BRUNA GONÇALVES GARCIA

COMPARAÇÃO DA ATIVIDADE DE PROLIFERAÇÃO CELULAR NOS FIBROMAS ODONTOGÊNICOS E OSSIFICANTES

BELO HORIZONTE
FACULDADE DE ODONTOLOGIA DA UNIVERSIDADE FEDERAL DE MINAS GERAIS
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Dissertação apresentada ao Programa de Pós-graduação da Faculdade de Odontologia-UFMG, como requisito parcial para obtenção do título de Mestre em Odondologia.
Área de concentração: Patologia Bucal
Orientador: Prof. Dr. Ricardo Alves de Mesquita
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SUMÁRIO

RESUMO

O fibroma odontogênico central (FOC) é uma condição rara classificada pela Organização Mundial da Saúde (OMS-2005) como uma neoplasia benigna de origem fibroblástica. O fibroma odontogênico periférico (FOP) é a contraparte periférica do FOC e, por se tratar de uma patologia rara, é objetivo de poucos estudos. O fibroma ossificante periférico (FOPE) não é considerado uma lesão neoplásica, e sim uma reação hiperplásica devido à presença de inflamação local, causada por fatores irritantes. O fibroma ossificante central (FOCE) é um tipo de lesão fibro-óssea caracterizada pela substituição do osso por tecido conjuntivo fibroso, contendo quantidades variáveis de tecido mineralizado. O objetivo deste estudo foi analisar a atividade de proliferação celular do FOC, FOP, FOPE e FOCE através da técnica do AgNOR e da imunoexpressão da proteína PCNA. A amostra deste estudo foi composta por casos diagnosticados de FOC (4 casos), FOP (9 casos), FOPE (8 casos) e FOCE (7 casos) pertencentes aos arquivos dos Serviços de Patologia Bucomaxilofacial das Faculdades de Odontologia da Universidade Federal de Minas Gerais e da Universidade de São Paulo. A análise quantitativa e morfométrica das AgNORs e índice de PCNA foram determinados. A análise estatística foi realizada com nível de significância de 0,05. As lesões centrais apresentaram maior média do número de AgNOR/núcleo e índice de PCNA do que as lesões periféricas, no componente mesenquimal ($p \leq 0,05$). A média do número de AgNOR/núcleo no componente epitelial foi maior no FOC do que no FOP ($p \leq 0,05$). Os componentes mesenquimal e epitelial apresentaram média do número de AgNOR/núcleo e índice de PCNA semelhantes no FOC e similar média do número de AgNOR/núcleo no FOP ($p \geq 0,05$). Assim, os dados de AgNOR e índice de PCNA observados no presente estudo demonstraram que: (1) as lesões avaliadas mostraram perfis de neoplasias benignas ou de lesões proliferativas não-neoplásicas; (2) os achados sugerem uma

atividade de proliferação celular semelhante nas lesões periféricas (FOP e FOPE), assim como, nos componentes epitelial e mesenquimal dos FOC e FOP.

Descritores: tumores odontogênicos, imunoistoquímica, AgNOR.

ABSTRACT

Title: Comparative cellular proliferative activity in ossifying and odontogenic fibromas

Central (COF) and peripheral (POF) odontogenic fibroma are benign odontogenic tumours. Peripheral ossifying fibroma (PEOF) is a non neoplastic reactive lesion. Central ossifying fibroma (CEOF) is a benign neoplasm fibro-osseous lesion. The aim of this study was to evaluate comparatively the cellular proliferative activity in these lesions. Immunohistochemistry to PCNA and the AgNOR protocol were performed in POF (9 cases), COF (4 cases), PEOF (8 cases) and CEOF (7 cases) belonging to the Oral Pathology Service of the Sao Paulo and Minas Gerais Universities. Quantitative and morphometric analysis of AgNOR and PCNA index were determined. Statistical analysis was performed with significance at the 0.05 level. Central lesions presented higher AgNOR number and PCNA index than peripheral lesions in the mesenchymal component ($p \leq 0,05$). AgNOR number in the epithelial component was higher in the central odontogenic fibroma than in the peripheral odontogenic fibroma ($p \leq 0,05$). Mesenchymal and epithelial components presented similar AgNOR number and PCNA index in the central odontogenic fibroma and similar AgNOR number in the peripheral odontogenic fibroma ($p \geq 0,05$). All the four types of lesions showed a profile of a benign neoplasm (COF and CEOF) or of a non neoplastic reactive lesion (POF and PEOF). These findings reflect that the POF may represent a non neoplasm reactive lesion in the resemblance of the PEOF. Also, the epithelial and the mesenchymal components of POF and COF showed similar cellular proliferative activity.

