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ESTUDO HISTOPATOLÓGICO DAS DISPLASIAS EPITELIAIS EM LESÕES INFLAMATÓRIAS CRÔNICAS DA CAVIDADE ORAL

Aracaju

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ESTUDO HISTOPATOLÓGICO DAS DISPLASIAS EPITELIAIS EM LESÕES INFLAMATÓRIAS CRÔNICAS DA CAVIDADE ORAL

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal de Sergipe, para obtenção do título de Mestre em Odontologia.

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A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê.” (Arthur Schopenhauer)

RESUMO

Introdução: A inflamação crônica tem um papel importante na transformação e progressão tumoral durante a carcinogênese oral. Muitas lesões inflamatórias crônicas (LIC) da cavidade oral estão relacionadas a processos displásicos do epitélio, à resposta imune e à mudança na deposição do colágeno. **Objetivos:** Investigar a presença de displasia e graduá-las histologicamente nas LIC de origem traumática, como também, avaliar a densidade de mastócitos e de diferentes tipos de fibras colágenas nas LIC com displasias epiteliais e comprar aos casos de carcinomas de células escamosas (CCE). **Material e Métodos**: Inicialmente 183 LIC foram avaliadas quanto à presença de displasia e classificadas em relação ao grau. Em seguida, 45 casos foram divididos em: Grupo controle (CCE), Grupo 1 (displasia leve- DL), Grupo 2 (displasia moderada/severa- DM/S). Foram corados com Azul de Toluidina para quantificar os mastócitos e Picrosirius Red para avaliação dos tipos de fibras colágenas I e III. **Resultados**: As LIC foram mais frequentes em mulheres (n=107) com idade de 36,6 anos. O sítio mais afetado foi a mucosa do lábio inferior (29,7%), já a lesão mais frequente foi o fibroma traumático (39,2%). A displasia leve esteve presente em 56,3% da amostra. Os mastócitos foram evidenciados nos três grupos: grupo controle (6,76 mastócitos/mm), grupo 1 (10,82 mastócitos/$mm^{2)}$ e grupo 2 (19,18 mastócitos/$mm^{2)}$.Quando analisadas as fibras colágenas, observou-se no grupo controle e no grupo 2 que as fibras tipo III foram mais prevalentes, já no grupo 1 prevaleceu-se as fibras tipo I. **Conclusão:** Lesões inflamatórias crônicas orais apresentaram alterações displásicas na maior parte dos casos. O estudo sugere uma participação dos mastócitos na fase de transformação tumoral. E a alteração gradativa dos colágenos tipo I e III indica alteração das células produtoras de colágeno, durante transformação tumoral.

Descritores: Displasia; Carcinoma de células escamosas, Mastócitos; Colágeno.

ABSTRACT

Introduction: Chronic inflammation plays an important role on the transformation and tumor progression during oral carcinogenesis. There is a great number of chronic inflammatory lesions (CIL) in the oral cavity which are related to dysplastic processes of the epithelium, immune response and changes on the collagen deposition. **Objectives:** To investigate the presence of dysplasia and to histologically grade them in the CIL of traumatic cause, as well as toaccessthe density of mast cells and different types of collagen fibers in cases of epithelial dysplasias and oral squamous cell carcinomas (OSCC). **Material and Method:** Initially, 183 CIL cases were evaluated as to the presence of dysplasia and also classified according to its degree of epithelial dysplasia. Among those lesions, 45 CIL cases were selected and divided into two groups: group 1 (15 cases of mild dysplasia), group 2 (15 cases of moderate/severe dysplasia). The control group was composed by 15 cases of OSCC.They were stained with toluidine blue in order to quantify the mast cells and picrosirius red to semi-quantify the collagen type fibers. **Results**: The mast cells were detected in all groups presenting a mean of 6,76 cells/mm^2, 10.82 cells/mm^2 and 19.18 cells/mm^2 in the control, group 1and 2 respectively. Regarding the collagen fibers, type III was more prevalent on groups 2 and control while type I fibers were more abundant on group 1. **Conclusion**: Oral chronic inflammatory lesions showed dysplastic changes in most analyzed cases. The results suggests an active participation of mast cells in the stage of tumor transformation, since it was detected a higher density onthe dysplasia cases when compared to the OSCC cases. Nevertheless, the gradual change of collagen type fibers indicates that collagen-producing cells become altered during the stages of dysplasia (tumor transformation).

Keywords: Dysplasia; Oral squamous cell carcinomas; Mast cells; Collagen.

SUMÁRIO

1 INTRODUÇÃO

O grande desafio da Odontologia no campo de atuação da Patologia Bucal refere-se em aumentar precocemente o diagnóstico de casos de câncer de boca, visto que a maior parte desses ocorre em estágio avançado, restringindo as possibilidades de tratamento com sucesso da doença[1].

Nos esforços em concretizar estes eventos, têm sido constatada a presença de lesões antes do aparecimento do câncer. Essa fase é denominada de displasia epitelial que se caracteriza por um diagnóstico histopatológico com alterações celulares e arquitetônicas do epitélio escamoso estratificado e podem ou não evoluir para o câncer, conferindo potencial de malignidade na lesão[2-4]. Quando removido o agente promotor da displasia, as alterações a nível celular e estrutural voltam ao normal, por isso a importância de conhecer quais fatores causam tais alterações[5].

Um dos fatores citado na literatura é o infiltrado inflamatório crônico, que se encontra presente no micro ambiente nos casos de displasia[8] e nos carcinomas[7,8], levando os autores sugerirem a participação ativa da inflamação no processo de iniciação, promoção e progressão do câncer[6-9].

A inflamação crônica é composta por células, citocinas e mediadores que atuam para eliminar os agentes que causam danos ao organismo e em seguida restabelecem as estruturas que foram comprometidas ou perdidas. Fatores externos (microorganismos, trauma, substancias tóxicas) e fatores internos (células danificadas ou necróticas, baixo teor de oxigênio nos tecidos) ativam a cascata da inflamação. Assim, durante a inflamação crônica pode-se encontrar várias células mononucleares inflamatórias, áreas lesionadas e áreas de reparo[10].

No entanto, sabe-se que o sistema imunológico é uma rede complexa formada por órgãos, células e citocinas, o que torna o entendimento do processo inflamatório mais complexo. Entre as diversas células, destaca-se a ação dos mastócitos, que são derivados de células hematopoéticas da medula óssea, os quais circulam na corrente sanguínea ainda na forma agranular (fase imatura), completando a sua maturação no tecido que foram recrutados[11]. Os mastócitos maduros apresentam citoplasma rico em grânulos e quando ativados liberam seus

mediadores químicos que promovem a defesa do organismo, participando do processo inflamatório, nas reações alérgicas e na expulsão de microorganismos[19]. Eles são responsáveis pela iniciação e transformação da inflamação aguda para crônica[12], atuam na angiogênese e no processo de cicatrização[14].

No entanto com a continuação prolongada da inflamação crônica, os mastócitos são expressamente ativados e como estão presentes nas displasias epiteliais e no CCE, os autores sugerem sua participação no processo da promoção do câncer[14-16]. Esta super ativação dos mastócitos pode causar diversas alterações biológicas, tais como efeitos mitogênicos, degradação da matriz extracelular, angiogênese e recrutamento de células inflamatórias, podendo contribuir para a formação e progressão do tumor[17].

Nesse contexto, a inflamação crônica persistente provoca também alterações na matriz extracelular (MEC), uma vez que promoverá a proliferação de fibroblastos e consequentemente o aumento da síntese de colágeno, podendo esse processo de reparo causar disfunções nos tecidos[10].

Durante o desenvolvimento do carcinoma de células escamosas (CCE), células epiteliais malignas invadem o tecido conjuntivo e a interação dessas células com a matriz extracelular (MEC) aumentam a deposição de fibras colágenas tipo I e tipo III. Essas alterações na arquitetura e na composição da matriz contribuem para o desenvolvimento e progressão do câncer[18].

Assim, sabendo-se que essas alterações no epitélio, na concentração dos mastócitos e na deposição do colágeno estão associadas à transformação maligna, e que podem ser encontradas nas lesões inflamatórias crônicas (LIC) da cavidade oral, tornou-se essencial avaliar a presença dessas alterações nas LIC e compará-las com os casos de CCE.

2 OBJETIVO

2.1 Objetivo Geral

Analisar a presença da displasia epitelial nas Lesões Inflamatórias Crônicas (LIC).

2.2 Objetivos Específicos

Avaliar os diferentes graus de displasia epitelial nas Lesões Inflamatórias Crônicas (LIC).

Avaliar a densidade de mastócitos e diferentes tipos de fibras colágenas na matriz extracelular das Lesões Inflamatória Crônicas com displasias e comparar com o grupo controle de Carcinoma de Células Escamosas (CCE).

3 METODOLOGIA

3.1 Implicações éticas e tipo de estudo

A pesquisa foi aprovada pelo Comitê de Ética em Pesquisa da Universidade Federal de Sergipe via Plataforma Brasil (CAE 080933/2015).

