Reverse - CATAAAGGAAGGCCAGGACA), *CYP2A6* (Forward - GAGACGTGATCCCCATGAGT, Reverse - GGTACACTTCGGTGCCCTTA), and *TUBA1c* (Forward - CCGGGCAGTGTTTGTAGACT, Reverse - TTGCCTGTGATGAGTTGCTC). Tests of efficiency of the primers and standardization of the amount of cDNA, concentration and annealing temperature were performed. All primers showed efficiency between 95 and 154%.

The qPCR method was applied to evaluate the amount of cDNA product in the exponential phase of the amplification reaction. The SYBR1 Green fluorophore reagent (Platinum1 SYBR1 Green qPCR SuperMix-UDG Applied Biosystems, Framingham, MA, USA) was used in the StepOnePlus ™ System (Applied Biosystems, Framingham, MA, USA), and the amplification conditions were: 50° C per 2 min, followed by 95 ° C for 2 min and over 40 cycles of 95 ° C for 15 s, followed by 30 s at 60 ° C. The analyses of the relative changes in gene expression were made using the $2^{-\Delta\Delta CT}$ methods.

Statistical analysis

Calculations of the real power of the Mann-Whitney comparison tests are made using the

sample size of each group, the difference between the mean values of the groups and the standard deviation. Thus, to estimate the power of the tests, graphs were obtained that weighted all the variants for each of the genes, and the power of the test for the CYP1A1 gene is between 53.0% and 80.4%, for the CYP1B1 gene it is between 94.9% and 99.9% and for the CYP2A6 gene it is between 56.33% and 83.60%.

For statistical analysis, the sample was divided regarding tobacco use into smokers and non-smokers, and cancer stages. The cancer samples were classified based on the 8th TNM classification (UICC/AJCC), into Initial (Stages I and II) and advanced stages (Stages III and IV). The normality of the data was evaluated by the D'Agostino Pearson test. The non-parametric data was represented by the median and interquartile rate (IQR), while parametric by mean and standard deviation (SD). Comparisons of gene expression between the groups were performed by the Mann-Whitney test. Spearman's correlation test was used for the correlation of gene expression with general data of the sample. A significance level of 5% was used for all tests. The GraphPad Prism software version 7.0, 2016 was used to analyze the data.

RESULTS

It was included 32 men (67%) and 16 women (33%). The mean age was 55 ± 14.5 years. Thirty-six participants were smokers, 28 men and 8 women. The demographic characteristics of the participants and the information regarding the smoking profile are described in Table 1. The analysis by the Mann-Whitney test has demonstrated that the expression of the *CYP1A1* gene was higher among smokers (P = 0.002), compared to non-smokers, and in men (P = 0.021), compared to women. No women expressed the CYP2A6 gene. Both genes were not expressed in nonsmokers. We found no significant difference in the expression of *CYP1B1* between men and women (P = 0.325), as between smokers and nonsmokers (P = 0.382).

A matrix of correlations (ρ) between the variables studied for the entire sample is given in Table 2. It was found significant correlations between the presence of OSCC with

Table 1 - Baseline characteristics of the study population

	Male	Female	p-value*
Participants n (%)	32 (67)	16 (33)	
Age, years (median ± IQR)	58±10.25	49.5±23.5	0.015
Cancer n (%)	24 (50)	9 (18.75)	0.161
Smokers n (%)	28 (58)	8 (16.7)	0.010
Age, years (median ± IQR)	57.5±10.75	61±17.75	0.379
Smoking initiation age	16±4.5	16±14.5	0.259
Cigarettes per day (CPD) (median ± IQR)	30±10	20±17	0.029
< 20	3	3	
20 – 40	22	5	
> 40	3	-	
Duration of smoking in years (median ± IQR)	40±18.5	27±15.25	0.024
< 20	2	2	
20 – 40	13	5	
> 40	13	1	
Pack-years (median ± IQR)	49±32.25	20.5±38.88	0.015
< 20	2	4	
20 – 40	6	2	
> 40	20	2	
Fagerström Test	6±2.25	3.5±4.25	0.015
< 4 points	4	4	
≥ 4 points	24	4	

*Statistically significant differences (P <0.05) were evaluated by Mann–Whitney test.

