JULIANA HINTZ GERMANOS SCHEIDT

IMUNODETECÇÃO DE VEGF, CASPASE-3 E p53 EM CARCINOMAS BUCAIS DE CÉLULAS ESCAMOSAS DE PACIENTES USUÁRIOS OU NÃO DE TABACO E ÁLCOOL

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RESUMO

O carcinoma de células escamosas (CCE) é a neoplasia maligna mais frequente da mucosa bucal e representa um problema de saúde pública devido aos altos índices de mortalidade e morbidade. Seu desenvolvimento está associado a fatores intrínsecos e extrínsecos, dentre os quais se destacam o alcoolismo e o tabagismo. O objetivo deste estudo foi comparar as características clínico-patológicas e a imunodetecção das proteínas VEGF, caspase-3 e p53 entre CCE bucais de pacientes usuários ou não de tabaco e álcool. Foram selecionados aleatoriamente 90 espécimes de carcinomas bucais, provenientes de pacientes diagnosticados no Serviço de Estomatologia e Prevenção do Câncer Bucomaxilofacial do Hospital São Lucas –PUCRS, entre 1991 e 2011. Os espécimes foram distribuídos em três grupos de acordo com a exposição aos fatores de risco: 30 casos de pacientes tabagistas, 30 de tabagistas/etilistas e 30 de indivíduos não tabagistas/não etilistas. As características clínicas das lesões foram avaliadas retrospectivamente, seu diagnóstico histopatológico foi revisado por um patologista e a detecção das proteínas VEGF, caspase-3 e p53 foi avaliada por meio de imunoistoquímica. No grupo de não tabagistas/não etilistas foram encontrados, principalmente, pacientes do sexo feminino ($p \leq 0,001$), com média de idade superior ($p=0,004$) em comparação aos demais. O grupo de tabagistas/etilistas exibiu tumores maiores, com diferença significativa quando comparado aos pacientes não expostos a esses fatores ($p=0,004$). A graduação histopatológica também diferiu entre estes grupos ($p=0,040$), uma vez que maior número de lesões grau I e menor número de lesões grau III foram encontradas nos pacientes não tabagistas/não etilistas. Não foi observada diferença significativa quanto à imunoreatividade das

proteínas VEGF (p=0,315), caspase-3 (p=0,860) e p53 (p=0,876) entre os grupos. Com base nos resultados obtidos, conclui-se que existem importantes diferenças clínico-patológicas entre CCE bucais de pacientes tabagistas e etilistas com os de indivíduos não expostos a esses fatores. Entretanto, a imunorreatividade das proteínas VEGF, caspase-3 e p53 não é influenciada pelo tabagismo e etilismo no carcinoma bucal, sugerindo que outros eventos moleculares estejam associados à maior agressividade biológica dessa lesão nos pacientes usuários de tabaco e álcool.

Palavras-chave: Câncer bucal. Tabagismo. Etilismo. Caspase-3. Proteína p53. VEGF.

ABSTRACT

Squamous cell carcinoma (SCC) is the most prevalent malignancy of the mouth and represents a public health problem due to high mortality and morbidity rates. Its development is associated with intrinsic and extrinsic factors, mainly with tobacco and alcohol. The objective of this study was to compare the clinicopathological characteristics and immunoreactivity of proteins VEGF, caspase-3 and p53 between oral SCC from users and non users of tobacco and alcohol. We randomly selected 90 specimens of oral carcinomas, from patients diagnosed between 1991 and 2011 in Oral Medicine Division - São Lucas Hospital. The specimens were distributed into three groups according to exposure to risk factors: 30 specimens from smokers, 30 from smokers/alcohol drinkers and 30 from individuals not exposed to these factors. The clinical characteristics of the lesions were evaluated retrospectively, the histopathological diagnoses were reviewed by a pathologist and the proteins VEGF, caspase-3 and p53 were detected by immunohistochemistry. The group of non-smokers/non-alcohol drinkers consisted mainly of women ($p \leq 0.001$), with a higher mean age ($p= 0.004$). The group of smokers/alcohol drinkers exhibited significantly larger tumors when compared to patients not exposed to smoking and alcohol ($p=0.004$). The histopathological grading also differed between these groups ($p = 0.040$), since a greater number of grade I lesions and fewer grade III lesions were found in patient non-smokers/non-alcohol drinkers. No significant difference was observed in relation to immunoreactivity for the proteins VEGF ($p= 0.315$), caspase-3 ($p= 0.860$) and p53 ($p= 0.876$) between the groups. Based on the results, we conclude that there are substantial clinicopathological differences between oral carcinomas in users and non

users of tobacco and alcohol. We found no difference in the immunodetection of the proteins VEGF, caspase-3 and p53 between the groups, suggesting that their levels are not influenced by smoking or alcohol consumption and that other molecular mechanisms are associated with the biological aggressiveness of oral carcinoma in patients exposed to these risk factors.

Key Words: Oral Cancer. Smoking. Alcoholism. Caspase-3. P53 proteins, VEGF

LISTA DE ILUSTRAÇÕES

Artigo de Pesquisa

LISTA DE TABELAS

Artigo de Pesquisa

SUMÁRIO

Introdução

1 INTRODUÇÃO

O câncer de boca é um problema de saúde pública em muitos países devido aos altos índices de morbi-mortalidade. No Brasil, representa o sétimo tipo de neoplasia maligna mais frequente, com cerca de 14.000 novos casos diagnosticados ao ano (INCA, 2010). O carcinoma de células escamosas (CCE) é a neoplasia maligna mais comum da cavidade bucal, origina-se do epitélio de revestimento, ocorre, preferencialmente, em língua e possui predileção por pacientes do sexo masculino, com média de idade de 57 anos (PITHAN et al., 2004).

O desenvolvimento do CCE bucal está associado a fatores intrínsecos e extrínsecos, dentre os quais se destacam o álcool e o tabaco. A exposição simultânea aos mesmos é significativamente associada a um risco mais elevado para o desenvolvimento do carcinoma bucal, uma vez que estas substâncias apresentam efeito sinérgico (TALAMINI et al., 2002 ; DE STEFANI et al., 2007; IDE et al., 2008 ; PELUCCHI et al., 2008). O tabaco constitui o fator primordial (90%) associado a essa neoplasia, pois provoca aumento do número de células aneuploides no epitélio oral (SOUTO et al., 2010) e diminuição da expressão de genes supressores tumorais (SMITH et al., 2006), dentre outras alterações. Métodos de citologia exfoliativa têm demonstrado que o fumo causa alterações no epitélio oral de pacientes clinicamente saudáveis como aumento da área nuclear (OGDEN; COWPE; GREEN, 1990; SCULLY; FIELD; TANZAWA, 2000) e da proliferação celular (CANÇADO; YURGEL; SANTANA-FILHO, 2001).

Alguns autores sugerem que o álcool tem menos importância do que o tabaco ou a associação de ambos para o desenvolvimento do carcinoma bucal (FELDMAN; HAZAN, 1975; MUWONGE et al., 2008). Entretanto, esta substância pode provocar

alterações na mucosa tais como hiperproliferação celular e mudanças no metabolismo do acido retinóico (ROSSING; VAUGHAN; MCKNIGHT, 1989). Reis et al. (2002) avaliaram células epiteliais exfoliadas da mucosa jugal e língua de 40 indivíduos etilistas e não tabagistas e de 20 indivíduos-controle (não etilistas e não tabagistas) e observaram um aumento significativo na frequência de micronúcleos nas células da língua dos pacientes alcoólatras. Além disso, a frequente ingestão de bebidas alcoólicas causa diminuição do fluxo salivar, deixando a mucosa mais suscetível a outros fatores extrínsecos (ZNAOR et al., 2003). Squier, Kremer e Wertz (2003) observaram que a ingestão de álcool aumentou significativamente a permeabilidade do epitélio oral de ratos após 120 dias de exposição.

O uso do álcool e do tabaco parece interferir também no curso da doença, uma vez que pacientes tabagistas e etilistas tendem a apresentar tumores mais agressivos, pior prognóstico, maior taxa de recidiva e pior resposta ao tratamento (BROWMAN et al., 1993; VAN OIJEN et al., 1998; KHURI et al., 2001; DO et al., 2003; PYTYNIA et al., 2004; FORTIN;. WANG; VIGNEAULT, 2009; CHEN et al., 2011-A; CHEN et al., 2011-B; KRUSE; BREDELL; GRÄTZ, 2010; HARRIS et al., 2010).

A expressão de determinadas proteínas pode estar associada à agressividade do CCE bucal e ser um marcador prognóstico em pacientes com a doença. A proteína p53 é um marcador bastante estudado na literatura, pois em sua forma selvagem possui meia-vida curta e é pouco detectada por meio de imunoistoquímica. Porém, quando sofre mutações, sua meia-vida torna-se mais longa, possibilitando sua imunodetecção nos tecidos (KATO et al., 2011). A expressão da p53 está associada a um pior prognóstico e a uma menor sobrevida em pacientes com CCE de cabeça e pescoço (SIEGELMANN-DANIELI et al., 2005;

BOSLOOPER et al., 2008; KATO et al., 2011). A inativação dessa proteína promove alterações no reparo do DNA e na apoptose, gerando aumento na instabilidade genética, que pode levar a um acúmulo de mutações (CHARI et al., 2009). Alguns estudos investigaram a associação da expressão da p53 com os hábitos de tabagismo e etilismo em CCE bucais. Siegelmann-Danieli et al. (2005) não encontraram associação entre a presença desses fatores de risco e a imunodetecção da p53. Por outro lado, Hsieh et al. (2001) observaram aumento da expressão gênica de p53 em lesões de pacientes expostos ao álcool e ao tabaco.

O VEGF (fator de crescimento endotelial vascular) induz a proliferação, migração e sobrevida das células endoteliais durante o crescimento tumoral pela ligação às quinases receptoras de tirosinas específicas, sendo considerado um regulador chave no processo de neovascularização (SIEMEISTER; MARTINY-BARON; MARMÉ, 1998; FERRARA; GERBER; LECOUTER, 2003). Mărgăritescu et al. (2009) sugerem que a imunodetecção deste marcador pode ser considerada uma ferramenta confiável na avaliação do prognóstico de pacientes com CCE bucais, prevendo menor sobrevida livre de doença, pior sobrevida geral e doença metastática. Kyzas et al.(2005) e Cheng et al. (2011) observaram que neoplasias malignas de boca em estágio mais avançado, com a presença de linfonodos metastáticos, apresentaram maior imunorreatividade do VEGF. Shao et al. (2008) constataram que a expressão do VEGF apresentou correlação positiva com o sítio do tumor, estágio clínico, recorrência, metástases à distância e comprometimento de linfonodos. Por outro lado, Chuang et al. (2006) não encontraram correlação entre a expressão do VEGF, em 94 casos de neoplasia maligna de língua (T1 e T2), e a idade dos pacientes, invasão vascular ou graduação histopatológica.

As caspases, pertencentes à família das proteases de cisteína, podem ser classificadas de acordo com a sua função em caspases iniciadoras, que estão associadas à iniciação da cascata proteolítica, e em caspases efetores, que são responsáveis pela clivagem de substratos (DEGTEREV; BOYCE; YUAN, 2003). A caspase-3 é uma caspase efetora, sendo a principal via de regulação da apoptose (ZIMMERMANN; BONZON; GREEN, 2001). A ativação destas proteínas provoca a inativação de enzimas reparadoras do DNA, dos reguladores do ciclo celular e das proteínas responsáveis pela manutenção da estrutura celular (afetando a adesão célula-célula e célula-matriz) (KRAJEWSKA et al., 1997). No linfoma de Hodgkin (CHHANABHAI et al., 1997) e no carcinoma de fígado (FUJIKAWA et al., 2000) sua expressão está reduzida em comparação ao tecido normal. Em contraste, em tumores do ducto pancreático a expressão da caspase-3 encontra-se aumentada (SATOH et al., 2000). Nos tumores da cavidade bucal, os resultados com relação à sua expressão são divergentes. Andressakis et al. (2008) compararam 87 espécimes de CCE de língua com áreas adjacente ao epitélio tumoral e observaram redução na expressão da caspase-3 e da caspase-8 nos tecidos neoplásicos. Por outro lado, Hague et al. (2004), ao compararem amostras de epitélio oral com espécimes de CCE bucais, encontraram maior imunodetecção da caspase-3 no tumor em comparação ao epitélio normal.

Na literatura há evidências de que o tabagismo e o etilismo não só participam da gênese do carcinoma bucal de células escamosas, como também estão associados a uma doença mais agressiva. Uma vez que a expressão de determinadas proteínas pode estar associada ao comportamento biológico do CCE bucal, este estudo teve por objetivo comparar as características clínico-patológicas e

a imunodetecção de VEGF, caspase-3 e p53 de carcinomas de pacientes etilistas e tabagistas com os de indivíduos não expostos a esses fatores de risco.