Trata-se de um estudo observacional, descritivo e retrospectivo. Todos os laudos histopatológicos arquivados no laboratório de Patologia Oral do Departamento de Odontologia da UFS, no período de 2009-2014 foram revisados para seleção da amostra.

3.2 População e Amostra

Foram selecionados 183 casos, cujos diagnósticos histopatológicos fazem parte do elenco de LIC de origem traumática ou traumatizada secundariamente e 56 casos com diagnóstico de CCE da cavidade oral.

Foram excluídas as lesões que, apesar de serem de origem inflamatória, já são reconhecidamente lesões potencialmente malignas, como por exemplo: queilite actínica, estomatite nicotínica entre outras, assim como os casos de câncer de lábio, já que estudos recentes mostraram que a radiação solar pode induzir o aumento do número de mastócitos[15,16]. As lesões intra-ósseas, granuloma piogênico e lâminas com ausência de epitélio ou quando o mesmo estava desgarrado do tecido conjuntivo, foram excluídas por impossibilitarem a avaliação de displasia. Por fim os casos cujos blocos de tecido parafinado não permitiram a confecção de três ou mais lâminas.

3.3 Método

A pesquisa foi dividida em duas etapas, a primeira foi composta apenas pelos casos de LIC, que foram avaliadas quanto à presença de displasia e graduadas. Para essas avaliações histopatológicas, as lâminas contendo cortes das lesões foram obtidas do arquivo do departamento de odontologia e estavam corados por hematoxilina e eosina (HE). Foram analisadas por dois observadores independentes, previamente calibrados, em microscópio óptico Olympus® CX31

(São Paulo, SP, Brasil). Nos casos de incompatibilidade entre os observadores, a classificação do grau da displasia foi decidida por um patologista experiente da equipe.

O grau de displasia foi padronizado utilizando-se a classificação de Smith and Pindborg[19], a qual determina uma pontuação numérica para cada uma das 13 características histológicas avaliadas. Cada característica foi graduada como: ausente, sutil e marcante. O índice foi definido como sendo a soma dessas 13 pontuações, considerado sem displasia as lesões com escore de 0 a 10; displasia discreta os casos entre 11 e 25; displasia moderada quando os valores se encontraram entre 26 e 45 e displasia severa, escore entre 46 e 75. Os casos de displasia moderada e severa foram agrupados juntos por apresentarem o mesmo tratamento clínico.

Tabela 1 – Classificação para avaliação do grau de displasia.

Alterações	Aumento	Escore
1-Projeções em gota	10x	0/2/4
2-Estratificação irregular	10x	0/2/6
3-Queratinização abaixo da camada de queratina	10x	0/2/5
4-Hiperplasia da camada basal	10x	0/1/3
5-Perda de adesão intercelular	10x	0/1/4
6-Perda de polaridade	10x	0/2/6
7-Hipercromatismo nuclear	10x	0/2/5
8-Relação núcleo/citoplasma alterada	40x	0/2/6
9-Anisocitose e anisocariose	40x	0/2/6
10-Pleomorfismo celular e nuclear	40x	0/2/6

11-Atividade mitótica	40x	0/1/5
12-Nível de atividade mitótica (localização)	40x	0/3/10
13-Mitoses atípicas	40x	0/6/10

1º escore: ausência de alteração; 2º escore: pouca alteração; 3º escore: muita alteração.

A segunda etapa foi iniciada com a seleção de 15 casos de displasia leve (Grupo 1) e 15 de displasia moderada/severa (Grupo 2) da amostra total das LIC (n = 158) previamente selecionadas na etapa 1. Os critérios de inclusão utilizados nesta etapa foram lesões com diagnóstico de fibroma traumático, hiperplasia fibrosa inflamatória ou fibroma de células gigantes. Um terceiro grupo, o grupo controle, foi composto de 15 casos da amostra inicial de CCE (n = 44).

Esta etapa foi avaliada por um observador devidamente calibrado por dois patologistas com experiências em avaliações de mastócitos e de fibras colágenas. Foi um estudo cego, no qual o observador não sabia o grupo que as lâminas pertenciam, uma vez que só continham um código para identificá-las.

Os três grupos foram avaliados quantitativamente para mastócitos e semiquantitativamente para fibras colágenas. Assim, foram confeccionadas três lâminas de cada bloco parafinado, cortados em micrótomo (cada secção de 5µm), onde 02 foram coradas, uma com Azul de Toluidina (Certistain, Darmstadt, Germany) para avaliação dos mastócitos e outra com Pricosirius Red (EasyPath, Rankonkoma, NY, EUA), a fim de avaliar as fibras colágenas. A terceira era reserva em caso de necessidade de repetir alguma coloração.

Para a avaliação da densidade dos mastócitos, os cortes foram desparafinados, desengordurados e hidratados gradativamente, de forma habitual e lavados com água destilada. Em seguida, seguindo o manual do fabricante, foram cobertos com azul de toluidina (0,1 g de Certistain® Azul de toluidina em 100 ml de água destilada) por vinte minutos e lavados em seguida com água destilada por um minuto. Por fim, o material foi desidratado em dois banhos de álcool a 95% (1minuto cada), dois banhos de álcool absoluto (1minuto cada) e dois banhos de xilol (1 minuto cada), e enfim montado com Bálsamo do Canadá.

A contagem dos mastócitos foi realizada em 6 campos: no caso das LIC, três campos foram superficiais e três profundos, selecionando a área de maior concentração da célula em questão; já nos casos de CCE, os seis campos selecionados foram ao redor das massas tumorais. Os mastócitos foram avaliados em objetiva de 40x, por um observador devidamente calibrado. Cada campo apresentou uma área de 1 mm^2, totalizando por lâmina 6 mm^2. O microscópio óptico Leica® (Leica Microsystems, Cambrige, Reino Unido) foi acoplado ao computador para capturar as imagens que foram analisadas pelo software de análise de imagens, ImageJ®. Em seguida foi feita a média de mastócitos por lâmina/lesão e expressa por células/mm^2.

Para a avaliação do tipo de fibras colágenas, os cortes foram corados com Picrosirius Red Staining EasyPath®, seguindo o manual do fabricante do kit. Assim, as lâminas foram desparafinizadas em xilol por cinco minutos, hidratadas em álcool 99%, 95% e 70% e, em seguida, lavadas em água corrente. Posteriormente, os cortes foram envolvidos com o reagente A por uma hora e lavados em água corrente por três minutos. Após a secagem, adicionou-se o reagente B por quatro minutos, seguido de lavagem por cinco minutos e então secos. Por fim, foram desidratados em sequência crescente de álcool até o xilol e montados em Bálsamo do Canadá.

Para as fibras colágenas, utilizou-se filtro polarizador adaptado ao microscópico óptico Olympus® CX31 (São Paulo, SP, Brasil), o qual permitiu distinguir as fibras colágenas tipo I das de tipo III, em virtude de apresentarem birrefringências diferentes. As do tipo I apresentam birrefringência laranja-avermelhado a laranja–amarelado; já as do tipo III variam do amarelo-esverdeado ao verde[20]. Foi avaliado também se as fibras eram curtas ou longas. Em cada lâmina foram selecionados seis campos, sendo três superficiais e três profundos, todos observados por apenas um observador devidamente calibrado. Para avaliar a concentração de cada fibra foi adaptada do estudo de Cirino et.al. (2016)[21], quadro escores como mostra a tabela 2.

Tabela 2. Classificação para a intensidade das fibras colágenas tipo I e III.

Escore	Concentração	Classificação
0	0	Ausente
1	< 10 %	Fraca
2	10 % < X < 50 %	Moderada
3	>50 %	Forte

3.4 Análise Estatística

Foram feitas análise descritivas nos casos de displasia. Os dados obtidos dos mastócitos e das fibras colágenas foram analisados estatisticamente por meio do programa GraphPad Prisma versão 7 para Windows. Os mastócitos foram avaliados pelo teste D'Agostino-Pearson, para avaliar a normalidade dos resultados e foi decidido aplicar o teste Kruskal Walis, sendo a correlação testada com o coeficiente de Dunn's. Os testes de Kruskal Walis e Dunn's também foram aplicados nas avaliações das fibras colágenas, sendo todos com significância de 5% ($p<0,05$). Para a avaliação intra-examinador foi aplicado o coeficiente de correlação intra-classe (ICC) para mastócitos e o Kappa para fibras colágenas.