Table 2 - Matrix of correlations (ρ) between the variables studied for the entire sample

	Age	Gender	SS	CPD	Duration	FTCD	CANCER	CYP1A1	CYP1B1	CYP2A6
Age										
Gender	0,316*									
[a] SS	0,325*	0,408**								
[b] CPD	0,262	0,471**	0,766**							
Duration	0,607	0,476**	0,756**	0,606**						
[c] FTCD	0,199	0,499**	0,740**	0,739**	0,645**					
CANCER	0,198	0,191	0,234	0,098	0,229	0,227				
CYP1A1	0,058	0,287*	0,414**	0,057	0,285#	0,196	0,309*			
CYP1B1	0,178	-0,068	-0,046	-0,078	-0,033	-0,003	0,522	0,209		
CYP2A6	0,244	0,445**	0,363*	0,204	0,500**	0,253	0,333*	0,324*	0,275#	

Spearman's correlation test analysis. SS: Smoking Status. CPD: Cigarettes Per Day. FTCD: Fagerström Test for Cigarettes Dependence. * P<0.05. ** P<0.01 indicating statistically significant correlations. # Borderline correlations (0.05 < P< 0.10).

CYP1A1 (P = 0.032) and *CYP2A6* expression (P = 0.021). The *CYP1B1* gene expression was correlated with the presence of OSCC for men (P = 0.011) and women (P = 0.002), as well as for smokers (P = 0.002) and nonsmokers (P = 0.022). Among men, *CYP2A6* expression was significant correlated to the duration of cigarette use (P<0.001), as well as with the expression of *CYP1A1* (P = 0.025) and *CYP1B1* (P = 0.006).

Thirty-three participants had OSCC (24 men and 9 women), among which 27 were smokers. Nineteen men and 3 women had ≥ 4 points at the FTCD. Smokers with cancer showed significant differences related to *CYP1A1* (P = 0.045), *CYP1B1* (P = 0.001) and *CYP2A6* (P = 0.026) expression, compared to smokers without cancer. However, we found no differences regarding the number of cigarettes smoked per day (0.185), duration of smoking (P = 0.285), pack-years (P = 0.268) and FTCD (P = 0.259).

Considering the stages analysis, we observed higher ND between individuals in advanced stages compared to participants at the early stages (P = 0.002). Participants in early stages had strongly correlation among CYP2A6 expression and the number of cigarettes smoked per day (P = 0.005), duration of smoking (P = 0.005) and FTCD (P = 0.014).

DISCUSSION

Phase I genes, *CYP1A1, CYP1B1,* and *CYP2A6* were more expressed in the samples of OSCC. *CYP1A1* gene was higher among men and *CYP2A6* was not expressed in women. Both genes were not expressed in nonsmokers. The *CYP1B1* gene was significantly expressed in the cancer presence despite the gender or tobacco use.

The OSCC ranks seventh among the top ten types of cancer worldwide with more than 350,000 deaths annually [14]. Numerous carcinogenic compounds have already been identified in cigarette smoke capable of being absorbed on the first contact with the oral mucosa. The cytochrome P450 family of enzymes is mainly responsible for the biotransformation of these substances and some genes such as *CYP1A1* and *CYP1B1* have already been identified in several transformations and bioactivation networks of carcinogens as possible markers of tobacco-related malignancies [15,16].

Potentially carcinogenic substances from tobacco and alcohol are metabolized by Phase I enzymes into more reactive and soluble components, while the Phase II enzymes can detoxify and eliminate those intermediate elements [10]. The potential carcinogenic effects of tobacco derivatives also occur with the use of smokeless presentations, such as sniffing, sucking, or chewing form. Mallery et al. (2014) [17] demonstrated that Phase I and II enzymes are present in the healthy oral mucosa and may induce the onset of OSCC by activating the potentially carcinogenic components found in smokeless tobacco. Some genes such as *CYP1A1* and *CYP1B1* have been considered as possible markers of tobacco-related malignancies due to their presence in several networks of carcinogenic transformation and bioactivation [15].

The *CYP1A1*, *CYP1B1* and *CYP2A6* genes encode specific proteins that are also involved in the metabolism of endogenous substrates such as cholesterol, steroid, and exogenous synthesis as drugs, ethanol, and chemotherapeutics. Furthermore, its expression can be induced at extrahepatic sites according to the need for bioactivation of xenobiotics and their relation to the carcinogenic process remains controversial in some types of cancer [18,19].