Artigo de Revisão

2 ARTIGO DE REVISÃO

O artigo a seguir intitula-se ***Clinicopathologic and molecular characteristics of oral squamous cell carcinoma in patients with and without known risk factors*** e foi formatado de acordo com as normas do periódico ***Medicina Oral Patología Oral y Cirugia Bucal*** (Anexo A).

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CLINICOPATHOLOGIC AND MOLECULAR CHARACTERISTICS OF ORAL SQUAMOUS CELL CARCINOMA IN PATIENTS WITH AND WITHOUT KNOWN RISK FACTORS

Running Title: INFLUENCE OF ALCOHOL AND TOBACCO IN CHARACTERISTICS OF ORAL SQUAMOUS CELL CARCINOMA

Juliana Hintz Germanos Scheidt[1]
Liliane Soares Yurgel[2]
Karen Cherubini[2]
Maria Antonia Zancanaro de Figueiredo[2]
Fernanda Gonçalves Salum[2]

Oral Medicine Division, Pontifical Catholic University of Rio Grande do Sul, Brazil.

1 MSc, student, Oral Medicine Division, Pontifical Catholic University of Rio Grande do Sul, Brazil

2 PhD in Oral Medicine, Pontifical Catholic University of Rio Grande do Sul, Brazil

Corresponding address:
Fernanda Gonçalves Salum
Pontifícia Universidade Católica do Rio Grande do Sul - PUCRS
Hospital São Lucas
Av. Ipiranga, 6690 – Sala 231 – 2º andar
CEP: 90610-000 - Porto Alegre – RS – Brazil
Tel/Fax: +55 51 3320-3254
E-mail: fesalum@terra.com.br

ABSTRACT

Alcohol and tobacco are the main extrinsic etiological factors for the genesis of oral squamous cell carcinoma (SCC), but it is still not clear if the presence of these factors interfere with clinical, pathologic and molecular characteristics or with the prognosis and treatment of the disease. In the present study, these characteristics were reviewed, establishing comparisons between the lesions of patients exposed and not exposed to tobacco and alcohol. We observed that oral SCC in non smokers and non alcohol drinkers occur mainly in female patients, under 50 or over 70 years old. The lesions tend to be less aggressive in this group of patients and have a better prognosis. The molecular characteristics of these malignant tumors also appear to be influenced by the presence of these habits, once mutations of p53 have been associated with tobacco and alcohol use. The understanding of the differences between the neoplasms of these two groups of patients can contribute to the management of this cancer, which could lead to advances in the determination of more appropriate therapeutic measures.

Kew words: Oral Cancer. Risk Factors. Prognosis.

INTRODUCTION

Oral cancer represents the 7th most common type of malignancy in Brazil, with about 14,000 new cases diagnosed per year. Squamous cell carcinoma (SCC) is the most common malignant tumor of this anatomic site, and in approximately 80% of cases, it is associated with extrinsic factors such as the use of tobacco, alcohol or both (1). This cancer occurs preferentially in the tongue, exhibiting predilection for men with a mean age of 57 years (2). In the last years, however, there has been an increase in the incidence of this lesion in younger patients, that is, under 45 years old (3,4).

Tobacco use constitutes the primary factor (90%) for the development of oral SCC. Tobacco contains about 50 substances with carcinogenic potential, such as nitrosamines and aromatic hydrocarbons (5). Besides, smoke raises the temperature of the mouth, which contributes to its deleterious effect. The involvement of alcohol is not so clear with respect to tobacco. While studies suggest that the risk of developing oral SCC in patients who are alcohol drinkers (non-smokers) is slightly higher (6), others demonstrate that excessive consumption of alcoholic beverages is an important factor for the occurrence of this cancer (7,8). Alcohol is associated with cell hyperproliferation (which increases vulnerability to inhaled or ingested carcinogens), production of metabolites with carcinogenic action, such as acetaldehyde, induction of enzymes that activate pro-carcinogens and reduction of retinoic acid (9). The consumption of alcohol, especially ethanol, interferes with DNA repair and can have an immunosuppressive effect (10).

Simultaneous exposure to tobacco and alcohol is significantly associated with a higher risk of developing oral SCC, because these substances show a synergistic

effect (11-14). Despite the strong connection with the genesis of oral cancer, there are controversies about whether drinking alcohol and tobacco use is associated with clinical and molecular patterns of this lesion and a better or worse prognosis in patients with the disease. In this study, we conducted a review of the clinicopathologic and molecular characteristics and biological behavior of head and neck SCC, with emphasis on oral cancer, comparing the lesions of smokers and alcohol drinkers with individuals not exposed to these risk factors.

CLINICOPATHOLOGIC AND PROGNOSTIC CHARACTERISTICS

Studies have demonstrated that head and neck SCC in non alcohol drinkers and non smokers develops more often in the more advanced age group and in women (15-18). Lo et al. (19) found that in individuals exposed to risk factors (chewing tobacco, smoking and alcohol), the lesion developed a mean of 12 years earlier than in those not exposed. Meanwhile, Dahlstrom et al. (20) on evaluating 1303 individuals with SCC, observed that the group of non smokers and non alcohol drinkers was significantly younger. Harris et al. (21) evaluated 78 young patients with SCC of this region, aged between 18 and 39 years. The non smokers and non alcohol drinkers had a lower mean age and women were more often affected in comparison to exposed individuals. However, in relation to age group for the development of SCC of head and neck in patients not exposed to these risk factors, age extremes seem to be more often observed, that is, individuals under 50 or over 70 years old are more affected (20).

In patients not exposed to the risk factors analyzed, the lesions develop primarily in the oral cavity, especially in the anterior tongue, alveolar ridge and

gingiva (18,20,22). In individuals who smoke and drink alcohol, the tumors occur mostly in the larynx, hypopharynx, posterior tongue, retromolar trigone and mouth floor (20,22).

In relation to the size of the lesion and clinical stage, the studies yielded conflicting results on comparing patients exposed and not exposed to risk factors. Schmidt et al. (22) e Bachar et al. (4) did not observe a significant difference with respect to these clinical parameters between smokers and non smokers. Meanwhile, Dahlstrom et al. (20) e Harris et al. (21) found a greater percentage of tumors in stage I in patients not exposed to the risk factors. Kruse et al. (18) also found that the majority of oral SCC cases in non smokers and non alcohol drinkers were T1 or T2.

Link et al. (15) observed in a group of patients who were non smokers, more cases of moderately and poorly differentiated SCC, indicating a more aggressive disease. Meanwhile, more recent studies have demonstrated that in patients not exposed to smoking and alcohol the lesions tended to be classified as well or moderately differentiated, while in exposed individuals, a lower degree of cell differentiation has been observed (4,18).

A lower five-year survival rate was observed in patients who chewed tobacco, but no significant difference was found in survival between smokers and non smokers, nor between alcohol drinkers and non alcohol drinkers (19). Harris et al. (21) did not find a difference between groups of smokers/alcohol drinkers and non smokers/non alcohol drinkers with respect to the disease-free survival rate. However, they suggested a better general survival in 10 years for the group of individuals not exposed to the risk factors.

On the other hand, Pytynia et al. (23) found that patients with head and neck SCC who did not smoke showed a longer mean time of general, disease-free survival

compared to smokers. Ide et al. (13) and Fortin et al. (24) also demonstrated that smokers and alcohol drinkers, showed survival rates and local control of the disease that were inferior to that in patients not exposed to these risk factors. According to Girod et al. (25), female smokers with a diagnosis of oral and oropharynx cancer show a worse prognosis.

Patients who smoke also show greater rates of recurrence of the lesion in comparison to non smokers. Ex-smokers, in turn, show intermediate rates between the above groups (26). Do et al. (27) observed that smokers and alcohol drinkers had a significantly greater risk of developing a second primary tumor in comparison to non smokers, especially if they continued the habit after diagnosis of the lesion. Meanwhile, on comparing the pattern of recurrence in cases of SCC of the tongue, Bachar et al. (4) did not observe a significant difference with respect to local and regional recurrence between patients exposed and not exposed to smoking and alcohol. The patients younger than 40 years, not exposed to the risk factors analyzed, showed a worse prognosis, suggesting that other factors besides smoking and alcohol drinking play a role in the pathogenesis of tumors of the tongue in this group of individuals.

Besides increasing the risk recurrence of the disease, smoking and alcohol drinking can reduce the efficacy of the treatment (28,29). Mayne et al. (30) followed 264 patients treated for oral, pharynx and larynx cancer in the early stages and analyzed the use of alcohol and tobacco in the pre- and post-diagnosis of the neoplasm. Patients who smoked showed a worse prognosis than those who did not have this habit. Alcohol use increased risk of mortality in a dose-dependent way in patients who drank more than five doses of beer or liquor per day. After diagnosis, those who continued with the alcohol habit exhibited worse survival. In relation to

smoking, no significant difference in survival was found between the patients who continued to smoke and those who quit.

Chen et al. (31) observed that non smokers showed a better prognosis after radiotherapy than did smokers who continued their habit. These results suggest that head and neck cancer in smokers can show a biologically more aggressive phenotype compared to patients who are non smokers. In another study, Chen et al. (32) evaluated post-radiotherapy survival comparing patients with SCC of the head and neck who quit smoking with those who continued the habit after diagnosis. The individuals who continued to smoke showed a significantly shorter survival, worse locoregional control of the disease and higher rate of complications with radiotherapy. Browman et al. (33) also observed that patients who smoked and continued the habit during radiotherapeutic treatment displayed significantly lower response and survival rates compared to patients who did not smoke.

MOLECULAR CHARACTERISTICS

The detection of p53 protein, which implies the presence of stabilized mutated protein, has been associated with a poor prognosis of SCC of the head and neck. Siegelmann-Danieli et al. (34) did not find a significant association between the immunodetection of p53 protein in SCC of the tongue with consumption of alcohol and tobacco. On the other hand, Van Oijen et al. (35) reported that the immunodetection of p53 protein in the mucosa adjacent to the tumor in head and neck SCC patients was significantly greater in those who were smokers.

Mutations of p53 protein have been associated with smoking and alcohol use in patients with head and neck SCC. Brennan et al. (36) found mutations of p53

protein in 58% of patients with head and neck SCC who used tobacco and alcohol. In patients who are smokers, the mutation occurred in 33% of cases, and in 17% of cases in individuals not exposed to the risk factors. In the latter, potentially endogenous mutations were observed, while in the patients who were smokers and alcohol drinkers, mutations of this type were uncommon. Hsieh et al. (37) also found that the neoplasms of patients who drank alcohol exhibited an increase in the incidence of mutation of p53. When alcohol consumption is associated with smoking, this mutation is more significant, demonstrating the synergistic effect of these risk factors.

Koch et al. (38) investigated mutations of protein p53, HPV infection and loss of heterozygosity in 305 cases of SCC of the head and neck. SCC in the patients who were smokers showed significantly higher rates of mutation of protein p53 and percentage of infection by HPV slightly lower. In addition, in this group, loss of heterozygosity in 3p, 4q, and 11q13 and the mean number of chromosome losses were greater. The tumors of patients who did not smoke exhibited a lower frequency of common genetic alterations, suggesting that subjacent mutations can be unknown in these neoplasms.

Van Oijen et al. (39) compared the immunodetection of the marker Ki67 in SCC of the upper aerodigestive tract (47 of the oral cavity, 12 of larynx, 8 of the hypopharynx and 4 of the oropharynx) and the oral epithelium of clinically healthy individuals. Each group was subdivided into smokers, non smokers and ex-smokers. Increase in cell proliferation was observed in the oral epithelium of smokers, both patients with carcinoma and healthy individuals. These epithelial alterations favor genetic events that culminate in the development of cancer. Ex-smokers in both

groups had a tendency toward increased cell proliferation, suggesting that even after quitting, the epithelial alterations persist.

Farshadpour et al. (40) analyzed the expression of p53 and Ki-67 in the mucosal epithelium adjacent to tumor in 29 head and neck SCC patients exposed to tobacco and alcohol and 33 not exposed to these factors. The expression of p53 was significantly higher in the patients who used tobacco and alcohol than in non-user patients. The immunodetection of the marker ki-67 was not influenced by the presence of these factors.

VEGF (vascular endothelial growth factor) have a positive association with a more advanced clinical stage of oral SCC and may have prognostic value in patients with this malignancy (41). Meanwhile, Faustino et al. (42) and Kyzas et al. (43) found no correlation between the immunoreactivity of that angiogenic marker with smoking and alcohol consumption in patients with oral SCC.

