UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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KI-67, TGF-β1 E ELASTINA NO COMPORTAMENTO E PROGNÓSTICO DE QUEILITE ACTÍNICA E CARCINOMA ESPINOCELULAR DE LÁBIO

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“Digo: o real não está na saída nem na chegada: ele se dispõe para a gente é no meio da travessia.”

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RESUMO

SALVADORI, Gabriela. **Ki-67, TGF-β1 e Elastina no comportamento e prognóstico de queilite actínica e carcinoma espinocelular de lábio.** 2013. 43f. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

As alterações epiteliais observadas nas queilites actínicas (QA) e carcinomas espinocelulares (CEC) de lábio vem sendo estudadas por meio de diferentes marcadores, a fim de observar os fatores de diagnóstico e prognóstico para ambas as lesões. O objetivo do presente estudo foi analisar a imunomarcação do Ki-67, TGF- β1 e elastina nas QA e CEC de lábio, com o intuito de verificar um possível papel destas proteínas na carcinogênese labial, bem como correlacioná-los com fatores de risco, graduação histológica e acompanhamento dos pacientes. Foram coletados dados sobre características demográficas, fatores de risco, aspectos clínicos, tratamento e evolução de 29 casos de QA e 53 casos de CEC de lábio. As QA foram classificados de acordo com a OMS e os CEC de lábio de acordo com Bryne et al. As imunomarcações para Ki-67, TGF-β1 e elastina foram analisadas quantitativa ou semi-quantitativamente. Os dados foram analisados pelo teste do qui-quadrado, teste exato de Fisher e análise de regressão logística . A QA mostrou aumento de células positivas para o Ki -67 conjuntamente com o aumento do grau de displasia epitelial ($p<0,01$). Observou-se uma correlação significativa entre o Ki-67 com o consumo de tabaco ($p < 0,05$), graduação histopatológica ($p<0,01$) e evolução ($p=0,01$). Nos casos de CEC de lábio houve associação entre maior número de células Ki-67 positivas com a recidiva do tumor ($p<0,01$). Correlação significativa entre Ki-67 com o consumo de tabaco ($p=0,009$), graduação histopatológica ($p<0,01$) e recidiva do tumor ($p<0,01$) também foi observada. A QA mostrou imunomarcação para o TGF-β1 em ambos os tecidos (parênquima e estroma), e uma correlação inversa foi observada com o Ki-67 e o grau de displasia epitelial ($p<0,01$). O CEC de lábio mostrou imunomarcação inversa em relação ao TGF-β1 e a graduação histopatológica do tumor ($p<0,01$). Quanto à expressão de elastina, todos os casos de QA demonstraram uma organização das fibras elásticas como massa difusa e compacta. Observou-se uma correlação significativa entre o grau de elastina com a exposição ao sol ($p<0,01$). No CEC de lábio a elastose foi mais fina e interrompida quando comparado com as QA, e esta diferença no padrão de imunomarcação elastina foi estatisticamente significativa entre os grupos ($p<0,01$). Em conclusão, os resultados deste estudo indicam que o alterações no perfil de Ki-67 e TGF-β1 contribuem para a carcinogênese labial. Além disso, a elastina reflete as alterações da matriz extracelular em QA e CEC de lábio.

ABSTRACT

SALVADORI, Gabriela. **Content of Ki-67 and TGF-β1, but no Elastin, is significantly altered in the lip carcinogenesis and associated to behavior of Actinic Cheilitis and Lip Squamous Cell Carcinoma.** 43f. Final Paper (Graduation in Dentistry) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2013.

Epithelial changes observed in actinic cheilitis (AC) and lip squamous cell carcinoma (LSCC) have been studied using different markers in order to observe diagnostic and prognostic factors for both lesions. The aim of the present study was to analyze the immunostaining of Ki-67, TGF-β1 and elastin in AC and LSCC, in order to verify a possible role of these proteins in lip carcinogenesis, as well as correlate them with risk factors, histological graduation and patient follow-up. Data were collected regarding demographic features, risk factors, clinical aspects, treatment and outcome in 29 cases of AC and 53 cases of LSCC. AC were classified according to WHO and LSCC according to Bryne *et al.* The immunostainings for Ki-67, TGF-β1 and elastin were analyzed quantitative and semi-quantitative. Data were analyzed by chi-square, Fisher's Exact Test and logistic regression analysis. The AC showed higher positive cells for Ki-67 with increase of epithelial dysplasia ($p<0.01$). A significant correlation between Ki-67 with tobacco consumption ($p<0.05$), histopathological graduation ($p<0.01$) and evolution ($p=0.01$) was observed. The LSCC exhibited an increase in the percentage of Ki-67 positive cells associated with worse tumor graduation ($p<0.01$). A significant correlation between Ki-67 with tobacco consumption ($p=0.009$), histopathological graduation ($p<0.01$) and tumor recurrence ($p<0.01$) also was observed. The AC showed immunolabeling for TGF-β1 in both epithelial (parenchyma) and stromal tissues, but an inverse correlation was observed with Ki-67 positive and degree of epithelial dysplasia ($p<0.01$). The LSCC showed that epithelial and stromal TGF-β1 labeling was reversed to tumor histopathological graduation ($p<0.01$). Regarding expression of elastin, all AC cases demonstrated an organization of elastic fibers as diffuse and compact mass. A significant correlation between elastin grade with sun exposure was observed ($p<0.01$). The elastosis in LSCC was thinner and discontinued when compared to AC, and this difference in elastin immunolabeling pattern was statistically significant between the groups ($p<0.01$). In conclusion, the results of this study indicate that altered content of Ki-67 and TGF-β1 contributes to lip carcinogenesis. Furthermore, elastin reflects the alterations of ECM in AC and LSCC.

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1 ANTECEDENTES E JUSTIFICATIVA

O câncer é uma das causas mais comuns de mortalidade e morbidade no mundo todo, tendo uma incidência anual de aproximadamente 10 milhões de casos novos (MARTINS-FILHO; DA SILVA; PIVA, 2011). No Brasil, segundo o Instituto Nacional do Câncer, o câncer de boca, especificamente, acomete cerca de 14.000 pacientes por ano sendo responsável por 6.510 mortes/ano (Instituto Nacional do Câncer, 2012). Dentre os diferentes tipos de câncer, o carcinoma espinocelular (CEC) que se origina do epitélio de revestimento, corresponde a cerca de 90% dos casos em boca (AIKEN et al., 2013).

O sítio mais frequente do CEC em boca é o lábio, representando 30% dos casos diagnosticados. Quando comparado aos CEC de língua e outras localizações em boca, o CEC de lábio exibe comportamento clínico menos agressivo e melhor prognóstico devido ao seu padrão de crescimento e invasão mais localizados e baixos índices de metástases regionais (BATISTA et al., 2010). A presença e extensão de metástase em linfonodos regionais tem sido o principal fator prognóstico para determinar a sobrevida do paciente portador de CEC de lábio. Outras informações como tamanho da lesão, sua localização, graduação clínica e história de recorrência são fatores prognósticos a serem considerados na avaliação do paciente (ZITSCH et al., 1995).

O processo de carcinogênese envolve várias etapas descritas como iniciação, promoção, progressão e manifestação clínica do tumor. A iniciação representa a fase na qual ocorre lesão ao DNA por carcinógenos. Na promoção, as células iniciadas, após a ação co-carcinógenos, são estimuladas a proliferar e passar o dano genético às células filhas até que se formem clones de células mutadas que invadam o tecido adjacente caracterizando as neoplasias malignas (CALIFANO; SIDRANSKY, 1999). No modelo aceito atualmente para descrever a carcinogênese em boca, pode-se verificar alterações celulares e arquiteturais que são chamadas de lesões potencialmente malignas ou cancerizáveis (EPSTEIN et al., 2002). Estas lesões apresentam ao longo do seu curso um risco aumentado de desenvolver um câncer quando comparado ao tecido normal. Nos lábios, a queilite actínica (QA) é a principal lesão potencialmente maligna e assim como o CEC, tem como principal

agente etiológico a exposição crônica a radiação ultra-violeta (UV) e o consumo de tabaco, sendo o processo de desenvolvimento destas doenças denominado de fotocarcinogênese labial (HUBER, 2010; MALASPINA et al., 2011; ROSSOE et al., 2011). Assim sendo, ambas as lesões são mais prevalentes em regiões tropicais e subtropicais, especialmente em indivíduos expostos ao sol como trabalhadores rurais, pescadores, marinheiros e surfistas (CARVALHO et al., 2010; JUNQUEIRA et al., 2011). O índice de progressão das QA para CEC varia entre 10 a 20% (SANTOS et al., 2003) e o principal fator é a presença de displasia epitelial.