The *CYP1A1* gene is responsible for the carcinogen's bioactivation found in tobacco, especially PAHs. Based on this point of view, it was expected that there would be an expression of this gene among smokers with OSCC. Due to this argument, it was plausible that it was not expressed at the non-smoker users [20,21].

The *CYP1B1* gene, as well as *CYP1A1*, is related to the metabolism of PAHs, in particular benzopyrenes. The study of this gene in smoking patients, therefore, is important due to its relation to tobacco use and its carcinogens activation [22]. In vitro studies have already demonstrated increased expression of *CYP1B1* in both SCC samples by immunohistochemistry technique and SCC cell cultures [23,24]. Since their function is to metabolize hormones such as estrogen, these genes are related to malignant neoplasms of the breast and endometrium [18]. We found an increased expression of *CYP1B1* in the OSCC group. Some studies have found similar results with a high expression of *CYP1B1* in SCC from other organs such as the lung and esophagus [25,26]. Additionally, we found a high correlation of *CYP1B1* expression with the cancer presence at all subgroups studied regarding gender and tobacco use, which seems to us a probable role as tumor marker as seen in other studies [27,28].

The *CYP2A6* gene has shown to be expressed in the respiratory tract epithelium from the trachea, indicating an important finding considering the possibility of carcinogenic effects and the development of lung cancer in smokers [19]. Although *CYP2A6* is related to nicotine metabolism and also nitrosamines and HAPs as well, the expression was not detected in woman at all, even the smokers. *CYP1A1* was not expressed in non-smokers for both gender which suggests that those specific enzyme production was not induced due the absence of tobacco compounds exposure [10]. On the other hand, it was strongly correlated to time of smoking, settling the statement that, at least for men, the cigarette smoke induces *CYP1A1* expression in oral lesions, principally in cancer sample.

The complex role of the tobacco compounds on the carcinogenesis process has been studied for a long time and processes enrolling multiple genes have been discovered. Based on this point of view, it was not a surprise that we had also found a synergic correlation between de *CYP1A1*, *CYP1B1,* and *CYP2A6* genes, mostly in men who also were the biggest tobacco consumers in our study. Furthermore, especially *CYP1A1* and *CYP2A6* expressions were related to the number of cigarettes smoked per day and length of smoking, even in the group without cancer. This result suggests that clinical and dental professionals, as well as oncologists, can use these practical clinical parameters to infer the gene expression and carcinogenic activity at the oral cavity in smokers without detectable malignant lesions.

The group of non-smoker patients with OSCC was composed mostly of young women in the early stages of the disease. These characteristics may be explained by the discomfort of an oral lesion discovery in people who never smoked and the quick search for health professional's evaluation, especially performed by women. Despite that this scenario should be the ideal pattern, oral cancer early diagnosis remains a challenge, not only in Brazil, but worldwide [29]. Best treatment outcomes can be influenced by the delay diagnoses that could, in turn be related to the lack of knowledge, fear or not concern of oral changes aspects [29,30]. Like the OSCC group, the control was composed by great tobacco consumers with behavior risk patterns and likelihood of cancer development in the future. This aspect lustrates the reality where educational alerts about the risk of oral diseases due to the use of tobacco as well as appropriate cessation intervention can change this panorama and expand the early diagnosis and proper treatment of oral cancer [30].

In our study, the FTCD was strongly correlated to *CYP2A6* expression in early stages of cancer in men indicating that, along with other simple tobacco profile parameters available like cigarettes per day (CPD) and pack-years, it is possible to predict the *CYP2A6* expression not only for the nicotine metabolism but also for carcinogenic compounds like specific tobacco nitrosamines, which is also substrate for this gene.

To the best of our knowledge, this is the first study to evaluate the main CYP genes expression in OSCC related to clinical aspects as well as tobacco consumption correlation. The complex process of carcinogenesis includes many steps and variables, but our study brings new research suggestions, such as the correlation of gene expression with the dosage of tobacco carcinogens in the saliva of patients with OSCC, as well as oxidative stress biomarkers that are able to estimate the role of each aspect in oral carcinogenesis.