DISCUSSION

It is established in the literature that among the etiological factors involved in the genesis of oral SCC, tobacco and alcohol use plays a major role. When these substances are combined, the carcinogenic effect becomes potentiated due to their synergistic effect (11-14). In the literature, it is not clear if the presence or absence of these habits affect the clinicopathologic and molecular characteristics of the tumor, as well as the prognosis of the patient. In the present study, these characteristics were reviewed. The studies demonstrated that SCC of head and neck in patients who are non smokers and non alcohol drinkers occurred predominately in females.

With respect to age range, age extremes are observed, that is, individuals under 50 or over 70 years are more affected (15-18,20,21).

The lesions of patients who are non smokers and non alcohol drinkers tend to show a less aggressive behavior, that is, the majority are classified as T1 or T2, and with respect to degree of histological differentiation, they are usually better differentiated (18,21,39). Besides, in these patients, the risk of tumor recurrence is lower and survival and prognosis are better (23,24,26,27). Response to radiotherapy also tends to be better in patients who are non smokers or who quit the habit during treatment (31-33).

The expression of proteins associated with the regulation of the cell cycle and mutations of various tumor suppressor genes have been investigated in oral cancer. However, the literature is sparse on the comparison of the molecular mechanisms of oral carcinoma in patients users or non users of tobacco and alcohol. p53 protein is an established marker in the literature; its inactivation affects DNA damage repair and apoptosis, causing an increase in genetic instability which can to lead to an accumulation of mutations. The expression of this protein appears to be influenced by tobacco and alcohol use, which causes its mutation (36-38). On the other hand, smoking and drinking habits do not seem to affect the immunodetection of VEGF, which is also considered a prognostic marker in patients with oral SCC (42, 43).

The clinicopathologic patterns and biological behavior of SCC of the head and neck in smokers and alcohol drinkers compared to non smokers and non alcohol drinkers are distinct. Oral cancer can show a biologically more aggressive phenotype in smokers and alcohol drinkers. The analysis of these patterns contribute to the understanding and management of this cancer, which could lead to advances in the

determination of more appropriate therapeutic measures and reduction of morbidity and mortality.

REFERENCES

1. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and unrelated oral squamous cell carcinomas in the United States. J Clin Oncol. 2008;26:612-9.

2. Pithan SA, Cherubini K, Figueiredo MAZ, Yurgel LS. Epidemiological profile of oral squamous cell carcinoma in patients attended at Stomatology Division of Hospital São Lucas - PUCRS. Rev Odonto Ciência. 2004;19:126-30.

3. Llewellyn CD, Linklater K, Bell J, Johnson NW, Warnakulasuriya S. An analysis of risk factors for oral cancer in young people: a case-control study. Oral Oncol. 2004;40:304-13.

4. Bachar G, Hod R, Goldstein DP, Irish JC, Gullane PJ, Brown D et al. Outcome of oral tongue squamous cell carcinoma in patients with and without known risk factors. Oral Oncol. 2011;47:45-50.

5. Gupta PC, Murti PR, Bhonsle RB. Epidemiology of cancer by tobacco products and the significance of TSNA. Crit Rev Toxicol. 1996;26:183-98.

6. Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J Natl Cancer Inst. 2007;99:777-89.

7. Bagnardi V, Blangiardo M, La Vecchia C, Corrao G. A meta-analysis of alcohol drinking and cancer risk. Br J Cancer. 2001;85:1700-5.

8. Polesel J, Dal Maso L, Bagnardi V, Zucchetto A, Zambon A, Levi F et al. Estimating dose-response relationship between ethanol and risk of cancer using regression spline models. Int J Cancer. 2005;114:836-41.

9. Rossing MA, Vaughan TL, McKnight B. Diet and pharyngeal cancer. Int J Cancer. 1989;44:593-7.

10. Gerson SJ. Oral cancer. Crit Rev Oral Biol Med. 1990;1:153-66.

11. Talamini R, Bosetti C, La Vecchia C, Dal Maso L, Levi F, Bidoli E et al. Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. Cancer Causes Control. 2002;13:957-64.

12. De Stefani E, Boffetta P, Deneo-Pellegrini H, Ronco AL, Acosta G, Ferro G, et al. The effect of smoking and drinking in oral and pharyngeal cancers: a case-control study in Uruguay. Cancer Lett. 2007;246:282-9.

13. Ide R, Mizoue T, Fujino Y, Hoshiyama Y, Sakata K, Tamakoshi A et al. Cigarette smoking, alcohol drinking, and oral and pharyngeal cancer mortality in Japan. Oral Dis. 2008;14:314-9.

14. Pelucchi C, Gallus S, Garavello W, Bosetti C, La Vecchia C. Alcohol and tobacco use, and cancer risk for upper aerodigestive tract and liver. Eur J Cancer Prev. 2008;17:340-4.

15. Link MJO, Kaugars GE, Burns JC. Comparison of oral carcinomas in smokeless tobacco users and nonusers. J Oral Maxillofac Surg. 1992;50:452-5.

16. Wiseman SM, Swede H, Stoler DL, Anderson GR, Rigual NR, Hicks WL Jr et al. Squamous cell carcinoma of the head and neck in nonsmokers and nondrinkers: an analysis of clinicopathologic characteristics and treatment outcomes. Ann Surg Oncol. 2003;10:551-7.

17. Farshadpour F, Hordijk GJ, Koole R, Slootweg PJ. Non-smoking and non-drinking patients with head and neck squamous cell carcinoma: a distinct population. Oral Dis. 2007;13:239-43.

18. Kruse AL, Bredell M, Grätz KW. Oral squamous cell carcinoma in non-smoking and non-drinking patients. Head Neck Oncol. 2010;2:24.

19. Lo WL, Kao SY, Chi LY, Wong YK, Chang RC. Outcomes of oral squamous cell carcinoma in Taiwan after surgical therapy: factors affecting survival. J Oral Maxillofac Surg. 2003;61:751-8.

20. Dahlstrom KR, Little JA, Zafereo ME, Lung M, Wei Q, Sturgis EM. Squamous cell carcinoma of the head and neck in never smoker-never drinkers: a descriptive epidemiologic study. Head Neck. 2008;30:75-84.

21. Harris SL, Kimple RJ, Hayes DN, Couch ME, Rosenman JG. Never-smokers, never-drinkers: unique clinical subgroup of young patients with head and neck squamous cell cancers. Head Neck. 2010;32:499-503.

22. Schmidt BL, Dierks EJ, Homer L, Potter B. Tobacco smoking history and presentation of oral squamous cell carcinoma. J Oral Maxillofac Surg. 2004;62:1055-8.

23. Pytynia KB, Grant JR, Etzel CJ, Roberts DB, Wei Q, Sturgis EM. Matched-pair analysis of survival of never smokers and ever smokers with squamous cell carcinoma of the head and neck. J Clin Oncol. 2004;22:3981-8.

24. Fortin A, Wang CS, Vigneault E. Influence of smoking and alcohol drinking behaviors on treatment outcomes of patients with squamous cell carcinomas of the head and neck. Int J Radiat Oncol Biol Phys. 2009;74:1062-9.

25. Girod A, Mosseri V, Jouffroy T, Point D, Rodriguez J. Women and squamous cell carcinomas of the oral cavity and oropharynx: is there something new? J Oral Maxillofac Surg. 2009;67:1914-20.

26. Khuri FR, Kim ES, Lee JJ, Winn RJ, Benner SE, Lippman SM, et al. The impact of smoking status, disease stage, and index tumor site on second primary tumor incidence and tumor recurrence in the head and neck retinoid chemoprevention trial. Cancer Epidemiol Biomarkers Prev. 2001;10:823-9.

27. Do KA, Johnson MM, Doherty DA, Lee JJ, Wu XF, Dong Q et al. Second primary tumors in patients with upper aerodigestive tract cancers: joint effects of smoking and alcohol (United States). Cancer Causes Control. 2003;14:131-8.

28. Berger MR, Zeller WJ. Interaction of nicotine with anticancer treatment. Klin Wochenschr. 1988;66:127-33.

29. Dasgupta P, Kinkade R, Joshi B, Decook C, Haura E, Chellappan S. Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. Proc Natl Acad Sci. USA 2006;103:6332-7.

30. Mayne ST, Cartmel B, Kirsh V, Goodwin WJ Jr. Alcohol and tobacco use prediagnosis and postdiagnosis, and survival in a cohort of patients with early stage cancers of the oral cavity, pharynx, and larynx. Cancer Epidemiol Biomarkers Prev. 2009;18:3368-74.

31. Chen AM, Chen LM, Vaughan A, Farwell DG, Luu Q, Purdy JA, et al. Head and neck cancer among lifelong never-smokers and ever-smokers: matched-pair analysis of outcomes after radiation therapy. Am J Clin Oncol. 2011;34:270-5.

32. Chen AM, Chen LM, Vaughan A, Sreeraman R, Farwell DG, Luu Q et al. Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavorable outcome. Int J Radiat Oncol Biol Phys. 2011;79:414-9.

33. Browman GP, Wong G, Hodson I, Sathya J, Russell R, McAlpine L et al. Influence of cigarette smoking on the efficacy of radiation therapy in head and neck cancer. N Engl J Med. 1993;328:159-63.

34. Siegelmann-Danieli N, Ben-Izhack O, Hanlon A, Ridge JA, Stein ME, Khandelwal V et al. P53 alteration in oral tongue cancer is not significantly associated with age at diagnosis or tobacco exposure. Tumori. 2005;91:346-50.

35. van Oijen MG, van de Craats JG, Slootweg PJ. P53 overexpression in oral mucosa in relation to smoking. J Pathol. 1999;187:469-74.

36. Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruban RH, Eby YJ et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. N Engl J Med. 1995;332:712-7.

37. Hsieh LL, Wang PF, Chen IH, Liao CT, Wang HM, Chen MC et al. Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. Carcinogenesis. 2001:22;1497-503.

38. Koch WM, Lango M, Sewell D, Zahurak M, Sidransky D. Head and neck cancer in nonsmokers: a distinct clinical and molecular entity. Laryngoscope. 1999;109:1544-51.

39. van Oijen MG, Gilsing MM, Rijksen G, Hordijk GJ, Slootweg PJ. Increased number of proliferating cells in oral epithelium from smokers and ex-smokers. Oral Oncol. 1998;34:297-303.

40. Farshadpour F, Hordijk GJ, Koole R, Slootweg PJ. Head and neck squamous cell carcinoma in non-smoking and non-drinking patients with multiple tumors: etiologic significance of p53 and Ki-67 in non-tumorous epithelium. J Oral Pathol Med. 2008;37:549-54.

41. Mărgăritescu C, Pirici D, Simionescu C, Mogoantă L, Raica M, Stîngă A et al. VEGF and VEGFRs expression in oral squamous cell carcinoma. Rom J Morphol Embryol 2009;50:527-48.

42. Faustino SE, Oliveira DT, Nonogaki S, Landman G, Carvalho AL, Kowalski LP. Expression of vascular endothelial growth factor-C does not predict occult lymph-node metastasis in early oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2008;37:372-8.

43. Kyzas PA, Geleff S, Batistatou A, Agnantis NJ, Stefanou D. Evidence for lymphangiogenesis and its prognostic implications in head and neck squamous cell carcinoma. J Pathol. 2005;206:170-7.

Artigo de Pesquisa

3 ARTIGO DE PESQUISA

O artigo a seguir intitula-se ***Immunodetection of VEGF, caspase-3 and p53 in oral squamous cell carcinomas of patients exposed and not exposed to smoking and alcohol*** e foi formatado de acordo com as normas do periódico ***Archives of Oral Biology*** (Anexo B).