HISTOPATHOLOGICAL STUDY OF EPITHELIAL DYSPLASIAS IN CHRONIC INFLAMMATORY LESIONS OF THE ORAL CAVITY

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ABSTRACT

The objective of this study was to investigate the presence of dysplasia and to histologically grade them in the CIL of traumatic cause, as well as to access the density of mast cells and different types of collagen fibers in cases of epithelial dysplasias and oral squamous cell carcinomas (OSCC). Initially, 183 CIL cases were evaluated as to the presence of dysplasia and also classified according to its degree of epithelial dysplasia. Among those lesions, 45 CIL cases were selected and divided into two groups: group 1 (15 cases of mild dysplasia), group 2 (15 cases of moderate/severe dysplasia). The control group was composed by 15 cases of OSCC.They were stained with toluidine blue in order to quantify the mast cells and picrosirius red to semi-quantify the collagen type fibers. The mast cells were detected in all groups presenting a mean of 6,76 cells/mm^2, 10.82 cells/mm^2 and 19.18 cells/mm^2 in the control, group 1and 2 respectively. Regarding the collagen fibers, type III was more prevalent on groups 2 and control while type I fibers were more abundant on group 1. Oral chronic inflammatory lesions showed dysplastic changes in most analyzed cases. The results suggests an active participation of mast cells in the stage of tumor transformation, since it was detected a higher density onthe dysplasia cases when compared to the OSCC cases. Nevertheless, the gradual change of collagen type fibers indicates that collagen-producing cells become altered during the stages of dysplasia (tumor transformation).

Keywords: Dysplasia; Oral squamous cell carcinomas; Mast cells; Collagen.

INTRODUCTION

The great challenge in Dentistry regarding the Oral Pathology field is to increase the diagnosis of oral cancer in early stages, since most diagnoses are done when the disease is in an advanced stage, which reduce the possibilities of a successful treatment (1).

In this context, a special attention has been given to identify and observe suspect lesions that exhibit epithelial dysplasia, characterised histopathologicallyas cellular and architectural changes throughout the oral stratified squamous epithelium. Such changes might evolve or not to cancer providinga malignant potential to the lesion (2-4). When the dysplasia causing agent is removed, changes on the cellular and structural levelreturn to the normal condition, justifying the importance of the knowledge of the factors that trigger such changes (5).

One of those factors is the chronic inflammatory infiltrate found frequently in the microenvironment of cases of epithelial dysplasia (6) and carcinomas (7,8), leading the authors to suggest an active participation of the inflammation in the process of initiation, promotion and progression of cancer (6-9).

The chronic inflammation is composed of cells, cytokines and cell mediators that act together to eliminate the agents that are causing damages to the organism and later reestablish the compromised or lost structures. External (microorganisms, trauma, toxic substances) and internal (damaged or necrotic cells, low level tissue oxygen) factors trigger the inflammation network. Therefore, during a chronic inflammation process there are several mononuclear cells along with injured areas and also sites of tissue repair (10).

However, it is known that the immune system is a complex network composed of organs, cells and cytokines, which difficults the complete understanding of the inflammatory process. The mast cells play an important role on this process: they are derived from hematopoietic precursors in the bone marrow, which flow into the blood stream when they are still in the agranular form (inmature phase), completing their maturation process in the local tissue (11). Finaly, the mature mast cells show a cytoplasm full of granules thatif activated, release its content that promotes the defense of the organism, contributing to the development of inflammatory process, allergic reactions as well as the expulsion of microorganisms (12). They are responsible for the initiation and transition of acute to chronic inflammation (13), play a role on the angiogenesis and on the healing processes (14).

Nevertheless, as the chronic inflammation process remains in the tissue, the mast cells are more and more activated and as they are present in epithelial dysplasias and in oral squamous cell carcinomas (OSCC), a few authors suggest that they have an important role in cancer development (14-16). This super activation of the mast cells can cause several biological changes, such as mitogenic effects, degradation of the extracellular matrix,

increased angiogenesis and recruitment of inflammatory cells which can contribute to tumor formation and progression (17).

The persistent chronic inflammation might also cause changes in the extracellular matrix (ECM), since it will promote the proliferation of fibroblasts and consequently the increase in collagen fibers synthesis which can cause tissue dysfunctions (10). During the growth of OSCC, malignant epithelial cells invade the connective tissue and the interaction of these cells with the ECM increase the deposition of collagen fibers types I and III. These changes on the architecture and composition of the matrix contribute to the tumor development and progression (18).

Considering the role of dysplastic changes in the epithelium, the density of mast cells in the connective underlying tissue and the deposition of different types of collagen fibers in the ECM in the malignant transformation process and also that all of those can be found in chronic inflammatory lesions (CIL) in the oral cavity, it becomespivotal to evaluate those factors on the CIL when compared to the OSCC cases.

MATERIALS AND METHODS

The research was approved by the Research Ethics Committee of Federal University of Sergipe (UFS) at Brazil (CAAE 080933/2015), characterized as an observational, descriptive and retrospective study. All of the archived histopathololological reports stored at the laboratory of Oral Pathology of the Department of Dentistry at UFS, in the period 2009-2014 were revisited for sample selection, resulting in 183 cases whose histopathological diagnoses are consistent with traumatic CIL and in 56 OSCC cases.

The lesions that are already recognized to be potentially malignant were excluded despite the fact of being of inflammatory origin such as actinic cheilitis, nicotinic stomatitis among others as well as lip cancer, due to the fact that recent studies showed that solar radiation can induce the increase of local mast cells density (15,16). Intraosseous lesions, pyogenic granuloma and histological slides without epithelium or when it was detached from the connective tissue were excluded because of the impossibility to evaluate epithelial dysplasia. Also, the cases whose blocks of paraffin-embedded tissue did not exhibited engough material to make three slides were eliminated.

Then, the research was divided into two phases. The first one comprised theCIL cases, that were evaluated as to the presence of dysplasia and graded. The slides containing the lesions sections were obtained in the Department of Dentistry and they were stained with hematoxylin and eosin (HE), analysed by two independent observers, previously calibrated, on an optical microscope Olympus® CX31 (São Paulo, SP, Brazil).In case there

was incompatibility between the observers, the classification of the dysplasia degree was decided after agreement/discussion between the pathologists.

The degree of dysplasia was standardized by using SMITH & PINDBORG (19) classification, which determines a numerical score for each of the 13 histological evaluated characteristics. Each characteristic was graded as: absent, weak and strong. The index was defined as the sum of these 13 scores, considered to be without dysplasia the lesions scored between 0–10; mild dysplasia (MD) for the cases scored between 11-25; moderate dysplasia when the values ranged from 26 to 45 and finally severe dysplasia for the ones between 46-75.The cases of moderate and severe dysplasia (M/SD) were analyzed together due to the fact that the clinical treatment is similar.

The second stage started with the selection of 15 cases of mild dysplasia (Group 1) and 15 cases of moderate/severe dysplasia (Group 2) of the total sample of CIL (n=158) previously selected, including lesions with diagnosis of traumatic fibroma, inflammatory fibrous hyperplasia or giant cell fibroma. A third group, the control group, was composed of 15 cases from the initial OSCC sample (n = 44).The slides were evaluated by the same observers, previously calibrated. In disagreement cases, the classification was decided after agreement/discussion between the pathologists. Moreover, it was a blind study, in which the observers did not know which group the slides belonged to.

The three groups were evaluated quantitatively for mast cell and semi-quantitatively for collagen fibers. Thus, three sections of each paraffin block were cut by a microtome (5μm thickness), where one section was stained with toluidine blue (Certistain, Darmstadt, Germany) for evaluation of the mast cells and the other one with pricosirius red (EasyPath, Rankonkoma, NY, EUA), in order to evaluate the collagen fibers, in a total of two slides, both following the manufacturer' instructions. A third slide with a 5 μm section was also made in case it was necessary to repeat one of the stainning techniques applied herein.

The mast cells density was performed in six fields: on CIL cases, three observed fields corresponded to superficial areas while the three other fileds analyzed corresponded to deep areas, always checking the entire section looking for áreas exhibitingthe greatest amount of the cell considered; as for the cases of OSCC, the six selected fields were around the tumor masses. The mast cells were evaluated in a 40x objective lense by a properly calibrated observer. Each field presented an area of 1 mm^2, resulting a total of 6 mm^2 for each slide/section. The Leica® optical microscope was attached to a computer to capture the images that were analyzed by ImageJ®, an image analysis software. The mean mast cell per slide/section/lesion was expressed as cells/mm^2.

For the collagen type fibers, a polarized light microscopy was used, which allowed to distinguish type I collagen fibers from type III, because they show different birefringences.

Type I birefringence presents reddish-orange to yellowish-orange polarizing colourswhile type III colours vary from greenish-yellow to Green (20). It was also evaluated the fibers length (short or longer). Likewise, in each slide six fields were selected, being three superficial and three deep areas, all observed by only one observer duly calibrated. In order to evaluate the density of each collagen fiber, scores were atributed according to the report of CIRINO et al. (21), as shown in table 1.

Tab 1. Classification of the collagen typefibers density.

Score	Concentration	Classification
0	0	Absent
1	< 10 %	Weak
2	10 % < X < 50 %	Moderate
3	>50 %	Strong

Data obtained from mast cells and collagen type fibers were statistically analysed using GraphPad Prisma version 7 for Windows®. The mast cells were evaluated by the D'Agostino-Pearson test to evaluate the normality of the results followed by the Kruskal Walis test, and the correlation was tested with the Dunn's coefficient. Kruskal Walis and Dunn's tests were also applied in the evaluations of collagen fibers, all with significance of 5% ($p < 0.05$).For intra-examiner evaluation the intra-class correlation coefficient (ICC) for mast cells and Kappa for collagen fibers were also applied.