A QA se caracteriza clinicamente por placas brancas associadas a áreas de eritema, erosão ou crostas no vermelhão do lábio inferior de forma localizada ou difusa (VIEIRA et al., 2012). O CEC de lábio mostra-se preferencialmente como úlcera entremeada com placas brancas. Histologicamente, a QA pode apresentar padrões variados de alterações celulares e arquiteturais no epitélio tais como: hiperceratose, hiperplasia, acantose ou atrofia do epitélio podendo exibir padrões variados de displasia epitelial. O tecido conjuntivo usualmente apresenta elastose solar e inflamação crônica. (HUBER, 2010; ARAÚJO et al., 2007; WOOD et al., 2011). Sabe-se que, à medida que ocorrem modificações gênicas frente à exposição a radiação UV e ao tabaco, as células epiteliais sofrem distúrbios de diferenciação e podem invadir os tecidos adjacentes, caracterizando a formação do CEC. Este por sua vez, é formado por uma população heterogênea de células tumorais com crescimento autossuficiente e autônomo, e destas, apenas uma pequena subpopulação exibe grande potencial proliferativo e são consideradas as células-tronco mutada (do inglês - *cancer stem cell* - CSC), o que influencia o crescimento tumoral e sua capacidade de resposta aos diferentes agentes terapêuticos (ZHANG; FILHO; NÖR, 2012).

As alterações epiteliais observadas na queilite actínica e no CEC têm sido estudadas na busca de fatores prognósticos de ambas as lesões por meio de diferentes marcadores de proliferação celular, apoptose, de inflamação dentre outros, tais como: Ki-67, p53, bcl-2, COX, mastócitos. (SITTEL et al., 1999; SCHLIEPHAKE, 2003; PEREA, VAL BERNAL, BUSTILO, 2004; MARTÍNEZ et al., 2005; RAJU et al., 2005; GONZALES-MOLES et al., 2010; SOUZA et al., 2011; ROJAS et al., 2012).

O antígeno Ki-67 vem sendo utilizado na rotina da patologia como marcador nuclear de células em proliferação refletindo a fração total de proliferação celular no tecido (GONZALEZ-MOLES et al., 2010). Isto porque esta proteína é observada em células nas fases G1, S, G2 e mitose do ciclo celular (WINKING et al., 2004). A análise do Ki-67 tem mostrado um valor preditivo do padrão de crescimento tecidual (SAITO et al., 1999) e um aumento na marcação de Ki-67 tem sido observado em lesões potencialmente malignas e malignas de boca (SLOOTWEG et al.,1994; KUROKAWA et al., 2003; TABOR et al., 2003). Todavia, o papel desta proteína como marcador prognóstico em carcinoma de boca ainda não está completamente definido e variações metodológicas podem ser responsáveis pelos diferentes resultados observados pelos autores. Analisando a marcação de Ki-67 em CEC de boca, Dragomir et al. (2012) mostraram correlação entre o grau de displasia de lesões cancerizáveis e o grau de diferenciação dos carcinomas, apresentando altos valores e intensidade de marcação na área do *front* de invasão.

Os membros da família do fator de crescimento e transformação beta (TGF-β1, β2 e β3) são citocinas envolvidas na proliferação, migração e diferenciação celular, assim como na apoptose (BIERIE; MOSES, 2006). Portanto, tem importante papel na homeostase tecidual, cicatrização de feridas, angiogênese e câncer (SAITO et al., 2013; CHEN et al., 2011). Assim como outros fatores de crescimento, o TGF-β pode atuar na carcinogênese de diferentes tecidos, tanto estimulando como inibindo a proliferação e diferenciação dependendo do tipo celular (MINCIONE et al., 2007).

O TGF-β1 é uma citocina que inclui mais de 100 diferentes proteínas e um número superior a 40 tem sido descrito em mamíferos (DERYNCK; ZHANG, 2003; GIEHL; IMAMICHI; MENKE, 2007). Esta citocina tem sido descrita em diferentes tipos de câncer em associação com a redução da resposta imunológica (BECK et al., 2001), estimulação da angiogênese (CHOI et al., 1997; DERYNCK et al., 2001; BERTOLINO et al., 2005), aumento da síntese de enzimas proteolíticas (SEOMUN et al., 2001; KIM et al., 2004) e estimulação da deposição de MEC (matriz extracelular) no microambiente tumoral (CHENG; LOVETT, 2003). Além disso, dados de estudos clínicos associam positivamente a expressão de TGF-β1 com o aumento da capacidade de invasão de tumores de mama (TAYLOR; LEE;

SCHIEMANN, 2011) e próstata (THOMPSON et al., 1992) e com redução da sobrevida em carcinomas pancreáticos (FRIESS et al., 1993). Foram evidenciadas altas concentrações deste fator de crescimento em câncer colorretal (HAWINKELS et al., 2009), carcinomas gástricos (MUTOH et al., 2010), carcinomas renais (KOMINSKY et al., 2007) e CEC de cabeça e pescoço (LOGULLO et al., 2003). Porém, outros estudos mostram que o TGF-β1 parece desempenhar uma função ambígua durante o processo de carcinogênese. Nos estágios mais iniciais da progressão tumoral parece atuar como inibidor da proliferação celular enquanto que, nos estágios mais avançados parece contribuir para a invasão e crescimento tumoral (MINCIONE et al., 2007; AKHURST; DERYNCK, 2001).

A exposição excessiva à radiação solar também resulta em acúmulo de material elástico que substitui o colágeno normal, gerando elastose (ROJAS et al., 2012). Todos os mecanismos envolvidos nas modificações da matriz extraceluar durante fotocarcinogênese ainda não estão esclarecidos, assim como o real papel da elastose no desenvolvimento e progressão tumoral (SGARBI et al., 2010) Araújo et al. (2012), se propuseram a estudar a elastina, principal componente da elastose solar, com o intuito de estabelecer uma relação entre a presença desta proteína e os diferentes graus de displasia epitelial das lesões de QA. Estes autores observaram um aumento na deposição da elastina nos casos de QA quando comparado com a mucosa labial normal, entretanto não encontraram correlação entre este aumento de marcação com o grau de displasia epitelial. Ainda não existem relatos na literatura sobre o estudo da elastina em CEC de boca.

Com intuito de compreender o comportamento e o prognóstico da QA e do CEC de lábio, vários estudos vem sendo realizados com diferentes proteínas teciduais (SCHILIEPHAKE, 2003; GONZALES-MOLES et al., 2010, SCHUSSEL; PINTO JR; MARTINS, 2011; GRIMMINGER; DANNENBERG, 2012). Neste sentido, pode-se perceber que o papel do TGF-β1 e elastina na carcinogênese de lábio ainda não foi estudado, bem como sua relação com marcadores de proliferação celular.

2 ARTIGO CIENTIFICO

Content of Ki-67 and TGF-β1, but no Elastin, is significantly altered in the lip carcinogenesis and associated to behavior of Actinic Cheilitis and Lip Squamous Cell Carcinoma

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Abstract

Epithelial changes observed in actinic cheilitis (AC) and lip squamous cell carcinoma (LSCC) have been studied using different markers in order to observe diagnostic and prognostic factors for both lesions. The aim of the present study was to analyze the immunostaining of Ki-67, TGF-β1 and elastin in AC and LSCC, in order to verify a possible role of these proteins in lip carcinogenesis, as well as correlate them with risk factors, histological graduation and patient follow-up. Data were collected regarding demographic features, risk factors, clinical aspects, treatment and outcome in 29 cases of AC and 53 cases of LSCC. AC were classified according to WHO and LSCC according to Bryne *et al.* The immunostainings for Ki-67, TGF-β1 and elastin were analyzed quantitative and semi-quantitative. Data were analyzed by chi-square, Fisher's Exact Test and logistic regression analysis. The AC showed higher positive cells for Ki-67 with increase of epithelial dysplasia The AC showed higher positive cells for Ki-67 with increase of epithelial dysplasia ($p<0.01$). A significant correlation between Ki-67 with tobacco consumption ($p<0.05$), histopathological graduation ($p<0.01$) and evolution ($p=0.01$) was observed. The LSCC exhibited an increase in the percentage of Ki-67 positive cells associated with worse tumor graduation ($p<0.01$). A significant correlation between Ki-67 with tobacco consumption ($p=0.009$), histopathological graduation ($p<0.01$) and tumor recurrence ($p<0.01$) also was observed. The AC showed immunolabeling for TGF-β1 in both epithelial (parenchyma) and stromal tissues, but an inverse correlation was observed with Ki-67 positive and degree of epithelial dysplasia ($p<0.01$). The LSCC showed that epithelial and stromal TGF-β1 labeling was reversed to tumor histopathological graduation ($p<0.01$). Regarding expression of elastin, all AC cases demonstrated an organization of elastic fibers as diffuse and compact mass. A significant correlation between elastin grade with sun exposure was observed ($p<0.01$). The elastosis in LSCC was thinner and discontinued when compared to AC, and this difference in elastin immunolabeling pattern was statistically significant between the groups ($p<0.01$). In conclusion, the results of this study indicate that altered content of Ki-67 and TGF-β1 contributes to lip carcinogenesis. Furthermore, elastin reflects the alterations of ECM in AC and LSCC

Keywords: Oral cancel, prognostic biomarkers, potentially malignant disorders.