Key words: odontogenic tumours, immunohistochemistry, AgNOR.

1. APRESENTAÇÃO

O fibroma odontogênico central (FOC) é uma condição rara classificada pela Organização Mundial da Saúde (OMS-2005) como uma neoplasia odontogênica benigna, caracterizada por quantidades variadas de epitélio odontogênico inativo presentes sobre um estroma fibroso e maduro. Esta lesão localiza-se nos maxilares e apresenta um crescimento lento e persistente, que resulta em expansão da cortical. Radiograficamente, sua aparência é variada podendo ser observada, na maioria das vezes, uma lesão radiolúcida unilocular que envolve algumas vezes dentes impactados. O aspecto histopatológico revela um tumor composto predominantemente por fibras colágenas maduras, apresentando ninhos ou cordões de epitélio odontogênico. O FOC é tratado com excisão cirúrgica e não apresenta nenhuma tendência de transformação maligna (Gardner, 1980). Os índices de recorrência foram citados em 13% dos casos (Svirsky et al., 1986).

O fibroma odontogênico periférico (FOP), considerado o correspondente periférico do FOC, por se tratar de uma patologia rara, é objetivo de poucos estudos. O seu comportamento biológico, características histopatológicas e clínicas foram estabelecidas recentemente, fazendo com que esta lesão obtivesse classificação e diagnóstico equivocados durante um longo período. Dentre as sinonímias reportadas pode-se citar: fibroma ossificante periférico, epúlide fibromatosa, epúlide angiomatosa, epúlide granulomatosa e hiperplasia gengival. Atualmente, o FOP é definido pela Organização Mundial da Saúde (OMS-2005) como uma neoplasia odontogênica benigna de origem fibroblástica. Esta lesão localiza-se na gengiva inserida, principalmente na mandíbula, e apresenta-se como um crescimento gengival exofítico, lento, único e assintomático. É caracterizado histopatologicamente por tecido conjuntivo denso, contendo aglomerado de epitélio odontogênico (Gardner, 1982). O tratamento mais freqüentemente utilizado é a excisão cirúrgica total, sendo que a lesão não apresenta nenhuma tendência de

transformação maligna. Os índices de recorrência foram relatados em 7,7% dos casos (Kenney et al., 1989).

O fibroma ossificante periférico (FOPE), também conhecido como epúlide ossificante fibrosa, se origina das células do ligamento periodontal. O FOPE não é considerado uma lesão neoplásica, e sim uma reação hiperplásica devido à presença de inflamação local, em decorrência de fatores irritantes como, microorganismos, forças mastigatórias, traumas, restos de alimentos, placa bacteriana, cálculos, e causas iatrogênicas (Buchner e Hansen, 1987). Clinicamente, apresenta-se, predominantemente, na gengiva com uma coloração esbranquiçada. Acomete mais frequentemente pacientes da segunda e terceira décadas de vida, principalmente, mulheres. É caracterizado pela proliferação de fibroblastos do ligamento periodontal que apresentam a capacidade de formar tecido mineralizado (osso e cemento). A lesão é tratada com excisão cirúrgica, não apresentando nenhuma evidência de transformação maligna. Os índices de recorrência foram relatados em 15,9% dos casos (Buchner e Hansen, 1987; Buduneli et al., 2001).

O fibroma ossificante central (FOCE) é um tipo de lesão fibro-óssea benigna, de caráter neoplásico, caracterizada pela substituição do osso por tecido conjuntivo fibroso, contendo quantidades variáveis de tecido mineralizado. Este se apresenta nos maxilares, acometendo mais mulheres da segunda década de vida (Su et al., 1997). O tratamento mais freqüentemente utilizado é a excisão cirúrgica total, sendo que não são observadas tendências de transformações malignas. O índice de recorrência varia entre 6-28% (Sciubba et al., 1989; Su et al., 1997).