Fator de impacto: 1.463

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IMMUNODETECTION OF VEGF, CASPASE-3 AND P53 IN ORAL SQUAMOUS CELL CARCINOMAS OF PATIENTS EXPOSED AND NOT EXPOSED TO SMOKING AND ALCOHOL

Running title: VEGF, CASPASE-3 AND P53 IN ORAL CARCINOMA

Key Words: Oral Cancer. Smoking. Alcoholism. Caspase-3. P53 proteins, VEGF

Juliana Hintz Germanos Scheidt

Liliane Soares Yurgel

Juliana Romanini

Karen Cherubini

Maria Antonia Zancanaro de Figueiredo

Fernanda Gonçalves Salum*

Oral Medicine Division, São Lucas Hospital - Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

Address for correspondence:
Fernanda Gonçalves Salum
Hospital São Lucas PUCRS
Av. Ipiranga, 6690 – Sala 231 – 2º andar
CEP: 90610-000
Porto Alegre – RS – Brazil
Tel/Fax: +55 51 3320-3254
E-mail: fesalum@terra.com.br

ABSTRACT

Objectives: The objective of this study was to compare the clinicopathological characteristics and immunoreactivity of VEGF, caspase-3 and p53 between oral squamous cell carcinomas (SCC) from users and non users of tobacco and alcohol. Design: We randomly selected 90 specimens of oral carcinomas, from patients diagnosed between 1991 and 2011 in Oral Medicine Division. The specimens were distributed into three groups according to exposure to risk factors: 30 specimens from smokers, 30 from smokers/alcohol drinkers and 30 from individuals not exposed to these factors. The clinicopathological characteristics of the lesions were evaluated and the proteins VEGF, caspase-3 and p53 were detected by immunohistochemistry. Results: The group of non-smokers/non-alcohol drinkers consisted mainly of women ($p \leq 0.001$), with a higher mean age ($p= 0.004$). The group of smokers/alcohol drinkers exhibited larger tumors when compared to patients not exposed to smoking and alcohol ($p=0.004$). The histopathological grading also differed between these groups ($p = 0.040$), since a greater number of grade I lesions and fewer grade III lesions were found in patient non-smokers/non-alcohol drinkers. No significant difference was observed in relation to immunoreactivity of VEGF ($p= 0.315$), caspase-3 ($p= 0.860$) and p53 ($p= 0.876$) between the groups. Conclusions: There are substantial clinicopathological differences between oral carcinomas in users and non users of tobacco and alcohol. In the present study, immunodetection of the proteins VEGF, caspase-3 and p53 was not influenced by smoking or alcohol consumption, suggesting that other molecular mechanisms are associated with the biological aggressiveness of oral carcinoma in patients exposed to these risk factors.

INTRODUCTION

Squamous cell carcinoma (SCC) accounts for approximately 95% of the malignancies of the oral cavity[1] and represents a public health problem in many countries due to the high morbi-mortality rates. Alcohol and smoking are primary factors for the genesis of this tumor, and when combined, their carcinogenic effects become potentiated.[2-5] Smoking is responsible for the increase of aneuploid cells in the oral epithelium, which are more likely to undergo malignant transformation.[6,7] Besides, smoke contains carcinogenic substances associated with decreased expression of tumor suppressor genes and genes involved in DNA repair and cell cycle control.[8,9] The participation of alcohol in the development of oral cancer is not too clear, but alcohol does have a toxic effect on the oral mucosa, can cause alterations in retinoic acid metabolism, and can induce cell hyperproliferation, increasing vulnerability to the action of other carcinogenic agents.[10] Besides participating in the genesis of oral SCC, smoking and alcohol affect the clinicopathological characteristics of the tumor. Response to treatment and the rates of recurrence, survival and prognosis are worse in patients exposed to these factors.[11-14]

Protein p53, which plays a major role in maintaining the integrity of the genetic code, contributes to tumor suppression through at least two mechanisms: induction of apoptosis and interruption of cell proliferation.[15-17] Its inactivation disrupts the DNA repair process, apoptosis and senescence, causing an increase in genetic instability, which can lead to the accumulation of mutations.[18,19] The mutation of the gene p53, which codes for the homologous protein, is the most common genetic anomaly in many types of cancer, where it is present in 35 to 67% of cases of oral SCC.[20,21]

The increase in the immunodetection and gene expression of p53 appear to be associated with a worse prognosis of oral cancer.[22-25]

Angiogenesis also shows an important role in tumor growth and metastasis. VEGF (vascular endothelial growth factor), considered a key regulator in the neovascularization process, increases vascular permeability and induces the proliferation, migration and survival of endothelial cells during tumor growth.[26,27] Cheng et al.[28] observed that malignant neoplasias of the mouth at more advanced stages, with the presence of metastatic lymphonodus, showed the highest VEGF immunoreactivity. Mărgăritescu et al.[29] suggested that the immunodetection of this marker can be considered a reliable tool in determining the prognosis of oral SCC, predicting shorter disease-free survival, worse general survival and metastatic disease.

Caspases belong to the family of cysteine proteases and are involved in apoptosis. As a consequence of their activation, there is inactivation of DNA repair enzymes, regulators of the cell cycle and proteins responsible for maintaining cell structure.[30] Caspase-3 is the main effector caspase, and depending on the type of tumor, its expression can be reduced, such as in Hodgkin lymphoma[31] and liver cancer,[32] or increased, such as in carcinomas of the breast.[33,34] Hague et al.[35] found a positive correlation between the immunodetection of caspase-3 and tumor staging in oral SCC, demonstrating greater expression in poorly differentiated lesions. On the other hand, Andressakis et al.[36] found a decrease in the expression of caspase-3 and -8 in carcinomas of the tongue when compared to normal epithelium and did not observe a correlation between the clinicopathological characteristics and the markers analyzed.

There is evidence that smoking and alcohol are not only involved in the genesis of oral cancer, but are also associated with a more aggressive disease. The aim of this study was to compare the clinicopathological characteristics and immunodetection of the proteins VEGF, caspase-3 and p53 between oral SCC from users and non users of tobacco and alcohol.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (Protocol 10/05052). A total of 90 specimens of oral squamous cell carcinoma, obtained from the pathology archive of the Oral Medicine Division, São Lucas Hospital (Brazil) and diagnosed between 1991 and 2011, were included. The histopathological diagnosis was reviewed and the histopathological grading of the lesions was determined by a pathologist in accordance with the criteria of the World Health Organization.[37] We excluded specimens from patients with recurrence of the lesion, of lip carcinoma, with proliferative verrucous leukoplakia, HIV positive, transplanted, chronic users of corticosteroids and from individuals who were receiving chemotherapy and/or radiotherapy. Carcinomas of patients with a history of smoking and alcohol consumption, but who had quit the habit prior to the diagnosis were also excluded from the study.

Information on sex and age of the patients, as well as on their smoking and alcohol consumption habits, was obtained from their medical charts. Patients were considered smokers if they smoked regularly (generally a pack a day) or who went through a total of six or more packs in a year. Positive exposure to alcohol was considered if the medical chart contained information about moderate or heavy

consumption of alcohol or if the patient had at least 14 drinks per week regularly.[22] In addition, we recorded data referring to the location and clinical aspects of the lesion.

A total of 90 specimens were distributed into three groups according to the presence or not of extrinsic factors related to the genesis of the oral cancer: 30 carcinomas from patient smokers and alcohol drinkers, 30 from patient smokers, and 30 from individuals not exposed to these risk factors.

Immunohistochemistry

We performed immunohistochemical assays using the Polymer-Based method. Proper formalin-fixed and paraffin-embedded tissues as 3-μm thick sections were obtained and mounted on slides coated with 3-aminopropiltrietoxisilano™ (Sigma-Aldrich, Brasil), and the slides were put in the oven at 60°C for 30 min. Slides were deparaffinized in xylene, rehydrated in a graded alcohol series, and washed in tap water. For antigen-retrieval, the sections were placed in a staining jar filled with Tris-EDTA solution, pH 9.0, and immersed in a water bath for 20 min at 100ºC. Slides were then placed at room temperature for 30 minutes and washed in tap water. Endogenous peroxidase activity was blocked with 5% peridrol for 30 min. Slides were again placed at room temperature for 30 minutes and washed in tap water. Non-specific reactions were blocked with 5% skim milk in phosphate-buffered saline (PBS) and washed. The sections were incubated overnight at 4ºC with mouse monoclonal antibodies against p53 (1:1.300 dilution, Dako, USA), VEGF (1:500 dilution, ZYMED, USA) e caspase-3 (1:500 dilution, LABSOURCE, USA). The slides were incubated with PicTure™-MAX (Zymed Laboratory, Carlsbad, USA) polymer conjugate and Dako Liquid DAB+ Substrate Chromogen System™ (3,3'-diaminobenzidine) (DAKO North America Inc., Carpinteria, USA) for the visualization

of antigen-antibody complexes. All sections were counterstained with Harris Hematoxylin. As a negative control, the primary antibodies were omitted from the reaction sequence. Tissue specimens of skin, in which we had detected positive immunoreactivity for VEGF were used as a positive control. Tonsillar tissue was used as positive control for caspase-3 and mammary gland tissue for p53.

Analysis of the images

Evaluation was conducted with the rater blinded to knowledge of the clinical and pathologic characteristics of the cases. Five equidistant fields were captured in each slide, at 200x magnification. The images were obtained with a conventional light microscope (Zeiss, model Axioshop 40, Germany), coupled to an image capture system (Media Cybernetics, model Cool SNAP-Procf, USA). The images were captured and saved in TIFF format. The program ImageJ version 1.41u (National Institute of Health, Bethesda, USA) was used for their analysis. The semi-automated segmentation method was utilized for the quantitative analysis of the immunodetection of p53, caspase-3 and VEGF. For each protein, one slide was selected, in which ten points corresponding to positive immunoreactivity were marked. This marking served as a model for automated analysis of the other slides. The mean percentage of immunodetection was established for the five fields analyzed.

Analysis of the data

The data were analyzed initially using descriptive statistics. The SPSS program version 15 (SPSS Inc., Chicago, USA) was used for statistical analysis. Analysis of variance (ANOVA) was utilized for comparison of the age of the patients

between the groups. The information referring to the sex of the patients and size and histopathological grading of the lesions were compared by the chi-square test. Immunopositivity for the markers VEGF, caspase-3 and p53 in the three groups was evaluated using the Kruskal-Wallis test. Spearman's correlation test was also used to correlate variables. Differences were considered significant when P was less than 0.05.

RESULTS

Of the 90 specimens selected, 36 were from female patients and 54 from male patients, with ages ranging from 34 to 86 years. The clinicopathological characteristics of the cases within the groups studied are presented Table 1.

A significant difference was observed between the group of non-smokers and non-alcohol drinkers in relation to others with respect to sex, since the majority (86.7%) of the patients of this group were women (chi-square test, $p \leq 0.001$). In addition, the mean age of the group of non-smokers and non-alcohol drinkers was significantly higher compared to the others (ANOVA, $p= 0.004$).

With regard to the size of the lesions, the group of smokers and alcohol drinkers exhibited significantly larger tumors, when compared to the group not exposed to smoking and alcohol (Kruskal Wallis, $p= 0.004$). The group of smokers did not differ from the others in relation to the size of the lesions. Histopathological grading also differed between the patient smokers and alcohol drinkers and those not exposed to these factors (Kruskal Wallis, $p = 0.040$), since a larger number of grade I lesions and smaller number of grade III lesions were found in patient non-smokers and non-alcohol drinkers. In this group, an inverse correlation was seen between

histopathological grading and age of the patients (Spearman's correlation test, p= 0.043).

Table 1. Demographic distribution of the patients and clinicopathological characteristics of the lesions within the groups studied.

	GROUPS			
	Smokers n=30	Smokers and alcohol drinkers n=30	Non-smokers and non-alcohol drinkers n=30	P
Sex				
Male	21 (70%)	29 (96.7%)	4 (13.3%)	≤ 0.001*
Female	9 (30%)	1 (3.3%)	26 (86.7%)*	
Age (years)				
Range	34-84	48-77	39-86	
Mean ± SD	56.53 (± 10.63)^{A}	58.10 (±7.97)^{A}	65.60 (±13.34)^{B}	0.004**
Size of lesion				
Up to 2 cm	11 (36.7%)	4 (13.8%)	18 (60.0%)	
2,1 cm to 4.0 cm	14 (46.7%)	17 (58.6%)	8 (26.7%)	0.004***
Greater than 4.1 cm	5 (16.7%)	8 (27.6%)	4 (13.3%)	
Mean Rank	44.47^{AB}	55.76^{A}	35.13^{B}	
Histopathological grading				
Grade I	11 (36.7%)	5 (16.7%)	14 (46.7%)	
Grade II	10 (33.3%)	15 (50.0%)	12 (40.0%)	0.04***
Grade III	9 (30.0%)	10 (33.3%)	4 (13.3%)	
Mean Rank	45.72^{AB}	53.42^{A}	37.37^{B}	
Localization				
Tongue				
Edge	6 (20%)	7 (23.3%)	11 (37.9%)	
Ventrum	5 (16.7%)	5 (16.7%)	8 (27.6%)	
Dorsum	1 (3.3%)	-	2 (6.9%)	
Base	-	1 (3.3%)	-	
Floor	11 (36.7%)	16(53.3%)	2 (6.8%)	
Gingiva/ridge	9 (30%)	12 (40%)	8 (27.6%)	
Buccal mucosa	3 (10%)	4 (13.3%)	2 (6.9%)	
Retromolar region	2 (6.7%)	2 (6.7%)	-	
Palate, hard/soft	3 (10%)	6 (20%)	-	
Tonsillar pillar	1 (3.3%)	2 (6.7%)	1 (3.4%)	
Oropharynx	1 (3.3%)	1 (3.3%)	-	
Uvula	-	1 (3.3%)	-	
Mandible	-	1 (3.3%)	-	

*Chi-Square Test

**Means followed by different letters differed significantly based on ANOVA complemented by Tukey's test for multiple comparisons

*** Mean Rank followed by different letters differed significantly based on Kruskal Wallis complemented by test for multiple comparisons.