Introduction

Oral carcinogenesis involves several steps described as: initiation, promotion, progression and clinical manifestation of the tumor. These alterations reflect accumulated genetic changes that lead to malignant transformation of normal mucosa epithelium (Chen *et al.,* 2011). In the currently accepted model for describing oral carcinogenesis, early steps of tumor progression are characterized by architectural and cellular changes that correspond to a group of conditions clinically known as potentially malignant disorder (Huber, 2010). Actinic cheilitis (AC) belongs to this group of conditions and has been associated to tobacco smoking and chronic exposure ultraviolet radiation (UV). Lip squamous cell carcinoma (LSCC) may be preceded by AC and is attributed to the same risk factors (Malaspina *et al.,* 2011).

Epithelial changes observed in AC and LSCC have been studied using markers linked to apoptosis, cell proliferation and inflammation, such as Ki-67, p53, bcl-2, BAX, mast cell and others in order to define diagnostic and prognostic factors for both lesions (Gonzáles-Moles *et al.,* 2010; Martínez *et al.,* 2005; RAJU *et a.l,* 2005*;* Araújo *et al.,* 2010*)*. As yet, a panel of molecular markers that allows a prognostic prediction of oral malignant transformation has not been determined.

Ki-67 antigen has been used in pathology routine as a nuclear marker of proliferating cell, reflecting total fraction of cell proliferation in tissue (Gonzáles-Moles *et al.,* 2010). This protein is expressed in G1, S, G2 and M phase of the cell cycle and absent in quiescent cells (G0) (Martínez *et al.,* 2005). The analysis of Ki-67 has shown a predictive value of standard tissue growth (Saito *et al.,* 1999). Furthermore, it has been reported an increase in Ki-67 immunolabeling in potentially malignant and malignant lesions of the oral mucosa (Kurokawa *et al.,* 2003).

TGF-β1 is a cytokine involved in many stages of cancer and it was shown to be related to a reduction of the immune response (Beck *et al.,* 2001), angiogenesis stimulation (Bertolino *et al.,* 2005) , increasing synthesis of proteolytic enzymes (Kim *et al.,* 2004)' induction of extracellular matrix (ECM) deposition in the tumor microenvironment as well as epithelial-mesenchymal transition (EMT) (Xu, Lamouille and Derynck, 2009). Previous studies show that TGF-β1 plays a dual role in carcinogenesis process. In earlier stages of tumor progression it seems to act as an inhibitor of cell proliferation whereas in later stages seems to contribute to tumor growth and invasion (Mincione *et al.,* 2007; Akhrust and Derynck, 2001).

Excessive exposure to UV also results in elastic material accumulation, which replaces normal collagen, resulting in elastosis. Furthermore, little is known about the relationship between epithelial dysplasia and the elastic fiber system (Sgarbi *et al.,* 2010; Araújo *et al.,* 2012).

Thus, the aim of the present study was to analyze the immunostaining of Ki-67, TGF-β1 and elastin in AC and LSCC, in order to verify a possible role of these proteins in lip carcinogenesis, as well as correlate them with risk factors, histological graduation and patient follow-up.

Material and Methods

Study Population

After approval by the Committee for Ethical Research of HCPA (process number 12-0176), a retrospective study was conducted and 96 cases of AC and 176 cases of LSCC diagnosed between 1996 and 2010 were selected from the archives of the Laboratory of Pathology of the Hospital de Clínicas, Porto Alegre, Brazil (HCPA).

The patients' records were evaluated and information about demographic features, risk factors, clinical presentation, treatment and outcome of injuries were manually collected. Gender, age, ethnic group, occupation, tobacco and sun exposure, clinical evaluation, extension, TNM staging and follow-up information (clinical outcome and survival time) were obtained from medical records. The follow-up period was defined as the time from diagnosis until the last visit to the HCPA.

Inclusion criteria were the completeness of all the information cited above in the medical records and enough material for specimens analysis. After that, 29 cases of AC and 53 cases of LSCC were included in this study.

Histopathological analysis

Histological sections stained with hematoxylin-eosin (HE) of AC cases were graded according to the World Health Organization (WHO) (Barnes et al., 2005) to determine the presence and degree of epithelial dysplasia. The histological grading of epithelial dysplasia was defined as: mild dysplasia, when the architectural disturbances were limited to the lower third of the epithelium, accompanied by cytologic atypia; moderate dysplasia, when the architectural disturbances were

spread up to the middle third of the epithelium, in which case the degree of cytologic atypia may be considered and severe dysplasia when more than two thirds of the epithelium presented architectural disorder, associated with cytologic atypia.

In cases of LSCC, the histological sections were graduated according to criteria described by Bryne *et al.* (1992) that evaluate the tumor front regarding degree of keratinization, nuclear polymorphism, pattern of invasion, lymphocytic infiltration. Each morphological feature was scored from 1 to 4, which upon summation resulted in a total malignancy score (low:4-8; moderate:9-12; high:13-16).

The presence of elastosis was evaluated in the connective tissue of AC and LSCC.

Immunohistochemistry

For immunohistochemical staining, the samples were serially sectioned into 3μm in silanized slides. The sections were deparaffinized in xylene, rehydrated in alcohol and immersed in a solution 3% hydrogen peroxide solution for 30 minutes to block endogenous peroxidase. Antigen retrieval was performed before the primary antibody incubation. The primary antibodies, sources, antigen retrieval, dilutions, and incubation times were as follows: Ki-67 (MIB-1, DAKO, low pH solution in water bath at 90^{o}C for 18h, 1:50, 1h), TGF-β1 (Santa Cruz, sc-146, pepsin pH1.8 1% for 10 min, 1:400, 18h) and Elastin (Novocastra, BA4, 1% tripsin solution at 37^{o}C for 30 min, 1:50, 1h). The detection system used was EnVision (DakoCytomation, Carpinteria, CA, USA). The sections were then incubated with diaminobenzidine tetrahydrochloride (DAB, Novocastra, Newcastle, UK) and counterstained with Mayer's hematoxylin. Negative controls were obtained by replacing the primary antibodies with non-immune serum. Positive control for Ki-67, TGF-β1 and elastin were, respectively, human appendix, rat uterus and normal human normal skin.

The cases of AC immunostained by Ki-67 were submitted to morphometric quantification. To this purpose, the entire length epithelial was analyzed, images of the selected fields were captured by conventional light microscopy CX41RF model (Olympus Latin America, Inc., Miami, Florida, USA) containing the camera QColor 5, Coolet, RTV (Olympus Latin America, Inc., Miami, Florida, USA) coupled and connected to a computer Dimension 5150 (Dell, Porto Alegre, RS, Brazil). The images were analyzed by software version 2.81 QCapture (Quantitative Imaging

Corporation, Inc.; Surrey, DC, Canada). The number and percentage of positive cells were assessed in each case.

Histological sections of SCC immunostained for Ki-67 were counted according to Martinez *et al.* (2005) Quantitative analysis was conducted from the capturing and analyzing images of the slides through the same imaging system described above. The number and percentage of positive cells were assessed in each case and 1000 cells were counted in a 400x magnification. The result is presented as percentage of 1000 positive cells (mean and standard deviation).

Histological sections immunostained by TGF-β1 were analyzed semi-quantitatively by a single observer using scores based on the percentage of positive cells in both the epithelium and connective tissue. Each case was classified as: score 0 (0 to 10% positive cells), score 1 (10 to 50%), score 2 (over 50%).