RESULTS

A total of 1152 biopsies were performed at Department of Dentistry (UFS), of which 183 had an histopathological diagnosis of chronic inflammatory lesions, also known as reactive or hyperplastic lesions (prevalence rate of 15.9%) and 56 were OSCC (prevalence of 4.9 %). After the exclusion criteria, remained were 158 cases of CIL and 44 cases of SCC.

In relation to the percentage of epithelial dysplasia of the total number of CIL diagnosed, 56.3% of the cases (n=89) presented mild dysplasia, 22.8% (n=36) moderate/severe dysplasia and 20.9% (n = 33) absence of epitelial dysplasia. Figure 1 shows the histological sections presentingmild and moderate/severe dysplasia.

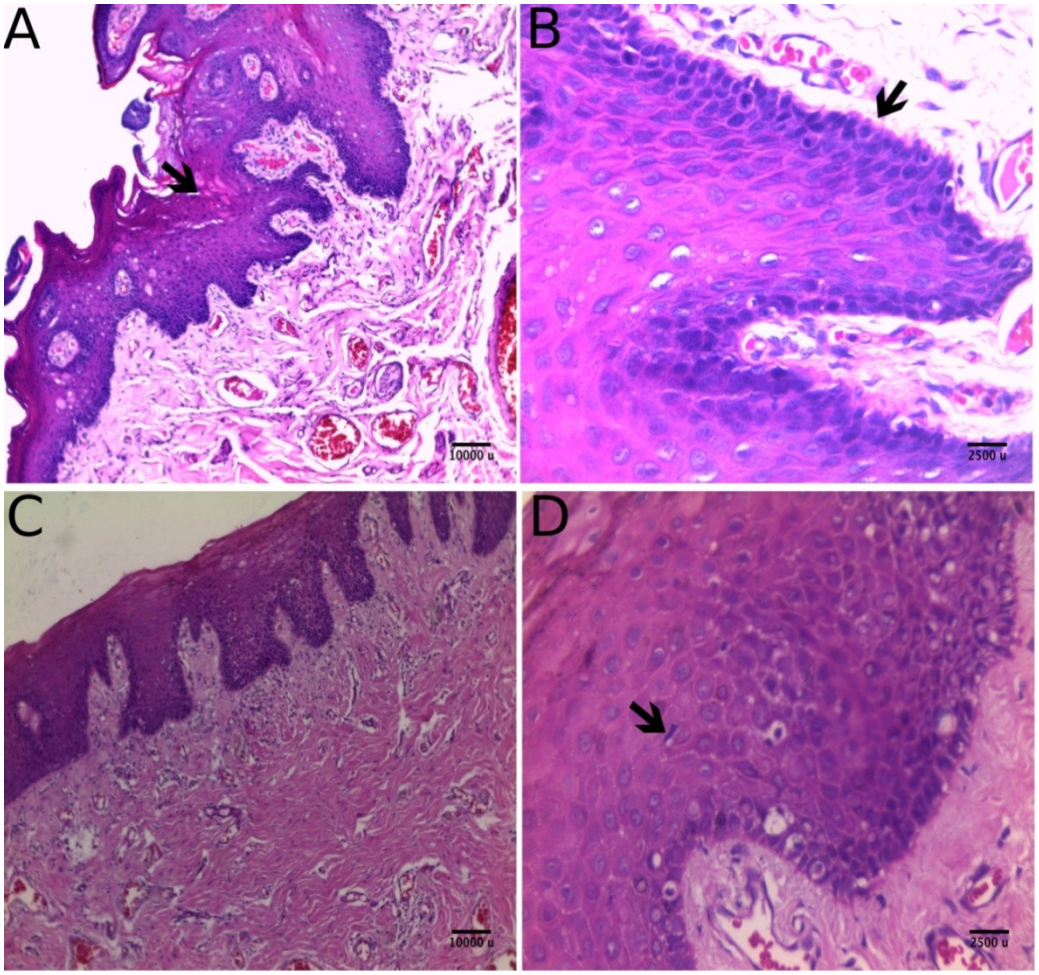

Fig 1: A, B - Traumatic fibroma with an histopathological diagnosis of mild epithelial dysplasia: A. Hyperkeratosis (arrow); B. Basal layer hyperplasia. C, D - Inflammatory fibrous hyperplasia with an histopathological diagnosis of moderate/severe epithelial dysplasia: C. Drop shaped rete-pegs and nuclear hyperchromatism; D. Atypical mitotic figure (arrow).

Regarding the toluidine blue staining at the second stage, the intra-examiner evaluation for mast cells presented excellent replicability (ICC = 0.997). The mast cells were evident in all groups (Figure 2) and the mean of the control group (OSCC) was 6.76 mast cells/mm^2, followed by the mild dysplasia group 1 with 10.82 mast cells/mm^2 and finally the moderate/severe dysplasia group 2, showing 19.18 mast cells/mm^2.

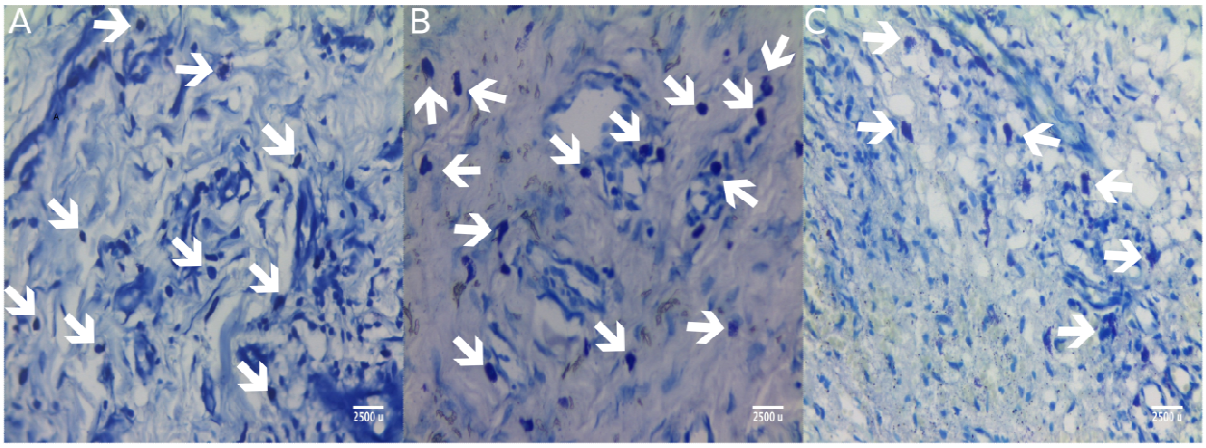

Fig 2: Stained sectionsby toluidine blue showing the mast cells in cases of mild dysplasia (A), moderate/severe dysplasia (B) and OSCC (C).

When comparing the mast cells means, a statistically significant difference was observed between the following groups (Kruskal-Wallis test p <0.0001): control vs mild dysplasia (Dunn's test p <0.01); control vs M/S dysplasia (Dunn's test p <0.001); and mild dysplasia vs M / S dysplasia (Dunn's test p <0.05).

In relation to the density of type III collagen fibers, 58.89% of thecontrol group (OSCC) exhibited aconcentration greater than 50% (score 3). As for the evaluation of fibers type I exclusively, only 30% of the cases presented a concentration greater than 50%. Thus, the predominant collagen fibers in the control group were type III. The same predominance was found in the M/SD group, showinga concentration of fibers III greater than 50% in 62.22% of the cases; type I fibers only predominate in 26.67% of the cases. In contrast, the MD group showed a predominance of type I collagen fibers, with 77.78% of cases presentingconcentration greater than 50%, whileonly 15.56% oftype III fibers had a score of 3, as shown in Figure 3 and Table 2.

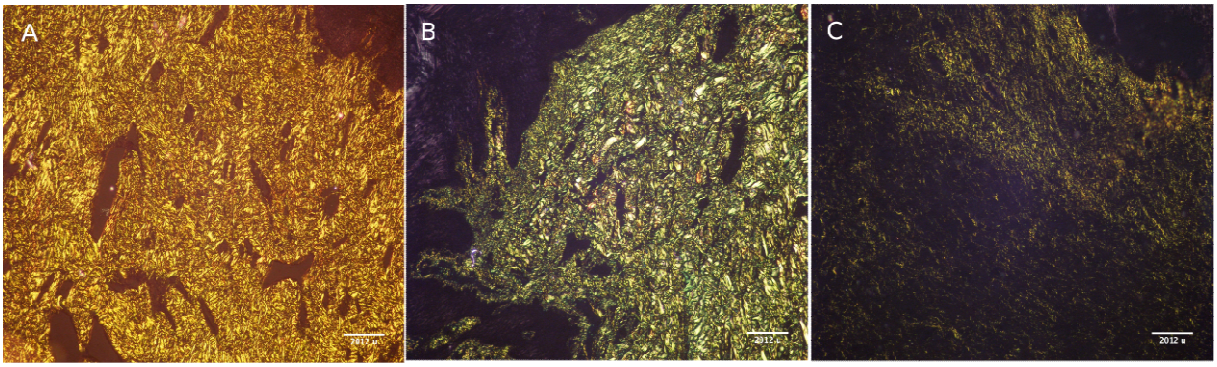

Fig 3: A) Photomicrograph of traumatic fibroma with mild dysplasia showing predominance of type I collagen fibers. B) Photomicrograph of traumatic fibroma with severe dysplasia showing a predominance of type III collagen fibers and small areas containing type I fibers.