Despite the sample size be considered a limitation, the carcinogenesis process is extremely complex and involves several steps, genes pathways, and complicated and expensive tests. However, the possibility of a correlation between clinical parameters easily assessed in the daily clinical routine together with a relatively simple method as qRT-PCR and the significant results obtained, we are aware that our study can contribute to better outcomes in the approach of individuals subject to important risk factors such as tobacco use. The various aspects raised in this study concerning the smoking profile may recommend changes in daily clinical practice care of patients with benign and malignant oral lesions. Simple information like ND, CPD, length of smoking and pack-years measurement can be useful evidence for patients that the changes caused by tobacco compounds at the oral mucosa are real and, in addition, can motivate them to a smoking quit attempt in near future.

This statement is based on the fact that, despite the smoking profile being similar between the groups, the expression of the genes *CYP1A1*, *CYP1B1,* and *CYP2A6* was the difference in participants with cancer. The strength of the correlation between *CYP1B1* expression and cancer, found mainly among smokers and non-smokers, might represent a probable marker of carcinogenic processes in benign lesions.

In conclusion, this study has illustrated that Phase I genes, *CYP1A1, CYP1B1,* and *CYP2A6,* were more expressed in OSCC samples. *CYP1A1* gene was higher among men and *CYP2A6* was not expressed in women, illustrating that although men and women may have similar tobacco consumption, in oral cancer they do not express tobacco carcinogens metabolizing genes in the same way. The absence of expression of *CYP1A1* and *CYP2A6* in nonsmokers may be justified by the lack of exposure to tobacco compounds. The *CYP1B1* gene was significantly expressed in the presence of cancer, regardless of gender or tobacco use. Smokers with OSCC are more dependent on cigarettes and certainly need help to quit smoking.

Acknowledgements

The authors would like to thank the Head and Neck Departments from the Celso Pierro Hospital from the Pontifícia Universidade Católica de Campinas, São Paulo and the Municipal Hospital José de Carvalho Florence in São José dos Campos, São Paulo for their valuable contribution for this study. We also thank Professor Flávio Peres and Prefeitura Municipal de São Sebastião, São Paulo for their support to this research.

Author Contributions

AAA: conception / design of the study, analysis / interpretation of data, drafting and revision of preliminary and final version. CMB: conception / design of the study, analysis / interpretation of data, drafting and revision of preliminary and final version. MGOA: data collection, data analysis / interpretation, approval of the final version. MBNP: data collection and approval of the final version. JFSC: data collection and approval of the final version. MBN: conception / design of the study, analysis / interpretation of data, drafting and revision of preliminary and final version. PPB: data collection, data analysis / interpretation, approval of the final version. FDN: conception / design of the study, analysis / interpretation of data, drafting and revision of preliminary and final version. CFLC: conception / design of the study, analysis / interpretation of data, drafting and revision of preliminary and final version. JDA: conception / design of the study, analysis / interpretation of data, drafting and revision of preliminary and final version.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

Funding

Funded by grant #2016/08633-0 from the Sao Paulo Research Foundation.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human

subject's oversight committee guidelines and policies of Head and Neck Departments from the Celso Pierro Hospital from the Pontifícia Universidade Católica de Campinas, São Paulo and the Municipal Hospital José de Carvalho Florence in São José dos Campos, São Paulo. The approval code for this study is (Protocol No. 1.033.312/2015).