As doenças anteriormente citadas caracterizam-se por exibir um padrão histológico semelhante, apresentando proliferação de fibroblastos que podem ser capazes de formar tecidos mineralizados (cemento, osso). A similaridade do padrão histológico indica a nomenclatura empregada para estas lesões. Sendo assim, torna-se importante a especificação de dados nos quais seja possível a realização da distinção das entidades.

O FOC e o FOP são lesões que apesar de serem consideradas entidades distintas, compartilham características semelhantes, sendo que a primeira é observada na região intra-óssea e a segunda na extra-óssea. Assim, o FOP é considerado a contraparte periférica do FOC.

Histologicamente, a lesão que mais se assemelha ao FOP é o FOPE (Buchner et al., 1987). As principais diferenças histológicas são: a presença de epitélio odontogênico no FOP e a presença de osso be formado no FOPE (Buchner, 1989). Apesar das semelhanças clínicas, o FOPE é uma lesão fibrosa reativa da gengiva, ao contrário do FOP. Em 1982, Gardner propôs a classificação do fibroma odontogênico periférico. Neste estudo foram sugeridos critérios para se diferenciar o FOP do fibroma ossificante periférico. Dentre eles pode-se citar: (1) o FOP geralmente exibe grande proliferação de epitélio odontogênico, ao contrário do fibroma ossificante periférico que raramente exibe este tipo de proliferação; (2) ulceração é comum no fibroma ossificante periférico, mas raramente está presente no FOP; (3) dentina displásica é freqüentemente encontrada no FOP, e osso bem formado no fibroma ossificante periférico; (4) células gigantes podem ser observadas no fibroma ossificante periférico e são raridades no FOP; (5) vasos bem formados encontram-se em maior número no FOP (Gardner, 1982).

Kenney et al. (1989) realizaram uma comparação entre o FOP e o FOPE e concluíram que as lesões representam entidades patológicas distintas sendo suas características clínicas e histológicas também diferentes.

O FOCE e o FOPE são considerados entidades distintas, classificadas, respectivamente, como lesão fibro-óssea neoplásica e lesão proliferativa não-neoplásica. Apesar de o aspecto histológico ser semelhante, as lesões apresentam tratamento e prognóstico distintos. Sendo assim, o diagnóstico diferencial adequado é essencial para uma correta abordagem.

Diante do padrão de crescimento destas lesões, suas distintas classificações, torna-se importante o estudo de seu comportamento biológico através de marcadores de atividade de proliferação celular, como o AgNOR e PCNA, visando ao melhor conhecimento destas entidades.

A observação de que certas proteínas ácidas não histonas ligadas às regiões organizadoras nucleares (NOR) possuíam grande afinidade pela prata levantou o interesse em pesquisas na área de patologia, uma vez que essas são consideradas marcadores de atividade de proliferação celular. As técnicas argirófilas de coloração desenvolvidas visam a localizar proteínas por um processo mais fácil. Através da análise quantitativa das AgNOR, pode-se obter, em associação com outros métodos, um diagnóstico diferencial entre lesões benignas e malignas, assim como, em alguns casos, associá-la ao prognóstico. Assim, nos últimos anos, as NOR em interfase têm sido muito estudadas e seu estudo tem se mostrado útil em patologia tumoral. Entretanto, os estudos das AgNOR em lesões fibro-ósseas, tumores odontogênicos e proliferações não neoplásicas são escassos (Coleman et al., 1996; Rosa et al , 1997; Do Carmo e Silva, 1998; Souza et al., 2000; Martins et al., 2001; Eslami et al., 2003).

Mesquita et al. (1998) avaliaram a atividade de proliferação celular presente no FOPE e no FOCE utilizando a técnica de coloração pela prata (AgNOR). O estudo concluiu que as duas lesões têm características benignas, o FOCE mostrando maior média do número de AgNOR/núcleo do que o FOPE. Isto sugeriu que o FOCE possui atividade proliferativa aumentada quando comparado com o FOPE.