C) OSCC photomicrograph showing predominance of type III collagen fibers and fibers more disorganized and shorter in lenght. (Picrosirius red staining).

Tab 2. Concentrations of type I and type III collagen fibers among the groups.

Groups	Concentrations	Type I Frequency	Type III Frequency
Controlgroup	Concentration< 10 %	3,33%	8,89%
	10%<concentration< 50%	66.6%	32,22%
	Concentration> 50 %	30%	58,89%
	Total	100%	100%
Mild dysplasia group	Concentration< 10 %	5,56%	25,56%
	10%<Concentration< 50%	16,65%	58,86%
	Concentration> 50 %	77,78%	15,56%
	Total	100%	100%
Moderate/severe dysplasia group	Concentration< 10 %	11,11%	7,78%
	10%<Concentration< 50%	62,22%	30%
	Concentration> 50 %	26,67%	62,22%
	Total	100%	100%

Score 1: concentration< 10 %; score 2: 10% <concentration< 50% and score 3: concentration> 50 %

Concerning the collagen fibers staining by picrosirius red, the replicability intra-examiner was considered good (Kappa = 0.71). When evaluating the birefringence of collagen fibers type I and type III under polarized light, it was found that in the fibers type I exhibited a gradual color change from reddish-orange to yellowish-orange, while the type III ranged from greenish-yellow to green. In all groups, the fibers were disorganized and short in lenght, specially in the cases of OSCC and moderate/severe dysplasia (M/SD). In relation to

the density of type I collagen fibers, a statistically significant difference (Kruskal-Wallis test p <0.0001) was observed, however, only between the following groups: control vs mild dysplasia(MD) (Dunn's test p = 0.001) and MD vs M/SD.The same was observed for the type III fibers, as shown in Table 3.

Tab 3. Statistical analysisof the collagen type fibers density among the groups.

Groups	Fibers type I	Fibers type III
Control vs Mild Dyspasia	P < 0.001*	P < 0.001*
Control vs Moderate/Severe Dysplasia	P > 0.05	P > 0.05
Mild Dysplasia vs Moderate/Severe Dysplasia	P < 0.001*	P < 0.001*

*Significant statistical difference

DISCUSSION

In the present study, the prevalence of reactive hyperplastic lesions was 15.9% of all cases diagnosed in the period studied. Similar values were found in the studies of KADEH et al. (22) e REDDY et al. (23), that of all oral lesions evaluated, 20.2% and 12.8%, respectively, were reactive hyperplastic.

The chronic inflammatory infiltrate was present in all lesions suggesting its participation in the epitelial changes. The chronic inflammation is a cyclical process, since proinflammatory cells will be attracted to the site of inflammation that will attract other inflammatory cells and cytokines (24).This, in turn, stimulate the production of signaling molecules to attract even more proinflammatory cells.Since the causing agent is not removed, the regulation process does not occur to end the inflammation, in its place there is a self-stimulation, creating a continuous cycle (24). Authors suggest that this imbalance becomes a suitable pathway for tumorigenesis, since chronic inflammation has been associated with cancer initiation, proliferation, survival and metastasis (8,25).

In their literature review, MOSS & BLASER (25) suggested that the persistent immune-inflammatory process promotes a proliferative reaction of the epithelial cells, increasing the selection process, which results in the appearance of cells adapted for survival, susceptible to the action of additional mutagenic agents. This genetic instability is due to mutations in tumor suppressor genes (26). The cells of the immune system under normal conditions are able to recognize and destroy tumor cells (via tumor antigens),

however, because of an imbalance, the defense cells do not destroy them and mediators of inflammation promote proliferation and survival, contributing to tumor promotion (27). In each mutation, the cells become less susceptible to the regulatory actions of the organism, thus, the proliferation of these and the accumulation of mutations that occur over the years, causes cancer development (28).

Some reports in the literature relate chronic inflammation to changes in epithelial cells (29), immune response (14) and extracellular matrix components (10). The relationship of inflammation with changes in epithelium was also observed in other studies (6,30), who evaluated the degree of dysplasia in the cases of actinic cheilitis and suggested that inflammatory cells play an active role in the disorganization of the epithelium, causing dysplastic changes and thus contributing to the malignant process. The changes in dysplasia include genetic, epigenetic and superficial changes, resulting in a malignant potential, since the accumulation of these alterations can generate tumor cells in the lesion (5).

In the researched literature there are only a few studies that have evaluated dysplasia in reactive hyperplastic lesions, especially the study by WANG et al. (31), who evaluated the malignancy potential of a few oral lesions. In 2641 cases of hyperplasia/hyperkeratosis, it was found in 64% (n=1684) epithelial dysplasia, using the World Health Organization (WHO) classification. This percentage is similar to the one described herein, in which the dysplastic alterations were found in 79.1% of the cases. This small difference can be justified by the different evaluation method applied (in the present study, the classification was Smith and Pindborg (19), as well as the types of oral lesions in the samples examined. Nevertheless, some authors reported that the epithelial changes found in reactive inflammatory lesions, although similar to dysplastic changes, should not be considered epithelial dysplasias and that in these cases the clinical history of the lesions should be taken into account (31-34). It is a fact that the gold standard for the diagnosis of dysplasia is the histopathological evaluation with Hematoxylin-Eosin (HE) staining (5,23,32). Therefore, the histological changes that by definition, characterize epithelial dysplasia, are the only criteria that can and should be considered for such diagnosis.

In this context, in the present study an inflammatory infiltrate was observed in all the lesions, and dysplasia was found in more than half the cases (79.1%), therefore the possibility of malignant transformation of the reactive hyperplastic lesions can not be ruled out, since they are the result of continuous trauma that activate the chronic inflammatory process, even though the literature considers them to be benign (21,35). In addtion, the giant cell fibroma lesions presented M/SD in 61.5% of the cases suggesting an increased risk of malignancy when compared to the other lesions studied. However, further studies are needed to evaluate this potential.

The presence of mast cells in all lesions evaluated suggests their participation during the chronic inflammation. Their function depend on the release of chemical mediators present in their granules, which are classified into preformed factors (heparin, histamine, hydrolases, oxidases, ECF-A, proteases and growth factors), neoformed factors (PGD2, LTC4, LTB4, PAF and thromboxanes) and neo-synthesized factors (IL3, IL4, IL5, IL6, IL8, IL12) (11).

Nowadays, the exact explanation of the mast cell's involvement in the malignant transformation is still unclear. This is due to release of tumor necrosis factor (TNF) by mast cells, which is responsible for chemotaxis of neutrophils, monocytes and T lymphocytes (TL) (36). Migration of TL to the area of inflammation allows a cyclic process of inflammation, since they secrete chemokines RANTES that promote the degranulation of the mast cells, which in turn release more TNF (36). The secretion of interleukin(IL)-12 by mast cells allows the differentiation of native T cells into Th1 cells; the release of IL-4 allows the transformation to Th2 lymphocytes (37). Recent studies show that the ratio between TCD4 and TCD8 lymphocytes may be related to the process of malignant transformation and tumor progression (29,38,39).

Such evidence is strengthened by the present study, where statistically significant differences were observed in the mast cell density of the three groups. It was observed that in the control OSCC group the mean concentration was 6.76 mast cell/mm^2; in the mild dysplasia was 10.82 mast cell/mm^2 and moderate/severe dysplasia presented the highest count, 19.18 mast cell/mm^2. These values differ from those found by PARIZI et al. (16), which evaluated the concentration of mast cells in 34 cases of oral cancer (22 intra-oral and 12 on the lip) and compared with 30 cases of skin cancer, describing a concentration of 13.1 mast cell/mm^2 on the intra-oral cases and 108.5 mast cell/mm^2 on the lip casescompared to 116.7 mast cell/mm^2 on the skin cases of cancer, indicating an induction of mast cell proliferation by solar radiation.

ARAUNAD (15) found a higher mast cell density in cases of carcinoma (27.57 cells/mm^2) related to actinic cheilitis, when compared to cases of epithelial dysplasia (17.4 cells/mm^2) and normal mucosa (1.78 cells/mm^2). However, this finding is entirely justifiable, since actinic cheilitis occurs on the lip, where solar radiation is the main risk factor. Nonetheless, consideringthe cases of epithelial dysplasia, his study corroborated the result described herein, since in both studies the concentration of mast cells was higher as the severity of dysplasiaincreased.