The elastin was analyzed, by a single observer, in up to 10 fields at 100x magnification, and the degree of elastosis was evaluated according to the scale of values (scores +3 to 0), adapted from Fukushima *et al.* (2000). The elastosis score +3 corresponded to a diffuse increase of elastic fibers with mass elastosis pattern; the score +2, was a diffuse increase of elastic fibers; the score +1, partial increase with focal clusters of elastic fibers; the score zero, corresponded to the same characteristics as the control group.

Differences between groups were evaluated using the Pearson chi-square followed by Fisher's exact test. All statistical calculations were performed using the SPSS Statistics 20.0 program. A p value < 0.05 was considered to be statistically significant.

Results

Demographic and Clinical Features

Of the twenty nine patients with AC, 24 were male with mean age of 66.78 years-old and 5 were females with mean age of 63.65 years-old. Of the 53 LSCC studied 45 were male with mean age of 59.6 years-old and 8 were female with mean age of 54.89 years-old. All the demographic and clinical features are described on Table 1.

The age distribution was similar in all groups ($p>0.05$). A decidedly male predominance was found, ranging around 82.8% and 84.9% in patients affected by AC and LSCC, respectively. All patients studied were Caucasian, and there was a predominance of patients that live in urban areas ($p>0.05$)(Table 1).

The documentation of tobacco consumption revealed a massive exposure in both AC and LSCC groups. No difference was found when comparing the groups ($p>0.05$). Sun exposure was high in patients of the both groups, ranging around 80.2% for AC and 64.2% for LSCC. ($p>0.05$) (Table 1). The use of sunscreen was almost never mentioned by patients.

The clinical aspects of AC and LSCC were divided into spot/plaque/nodule and ulcerated lesions. AC group occurred predominantly as spot/plaque/nodule lesions while LSCC presented more ulcerated lesions ($p<0.01$). Pain was reported by a few patients in both groups. However, when it was present, the clinical presentation of the lesion was an ulcer (Table 1).

Lesion size and TNM staging were collected for, respectively, AC and LSCC. The majority of AC lesions revealed small sizes as well as the most part of LSCC lesions were classified in initial TNM stages (I/II) (Table 1).

Daily use of sunscreen was recommended for all the AC patients. Besides, 13 (44.8%) were submitted to partial lesion removal, 7 (24.1%) to total lesion removal, 5 (17.4%) to cryotherapy and 4 (13.8%) submitted to non-surgical approach and sunscreen use was adopted. The LSCC patients were treated in 46 (86.8%) cases with surgery and 7 (13.2%) cases with surgery plus radiotherapy.

The average follow-up of AC patients was 6.4 years, ranging from 3 years to 10 years. The information about AC evolution according to lesion size and treatment are described on table 2. Only 4 (13.4%) cases of AC became LSCC. All these lesions were ulcerated, in smoker patients that did not use sunscreen, smaller that <2cm, treated with partial removal and the transformation occurred in a period less than 5 years of monitoring. Regarding epithelial dysplasia in the 4 cases that transformed into LSCC, 1 (3.4%) had mild dysplasia, 1 (3.4%) had moderate dysplasia and 2 (6.9%) presented severe dysplasia.

Logistic regression analysis comparing sun exposure in AC lesions with follow-up showed that patients who were exposed to UV radiation had 20.2 times higher chances of transforming into LSCC ($p=0.03$). The analysis of sunscreen use revealed patients that did not use had 15.7 ($p=0.04$) more chances to transform into LSCC.

The follow-up of LSCC patients showed that 4 (7.5%) cases exhibited recurrence. All cases were observed in smokers patients that did not use sunscreen and the recurrence occurred in a period less than 5 years of monitoring.

Table1. Demographic and clinical features of AC and LSCC.

Clinical Features	AC* (n=29)	LSCC** (n=53)	p-value×
Age	64.27±15.59	63.31±13.04	p=0.9
Gender			
Male	24 (82.8%)	45 (84.9%)	p=0.79
Female	5 (17.2%)	8 (15.1%)	
Ethnic Group			
Caucasian	29 (100%)	53 (100%)	-
Residence			
Urban	24 (82.8%)	39 (73.6%)	p=0.34
Rural	5 (17.2%)	14 (26.4%)	
Sun Exposure			
Yes	25 (80.2%)	34 (64.2%)	p=0.15
No	4 (19.8%)	19 (35.8%)	
Tobacco Consumption			
Yes	21 (72.4%)	36 (67.9%)	p=0.4
No	8 (27.6%)	17 (32.1%)	
Clinical Aspects			
Ulcer	9 (31%)	41 (77.4%)	p<0.01
Spot/Plaque/nodule	20 (69%)	12 (22.6%)	
Pain			
Yes	2 (6,9%)	12 (22.6%)	p=0.07
No	27 (93.1%)	41 (77.4%)	
TNM			
I/II	-	35 (66%)	-
III/IV	-	18 (34%)	
Size			
<2cm	26 (89.7%)	-	
2-4cm	2 (6.9%)	-	-
> 4cm	1 (3.4%)	-	

*Actinic Cheilitis

**Lip Squamous Cell Carcinoma

×Chi-square test

Table 2. Description of lesion size and treatment of AC according to clinical outcome.

		Clinical outcome			
		Absence	Presence	Transformation into LSCC	Total
Lesion Size	< 2cm	6	16	4	26
	2-4cm	1	1	0	2
	>4 cm	1	0	0	1
	Total	8	18	4	29
Treatment	Parcial Removal	0	9	4	13
	Total Removal	7	0	0	7
	Sunscreen use	0	4	0	4
	Cryotherapy	2	3	0	5
	Total	9	16	4	29

Histopathological analysis

The evaluation of epithelial lining and degree of epithelial dysplasia of 29 cases of AC showed that 5 (17.2%) cases had absence of dysplasia (non dysplastic), 10 (34.5%) mild dysplasia, 6 (20.7%) cases had moderate dysplasia and 8 (27.6%) had severe dysplasia. All cases showed some degree of elastosis.

In the 53 cases of LSCC, the tumor front was evaluated and 34 (64.2%) cases showed low-level of malignancy, 16 (30.2%) moderate and 3 (5.7%) high degree.

No correlation between the histological graduation of AC and LSCC was observed with clinical and demographic features.

Immunohistochemical analysis

The main data of Ki-67, TGF-β1 and elastin immunolabeling are summarized on Table 3.

Table 3. Absolute and percentage number of AC and LSCC cases distributed according to Ki67, TGF- β1 and elastin graduation.

		AC*					LSCC**		
	Graduation	Non-dysplastic 5 (17.2%)	Low dysplasia 9 (31.1%)	Moderate dysplasia 6 (20.6%)	Severe Dysplasia 9 (31.1%)	TOTAL 29 (100%)	Low 34 (64.2%)	Moderate and High 19 (35,8%)	TOTAL 53 (100%)
Ki-67	<30%	5 (100%)	7 (77.7%)	0 (0%)	0 (0%)	12 (43.3%)	32 (94.1%)	4 (21.1%)	36 (67.9%)
	30-50%	0 (0%)	0 (0%)	5 (83.3%)	1 (11.2%)	6 (20%)	2 (5.9%)	12 (63.2%)	14 (26.5%)
	>50%	0 (0%)	2 (33.3%)	1 (16.7%)	8 (88.8%)	11 (36.6)	0 (0%)	3 (15.7%)	3(5.6%)
	p-value+					<0.001			<0.001
TGF-β1 Epithelial	<10%	1 (20%)	3 (33.3%)	6 (100%)	9 (100%)	19 (65.5%)	6 (17.6%)	17(89.4%)	23 (43.4%)
	10-50%	4 (80%)	4 (44.5%)	0 (0%)	0 (0%)	8 (27.6%)	24 (70.5%)	2 (10.6%)	26 (40.1%)
	>50%	0 (0%)	2 (22.2%)	0 (0%)	0 (0%)	2 (6.9%)	4 (11.7%)	0 (0%)	4 (7.5%)
	p-value+					=0.001			=0.001
TGF-β1 Connective	<10%	1 (20%)	4 (44.5%)	6 (100%)	9 (100%)	20 (69%)	4(11.7%)	17(89.4%)	21(39.6%)
	10-50%	2 (40%)	3 (33.3%)	0 (0%)	0 (0%)	5 (17.2%)	17 (50%)	2 (10.6%)	19 (35.8%)
	>50%	2 (40%)	2 (22.2%)	0 (0%)	0 (0%)	4 (13.8%)	13 (38.3%)	0 (0%)	13 (24.6%)
	p-value+					<0.001			<0.001
Elastin	Score 0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	10 (29.4%)	4 (21%)	14 (26.4%)
	Score +1	1 (20%)	2 (22.2%)	0 (0%)	1 (11.1%)	4 (13.8%)	21 (61.8%)	14 (73.7%)	35 (66%)
	Score +2	1 (20%)	5 (55.5%)	5 (83.3%)	4 (44.4%)	15 (51.7%)	3 (8.8%)	1 (5.3%)	4 (7.6%)
	Score +3	3 (60%)	3 (33.3%)	1 (16.7%)	3 (33.3%)	10 (34.5%)	0 (0%)	0 (0%)	0 (0%)
	p-value+					>0.05			>0.05

*AC- Actinic Cheilitis
*LSCC – Lip Squamous Cell Carcinoma
×Chi-square test

Ki-67

All the AC and LSCC cases were positive for anti-Ki-67 antibody.