Table 2 shows the percentage of immunoreactivity for the markers studied. No significant difference was observed between the groups with respect to immunoreactivity for VEGF, caspase-3 and p53 (Fig. 1). There was also no significant correlation between the immunodetection of VEGF, caspase-3 and p53 and the clinicopathological characteristics investigated (data not shown).

Table 2. Percentage of immunoreactivity for the markers VEGF, p53 and caspase-3 in the three groups studied (median, p25-p75)

	GROUPS			
MARKER	Smokers	Smokers and alcohol drinkers	Non-smokers and non-alcohol drinkers	**P**
VEGF	29.57 (11.35-46.99)	44.31 (12.03-61.09)	36.76 (26.73-59.66)	0.315
p53	8.78 (2.43-14.67)	6.95 (1.84-12.12)	8.65 (4.20-13.13)	0.876
caspase-3	10.52 (3.24-69.53)	23.35 (1.95-61.55)	9.32 (2.07-71.13)	0.860

Kruskall Wallis

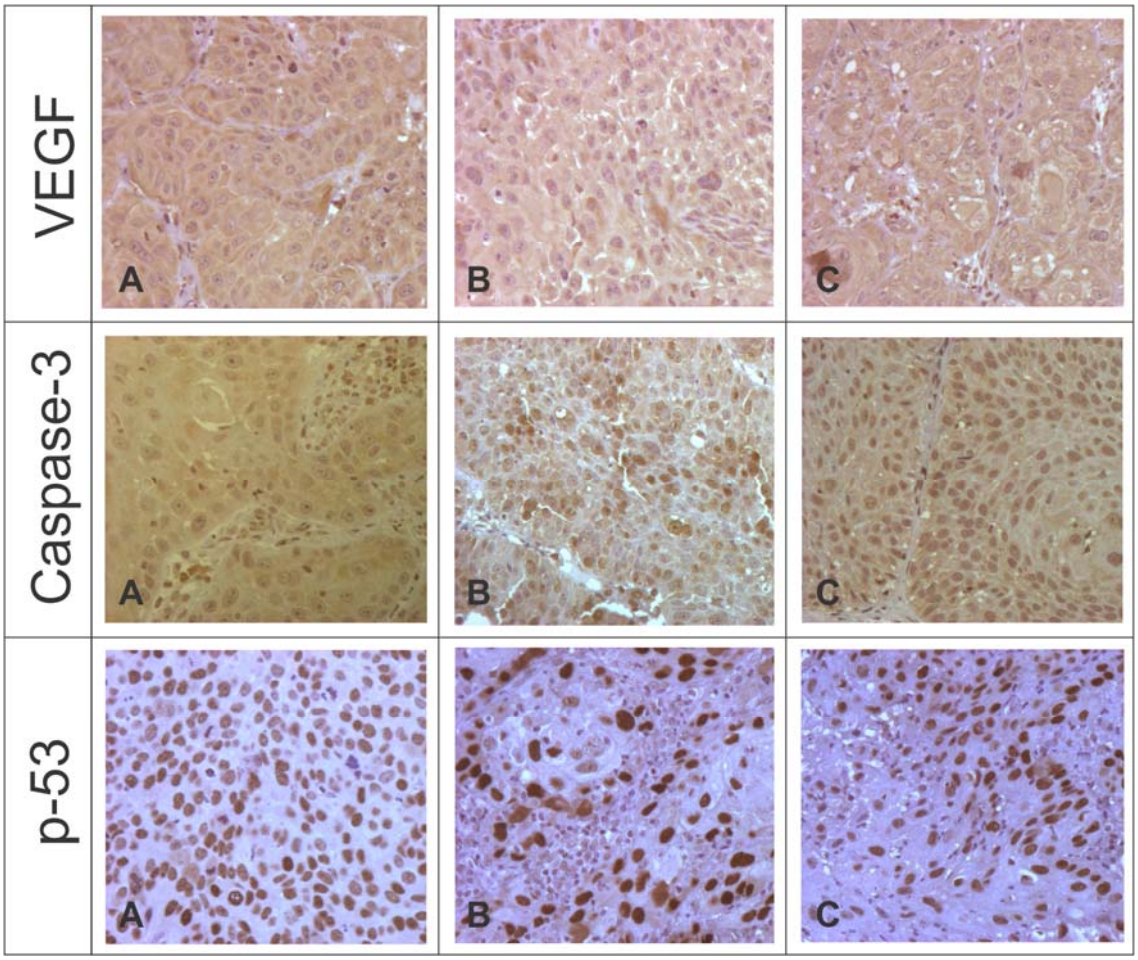


Figure 1. Immunoreactivity of proteins VEGF, caspase-3 and p53 in the groups of smokers (A), smokers/alcohol drinkers (B) and non-smokers/ non-alcohol drinkers (C) in oral squamous cell carcinoma.

DISCUSSION

In the present study, the clinicopathological characteristics and immunodetection of the proteins VEGF, caspase-3 and p53 in oral SCC were compared between smokers and/or alcohol drinkers and patients who had not been exposed to these risk factors. There is evidence that smoking and alcohol consumption are associated with a more aggressive biological behavior of oral carcinoma and, consequently, a worse prognosis of the disease.[11-14] In addition, studies suggest that the immunodetection of p53, VEGF and caspase-3 correlates

with a more advanced clinical stage of the disease and shows a prognostic value in head and neck SCC. [25,29,35,38-41]

No significant difference was found between the groups with respect to the immunohistochemical detection of these three proteins. Siegelmann-Danieli et al.[22] also found no association between the immunodetection of the protein p53 with smoking and alcohol in studying carcinomas of the tongue. Other authors suggest that the mutation of p53 is an early event of oral carcinogenesis, generally detected in cancerous lesions. On the other hand, in evaluating carcinomas of the head and neck and adjacent tissues, Brennan et al.[42] and Van Oijen, Van de Craats and Slootweg[43] found greater immunoreactivity for p53 in patient smokers. However, these studies involved tumors located in the larynx, hypopharynx and oropharynx, while the present study was restricted to lesions of the oral cavity.

Several studies have associated the expression of the angiogenesis marker VEGF with prognosis or oral cancer.[29,39-41] Swenson et al.[44] observed that condensed smoke form cigarettes interferes with the expression of VEGF. In a retrospective study, Kyzas et al.[39] analyzed 69 patients with malignant lesions of the head and neck and observed a significant correlation between the expression of this marker and a more advanced clinical stage of the disease, demonstrating that the expression of VEGF can have a prognostic value for patients with this neoplasm. However, these authors did not find a correlation between VEGF and clinicopathological characteristics such as age, histological grading and smoking, which was also found in the present study. Faustino et al.[45] evaluated the correlation between immunoreactivity for VEGF-C with clinicopathological findings in 87 cases of oral SCC and also did not find a significant association between this marker and

gender, age, location of the lesion, local and regional recurrence, metastatic lymphonodus, or smoking and alcohol consumption habits.

There are few studies that have determined an association between expression of caspase-3 and alcohol consumption and smoking in oral SCC. Hague et al.[35] found higher expression of this marker in poorly differentiated tumors. On the other hand, Andressakis et al.[36] did not observe a correlation between the clinicopathological characteristics of the lesion and the expression of caspase-3. Elias et al.[46] analyzed the cytotoxic effect of cigarette smoke extract on cultured cells of oral SCC and observed that the smoke significantly increased apoptosis in this malignant neoplasm, which was demonstrated by activation of caspase-3. In our study, greater expression of caspase-3 was found in the groups of smokers and/or alcohol drinkers when compared with the control, although the difference was not significant.

Corroborating the data in the literature, in the group of non-smokers and non-alcohol drinkers, the majority of the patients were women with a higher mean age when compared to the groups exposed to the risk factors investigated.[47-51] In this study, patients not exposed to the risk factors exhibited better differentiated lesions when compared to patients who were smokers and alcohol drinkers. Besides, the majority of the tumors of non-exposed patients measured less than 2 cm in diameter, while in patient smokers and alcohol drinkers, most of the lesions exhibited a larger diameter. Dahlstrom et al.,[52] Harris et al.[53] and Kruse et al.[51] also found that the majority of the oral SCC in patients who were non-smokers and non-alcohol drinkers were grade I lesions, T1 or T2.

The present study showed that there are substantial clinicopathological differences in oral carcinomas between users and non users of tobacco and alcohol.

However, despite the distinct clinicopathological characteristics, we did not find a significant difference in the immunodetection of the proteins p53, caspase-3 and VEGF between the groups. These proteins have a prognostic value for oral SCC, but their immunodection is not influenced by smoking and alcohol consumption, suggesting that other molecular mechanisms are associated with the biological aggressiveness of oral cancer in patients exposed to these risk factors.

REFERENCES

1. Andisheh-Tadbir A, Mehrabani D, Heydari ST. Epidemiology of squamous cell carcinoma of the oral cavity in Iran. J Craniofac Surg 2008;19(6):1699-1702.

2. Talamini R, Bosetti C, La Vecchia C, Dal Maso L, Levi F, Bidoli E et al. Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. Cancer Causes Control 2002;13(10):957-64.

3. De Stefani E, Boffetta P, Deneo-Pellegrini H, Ronco AL, Acosta G, Ferro G, et al. The effect of smoking and drinking in oral and pharyngeal cancers: a case-control study in Uruguay. Cancer Lett 2007;246(1-2):282-9.

4. Ide R, Mizoue T, Fujino Y, Hoshiyama Y, Sakata K, Tamakoshi A et al. Cigarette smoking, alcohol drinking, and oral and pharyngeal cancer mortality in Japan. Oral Dis 2008;14(4):314-9.

5. Pelucchi C, Gallus S, Garavello W, Bosetti C, La Vecchia C. Alcohol and tobacco use, and cancer risk for upper aerodigestive tract and liver. Eur J Cancer Prev 2008;17(4):340-4.

6. Dey P. Aneuploidy and malignancy: an unsolved equation. J Clin Pathol 2004;57(12):1245-9.

7. Souto GR, Caliari MV, Lins CE, de Aguiar MC, de Abreu MH, Mesquita RA. Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. J Oral Pathol Med 2010;39(8):605-10.

8. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma 2: chromosomal aberrations. Oral Oncol 2000;36(4):311-27.

9. Smith RV, Schlecht NF, Childs G, Prystowsky MB, Belbin TJ. Pilot study of mucosal genetic differences in early smokers and nonsmokers. Laryngoscope 2006;116(8):1375-9.

10. Homann N, Kärkkäinen P, Koivisto T, Nosova T, Jokelainen K, Salaspuro M. Effects of acetaldehyde on cell regeneration and differentiation of upper gastrointestinal tract mucosa. J Natl Cancer Inst 1997;89(22):1692-7.

11. Browman GP, Wong G, Hodson I, Sathya J, Russell R, McAlpine L et al. Influence of cigarette smoking on the efficacy of radiation therapy in head and neck cancer. N Engl J Med 1993;328(3):159-63.

12. Do KA, Johnson MM, Doherty DA, Lee JJ, Wu XF, Dong Q et al. Second primary tumors in patients with upper aerodigestive tract cancers: joint effects of smoking and alcohol (United States). Cancer Causes Control 2003;14(2):131-8.

13. Pytynia KB, Grant JR, Etzel CJ, Roberts DB, Wei Q, Sturgis EM. Matched-pair analysis of survival of never smokers and ever smokers with squamous cell carcinoma of the head and neck. J Clin Oncol 2004;22(19):3981-8.

14. Fortin A, Wang CS, Vigneault E. Influence of smoking and alcohol drinking behaviors on treatment outcomes of patients with squamous cell carcinomas of the head and neck. Int J Radiat Oncol Biol Phys 2009;74(4):1062-9.

15. Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 1991;352(6333):345-7.

16. Hartwell L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell 1992;71(4):543-6.

17. Mithani SK, Mydlarz WK, Grumbine FL, Smith IM, Califano JA. Molecular genetics of premalignant oral lesions. Oral Dis 2007;13(2):126-33

18. Vousden KH. Outcomes of p53 activation--spoilt for choice. J Cell Sci 2006;119(Pt 24):5015-20.

19. Chari NS, Pinaire NL, Thorpe L, Medeiros LJ, Routbort MJ, McDonnell TJ. The p53 tumor suppressor network in cancer and the therapeutic modulation of cell death. Apoptosis 2009;14(4):336-47.

20. Holm R, Skomedal H, Helland A, Kristensen G, Børresen AL, Nesland JM. Immunohistochemical analysys of p53 protein overexpression in normal, premalignant, and malignant tissues of the cervix uteri. J Pathol 1993;169(1):21-6.