BERHANE et al. (7) evaluated inflammatory cells in different degrees of dysplasia and in cases of OSCC and found a decrease after malignant conversion, suggesting that inflammation plays an active role in the malignant transformation process.Thus, considering

also the findings of the present study, it is suggested that the gradual increase of mast cells occurs in the stage of tumor transformation, decreasing when the lesion becomes self-sufficient, irreversible.

In addition to epithelial and inflammatory changes, changes in the quality and organization of ECM collagen fibers were also observed.These alterations can have diverse effects on cell differentiation, gene expression, proliferation/survival and migration of tumor cells (40,41).

During epithelial dysplasia, basal layer cells are disorganized, allowing neoplastic epithelial cells to interact with ECM components (42). Excessive proliferation of cells and their interaction with matrix structures promote a decrease in the oxygen content of the tissue and may cause genetic instability of the cells, and thus with the evolution of the carcinoma, they induce the formation of altered collagen fibers and increase collagenase activity (43). Other authors suggest that the stroma becomes a conducive medium for the evolution of cancer, since increased angiogenesis provides nutrients, allows the exchange of gases and eliminates the metabolic residues of neoplastic cells.In addition, excessive synthesis of collagen fibers might difficult the contact of inflammatory cells with tumor cells, preventing them from being destroyed, and thus enabling tumor growth (44).The mobilization of tumor cells can be facilitated by the protease-induced degradation of the fibers, such as collagenases (42).

Regarding the changes of collagen fibers in the different groups, this study verified that the birefringence of collagen fibers type I varied from eddish-orange to yellowish-orange. However, the type III fibers varied from greenish-yellow to green, in accordance with other studies reported in the literature (20,45-50).

In relation to the organization of the fibers, it was observed that the fibers were disorganized in the three groups, however, in the control OSCC group and in group 2 (M/SD), the disorganization and the presence of short fibers in lenght were more evident.This degradation of collagen fibers occurs due to the action of collagenases and is fundamental for carcinogenesis and tumor progression (50).

Thus, it was observed that type I collagen fibers were more present in the less severe cases of dysplasia, and type III, in the most advanced cases. This gradual replacement of the fibers associated with the evolution of the malignant transformation, corroborates with some studies (42,50,51), indicating a possible alteration of collagen-producing cells, which becomes permanent, contributing to tumor progression. However, they disagree with thereported by GANGANNA et al. (46).

A study showed that the increase in the density of collagen fibers in the mammary tissue of rats presented a three times greater risk of malignant tumor development, so the

authors suggested that this alteration may be involved in the process of tumor initiation, progression and metastasis (52). Other studies have evaluated only stromal changes related to OSCC and observed that the collagen polarization colors were yellowish red, in well-differentiated cases and green, in poorly differentiated cases (9,45,48,49).Thus, the change in the polarization color of the collagen fibers in the different degrees of dysplasia in the CIL and in the OSCC cases, denotes a relation with the evolution and progression of the malignant tumor.

Epithelial dysplasia was present in most cases of chronic inflammatory oral lesions. The study suggests a participation of mast cells in the stage of tumor transformation and also concludes that the gradual change of type I to type III collagen fibers indicates a change in the collagen-producing cells during the phases of dysplasia (tumor transformation).

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

1. Instituto Nacional do Câncer [homepage na internet]. Câncer [acesso 15 set 2016]. Disponível em: www.inca.gov.br
2. Manchanada A, Shetty DC. Reproducibility of grading systems in oral epithelial dysplasia. Medicina Oral Patología Oral y Cirugía Bucal. 2012; 17(6): 935-42.
3. Miyazaki Fujinami M, Inoue H, Kikuchi K, Ide K, Kusama K. Expression of activation-induced cytidine deaminase in oral epithelial dysplasia and oral squamous cell carcinoma. Journal of Oral Science 2013; **55**: 293-9.
4. Shirani S; Kargahi N; Razavi SM; Homayon S. Epithelial Dysplasia in Oral Cavity. IJMS 2014; **39(5)**: 406-417.
5. Rastogi V, Puri N, Mishara S, Arora S, Kaur G, Yadav L. An Insight to Oral Epithelial Dysplasia. Int J Head Neck Surg 2013; **4(2)**: 74-82.
6. Paiva MAF, Soares MSM, Figueiredo CRLV, Luna AH, Oliveira VEM, Júnior OB. Associação entre displasia e inflamação em queilite actínica. J Bras Patol Med Lab 2012; **48 (6)**: 455-458.

7. Berhane T, Halliday GM, Cooke B, Barnetson RS. Inflammation is associated with progression of actinic keratoses to squamous cell carcinomas in humans. Br J Dermatol 2002; **146 (5)**: 810-815, 2002.
8. Guo L, Zhang Y, Zhang L, Huang F, Li J, Wang S. MicroRNAs, TGF-β signaling, and the inflammatory microenvironment in câncer. Tumour Biol 2016; **37(1)**: 115–125.
9. Martins-Filho PRS, Santos LCF, Piva MR. The prevalence of actinic cheilitis in farmers in a semi-arid northeastern region of Brazil. International Journal of Dermatology 2011; **50 (9)**: 1109-14.
10. Kumar V, Abbas AK, Fausto N, Aster JC.Patologia bases patológicas das doenças. Rio de Janeiro: Elsevier. 2010; 79-110.
11. Jamur MC. Mastócitos. In: Hernandes FC, Colares-Buzato CB. Células: uma abordagem multidisciplinary. Barueri, Sao Paulo: Manole, 2005. 391-402.
12. Junqueira LCU. Biologia Celular e Molecular. Rio de Janeiro: Guanabara Koogan 2005; 8ed.: 289-297.
13. Walsh, LJ. Mast Cells and Oral Inflammation. Crit Rev Oral Biol Med 2003; **14(3)**: 188-98.
14. Correia KVD, Medrado ARAP. Participação dos Mastócitos no Reparo Tecidual em Lesões Inflamatória Bucais – Revisão de Literatura. Revista Bahiana de Odontologia 2013; **4 (1)**: 27-36.
15. Aranaud RR, Soares MSM, Santos MGCS, Lira CC. Densidade dos mastócitos em lesões de quelite actínica. Rev. Cubana de Estomatologia 2014; **51(3)**: 378-387.
16. Parizi ACG, Parizi JLS, Barbosa, RL, Nai, GA. Comparação entre a concentração de mastócito em carcinomas espinocelulares da pele e da cavidade oral. An. Bras. Dermatol 2010; **85(6)**: 811-818
17. Telagi N, Mujib BRA, Kulkarni PG, Naik R. The master switch: Comparative study of mast cell in oral epithelial dysplasia, oral submucous fibrosis and oral squamous cells carcinoma and their association with inflammation and angiogenesis. J Oral Maxillof Pathol 2015: Jan-Apr; **19 (1)**: 25-29.
18. Lin Q, Yun Z. Impact of the hypoxic tumor microenvironment on the regulation of cancer stem cell characteristics. Cancer Biol. Ther 2010; **9**: 949-56.
19. Pindborg JJ, Reichart P, Smith CJ, Van der Waal I. World Health Organization: Histological Typing of Cancer and Precancer of the Oral Mucosa. Berlin: Springer-Verlag. 1997; 2ª ed.
20. Coleman R. Picrosirius red staining revisited. Acta Histochem 2011; **113**: 231-233.
21. Cirino LMD, Vergne DMC, Santana PF, Almeida E, Costa LP, Albuquerque-Junior RLC, Lima-Verde IB, Padilha FF, Cardoso JC. Decreased inflammatory response in

rat bladder after intravesical administration of capsaicin-loaded liposomes. Anais da Academia Brasileira de Ciências 2016; **88(3)**: 1539-1547.