The AC showed different percentage of positive cells according to the evaluation of epithelial lining and degree of epithelial dysplasia (Figure 1 A,B and C). The mean of Ki-67 positive cells in AC was 19.1% (±4.7) in non dysplastic, 22,4% (±3.8) in mild dysplasia, 44.5% (±9.6) in moderate dysplasia and 55.7% (±10.1) in severe dysplasia. A significant correlation between Ki-67 with tobacco consumption ($p<0.05$), histopathological grading ($p<0.01$) and evolution ($p=0.01$) was observed. Higher levels of Ki-67 were detected in massive tobacco smokers, in cases with higher grades of epithelial dysplasia and in those AC cases that had malignant transformation.

The LSCC exhibited an association between the percentage of Ki-67 positive cells and the graduation of tumor (Figure 2 A,B and C). Low-level malignancy cases showed 29.7% (±10.3) of cells positive to Ki-67, moderate and high level of malignancy in combination showed a mean of 49.8% (±12.9) of positive cells. To allow the samples statistical analysis, moderate and high level cases were taken together based in the small number of cases in the higher level of malignancy. A significant correlation between Ki-67 with tobacco consumption ($p=0.009$), histopathological graduation ($p<0.01$) and tumor recurrence ($p<0.01$) was observed. The higher the levels of Ki-67 were detected in massive consumption of tobacco, higher the levels of malignancy and in cases of tumor recurrence.

Moreover, regardless the histopatological graduation, Ki-67 imunolabeling in LSCC was statistically higher when compared to AC ($p<0.01$).

Epithelial and Stromal TGF-β1

All the AC and LSCC cases were positive for antibody. The positivity was interpreted as cytoplasmic labeling in epithelial and connective cells.

The AC showed immunolabeling for TGF-β1 in both epithelial (parenchyma) and stromal tissues, but an inverse correlation was observed when keratinocytes ki-67 positive were compared to the degree of epithelial dysplasia (Table 3). Severe dysplasia revealed lower immunolabeling of epithelial and stromal TGF-β1 scores (Table 3, Figure 1 D, E and F). A significant correlation between tobacco with epithelial TGF-β1 ($p=0.01$) and with stromal TGF-β1 ($p=0.007$) was found. Lower

levels of epithelial and stromal TGF-β1 labeling were related to massive consumption of tobacco.

The LSCC showed that epithelial and stromal TGF-β1 labeling were reversed to tumor histopathological graduation (Figure 2 D, E and F). Higher level of malignancy cases revealed lower immunolabeling of epithelial and stromal TGF-β1 scores (Table 3, Figure 2F). No significant correlation between epithelial and stromal TGF-β1 labeling and clinical and demographic features was detected.

Comparing the AC and LSCC groups, we found significant difference only for stromal TGF-β1 immunolabeling, which was lower in AC ($p<0.05$).

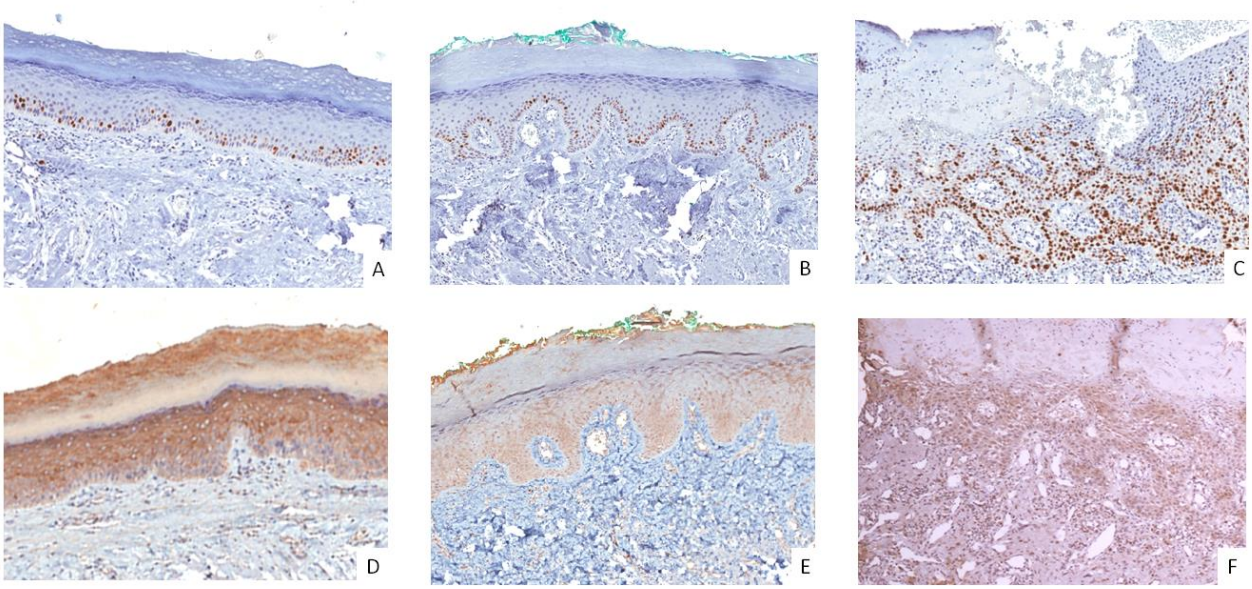

Figure 1. Immunohistochemical labeling of Ki-67 (A,B and C) and TGF-β1 (D,E and F) in AC with different degree of epithelial dysplasia (original magnification, x100). Non dysplastic AC (A) showed lower mean number of Ki-67 positive cells as compared with mild dysplasia (B) and moderate dysplasia (C). TGF-β1 exhibited an inverse correlation with Ki-67. Non dysplastic AC (D) showed higher labeling than mild dysplasia (E) and severe dysplasia(F).

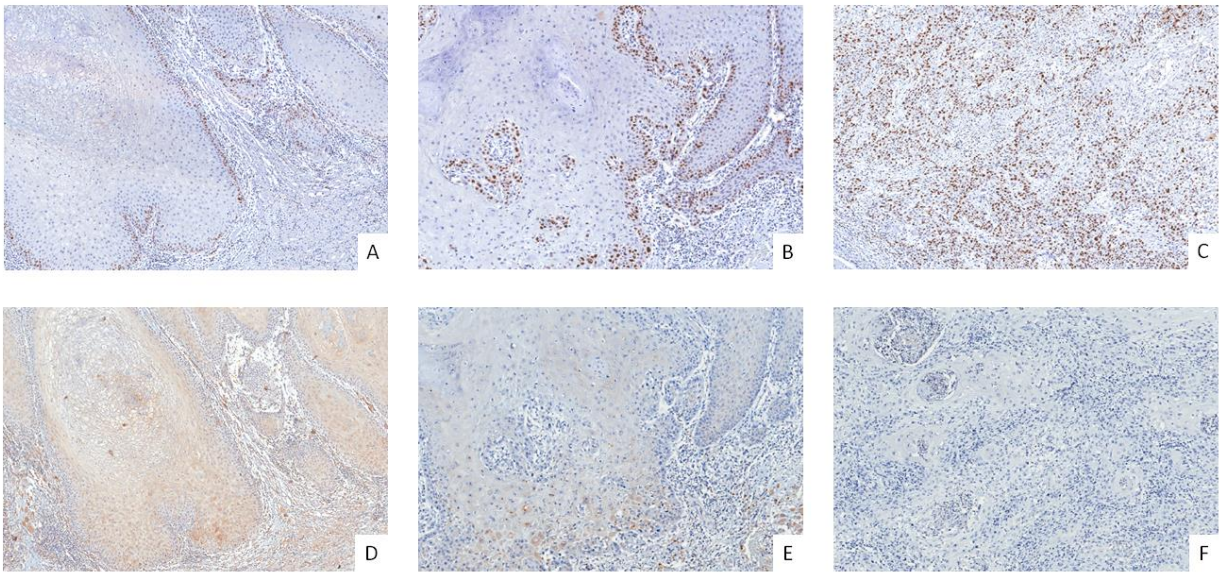

Figure 2. Immunohistochemical labeling of Ki-67 (A,B and C) and TGF-β1 (D,E and F) in LSCC with different tumor histological graduation (original magnification, x100). A. LSCC with low-level of malignancy exhibited lower mean number of Ki-67 positive cells as compared with moderate-level(B) and high-level(C). TGF-β1 exhibited an inverse correlation with Ki-67. Low-level of malignancy carcinomas (D) showed higher labeling than moderate-level (E) and high-level(F).