REFERENCES

1. World Health Organization [Internet]. Cancer prevention. Geneva: WHO; 2020 [cited 2021 Apr 15]. Available from: https://www.who.int/cancer/prevention/en/
2. Winn DM, Lee Y-CC, Hashibe M, Boffetta P. The INHANCE consortium: toward a better understanding of the causes and mechanisms of head and neck cancer. Oral Dis. 2015;21(6):685-93. http://dx.doi.org/10.1111/odi.12342. PMid:25809224.
3. Ali J, Sabiha B, Jan HU, Haider SA, Khan AA, Ali SS. Genetic etiology of oral cancer. Oral Oncol. 2017;70:23-8. http://dx.doi.org/10.1016/j.oraloncology.2017.05.004. PMid:28622887.
4. Paul CL, Tzelepis F, Boyes AW, D'Este C, Sherwood E, Girgis A. Continued smoking after a cancer diagnosis: a longitudinal study of intentions and attempts to quit. J Cancer Surviv. 2019;13(5):687-94. http://dx.doi.org/10.1007/s11764-019-00787-5. PMid:31332721.
5. Morgan G, Schnoll RA, Alfano CM, Evans SE, Goldstein A, Ostroff J, et al. National Cancer Institute conference on treating tobacco dependence at cancer centers. J Oncol Pract. 2011;7(3):178-82. http://dx.doi.org/10.1200/JOP.2010.000175. PMid:21886500.
6. Cinciripini P. Smoking cessation in patients with cancer: treatment advances and the oncologist's role. J Natl Compr Cancer Netw. 2017;15(5S):748-50. http://dx.doi.org/10.6004/jnccn.2017.0091. PMid:28515262.
7. Fluharty M, Taylor AE, Grabski M, Munafò MR. The association of cigarette smoking with depression and anxiety: a systematic review. Nicotine Tob Res. 2017;19(1):3-13. http://dx.doi.org/10.1093/ntr/ntw140. PMid:27199385.
8. Smith J, Nastasi D, Tso R, Vangaveti V, Renison B, Chilkuri M. The effects of continued smoking in head and neck cancer patients treated with radiotherapy: a systematic review and meta-analysis. Radiother Oncol. 2019;135:51-7. http://dx.doi.org/10.1016/j.radonc.2019.02.021. PMid:31015170.
9. Almeida AA, Bandeira CM, Goncalves AJ, Araujo AJ. Nicotine dependence and smoking habits in patients with head and neck cancer. J Bras Pneumol. 2014;40(3):286-93. http://dx.doi.org/10.1590/S1806-37132014000300012. PMid:25029652.
10. Rendic S, Guengerich FPP. Contributions of human enzymes in carcinogen metabolism. Chem Res Toxicol. 2012;25(7):1316-83. http://dx.doi.org/10.1021/tx300132k. PMid:22531028.
11. Scully C. Oral cancer aetiopathogenesis; past, present and future aspects. Med Oral Patol Oral Cir Bucal. 2011;16(3):e306-11. http://dx.doi.org/10.4317/medoral.16.e306. PMid:21441876.
12. Wassenaar CA, Zhou Q, Tyndale RF. CYP2A6 genotyping methods and strategies using real-time and end point PCR platforms. Pharmacogenomics. 2016;17(2):147-62. http://dx.doi.org/10.2217/pgs.15.156. PMid:26670214.
13. Huang CL, Lin HH, Wang HH. Evaluating screening performances of the Fagerstrom tolerance questionnaire, the Fagerstrom test for nicotine dependence and the heavy smoking index among Taiwanese male smokers. J Clin Nurs. 2008;17(7):884-90. http://dx.doi.org/10.1111/j.1365-2702.2007.02054.x. PMid:18321287.
14. Bosetti C, Carioli G, Santucci C, Bertuccio P, Gallus S, Garavello W, et al. Global trends in oral and pharyngeal cancer incidence and mortality. Int J Cancer. 2020;147(4):1040-9. http://dx.doi.org/10.1002/ijc.32871. PMid:31953840.
15. Cao C, Chen J, Lyu C, Yu J, Zhao W, Wang Y, et al. Bioinformatics analysis of the effects of tobacco smoke on gene Expression. PLoS One. 2015;10(12):e0143377. http://dx.doi.org/10.1371/journal.pone.0143377. PMid:26629988.
16. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138(1):103-41. http://dx.doi.org/10.1016/j.pharmthera.2012.12.007. PMid:23333322.