22. Kadeh H, Saravani S, Tajik M. Reactive Hyperplastic Lesions of the Oral Cavity. Iranian Journal of Otorhinolaryngology 2014; **27(79)**: 137-144.
23. Reddy V, Saxena S, Saxena S, ReddyM. Reactive hyperplastic lesions of the oral cavity: A ten year observational study on North Indian Population. Journal section: Oral Medicine and Pathology 2012; **4 (3)**: 136-140.
24. Patterson, WI; Georgel, PT. Breaking the cycle: the role of omega-3 polyunsaturated fatty acids in inflammation-driven cancers. Biochem Cell Biol 2014; **92(5)**: 321-328.
25. Moss SF; Blaser MJ. Mechanisms of disease: inflammation and the origins of cancer. Nat Clin Pract Oncol 2005; **2**: 90-7.
26. Feller L, Altini M, Lemmer J. Inflammation in the Context of Oral Cancer. Oral Oncol 2013; 887-892.
27. Stricker T P; Kumar V. Neoplasia. In: Kumar, V.; Abbas, A.K; Fausto, N.; Aster, J.C. Patologia bases patológicas das doenças. Rio de Janeiro: Elsevier. 2010; 79-110
28. Junqueira LCU. Histologia Básica. Rio de Janeiro: Guanabara Koogan 2012; 1ed.: 91-102.
29. Piva MR, Souza LB, Martins-Filho PRS, Nonaka CFW, Santos TS, Andrade ESS, Piva D. Role of Inflammation in Oral Carcinogenesis (Part II): CD8, FOXP3, TNF-α, TGF-β and NF-κB expression. Oncol Let 2013; **5**: 1909-14.
30. Martins GB, Reis SR de A, Sil va TMC. Expressão do colágeno I em carcinomas epidermóides da cavidadeoral. Pesqui Odontol Bras 2003; **17(1)**: 82-8.
31. Wang Y, Tail Y, Wang W, Chen C, Kao Y, Chen Y, Chen C. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health 2014; **99**: 1-9.
32. Aguirre EP, Aguirre UJM. Displasia epitelial. Concepto y significación. Av. Odontoestomatol 2008; **24 (1)**: 81-88.
33. Shimamura Y; Abe T; Nakahira; Yoda T; Murata S; Sugasawa M. Immunohistochemical analysis of oral dysplasia: diagnostic assessment by fascin and podoplanin expression. Acta Histochem. Cytochem 2011: **44 (6)**: 239–245.
34. Speight PM. Update on Oral Epithelial Dysplasia and Progression to Cancer. Head Neck Pathol 2007; **1 (1)**: 61-6.
35. Effiom OA, Adeyemo WL, Soyele OO. Focal Reactive lesions of the Gingiva: an analysis of 314 cases at a tertiary Health Institution in Nigeria. Nigerian Medical Journal 2011; **45(1)**: 35-40.

36. Zhou XJ, Sugerman PB, Savage NW, Walsh LJ, Seymour GJ . Intra-epithelial CD8+ T cells and basement membrane disruption in oral lichen planus. J Oral Pathol Med 2012; **31**: 23-27.
37. Santos PPA, Freitas VS, Freitas RA, Pinto LP, Souza LB. Relação entre Mastócitos e Células T na Inflamação. OdontolClin-Cient 2010; **9(3)**: 215-217.
38. Daniel D, Meyer-Morse N, Bergsland EK, Dehne K, Coussens LM, Hanahan D. Immune Enhancement of Skin Carcinogenesis by CD4 T Cells. JEM 2003; 197 **(8)**: 1017–1028.
39. Mesquita Júnior D, Araujo JAP, Catelan TTT, Souza AWS, Cruvinel WM, Andrade LEC, Silva NP. Fundamentos da Resposta Imunológica mediada por Linfócitos T e B. RevBrasReumatol 2010; **50(5)**: 552-580.
40. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. Curr. Opin. Cell. Biol 2010; **22**: 697-706.
41. Li A, Zhou T, Guo L, Si J. Collagen type I regulates ß-catenin tyrosine phosphorylation and nuclear translocation to promote migration and proliferation of gastric carcinoma cells. Oncol. Rep 2010; **23**: 1247-1255.
42. Fuentes B, Duaso J, Droguett D, Castillo C, Donoso W, Riviera C, Venegas B, Kemmerling U. Progressive extracellular matrix disorganization in chemically induced murine oral squamous cell carcinoma. ISRN Pathology 2012; **7**: 1-7.
43. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008; **121(3)**: 255–64.
44. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumour stroma generation and wound healing, The New England J Med 1986; **315**: 1650-1656.
45. Aparna V; Charu S. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the Picrosirius red stain- a histochemical study. Journal of Clinical and Diagnostic Research 2010; **4**: 3444 - 3449.
46. Ganganna K, Shetty P, Shroff SE. Collagen in histologic stages of oral submucous fibrosis: A polarizing microscopic study. J Oral Maxillofac Pathol 2012; **16**:162-166.
47. Junqueira LCU, Cossermelli W, Brentani R. Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. Arch Histol Jpn 1978; **41**: 267-74.
48. Kalele KK, Managoli NA, Roopa NM, Kulkarni M, Bagul N, Kheur S. Assessment of collagen fiber nature, spatial distribution, hue and its correlation with invasion and metastasis in oral squamous cell carcinoma and surgical margins using Picro Sirius red and polarized microscope. J Dent Res Ver 2014; **1**: 14-7.

49. Manjunatha B, AgrawalA, Shah V. Histopathological evaluation of collagen fibers using picrosirius red stain and polarizing microscopy in oral squamous cell carcinoma 2015; **11(2)**: 272-276.
50. Sharma R, Rehani S, Mehendiratta M, Kardam P, Kumra M, Mathias Y, Yadav J, Sahay K. Architectural Analysis of Picrosirius Red Stained Collagen in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma using Polarization Microscopy. Journal of Clinical and Diagnostic Research 2015; **9(12):** 13-16.
51. Yokoyama M. Alterations in stromal reaction during tumour progression in oral mucosa. J Hard Tissue Biology 2011; **20(1)**: 23-30.
52. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, White JG, Keely PG. Collagen density promotes mammary tumor initiation and progression. BMC Med 2008; **6**: 1-15.

5 CONSIDERAÇÕES FINAIS

A displasia epitelial esteve presente na maior parte dos casos de lesões inflamatórias crônicas orais.

O estudo sugere participação dos mastócitos na fase de transformação tumoral. A alteração gradativa dos colágenos tipo I e III indica uma alteração das células produtoras de colágeno, durante as fases da displasia (transformação tumoral).

6 COMUNICADO DE IMPRENSA

O diagnóstico precoce do câncer de boca tem sido um desafio para a pesquisa, já que o mesmo restringe a possibilidade de tratamento. Algumas lesões como a “QueiliteActínica” e as “Leucoplasias”, associadas a fatores de risco como radiação solar e fumo, apresentam, com frequência, a Displasia Epitelial e são reconhecidamente, precursoras do câncer de boca. No entanto, outras lesões benignas, aparentemente inócuas, estão associadas a processos irritativos constantes, sendo consideradas como lesões reacionais, ou mais precisamente, lesões inflamatórias crônicas.

Porém, o que vem chamando a atenção dos pesquisadores é a presença da inflamação crônica associada, tanto às displasias epiteliais, como aos cânceres, o que levou vários estudos da literatura a sugerirem a participação ativa da mesma, na transformação maligna. Sendo assim, por que não avaliar as lesões causadas por fatores irritantes como: dentes fraturados, próteses mal adaptadas, biofilmes bacterianos e etc., como é o caso dos “Fibromas e Hiperplasias Fibrosas”. Feito isso, o estudo detectou que 16% de todas as lesões avaliadas, foram lesões reacionais, e 79% destas, apresentavam displasia epitelial. O estudo sugere ainda que os mastócitos, uma das células inflamatórias presentes nessas lesões, têm participação no processo de malignização; e que a alteração gradativa dos colágenos tipos I e III, indicam alterações nos fibroblastos e contribuem com o avanço do câncer. Sendo assim, é importante eliminar todas as lesões reacionais, que devem ser avaliadas por um Patologista Oral, evitando que tais lesões benignas evoluam para o câncer, sem que se tenha a chance de conhecer como tudo começou.

REFERÊNCIAS

1. Instituto Nacional do Câncer [homepage na internet]. Câncer [acesso 15 set 2016]. Disponível em: www.inca.gov.br
2. Manchanada A, Shetty DC. Reproducibility of grading systems in oral epithelial dysplasia. Medicina Oral Patología Oral y Cirugía Bucal. 2012; 17(6): 935-42.
3. Miyazaki Fujinami M, Inoue H, Kikuchi K, Ide K, Kusama K. Expression of activation-induced cytidine deaminase in oral epithelial dysplasia and oral squamous cell carcinoma. Journal of Oral Science 2013; **55**: 293-9.
4. Shirani S; Kargahi N; Razavi SM; Homayon S. Epithelial Dysplasia in Oral Cavity. IJMS 2014; **39(5)**: 406-417.
5. Rastogi V, Puri N, Mishara S, Arora S, Kaur G, Yadav L. An Insight to Oral Epithelial Dysplasia. Int J Head Neck Surg 2013; **4(2)**: 74-82.
6. Paiva MAF, Soares MSM, Figueiredo CRLV, Luna AH, Oliveira VEM, Júnior OB. Associação entre displasia e inflamação em queilite actínica. J Bras Patol Med Lab 2012; **48 (6)**: 455-458.
7. Berhane T, Halliday GM, Cooke B, Barnetson RS. Inflammation is associated with progression of actinic keratoses to squamous cell carcinomas in humans. Br J Dermatol 2002; **146 (5)**: 810-815, 2002.
8. Guo L, Zhang Y, Zhang L, Huang F, Li J, Wang S. MicroRNAs, TGF-β signaling, and the inflammatory microenvironment in câncer. Tumour Biol 2016; **37(1)**: 115–125.
9. Martins-Filho PRS, Santos LCF, Piva MR. The prevalence of actinic cheilitis in farmers in a semi-arid northeastern region of Brazil. International Journal of Dermatology 2011; **50 (9)**: 1109-14.
10. Kumar V, Abbas AK, Fausto N, Aster JC.Patologia bases patológicas das doenças. Rio de Janeiro: Elsevier. 2010; 79-110.
11. Jamur MC. Mastócitos. In: Hernandes FC, Colares-Buzato CB. Células: uma abordagem multidisciplinary. Barueri, Sao Paulo: Manole, 2005. 391-402.
12. Junqueira LCU. Biologia Celular e Molecular. Rio de Janeiro: Guanabara Koogan 2005; 8ed.: 289-297.
13. Walsh, LJ. Mast Cells and Oral Inflammation. Crit Rev Oral Biol Med 2003; **14(3)**: 188-98.
14. Correia KVD, Medrado ARAP. Participação dos Mastócitos no Reparo Tecidual em Lesões Inflamatória Bucais – Revisão de Literatura. Revista Bahiana de Odontologia 2013; **4 (1)**: 27-36.
15. Aranaud RR, Soares MSM, Santos MGCS, Lira CC. Densidade dos mastócitos em

lesões de quelite actínica. Rev. Cubana de Estomatologia 2014; **51(3)**: 378-387.