Correlation between TGF-β1 and Ki-67 in epithelial tissue

To verify if TGF-β1 could be implicated on cell proliferation changes, the Pearson correlation test was used. It was observed that epithelial TGF-β1 was inversely correlated with Ki-67 in AC (Figure 3B, $R = -0,792$ $p<0.01$) and in LSCC (Figure 3A, $R = -0,601$; $p <0.01$).

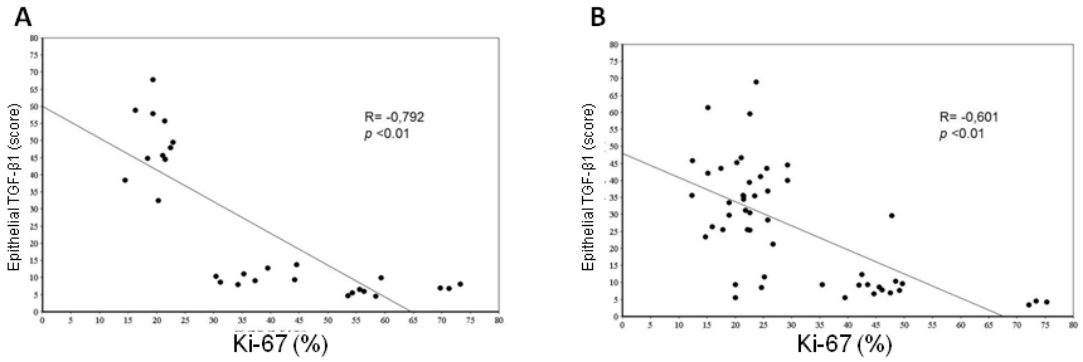

Figure 3. Correlation between Ki-67 and TGF-β1 expression in AC and LSCC epithelium (Pearson's correlation coefficient, $p < 0.05$).

Elastin

All 29 cases of AC were positive for elastin antibody and from the 53 cases of LSCC, 14 (26.4%) were negative.

Regarding expression of elastin, all AC cases demonstrated an organization of elastic fibers as diffuse and compact mass. None of the cases showed score 0 (Table 3). A significant correlation between elastin grade with sun exposure was observed ($p<0.01$). From 29 cases of AC, 25 had sun exposure history and these cases revealed a diffuse increase of elastic fibers (score +2 and +3).

The LSCC exhibited similar percentage of elastin in the different degrees of tumor histological graduation (Figure 4). No case with score +3 was observed in LSCC as well as no correlation between elastin and all clinical and demographic features. The elastosis in LSCC was thinner and discontinued when compared to AC, and this difference in elastin immunolabeling pattern was statistically significant between the groups ($p<0.01$).

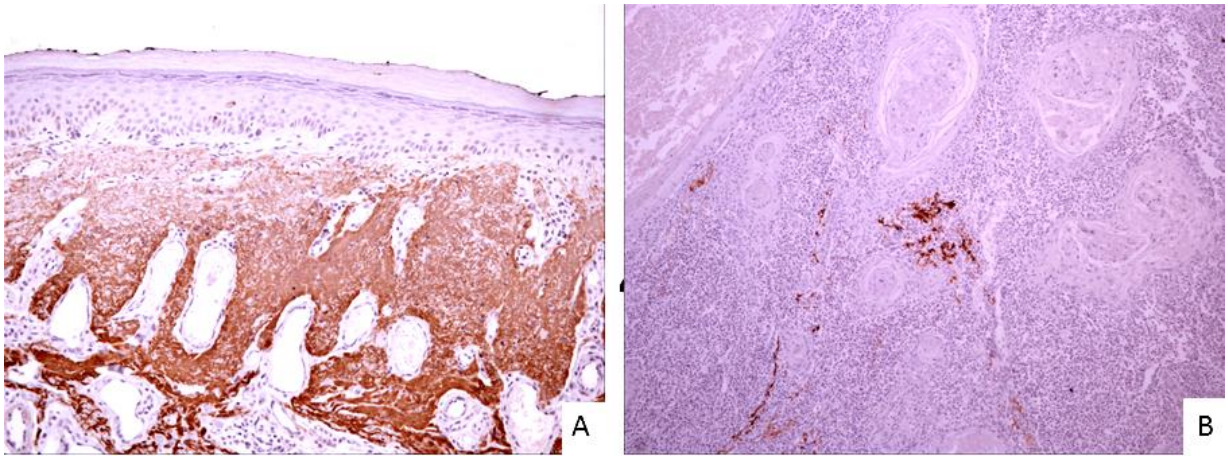

Figure 4. A. AC with mild epithelial dysplasia showing compact mass of elastic fibers concentration subjacent to the epithelial lining representing an extensive elastosis pattern (score +3). B. LSCC showing thinner and discontinued elastin near to tumor infiltration (score +1).

Discussion

The oral carcinogenesis is a multistep process in which genetic damage may lead to deregulation of the cell cycle associated to proteins alterations and malignant transformation. During lip carcinogenesis, the AC appears as an important potentially malignant disorder that could develop into invasive LSCC. Both lesions have been associated with regular and prolonged exposure to sun and tobacco (Huber, 2010; Malaspina *et al.,* 2001; Rossoe *et al.,* 2011). Many molecular markers have been proposed for AC and LSCC, however none showed an essential role for the assessment of individual prognosis in addition to the traditional clinicopathological evaluation (Mincione *et al.,* 2008). We studied Ki-67, a cell proliferation marker; TGF-β1**,** a potent growth inhibitor of epithelial cell proliferation; and elastin, the main component of solar elastosis in AC and LSCC lesions in order to provide information of a possible role of these biomarkers in malignant transformation and prognosis of these diseases.

Regarding demographic aspects, our findings are in agreement with the literature in both AC and LSCC groups. There was a predominance of men with a mean age of 60 years-old, Caucasian ethnicity and massive exposure to sun and tobacco (Czerninski, Zini and Sgan-Cohen, 2010.) Chronic exposure to ultraviolet

radiation is an important etiologic factor associated with the development of LSCC (Martínez *et al.,* 2005; López *et al.,* 2003). This fact is relevant to Brazil (latitude 10.00° S) and particularly the state of Rio Grande do Sul (latitude 30.00° S), that had large European colonization in the nineteenth century, and whose population has its economic activities based on agriculture (vegetables plantation and viticulture). Twenty-three patients of AC group and twenty-seven patients of LSSC group were farmers during the most part of their lives, working exposed to the sun light without any kind of protection.

Some studies have attempted to quantify the percentages of malignant transformation of AC, finding percentages that ranged from 1-20% of the cases (Santos *et al.,* 2003; Cavalcante, Anbinder and Carvalho, 2008; Marks, Rennie and Selwood, 1998). In our study, 13.4% of AC became LSCC and the transformation was observed in small lesions with ulcerative aspect, in smoker patients that did not use sunscreen and submitted to partial lesion removal. The main predictive factor for malignant transformation of potentially malignant disorders has been the severity of epithelial dysplasia. Interestingly, it was observed that the majority of our sample presented mild dysplasia and moderate dysplasia, which is in agreement with Kaugars *et al.* (2004) and Santos *et al.* (2003). In spite of that, the 4 cases that transformed into LSCC, one had mild dysplasia, one had moderate dysplasia and two presented severe dysplasia. These results corroborate that histopathological analysis of AC is very important, because epithelial dysplasia is significant to predict the potentially biological behavior of this lesion.