21. Cruz I, Napier SS, van der Waal I, Snijders PJ, Walboomers JM, Lamey PJ et al. Suprabasal p53 immunoexpression is strongly associated with high grade dysplasia and risk for malignant transformation in potentially malignant oral lesions from Northern Ireland. J Clin Pathol 2002;55(2):98-104.

22. Siegelmann-Danieli N, Ben-Izhack O, Hanlon A, Ridge JA, Stein ME, Khandelwal V et al. P53 alteration in oral tongue cancer is not significantly associated with age at diagnosis or tobacco exposure. Tumori 2005;91(4):346-50.

23. Kato K, Kawashiri S, Tanaka A, Noguchi N, Nakaya H, Hase T et al. Predictive value of measuring p53 labeling índex at the invasive front of oral squamous cell carcinomas. Pathol Oncol Res 2008;14(1):57-61.

24. Tanuma J, Izumo T, Hirano M, Oyazato Y, Hori F, Umemura E et al. FGFR4 polymorphism, TP53 mutation, and their combinations are prognostic factors for oral squamous cell carcinoma. Oncol Rep 2010;23(3):739-44.

25. Kato K, Kawashiri S, Yoshizawa K, Kitahara H, Okamune A, Sugiura S et al. Expression formo f p53 and PCNA at the invasive front in oral squamous cell carcinoma: correlation with clinicopathological features and prognosis. J Oral Pathol Med 2011.DOI:10.1111/j.1600-0714.2011.01032.x.

26. Siemeister G, Martiny-Baron G, Marmé D. The pivotal role of VEGF in tumor angiogenesis: molecular facts and therapeutic opportunities. Cancer Metastasis Rev 1998;17(2):241-8.

27. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9(6):669-76.

28. Chen AM, Chen LM, Vaughan A, Sreeraman R, Farwell DG, Luu Q et al. Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavorable outcome. Int J Radiat Oncol Biol Phys 2011;79(2):414-9.

29. Mărgăritescu C, Pirici D, Simionescu C, Mogoantă L, Raica M, Stîngă A et al. VEGF and VEGFRs expression in oral squamous cell carcinoma. Rom J Morphol Embryol 2009;50(4):527-48.

30. Krajewska M, Wang HG, Krajewski S, Zapata JM, Shabaik A, Gascoyne R et al. Immunohistochemical analysis of in vivo patterns of expression of CPP32 (Caspase-3), a cell death protease. Cancer Res 1997;57(8):1605-13.

31. Chhanabhai M, Krajewski S, Krajewska M, Wang HG, Reed JC, Gascoyne RD. Immunohistochemical analysis of interleukin-1 beta-converting enzyme/Ced-3 family protease, CPP32/Yama/Caspase-3, in Hodgkin´s disease. Blood 1997;90(6):2451-5.

32. Fujikawa K, Shiraki K, Sugimoto K, Ito T, Yamanaka T, Takase K, et al. Reduced expression of ICE/caspase1 and CPP32/caspase3 in human hepatocellular carcinoma. Anticancer Research 2000;20(3B):1927-32.

33. Vakkala M, Pääkkö P, Soini Y. expression of caspases 3, 6 and 8 is increased in parallel with apoptosis and histological aggressiveness of the breast lesion. Br J Cancer 1999;81(4):592-9.

34. O'Donovan N, Crown J, Stunell H, Hill AD, McDermott E, O'Higgins N et al. Caspase 3 in breast cancer. Clin Cancer Res 2003;9(2):738-42.

35. Hague A, Eveson JW, MacFarlane M, Huntley S, Janghra N, Thavaraj S. Caspase-3 expression is reduced, the absence of cleavage, in terminally differentiated normal oral epithelium but is increased in oral squamous cell carcinomas and correlates with tumour stage. J Pathol 2004;204(2):175-82.

36. Andressakis D, Lazaris AC, Tsiambas E, Kavantzas N, Rapidis A, Patsouris E. Evaluation of caspase-3 and caspase-8 deregulation in tongue squamous cell carcinoma, based on immunohistochemistry and computerized image analysis. J Laryngol Otol 2008;122(11):1213-8.

37. Pindborg JJ, Wahi PN. Histological Typing of Cancer and Precancer of the Oral Mucosa. 2th ed . Springer; 1997

38. Boslooper K, King-Yin Lam A, Gao J, Weinstein S, Johnson N. The clinicopathological roles of alpha-B-crystallin and p53 expression in patients with head and neck squamous cell carcinoma. Pathology 2008;40(5):500-4.

39. Kyzas PA, Geleff S, Batistatou A, Agnantis NJ, Stefanou D. Evidence for lymphangiogenesis and its prognostic implications in head and neck squamous cell carcinoma. J Pathol 2005;206(2):170-7.

40. Boonkitticharoen V, Kulapaditharom B, Leopairut J, Kraiphibul P, Larbcharoensub N, Cheewaruangroj W et al. Vascular endothelial growth factor a and proliferation marker in prediction of lymph node metastasis in oral and pharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2008;134(12):1305-11.

41. Cheng SJ, Lee JJ, Kok SH, Chou CH, Chang HH, Yang H et al. Expression of vascular endothelial growth factor is significantly associated with progression and prognosis of oral squamous cell carcinomas in Taiwan. J Formos Med Assoc. 2011;110(1):50-7.

42. Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruban RH, Eby YJ et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. N Engl J Med 1995;332(11):712-7.

43. Van Oijen MG, Van de Craats JG, Slootweg PJ. P53 overexpression in oral mucosa in relation to smoking. J Pathol 1999;187(4):469-74.

44. Swenson WG, Wuertz BR, Ondrey FG. Tobacco carcinogen mediated up-regulation of AP-1 dependent pro-angiogenic cytokines in head and neck carcinogenesis. Mol Carcinog 2011. DOI: 10.1002/mc.20775

45. Faustino SE, Oliveira DT, Nonogaki S, Landman G, Carvalho AL, Kowalski LP. Expression of vascular endothelial growth factor-C does not predict occult lymph-node metastasis in early oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2008;37(4):372-8.

46. Elias ST, Diniz J, Almeida RS, Alvarenga N, Simeoni LA, Silveira D et al. Cytotoxic effect of tobacco extracts on human oral squamous cell carcinoma cell-line. Oral Oncol 2010;46(12):869-73.

47. Link MJO, Kaugars GE, Burns JC. Comparison of oral carcinomas in smokeless tobacco users and nonusers. J Oral Maxillofac Surg. 1992:50:452-5.

48. Lo WL, Kao SY, Chi LY, Wong YK, Chang RC. Outcomes of oral squamous cell carcinoma in Taiwan after surgical therapy: factors affecting survival. J Oral Maxillofac Surg 2003;61(7):751-8.

49. Wiseman SM, Swede H, Stoler DL, Anderson GR, Rigual NR, Hicks WL Jr et al. Squamous cell carcinoma of the head and neck in nonsmokers and nondrinkers: an analysis of clinicopathologic characteristics and treatment outcomes. Ann Surg Oncol 2003;10(5):551-7.

50. Farshadpour F, Hordijk GJ, Koole R, Slootweg PJ. Non-smoking and non-drinking patients with head and neck squamous cell carcinoma: a distinct population. Oral Dis 2007;13(2):239-43.

51. Kruse AL, Bredell M, Grätz KW. Oral squamous cell carcinoma in non-smoking and non-drinking patients. Head Neck Oncol. 2010:2:1-3.

52. Dahlstrom KR, Little JA, Zafereo ME, Lung M, Wei Q, Sturgis EM. Squamous cell carcinoma of the head and neck in never smoker-never drinkers: a descriptive epidemiologic study. Head Neck 2008;30(1):75-84.

53. Harris SL, Kimple RJ, Hayes DN, Couch ME, Rosenman JG. Never-smokers, never-drinkers: unique clinical subgroup of young patients with head and neck squamous cell cancers. Head Neck 2010;32(4):499-503.

Discussão Geral

4 DISCUSSÃO GERAL

O carcinoma de células escamosas é a neoplasia maligna mais prevalente da mucosa bucal e seu desenvolvimento está associado a fatores intrínsecos e extrínsecos, principalmente, ao tabaco e ao álcool. Na literatura há evidências de que estes agentes não só participam da gênese do câncer de boca, como também estão associados a um comportamento biológico mais agressivo e, consequentemente, a um pior prognóstico da doença (BROWMAN et al., 1993; Do et al., 2003, PYTYNIA et al., 2004; FORTIN; WANG; VIGNEAULT, 2009). Alguns estudos sugerem que o aumento da imunorreatividade de p53, VEGF e caspase-3 pode estar associado a um estágio clínico mais avançado da doença e apresentar valor prognóstico no CCE de cabeça e pescoço (HAGUE et al., 2004; KYZAS et al., 2005; BOONKITTICHAROEN et al., 2008; BOSLOOPER et al., 2008; MĂRGĂRITESCU et al., 2009; CHENG et al., 2011; KATO et al., 2011). Neste estudo retrospectivo, as características clínico-patológicas de CCE bucais, bem como a imunodetecção das proteínas VEGF, caspase-3 e p53 foram comparadas entre lesões de pacientes tabagistas e etilistas com as de indivíduos não expostos a estes fatores de risco.

Corroborando os dados da literatura, no grupo de não tabagistas e não etilistas, a maioria dos pacientes era do sexo feminino, com média de idade superior quando comparados aos indivíduos expostos aos fatores investigados (LINK; KAUGARS; BURNS, 1992; LO et al., 2003; WISEMAN et al., 2003; FARSHADPOUR et al., 2007; KRUSE; BREDELL; GRÄTZ, 2010). Além disso, pacientes não tabagistas e não etilistas apresentaram, principalmente, lesões grau I, enquanto indivíduos tabagistas e etilistas exibiram maior número de lesões grau III. Estes

dados foram também observados por Dahlstrom et al. (2008) e Harris et al. (2010) que encontraram maior percentual de lesões bem diferenciadas em pacientes não expostos aos principais fatores extrínsecos. Outra característica distinta entre os grupos foi o tamanho das lesões, uma vez que pacientes não expostos apresentaram, em sua maioria, tumores com menos de 2 cm de diâmetro, enquanto nos indivíduos tabagistas e etilistas as lesões exibiram diâmetro superior. Kruse, Bredell e Grätz (2010) constataram que a maioria das neoplasias de boca em não fumantes e não etilistas foi T1 ou T2 indo ao encontro dos resultados encontrados no presente estudo. A disseminação metastática não foi avaliada, pois na maioria dos prontuários selecionados estas informações não constavam, não sendo possível investigar o estadiamento clínico da doença.

Apesar das características clínico-patológicas distintas observadas entre os grupos de pacientes com CCE bucal, não foi encontrada diferença significativa quanto à detecção imunoistoquímica de VEGF, caspase-3 e p53. Siegelmann-Danieli et al. (2005) também não encontraram associação da imunodetecção da proteína p53 com o uso do tabaco e do álcool. Foram estudados 45 casos de carcinoma de língua, 30 de pacientes tabagistas (a maioria também consumia álcool) e 15 de não tabagistas. Os autores sugerem que a mutação da p53 seja um evento precoce da carcinogênese bucal. Por outro lado, ao avaliarem carcinomas de cabeça e pescoço e tecidos adjacentes, Brennan et al. (1995) e Van Oijen, Van de Craats e Slootweg (1999) encontraram imunorreatividade superior da p53 em pacientes fumantes. Estes estudos envolveram tumores localizados em laringe, hipofaringe e orofaringe, enquanto o presente restringiu-se a lesões da cavidade bucal.

São escassos os trabalhos que fazem a correlação entre a expressão do VEGF com o alcoolismo e o tabagismo no CCE bucal. Ao estudarem o CCE de boca, Faustino et al. (2008) não encontraram associação significativa entre a expressão do VEGF e características clínico-patológicas como gênero, idade, localização da lesão, grupo étnico, recorrência local e regional, metástases linfonodais ou com os hábitos de tabagismo e etilismo. Kyzas et al. (2005), em um estudo retrospectivo com 69 pacientes com lesões malignas de cabeça e pescoço, observaram correlação significativa entre a expressão deste marcador e um estágio clínico mais avançado da doença. Entretanto, também não encontraram correlação entre a imunorreatividade do VEGF e características clínico-patológicas como idade, graduação histológica, metástases em linfonodos e hábito de tabagismo.