16. Parizi ACG, Parizi JLS, Barbosa, RL, Nai, GA. Comparação entre a concentração de mastócito em carcinomas espinocelulares da pele e da cavidade oral. An. Bras. Dermatol 2010; **85(6)**: 811-818
17. Telagi N, Mujib BRA, Kulkarni PG, Naik R. The master switch: Comparative study of mast cell in oral epithelial dysplasia, oral submucous fibrosis and oral squamous cells carcinoma and their association with inflammation and angiogenesis. J Oral Maxillof Pathol 2015: Jan-Apr; **19 (1)**: 25-29.
18. Lin Q, Yun Z. Impact of the hypoxic tumor microenvironment on the regulation of cancer stem cell characteristics. Cancer Biol. Ther 2010; **9**: 949-56.
19. Pindborg JJ, Reichart P, Smith CJ, Van der Waal I. World Health Organization: Histological Typing of Cancer and Precancer of the Oral Mucosa. Berlin: Springer-Verlag. 1997; 2ª ed.
20. Coleman R. Picrosirius red staining revisited. Acta Histochem 2011; **113**: 231-233.
21. Cirino LMD, Vergne DMC, Santana PF, Almeida E, Costa LP, Albuquerque-Junior RLC, Lima-Verde IB, Padilha FF, Cardoso JC. Decreased inflammatory response in rat bladder after intravesical administration of capsaicin-loaded liposomes. Anais da Academia Brasileira de Ciências 2016; **88(3)**: 1539-1547.
22. Kadeh H, Saravani S, Tajik M. Reactive Hyperplastic Lesions of the Oral Cavity. Iranian Journal of Otorhinolaryngology 2014; **27(79)**: 137-144.
23. Reddy V, Saxena S, Saxena S, ReddyM. Reactive hyperplastic lesions of the oral cavity: A ten year observational study on North Indian Population. Journal section: Oral Medicine and Pathology 2012; **4 (3)**: 136-140.
24. Patterson, WI; Georgel, PT. Breaking the cycle: the role of omega-3 polyunsaturated fatty acids in inflammation-driven cancers. Biochem Cell Biol 2014; **92(5)**: 321-328.
25. Moss SF; Blaser MJ. Mechanisms of disease: inflammation and the origins of cancer. Nat Clin Pract Oncol 2005; **2**: 90-7.
26. Feller L, Altini M, Lemmer J. Inflammation in the Context of Oral Cancer. Oral Oncol 2013; 887-892.
27. Stricker T P; Kumar V. Neoplasia. In: Kumar, V.; Abbas, A.K; Fausto, N.; Aster, J.C. Patologia bases patológicas das doenças. Rio de Janeiro: Elsevier. 2010; 79-110
28. Junqueira LCU. Histologia Básica. Rio de Janeiro: Guanabara Koogan 2012; 1ed.: 91-102.
29. Piva MR, Souza LB, Martins-Filho PRS, Nonaka CFW, Santos TS, Andrade ESS, Piva D. Role of Inflammation in Oral Carcinogenesis (Part II): CD8, FOXP3, TNF-α, TGF-β and NF-κB expression. Oncol Let 2013; **5**: 1909-14.

30. Martins GB, Reis SR de A, Sil va TMC. Expressão do colágeno I em carcinomas epidermóides da cavidadeoral. Pesqui Odontol Bras 2003; **17(1)**: 82-8.
31. Wang Y, Tail Y, Wang W, Chen C, Kao Y, Chen Y, Chen C. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health 2014; **99**: 1-9.
32. Aguirre EP, Aguirre UJM. Displasia epitelial. Concepto y significación. Av. Odontoestomatol 2008; **24 (1)**: 81-88.
33. Shimamura Y; Abe T; Nakahira; Yoda T; Murata S; Sugasawa M. Immunohistochemical analysis of oral dysplasia: diagnostic assessment by fascin and podoplanin expression. Acta Histochem. Cytochem 2011: **44 (6)**: 239–245.
34. Speight PM. Update on Oral Epithelial Dysplasia and Progression to Cancer. Head Neck Pathol 2007; **1 (1)**: 61-6.
35. Effiom OA, Adeyemo WL, Soyele OO. Focal Reactive lesions of the Gingiva: an analysis of 314 cases at a tertiary Health Institution in Nigeria. Nigerian Medical Journal 2011; **45(1)**: 35-40.
36. Zhou XJ, Sugerman PB, Savage NW, Walsh LJ, Seymour GJ . Intra-epithelial CD8+ T cells and basement membrane disruption in oral lichen planus. J Oral Pathol Med 2012; **31**: 23-27.
37. Santos PPA, Freitas VS, Freitas RA, Pinto LP, Souza LB. Relação entre Mastócitos e Células T na Inflamação. OdontolClin-Cient 2010; **9(3)**: 215-217.
38. Daniel D, Meyer-Morse N, Bergsland EK, Dehne K, Coussens LM, Hanahan D. Immune Enhancement of Skin Carcinogenesis by CD4 T Cells. JEM 2003; 197 **(8)**: 1017–1028.
39. Mesquita Júnior D, Araujo JAP, Catelan TTT, Souza AWS, Cruvinel WM, Andrade LEC, Silva NP. Fundamentos da Resposta Imunológica mediada por Linfócitos T e B. RevBrasReumatol 2010; **50(5)**: 552-580.
40. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. Curr. Opin. Cell. Biol 2010; **22**: 697-706.
41. Li A, Zhou T, Guo L, Si J. Collagen type I regulates ß-catenin tyrosine phosphorylation and nuclear translocation to promote migration and proliferation of gastric carcinoma cells. Oncol. Rep 2010; **23**: 1247-1255.
42. Fuentes B, Duaso J, Droguett D, Castillo C, Donoso W, Riviera C, Venegas B, Kemmerling U. Progressive extracellular matrix disorganization in chemically induced murine oral squamous cell carcinoma. ISRN Pathology 2012; **7**: 1-7.
43. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 2008; **121(3)**: 255–64.

44. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumour stroma generation and wound healing, The New England J Med 1986; **315**: 1650-1656.
45. Aparna V; Charu S. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the Picrosirius red stain- a histochemical study. Journal of Clinical and Diagnostic Research 2010; **4**: 3444 - 3449.
46. Ganganna K, Shetty P, Shroff SE. Collagen in histologic stages of oral submucous fibrosis: A polarizing microscopic study. J Oral Maxillofac Pathol 2012; **16**:162-166.
47. Junqueira LCU, Cossermelli W, Brentani R. Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. Arch Histol Jpn 1978; **41**: 267-74.
48. Kalele KK, Managoli NA, Roopa NM, Kulkarni M, Bagul N, Kheur S. Assessment of collagen fiber nature, spatial distribution, hue and its correlation with invasion and metastasis in oral squamous cell carcinoma and surgical margins using Picro Sirius red and polarized microscope. J Dent Res Ver 2014; **1**: 14-7.
49. Manjunatha B, AgrawalA, Shah V. Histopathological evaluation of collagen fibers using picrosirius red stain and polarizing microscopy in oral squamous cell carcinoma 2015; **11(2)**: 272-276.
50. Sharma R, Rehani S, Mehendiratta M, Kardam P, Kumra M, Mathias Y, Yadav J, Sahay K. Architectural Analysis of Picrosirius Red Stained Collagen in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma using Polarization Microscopy. Journal of Clinical and Diagnostic Research 2015; **9(12):** 13-16.
51. Yokoyama M. Alterations in stromal reaction during tumour progression in oral mucosa. J Hard Tissue Biology 2011; **20(1)**: 23-30.
52. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, White JG, Keely PG. Collagen density promotes mammary tumor initiation and progression. BMC Med 2008; **6**: 1-15.