Analyzing our immunohistochemical results, we observed that Ki-67 and TGF-β1 (epithelial and connective) were important markers of behavior and prognosis of AC and LSCC. These two proteins showed an inverse correlation with higher levels of Ki-67 being associated to lower TGF-β1 labeling. Other important finding was the correlation of both markers with epithelial histopathological alterations (AC and LSCC grading), tobacco consumption and lesions behavior. Higher levels of Ki-67 and lower levels of TGF-β1 were detected in massive tobacco smokers, in cases with severe grade of epithelial dysplasia and in those that AC with malignant transformation along time as well as high level of malignancy of LSCC.

Ki-67 is an important biomarker of cell proliferation that could predict growth status and aggressive behavior of potentially malignant lesions and oral cancer. It has been shown that the number of Ki-67 positive cells increases progressively from

normal mucosa, epithelial dysplasia and squamous cell carcinoma, suggesting that malignant progression have an increased number of cells licensed to proliferate (Gonzáles-Moles *et al.,* 2010; Rendon *et al.,* 2009; Dwivedi *et al.,* 2013; Perisanidis *et al.,* 2011). Recent studies have reported expression of Ki-67 at the tumour infiltrating front of SCC with a strong positive correlation to the histological grading of the carcinoma (Dissanayake, Johnson and Warnakulasuria, 2003). Other studies indicate that Ki-67 index is higher in the epithelial dysplasias when comparing with normal epithelia (Raju *et al.,* 2005; Schoelch *et al.,* 1999), is associated to tobacco consumption (Raju *et al.,* 2005) and useful for predicting recurrence (Wangsa *et al.,* 2008).

TGF-β1 is a member of a large family of cytokines that regulate a variety of cellular processes including cell proliferation, differentiation, motility, extracellular matrix production, angiogenesis and immune response (Beck, Schreiber and Rowley, 2001; Bertolino *et al.,* 2005; Kim *et al.,* 2004). Evidence suggests that alteration in TGF-β1 signaling pathway contributes to development of several types of cancer (Coffey *et al.,* 1998; Chambard and Pouyssegur, 1998). However, a contrasting effects of TGF-β1 have been described indicating a dual role of this protein in tumors: acting mainly as a growth inhibitor in early tumor stages, but contributing to tumor progression and invasiveness in more advanced diseases (Akhrust and Derynck, 2001). In some studies, increased levels of TGF-β1 are associated with poor prognosis of cancer (Kominski *et al.,* 2007); Logullo *et al.,* 2003). However, in others, a decrease in TGF-β1 level has been associated with tumor progression, loss of tumor cell differentiation and more aggressive behavior (Muro-Cacho *et al.,* 1999; Mincione *et al.,* 2008). In this study high levels of TGF-β1 were observed in non-dysplastic epithelia and its labeling reduced gradually with the dysplasia progression. Lower labeling of TGF-β1 was associated to AC malignant transformation. Concurrently with the decrease of TGF-β1 an elevation of Ki-67 level was observed. Mincione *et al.* (2008) demonstrated that TGF-β1system molecules and involucrin were intensively and homogeneously expressed in all normal epithelia surrounding oral squamous cell carcinoma, but the tumor cells exhibited a decreased expression of these proteins in the majority of neoplastic tissues. Similar to our results, these authors observed a reduction of the TGF-β1system molecules levels correlated with the tumor grading and no correlation with other clinicopathological parameters.

TGF-β1 is a potent inducer of differentiation for normal epithelial cells and its expression is increased in terminal differentiation of mucosal keratinocytes (Min *et al.,* 1999). On the other hand, Ki-67 is a proliferative marker with lower levels detected in normal epithelia and increases in their label are observed in dysplastic and malignant epithelia. Analyzing the Ki-67 and TGF-β1 results of this study, we could infer that during malignant transformation, keratinocytes acquire several characteristics including loss of differentiation, infiltrative and proliferative behavior associated to modifications of Ki-67 and TGF-β1. According to several authors, human keratinocytes treated with TGF-β1 do not proliferate, but differentiate as monitored by an increased level of involucrin, induced by the extracellular matrix protein through the PI3K/Akt signalling pathway (Piazza, Ritter and Baracka, 1995; Oh, Kook and Min, 2005).

A general feature of AC and LSCC is the accumulation of elastotic material, which suggests an insufficient formation and/or deficient fragmentation of elastic fibers (Knott *et al.,* 2009). This aspect has been associated to chronic sun exposure that lead to modifications in the extracellular matrix (ECM) (Philips *et al.*, 2007). Therefore, the interaction between mesenchymal cells and the ECM may also be altered in tumors, and thus influence tumor proliferation and invasion (Liotta*,* 1984). In our study, significant correlation between elastin grade and sun exposure was observed. The elastin labeling was detected in 100% of AC cases with a diffuse and compact mass pattern, however, LSCC exhibited focal clusters of elastic fibers and 26.4% were negative. The AC results are in accordance with Araújo *et al*. (2010) that indicate no association between this protein with different grades of epithelial dysplasia. However, this was the first study that analyzed the elastin in LSCC and we could observe difference in elastin distribution comparing AC. The elastosis in oral cancer was thinner and discontinued when compared to AC, and this difference in elastin immunolabeling pattern was statisticaly significant. In LSCC, ECM is disrupted with the invasion of neoplastic epithelial cells and the elastic fibers is degraded. These results indicated that the degradation of elastosis increases the risk of developing cancers (Yano *et al*., 2005).

To the best of our knowledge, this is the first study in which Ki-67, TGF-β1 and elastin have been evaluated in AC and LSCC, especially associating with clinicopathological features and outcome. Overall, TGF-β1 showed a clear inverse association with Ki-67 proliferation index, and both were correlated with histological

grading and clinical outcome. Elastin accumulation was associated with AC while its decrease and discontinuity was related to LSCC.

Conclusion

In conclusion, the results of this study indicate that altered content of Ki-67 and TGF-β1 contributes to lip carcinogenesis. Furthermore, elastin reflects the alterations of ECM in AC and LSCC.

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3 CONSIDERAÇÕES FINAIS

Este é o primeiro estudo em que Ki-67, TGF-β1 e elastina foram avaliados em QA e CEC de lábio, especialmente associando as características clínico-patológicas e resultados. No geral, o TGF-β1 mostrou uma associação inversa com a imunomarcação de Ki-67, e ambos foram correlacionados com grau de diferenciação e evolução clínica. O acúmulo de elastina foi associado com a QA, enquanto sua diminuição e descontinuidade esteve relacionada com o CEC de lábio. Em conclusão, os resultados deste estudo indicam que o conteúdo alterado de Ki-67 e TGF-β1 em lábio contribui para a carcinogênese. Além disso, a elastina reflete as alterações da matriz extracelular em QA e CEC de lábio.

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ANEXO A – CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

Plataforma Brasil - Ministério da Saúde

Hospital de Clínicas de Porto Alegre - HCPA / UFRGS

PROJETO DE PESQUISA

Título: ANÁLISE IMUNOISTOQUÍMICA DO PAPEL DO TGF-ß1 E SUA CORRELAÇÃO COM A PROLIFERAÇÃO CELULAR EM QUEILITE ACTÍNICA E CARCINOMA ESPINOCELULAR DE LÁBIO

Área Temática:

Pesquisador: Manoela Domingues Martins **Versão:** 2

Instituição: Hospital de Clínicas de Porto Alegre - HCPA / UFRGS **CAAE:** 02584012.7.0000.5327

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 66845

Data da Relatoria: 01/08/2012

Apresentação do Projeto:

Esta pesquisa trata-se de um estudo de casos. Será feita uma análise de prontuários avaliados manualmente e serão coletadas informações quanto aos dados demográficos, características clínicas e histopatológicas, tratamento e acompanhamento dos pacientes com queilite actínica e CEC. A análise morfológica será feita com as lâminas coradas por H/E revisadas por dois patologistas experientes, sem conhecimento prévio dos dados demográficos e características clínicas relativas às amostras. A análise imunohistoquímica (TGF-ß1, anti-Ki-67 e anti-elastina) será realizada em cortes histológicos de 3cm de bloco de parafina já disponível.

Objetivo da Pesquisa:

Objetivo Geral:

O objetivo do presente estudo será verificar a imunomarcação do TGF-ß1 em queilite actínica e CEC de lábio com intuito de verificar um possível papel desta proteína na carcinogênese labial.