O valor prognóstico da imunodetecção da caspase-3 no carcinoma bucal não é tão claro quanto o das demais proteínas investigadas. Hague et al. (2004) encontraram maior expressão deste marcador em tumores malignos bucais pobremente diferenciados, enquanto Andressakis et al. (2008) não observaram correlação entre as características clínico-patológicas e este marcador em carcinomas de língua. Neste estudo, a imunodetecção da caspase-3 foi superior nos grupos de tabagistas e etilistas quando comparados aos pacientes-controle, embora esta diferença não tenha sido significativa. A literatura é escassa quanto à análise da imunorreatividade dessa proteína em carcinomas bucais, investigando o efeito do fumo e do álcool. Elias et al. (2010) analisaram o efeito citotóxico do extrato do tabaco em culturas de células de tumores malignos epiteliais de boca e observaram que o fumo aumentou significativamente a apoptose nesta neoplasia maligna, o que foi constatado pela ativação da caspase-3.

Para análise quantitativa da imunodetecção dos marcadores estudados foi empregado o método de segmentação semi-automatizada, uma vez que o VEGF e a caspase-3 são detectados no citoplasma das células, o que facilita a ocorrência de vieses ao realizar-se a contagem manual. Utilizando este método foi possível padronizar-se a avaliação da imunorreatividade.

Em nosso estudo retrospectivo foram incluídos casos de carcinoma bucal diagnosticados entre 1991 e 2011, provenientes do arquivo do laboratório do Serviço de Estomatologia do Hospital São Lucas-PUCRS. Uma dificuldade encontrada para avaliação dos hábitos de tabagismo e etilismo foi a falta de informações em alguns dos prontuários analisados, principalmente, em relação ao tipo de bebida alcoólica ingerida e a quantidade de tabaco e álcool consumidos. Por essa razão, não foi possível verificar a correlação entre a quantidade de fumo e de bebidas alcoólicas utilizados com a imunorreatividade de VEGF, caspase-3 e p53.

Foram selecionados pacientes usuários de tabaco e álcool e aqueles que não apresentaram histórico de utilização destas substâncias. Para evitar vieses nos resultados obtidos, foram excluídos pacientes que, no momento do diagnóstico, relataram ser ex-tabagistas e ex-etilistas. Foram excluídos também casos de CCE de lábio, pois esta neoplasia maligna apresenta comportamento distinto ao das demais regiões da cavidade bucal e sua gênese tem forte associação com a exposição solar. Lesões de pacientes imunossuprimidos por HIV, transplantados, usuários de corticosteróides também não foram incluídas, pois esses fatores, além de aumentar as chances de aparecimento de neoplasias malignas, estão relacionados a uma doença mais agressiva. Também foram excluídos tumores que representaram recidivas e lesões de pacientes portadores de leucoplasia verrucosa

proliferativa, que se caracteriza por lesões progressivas, as quais evoluem frequentemente para o CCE e possuem fraca relação com o tabaco e o álcool.

Nosso estudo não demonstrou associação entre exposição ao tabaco e ao álcool com a imunodetecção de p53, VEGF e caspase-3 em CCE bucais. Seria interessante confirmar os resultados apresentados por meio de técnicas que avaliassem também a expressão dos genes VEGF, caspase-3 e p53 como, por exemplo, a PCR (*Polymerase Chain Reaction*) em tempo real. Entretanto, a extração de RNA dos espécimes da amostra estudada poderia ser inviável, uma vez que muitos dos casos foram diagnosticados há quase 20 anos. Para a realização dessa investigação seria necessário um estudo prospectivo, sendo o material coletado a fresco e congelado a -80°C. A utilização da técnica de imunoistoquímica no presente estudo permitiu que se obtivesse uma amostra de 90 casos, 30 dos quais provenientes de pacientes não fumantes e não etilistas.

Este estudo mostrou que existem diferenças clínico-patológicas importantes entre carcinomas bucais de células escamosas de pacientes tabagistas e etilistas e de não tabagistas e não etilistas. Neste grupo, as lesões ocorreram preferencialmente no sexo feminino, em uma faixa etária mais avançada, além de predominarem tumores bem diferenciados e de tamanho menor. No entanto estas diferenças não foram confirmadas na análise imunoistoquímica pela detecção das proteínas p53, caspase-3 e VEGF, sugerindo que esta não é influenciada pelo tabagismo e etilismo. Apesar das três proteínas estudadas serem consideradas marcadores prognósticos para o carcinoma bucal de células escamosas, outros eventos moleculares podem estar associados à maior agressividade biológica tumoral nos pacientes usuários de tabaco e álcool.

Referências

REFERÊNCIAS

ANDRESSAKIS, D. et al. Evaluation of caspase-3 and caspase-8 deregulation in tongue squamous cell carcinoma, based on immunohistochemistry and computerised image analysis. **J Laryngol Otol**, London, v. 122, n. 11, p. 1213-8, May 2008.

BOONKITTICHAROEN, V. et al. Vascular endothelial growth factor a and proliferation marker in prediction of lymph node metastasis in oral and pharyngeal squamous cell carcinoma. **Arch Otolaryngol Head Neck Surg**, Chicago, v. 134, n. 12, p. 1305-11, Dec. 2008.

BOSLOOPER, K. et al. The clinicopathological roles of alpha-B-crystallin and p53 expression in patients with head and neck squamous cell carcinoma. **Pathology**, Surry Hills, v. 40, n. 5, p. 500-4, Aug. 2008.

BRENNAN, J. A. et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. **N Engl J Med**, Boston, v. 332, n. 11, p. 712-7, Mar. 1995.

BROWMAN, G. P. et al. Influence of cigarette smoking on the efficacy of radiation therapy in head and neck cancer. **N Engl J Med**, Boston, v. 328, n. 3, p. 159-63, Jan. 1993.

CANÇADO, R.P.; YURGEL, L.S.; SANTANA-FILHO, M. Evaluation of the nucleolar organizer region associated proteins in exfoliative cytology of normal buccal mucosa. Effect of smoking. **Oral Oncol**, **Oxford**, v. 37, n. 5, p. 446-54, July 2001.

CHARI, N. S. et al. The p53 tumor suppressor network in cancer and the therapeutic modulation of cell death. **Apoptosis**, London, v. 14, n. 4, p. 336-47, Apr. 2009.

CHEN, A. M. et al. Head and Neck cancer among lifelong never-smokers and ever-smokers: matched-pair analysis of outcomes after radiation therapy. **Am J Clin Oncol**, New York, v. 34, n. 3, p. 270-5, June 2011A.

CHEN, A. M. et al. Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavorable outcome. **Int J Radiat Oncol Biol Phys**, Elmsford, v. 79, n. 2, p. 414-9, Feb. 2011B.

CHENG, S. J. Expression of vascular endothelial growth factor is significantly associated with progression and prognosis of oral squamous cell carcinomas in Taiwan. **J Formos Med Assoc**, Taipei, v. 110, n. 1, p. 50-7, Jan. 2011.

CHHANABHAI, M. et al. Immunohistochemical analysis of interleukin-1beta-converting enzyme/Ced-3 family protease, CPP32/Yama/Caspase-3, in Hodgkin's disease. **Blood**, New York, v. 90, n. 6, p. 2451-5, Sept. 1997.

CHUANG, H. C. et al. High expression of CD 105 as a prognostic predictor of early tongue cancer. **Laryngoscope**, St. Louis, v. 116, n. 7, p. 1175-9, July 2006.

DAHLSTROM, K. R. et al. Squamous cell carcinoma of the head and neck in never smoker-nerver drinkers: a descriptive epidemiologic study. **Head Neck**, New York, v. 30, n. 1, p. 75-84, Jan. 2008.

DEGTEREV, A.; BOYCE, M.; YUAN, J. A decade of caspases. **Oncogene**, Basingstoke, v. 22, n. 53, p. 8543-67, Nov. 2003.

DE STEFANI, E. et al. The effect of smoking and drinking in oral and pharyngeal cancers: a case-control study in Uruguay. **Cancer Lett**, Amsterdam, v. 246, n. 1-2, p. 282-9, Feb. 2007.

DO, K. A. et al. Second primary tumors in patients with upper aerodigestive tract cancers: joint effects of smoking and alcohol (United States). **Cancer Causes Control**, Oxford, v. 14, n. 2, p. 131-8, Mar. 2003.

ELIAS, S.T. et al. Cytotoxic effect of tobacco extracts on human oral squamous cell carcinoma cell-line. **Oral Oncol**, Oxford, v. 46, n. 12, p. 869-73, Dec. 2010.

FARSHADPOUR, F. et al.Non-smoking and non-drinking patients with head and neck squamous cell carcinoma: a distinct population. **Oral Dis**, Houndmills, v. 13, n. 2, p. 239-43, Mar. 2007.

FAUSTINO, S. E. et al. Expression of vascular endothelial growth factor-C does not predict occult lymph-node metastasis in early oral squamous cell carcinoma. **Int J Oral Maxillofac Surg**, Copenhagen, v. 37, n. 4, p. 372-8, Apr. 2008.

FELDMAN, J. G.; HAZAN, M. A case-control investigation of alcohol, tobacco and diet in head and neck cancer. **Prev Med.**, Baltimore, v. 4, n. 4, p. 444-63, Dec. 1975.

FERRARA, N.; GERBER, H.P.; LECOUTER, J. The biology of VEGF and its receptors. **Nat Med**, New York, v. 9, n. 6, p. 669-76, June 2003.

FORTIN, A.; WANG, C.S.; VIGNEAULT, E. Influence of smoking and alcohol drinking behaviors on treatment outcomes of patients with squamous cell carcinomas of the head and neck. **Int J Radiat Oncol Biol Phys**, Elmsford, v. 74, n. 4, p. 1062-9, July 2009.

FUJIKAWA, K. et al. Reduced expression of ICE/caspase1 and CPP32/caspase3 in human hepatocellular carcinoma. **Anticancer Res**, Athens, v. 20, n. 3B, p. 1927-32, May-June 2000.

HAGUE, A. et al. Caspase-3 expression is reduced, in the absence of cleavage, in terminally differentiated normal oral epithelium but is increased in oral squamous cell carcinomas and correlates with tumour stage. **J Pathol**, London, v. 204, n. 2, p. 175-82, Oct. 2004.

HARRIS, S. L. et al. Never-smokers, never-drinkers: unique clinical subgroup of young patients with head and neck squamous cell cancers. **Head Neck**, New York, v. 32, n. 4, p. 499-503, Apr. 2010.

HSIEH, L. L. et al. Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. **Carcinogenesis**, New York, v. 22, n. 9, p. 1497-503, Sept. 2001.

IDE, R. et al. Cigarette smoking, alcohol drinking, and oral and pharyngeal cancer mortality in Japan. **Oral Dis**, Houndmills, v. 14, n. 4, p. 314-9, May 2008.

INSTITUTO NACIONAL DE CÂNCER (INCA). Rio de Janeiro, [2008]. Disponível em: <http://www.inca.gov.br/>. Acesso em: 22 dez. 2010.

KATO, K. et al. Expression form of p53 and PCNA at the invasive front in oral squamous cell carcinoma: correlation with clinicopathological features and prognosis. **J Oral Pathol Med**, Copenhagen, Apr. 2011. Doi 10.1111/j.1600-0714.2011.01032.x.

KRAJEWSKA, M. et al. Immunohistochemical analysis of in vivo patterns of expression of CPP32 (Caspase-3), a cell death protease. **Cancer Res**, Chicago, v. 57, n. 8, p. 1605-13, Apr. 1997.

KHURI, F. R. et al. The impact of smoking status, disease stage, and index tumor site on second primary tumor incidence and tumor recurrence in the head and neck retinoid chemoprevention trial. **Cancer Epidemiol Biomarkers Prev**, Philadelphia v. 10, n. 8, p. 823-9, Aug. 2001.

KRUSE, A.L.; BREDELL, M.; GRÄTZ, K.W. Oral squamous cell carcinoma in non-smoking and non-drinking patients. **Head Neck Oncol**, London, v. 4, n. 2, p. 1-3, Oct. 2010.

KYZAS, P. A. et al. Evidence for lymphangiogenesis and its prognostic implications in head and neck squamous cell carcinoma. **J Pathol**, London, v. 206, n. 2, p. 170-7, June 2005.

LINK, M. J. O.; KAUGARS, G. E.; BURNS, J. C. Comparison of oral carcinomas in smokeless tobacco users and nonusers. **J Oral Maxillofac Surg**, Philadelphia, v. 50, n. 5, p. 452-5, May 1992.

LO, W. L. et al. Outcomes of oral squamous cell carcinoma in Taiwan after surgical therapy: factors affecting survival. **J Oral Maxillofac Surg**, Philadelphia, v. 61, n. 7, p. 751-8, July 2003.

MĂRGĂRITESCU, C. et al. VEGF and VEGFRs expression in oral squamous cell carcinoma. **Rom J Morphol Embryol**, Bucuresti, v. 50, n. 4, p. 527-48, 2009.