17. Mallery SRR, Tong M, Michaels GCC, Kiyani ARR, Hecht SSS. Clinical and biochemical studies support smokeless tobacco's carcinogenic potential in the human oral cavity. Cancer Prev Res. 2014;7(1):23-32. http://dx.doi.org/10.1158/1940-6207.CAPR-13-0262. PMid:24265177.
18. Go REE, Hwang KAA, Choi KCC. Cytochrome P450 1 family and cancers. J Steroid Biochem Mol Biol. 2015;147:24-30. http://dx.doi.org/10.1016/j.jsbmb.2014.11.003. PMid:25448748.
19. Kumar S, Sinha N, Gerth KAA, Rahman MAA, Yallapu MMM, Midde NMM. Specific packaging and circulation of cytochromes P450, especially 2E1 isozyme, in human plasma exosomes and their implications in cellular communications. Biochem Biophys Res Commun. 2017;491(3):675-80. http://dx.doi.org/10.1016/j.bbrc.2017.07.145. PMid:28756226.
20. Vasconcelos GM, Struchiner CJ, Suarez-Kurtz G. CYP2A6 genetic polymorphisms and correlation with smoking status in Brazilians. Pharmacogenomics J. 2005;5(1):42-8. http://dx.doi.org/10.1038/sj.tpj.6500290. PMid:15534625.
21. Rossini A, Simao TA, Albano RM, Pinto LF. CYP2A6 polymorphisms and risk for tobacco-related cancers. Pharmacogenomics. 2008;9(11):1737-52. http://dx.doi.org/10.2217/14622416.9.11.1737. PMid:19018727.
22. Thum T, Erpenbeck VJJ, Moeller J, Hohlfeld JMM, Krug N, Borlak J. Expression of xenobiotic metabolizing enzymes in different lung compartments of smokers and nonsmokers. Environ Health Perspect. 2006;114(11):1655-61. http://dx.doi.org/10.1289/ehp.8861. PMid:17107849.
23. Nagaraj NSS, Beckers S, Mensah JKK, Waigel S, Vigneswaran N, Zacharias W. Cigarette smoke condensate induces cytochromes P450 and aldo-keto reductases in oral cancer cells. Toxicol Lett. 2006;165(2):182-94. http://dx.doi.org/10.1016/j.toxlet.2006.03.008. PMid:16713138.
24. Chi AC, Appleton K, Henriod JB, Krayer JW, Marlow NM, Bandyopadhyay D, et al. Differential induction of CYP1A1 and CYP1B1 by benzo[a]pyrene in oral squamous cell carcinoma cell lines and by tobacco smoking in oral mucosa. Oral Oncol. 2009;45(11):980-5. http://dx.doi.org/10.1016/j.oraloncology.2009.05.562. PMid:19576839.
25. Spivack SDD, Hurteau GJJ, Reilly AAA, Aldous KMM, Ding X, Kaminsky LSS. CYP1B1 expression in human lung. Drug Metab Dispos. 2001;29(6):916-22. PMid:11353763.
26. Su J-MM, Lin P, Wang C-KK, Chang H. Overexpression of cytochrome P450 1B1 in advanced non-small cell lung cancer: a potential therapeutic target. Anticancer Res. 2009;29(2):509-15. PMid:19331196.
27. Alsubait A, Aldossary W, Rashid M, Algamdi A, Alrfaei BM. CYP1B1 gene: implications in glaucoma and cancer. J Cancer. 2020;11(16):4652-61. http://dx.doi.org/10.7150/jca.42669. PMid:32626511.
28. Verma S, Saxena R, Siddiqui MH, Santha K, Sethupathy S. Evaluation of CYP1B1 expression, oxidative stress and phase 2 detoxification enzyme status in oral squamous cell carcinoma patients. J Clin Diagn Res. 2017;11(3):BC01-05. http://dx.doi.org/10.7860/JCDR/2017/22196.9324. PMid:28511368.

29. Bandeira CM, Almeida AÁ, Carta CFL, Almeida JD, Tango EK. How to improve the early diagnosis of oral cancer? Braz Dent Sci. 2017;20(4):25-31. http://dx.doi.org/10.14295/bds.2017.v20i4.1439.

30. Baumann E, Koller M, Wiltfang J, Wenz HJJ, Möller B, Hertrampf K. Challenges of early detection of oral cancer: raising awareness as a first step to successful campaigning. Heal Educ Res. 2016;31(2):136-45. http://dx.doi.org/10.1093/her/cyv099. PMid:26850059.

Janete Dias Almeida
(Corresponding address)
Department of Bioscience and Oral Diagnosis, Institute of Science and Technology of São José dos Campos, São Paulo State University, São José dos Campos, SP, Brazil.
Email: janete.almeida@unesp.br

Date submitted: 2021 Apr 15
Accept submission: 2021 Jul 01