Objetivos Específicos:

1) Correlacionar a imunomarcação do TGF-ß1 em queilite actínica e CEC de lábio e correlaciona-la com a proliferação celular e alterações no tecido conjuntivo adjacente.
2) Correlacionar a graduação histológica dos casos de queilite e de CEC com a imunomarcação pelo TGF-ß1 e Ki-67.
3) Correlacionar a graduação histológica dos casos de queilite e de CEC com a imunomarcação pelo TGF-ß1 e alterações conjuntivas detectadas pela elastina.
4) Correlacionar a evolução dos casos de queilite actínica e CEC com a imunomarcação do TGF-ß1, Ki-67 e elastina.
5) Correlacionar os dados de fatores de risco (fumo e exposição solar) com a imunomarcação do TGF-ß1, Ki-67 e elastina.

Avaliação dos Riscos e Benefícios:

Não há riscos nem benefícios diretos para os pacientes. Serão selecionados prontuários médicos de pacientes com diagnóstico histopatológico de queilite actínica (n=50) e de CEC de lábio (n=50) diagnosticados no Serviço de Patologia do HCPA atendidos no período de Janeiro 2001 a dezembro de 2006. Serão incluídos casos (n=10) de mucosa de lábio sem alteração epitelial.

Comentários e Considerações sobre a Pesquisa:

Todas as pendências foram devidamente esclarecidas.

Considerações sobre os Termos de apresentação obrigatória:

Os pesquisadores adicionaram Termo de Compromisso para Uso de Dados.

Recomendações:

Sem recomendações.

Conclusões ou Pendências e Lista de Inadequações:

1) Não é necessário solicitar autorização para uso do material biológico/histológico?
Resposta dos pesquisadores: Foi adicionado na Plataforma Brasil Termo de Compromisso para Uso de Dados (TCUD).
PENDÊNCIA ATENDIDA.

Todas as pendências foram respondidas pelos pesquisadores.

2) Qual a hipótese do trabalho?
Resposta dos pesquisadores: O TGF-ß1 e a elastina apresentam associação com a proliferação celular em queilite actínica e carcinoma espinocelular de lábio e tem valor preditivo para a transformação maligna de queilite actínica.
PENDÊNCIA ATENDIDA.

3) Levando-se em consideração que as amostras biológicas não serão identificadas e que os pacientes cujo material biológico será analisado não terão benefícios diretos, quais seriam as hipóteses sobre os possíveis achados e os benefícios esperados para os pacientes após um futuro diagnóstico nesta área? Os mesmos teriam indicação de realizar estas análises para prever a progressão e desenvolvimento da doença?
Resposta dos pesquisadores: O objetivo do presente estudo será verificar a imunomarcação do TGF-ß1 em queilite actínica e CEC de lábio com intuito de verificar um possível papel desta proteína na carcinogênese labial. Assim como, verificar a existência de associação entre esses marcadores com a evolução dos casos de queilite actínica e CEC. Pretende-se verificar se estes marcadores possuem papel preditivo na transformação maligna de lesões de queilite actínica.
PENDÊNCIA ATENDIDA.

4) No resumo são 50 casos com diagnóstico histopatológico de queilite actínica e 50 com
CEC de lábio e no corpo do projeto são 40 casos de cada. O orçamento também está baseado em 40 de cada grupo.
Resposta dos pesquisadores: Inicialmente tinha sido pensado em 50 casos, porém, ao consultar o grupo de estatístico do GPPG foi feito o cálculo amostral que indicou o número de 40 casos. O resumo foi alterado para que represente o valor descrito no corpo do texto no item material e métodos.
PENDÊNCIA ATENDIDA.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Projeto aprovado versão 27/07/2012.

ANEXO B – CARTA DE APROVAÇÃO DA COMPESQ-ODONTOLOGIA

UFRGS

Linhas de Pesquisa
Projetos de Pesquisa
Áreas de Atuação
Bolsas de Pesquisa
Programa de Iniciaçã Científica Voluntário
Programa de Fomento Pesquisa(auxílio)

Sistema Pesquisa - Pesquisador: Manoela Domingues Martins

Retornar

Fechar Imprimir

Projeto Nº: 22708

Título: ANALISE IMUNOISTOQUIMICA DO PAPEL DO TGF-β1 E SUA CORRELACAO COM A PROLIFERACAO CELULAR EM QUEILITE ACTINICA E CARCINOMA ESPINOCELULAR DE LABIO

COMISSAO DE PESQUISA DE ODONTOLOGIA: Parecer

O objetivo do projeto será verificar o papel da imunomarcação do TGF-1em queilite actínica e CEC de lábio e correlacioná-lo com a proliferação celular e alterações no tecido conjuntivo adjacente. Serão selecionados prontuários médicos de pacientes com diagnóstico histopatológico de queilite actínica (n=50) e de CEC de lábio (n=50) diagnosticados no Serviço de Patologia do HCPA atendidos entre Janeiro 2001 a dezembro de 2006. Serão incluídos casos (n=10) de mucosa de lábio sem alteração epitelial. Serão coletadas informações quanto aos dados demográficos, exposição a fatores de risco, características clínicas das lesões, tratamento e evolução das lesões. As lâminas de cada caso coradas em H&E serão revisadas por dois patologistas experientes calibrados e classificadas de acordo com a OMS nas queilites actinicas e pelo método de Bryne nos casos de CEC. Os cortes histológicos imunomarcados com anticorpo primário anti-TGF- 1 e Ki-67. Será realizada inicialmente uma análise descritiva para as variáveis consideradas, calculando-se média, desvio padrão, máximo, mínimo e mediana para as variáveis quantitativas e freqüências e porcentagens para as variáveis qualitativas. A existência de associação entre as variáveis independentes e os desfechos será avaliada por meio do teste qui- quadrado. A sobrevida será calculada pelo método de Kaplan-Meier e as curvas de sobrevida serão comparadas usando o teste estatístico de log-rank. O projeto possui mérito científico e está bem delineado, contemplando critérios metodológicos como eligibilidade, calibração, cegamento e cálculo amostral.

ANEXO C - FICHA DE LEVANTAMENTO DE DADOS

Ficha de Levantamento de dados
Prontuário:_____ **Nº AP**_____
Nome do paciente:_____

Idade: _____
Sexo: 1 ( ) masculino 2 ( ) feminino
Ocupação: _____
Cor: 1 ( ) branca 2 ( ) preta 3 ( ) amarela 4 ( ) outra
Residência: 1 ( ) urbana 2 ( ) rural

Fumo: 1 ( ) sim 2 ( ) não
6.1- Tipo: ( ) cigarro ( )charuto ( ) cachimbo ( ) palheiro ( ) outros.
6.2- Quantidade: _____
6.3- Periodo de uso: _____
6.4- Ex-fumante há quanto tempo:_____

Exposição solar: 1( ) sim 2 ( ) não
7.1- Tempo de exposição ao sol em anos ----------
7.2- Uso de proteção solar 1( ) sim 2 ( ) não
Dor: 1 ( ) sim 2 ( ) não

Lesão:
Sitio: 1 ( ) Lábio Inferior
Aspecto clínico: ( )mancha branca ( )mancha vermelha ( )placa ( )nódulo ( ) úlcera ( )úlcero-vegetante ( )úlcero-infiltrativo
Tamanho: _____mm
( )Tx ( )T0 ( )carcinoma “in situ” ( ) T1 ( )T2 ( )T3 ( )T4 ( )T4a-Lábio ( )T4a-Cavidade Oral ( )T4b-Lábio e cavidade oral
Metástase regional: ( )NX ( )N0 ( )N1 ( )N2 ( )N2a ( )N2b ( )N2c ( ) N3
Metástase à distância: ( )MX ( )M0 ( )M1
TNM: ( )Estádio 0 ( )Estádio I ( )Estádio II ( )Estádio III ( )Estádio IVa ( )Estádio IVb ( )Estádio IVc

Tratamento:
a. Queilite actínica: ( )filtro solar, ( )chapéu, ( )remoção parcial, ( )remoção total, ( )outros.
b. CEC: ( )cirurgia, ( )radioterapia, ( )quimioterapia e as associações.

Evolução:
Queilite actínica: 1( ) sem lesão, 2 ( ) com lesão, 3 ( ) evoluiu para CEC
CEC: 1 ( ) vivo 2 ( ) falecido pelo tumor 3 ( ) falecido por outra causa 4 ( ) falecido sem saber a causa
Há quantos anos:_____
Recidiva: 1 ( ) sim 2 ( ) não