MUWONGE, R. et al. Role of tobacco smoking, chewing and alcohol drinking in the risk of oral cancer in Trivandrum, India: A nested case-control design using incident cancer cases. **Oral Oncology**, Oxford, v. 44, n. 5, p. 446-54, May 2008.

PELUCCHI, C. et al. Alcohol and tobacco use, and cancer risk for upper aerodigestive tract and liver. **Eur J Cancer Prev**, Oxford, v. 17, n. 4, p. 340-4, Aug. 2008.

PITHAN, S. A. et al. Perfil epidemiológico do carcinoma espinocelular de boca em pacientes do Serviço de Estomatologia do Hospital São Lucas da PUCRS. Rev Odonto Ciência, Porto Alegre, v. 19, n. 44, p. 126-30, abr-jun. 2004.

PYTYNIA, K. B. et al. Matched-pair analysis of survival of never smokers and ever smokers with squamous cell carcinoma of the head and neck. **J Clin Oncol**, New York, v. 22, n. 19, p. 3981-8, Oct. 2004.

REIS, S. R. et al. Genotoxic effect of ethanol on oral mucosa cells. **Pesqui Odontol Bras**, São Paulo, v. 16, n. 3, p. 221-5, July-Sept. 2002.

ROSSING, M. A.; VAUGHAN, T. L.; MCKNIGHT, B. Deit and pharyngeal cancer. **Int J Cancer**, Genève, v. 44, n. 4, p. 593-7, Oct. 1989.

SATOH, K. et al. The pattern of CPP32/caspase-3 expression reflects the biological behavior of the human pancreatic duct cell tumors. **Pancreas**, New York, v. 21, n. 4, p. 352-7, Nov. 2000.

SCULLY, C.; FIELD, J. K.; TANZAWA, H. Genetic aberrations in oral or head and neck squamous cell carcinoma 2: chromosomal aberrations. **Oral Oncol**, **Oxford**, v. 36, n. 4, p. 311-27, July 2000.

SIEGELMANN-DANIELI, N. et al. P53 alteration in oral tongue cancer is not significantly associated with age at diagnosis or tobacco exposure. **Tumori**, Milano, v. 91, n. 4, p. 346-50, July-Aug. 2005.

SIEMEISTER, G.; MARTINY-BARON, G.; MARMÉ, D. The pivotal role of VEGF in tumor angiogenesis: molecular facts and therapeutic opportunities. **Cancer Metastasis Rev**, The Hague, v. 17, n. 2, p. 241-8, June 1998.

SHAO, Z. et al. Expression of EphA2 and VEGF in squamous cell carcinoma of the tongue: correlation with the angiogenesis and clinical outcome. **Oral Oncol**, Oxford, v. 44, n. 12, p. 1110-7, May 2008.

SMITH, R. V. et al. Pilot study of mucosal genetic differences in early smokers and nonsmokers. **Laryngoscope**, St. Louis, v. 116, n. 8, p. 1375-9, Aug. 2006.

SOUTO, G. R. et al. Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. **J Oral Pathol Med**, Copenhagen, v. 39, n. 8, p. 605-10, Sept. 2010.

SQUIER, .CA.; KREMER, M.J.; WERTZ, P.W. Effect of ethanol on lipid metabolism and epithelial permeability barrier of skin and oral mucosa in the rat. **J Oral Pathol Med**, Copenhagen, v. 32, n. 10, p. 595-9, Nov. 2003.

OGDEN, G.R.; COWPE, J.G.; GREEN, M.W. Quantitative exfoliative cytology of normal buccal mucosa: effect of smoking. **J Oral Pathol Med**, Copenhagen, v. 19, n. 2, p. 53-5, Feb. 1990.

TALAMINI, R. et al. Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. **Cancer Causes Control**, Oxford, v. 13, n. 10, p. 957-64, Dec. 2002.

VAN OIJEN, M. G. et al. Increased number of proliferating cells in oral epithelium from smokers and ex-smokers. **Oral Oncol**, Oxford, v. 34, n. 4, p. 297-303, July 1998.

VAN OIJEN, M. G.; VAN DE CRAATS, J.G.; SLOOTWEG, P.J. P53 overexpression in oral mucosa in relation to smoking. **J Pathol**, Oxford, v. 187, n. 4, p. 469-74, Mar. 1999.

WISEMAN, S. M. et al. Squamous cell carcinoma of the head and neck in nonsmokers and nondrinkers: an analysis of clinicopathologic characteristics and treatment outcomes. **Ann Surg Oncol**, New York, v. 10, n. 5, p. 551-7, June 2003.

ZIMMERMANN, K.C.; BONZON, C.; GREEN, D,R. The machinery of programmed cell death. **Pharmacol Ther,** Oxford, v.92, n.1, p. 57-70, Oct. 2001.

ZNAOR, A. et al. Independent and combined of tobacco smoking, chewing, and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. **Int J Cancer**, Genève, v. 105, n. 5, p. 681-6, July 2003.

Anexos e Apêndice

ANEXO A

SUBMISSÃO DO ARTIGO DE REVISÃO NO PERIÓDICO *MEDICINA ORAL, PATOLOGÍA ORAL Y CIRURGÍA BUCAL*

De: medoral.es [mailto:medoral@medoral.es]
Enviada: sex 1/7/2011 14:59
Para: Fernanda Goncalves Salum
Assunto: Med Oral Patol Oral Cir Bucal, Ref. 17777, 2011-07-01

2011-07-01

Reference: 17777

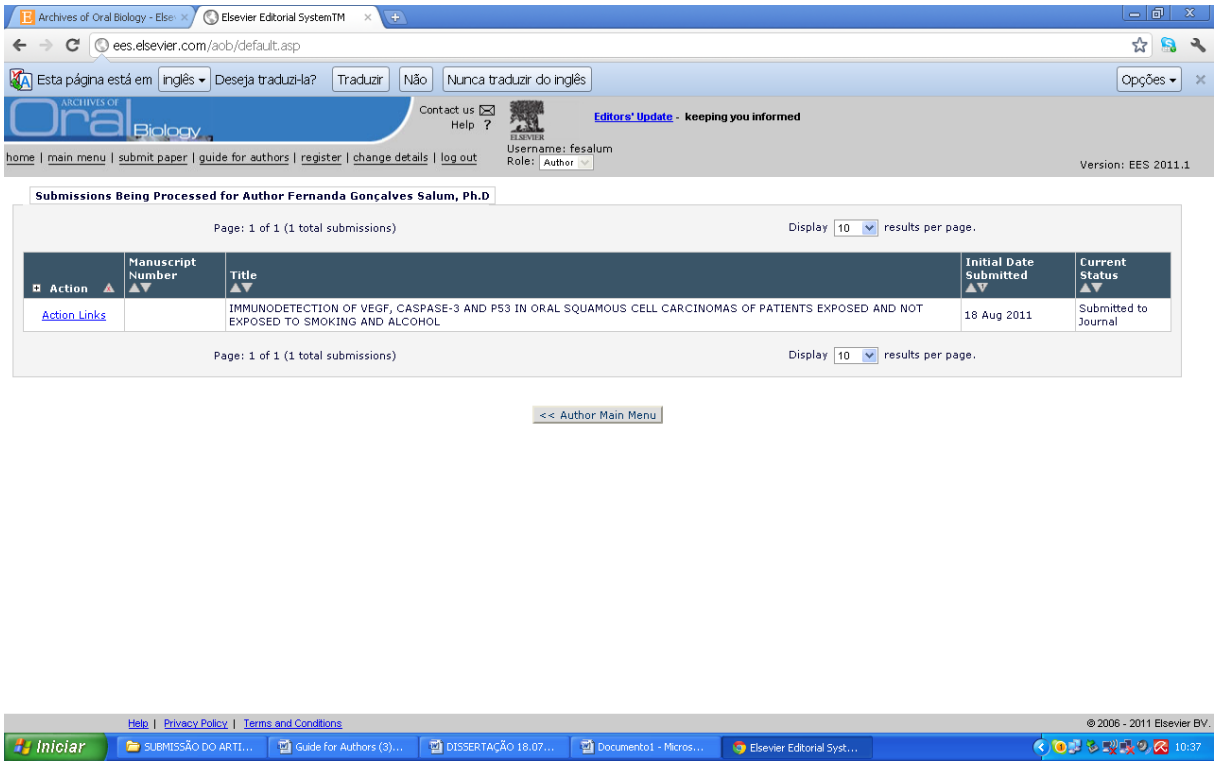
Dear Dr. Fernanda Salum,
Your manuscript entitled "CLINICOPATHOLOGIC AND MOLECULAR CHARACTERISTICS OF ORAL SQUAMOUS CELL CARCINOMA IN PATIENTS WITH AND WITHOUT KNOWN RISK FACTORS " has been successfully submitted online and has been forwarded to the referees for evaluation. In due time, you will be informed as to its possible publication in Med Oral Patol Oral Cir Bucal.

Yours sincerely.

Professor Jose V. Bagan
Editor Med Oral Patol Oral Cir Bucal
Indexed in: SCI-JCR, INDEX MEDICUS, MEDLINE, PUBMED, EXCERPTA MEDICA, EMBASE, SCOPUS, IME

ANEXO B

SUBMISSÃO DO ARTIGO DE PESQUISA NO PERIÓDICO *ARCHIVES OF ORAL BIOLOGY*



ANEXO C

APROVAÇÃO PELA COMISSÃO CIENTÍFICA E DE ÉTICA DA FACULDADE DE ODONTOLOGIA - PUCRS

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 29 de abril de 2010

O Projeto de: Dissertação

Protocolado sob nº:	0037/10
Intitulado:	Imunodetecção de VEGF, CASPASE-3 e p53 em carcinomas espinocelulares de pacientes etilistas e tabagistas e de não-etilistas e não tabagistas
Pesquisador Responsável:	Profa. Dra. Fernanda Gonçalves Salum
Pesquisadores Associados	Juliana Hintz Germanos; Liliane Soares Yurgel
Nível:	Mestrado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *28 de abril de 2010.*

Este projeto deverá ser imediatamente encaminhado ao CEP/PUCRS

05 05 2010
Juliana Hintz Germanos

Profa. Dra. Ana Maria Spohr
Presidente da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

Av. Ipiranga, 6681, Prédio 06 sala 210
Porto Alegre /RS – Brasil – Cx. Postal:1429
90619-900
Fone/Fax: (51) 3320-3538
e-mail: odontologia-pg@pucrs.br

ANEXO D

APROVAÇÃO PELO COMITÊ DE ÉTICA DA PUCRS

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA EM PESQUISA

OF.CEP-771/10 Porto Alegre, 11 de agosto de 2010.

Senhora Pesquisadora,

O Comitê de Ética em Pesquisa da PUCRS apreciou e aprovou seu protocolo de pesquisa (registro CEP 10/05052) intitulado "**Imunodetecção de VEGF, CASPASE-3, e p53 em carcinomas espinocelulares bucais de pacientes alcoolistas/tabagistas e de não-alcoolistas/não-tabagistas**".

Salientamos que seu estudo pode ser iniciado a partir desta data.

Os relatórios parciais e finais deverão ser encaminhados a este CEP.

Atenciosamente,

Prof. Dr. Rodolfo Herberto Schneider
Coordenador do CEP-PUCRS

Ilma. Sra.
Profa. Dra. Fernanda Gonçalves Salum
FO
Nesta Universidade

PUCRS
Campus Central
Av. Ipiranga, 6690 – 3ºandar – CEP: 90610-000
Sala 314 – Fone Fax: (51) 3320-3345
E-mail: cep@pucrs.br
www.pucrs.br/prppg/cep

APÊNDICE

FICHA DE COLETA DE DADOS

FICHA

Dados pessoais:

Nome:_____

Idade: _____ **Sexo:** M ( ) F ( ) **Nº da ficha clínica**:____:_____

Endereço:_____

Telefone: _____ **Profissão**: _____

Características da lesão:

Localização: _____

Aspecto clínico: _____

Tamanho da lesão:_____

Diagnóstico histopatológico:_____

Local da biópsia: _____

Data da biópsia: _____ **Número do anatomo-patológico**:_____

Fatores extrínsecos:

1. ( ) **tabaco** tipo:_____ quantidade_____ tempo: _____
   ( ) **ex-tabagista** há quanto tempo:_____ por quanto tempo_____

Classificação:

Leve ( ) Moderado ( ) Pesado ( )

2. ( ) **álcool** tipo:_____ quantidade:_____ tempo: _____
   ( ) **ex-etilista** há quanto tempo:_____por quanto tempo:_____

Classificação:

Leve ( ) Moderado ( ) Pesado ( )

3. ( ) **Chimarrão** freqüência:_____há quanto tempo:_____
4. ( ) **outros**, quais: _____