O antígeno nuclear de proliferação celular (PCNA) é a proteína auxiliar 36 Kd da DNA polimerase. Esta proteína mostra um aumento de sua expressão nas fases G1 e S do ciclo celular, embora níveis elevados de PCNA na ausência de ciclo possam ser observados. O PCNA tem sido largamente utilizado no estudo do comportamento biológico de diversos tipos de lesões. Dentre elas pode-se ressaltar: tumores

odontogênicos, lesões fibro-ósseas benignas e proliferações não neoplásicas (Funaoka et al., 1996; Piattelli et al., 1998; Souza et al., 2000; Sandra et al., 2001; Shear et al., 2002; Meer et al., 2003; Gomes et al., 2006).

Mesquita et al. (1998) avaliaram a atividade proliferativa presente no FOPE e no FOCE, utilizando a expressão imunoistoquímica do PCNA. O estudo concluiu que as duas lesões têm características benignas, o FOCE mostrando maior índice de PCNA do que o FOPE. Isto sugeriu que o FOCE possui atividade proliferativa aumentada quando comparado com o FOPE.

Ono et al. (2007) avaliaram através da técnica de imunoistoquímica diferenças entre o FOCE e FOPE. O estudo utilizou proteínas morfogenéticas do osso (BMP) –2 e –4, osteopontina (OPN), osteocalcina (OCN) e o antígeno de proliferação celular (PCNA) para o exame imunoistoquímico. Os resultados mostraram que o índice de PCNA foi significativamente maior no FOCE do que no FOPE. O estudo concluiu que o FOPE possui pequena habilidade de formar tecido duro, sendo considerada também apenas uma lesão reacional.

Considerando que os trabalhos descritos na literatura não avaliam e comparam, simultaneamente, o PCNA e AgNOR nas lesões mencionadas acima e que elas são de naturezas diferentes, o objetivo desse estudo foi avaliar e comparar a atividade de proliferação celular no FOP, FOC, FOPE e FOCE através da utilização da técnica de coloração pela prata (AgNOR) e da técnica de imunoistoquímica para avaliação de PCNA.

Optou-se por redigir esta dissertação em forma de artigo científico, seguindo as normas preconizadas pelo periódico de escolha, considerações finais e referências.

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2. ARTIGO

Title: Comparative cellular proliferative activity in ossifying and odontogenic fibromas

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Abstract

Objective: The aim was to compare the cellular proliferative activity in odontogenic and ossifying fibromas.

Study Design: Immunohistochemistry to PCNA and the AgNOR protocol were performed in peripheral odontogenic fibroma (9 cases), central odontogenic fibroma (4 cases), peripheral ossifying fibroma (8 cases) and central ossifying fibroma (7 cases). The Kruskal-Wallis and the Mann-Whitney tests were used in the statistical analysis.

Results: Central lesions presented higher AgNOR number and PCNA index than peripheral lesions in the mesenchyma. AgNOR number in the epithelial component was higher in the central than in the peripheral odontogenic fibroma. Mesenchymal and epithelial components presented similar AgNOR number and PCNA index in the central odontogenic fibroma and similar AgNOR number and area in peripheral odontogenic fibroma.

Conclusions: Peripheral odontogenic and ossifying fibromas demonstrate a similar cellular proliferative activity. The epithelial and the mesenchymal components of peripheral and central odontogenic fibroma show similar cellular proliferative activity.

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Introduction

The central odontogenic fibroma (COF) is a rare odontogenic tumour classified by the World Health Organization (WHO-2005) as a benign fibroblastic neoplasm containing a variety of amount apparently inactive odontogenic epithelium.[1] Peripheral odontogenic fibroma (POF) is considered the extraosseous counterpart of the COF.[1] The peripheral ossifying fibroma (PEOF) is considered to be a non neoplastic reactive lesion that represents a hyperplastic reaction due to inflammation. PEOF is associated to mineralization and it is derived from the periodontal ligament cells. Dental calculus, plaque, dental appliances, ill-fitting crows, and rough restorations are considered to be irritants factors.[2,3] The central ossifying fibroma (CEOF) is a benign neoplasm fibro-osseous lesion consisted of a benign connective-tissue matrix and islands or trabeculae of new bone.[4,5]

Nucleolar organizer regions (NORs) represent the loops of DNA actively transcribing to ribosomal RNA and thus to ribosomes and ultimately to protein.[6] The NOR are associated with acidic, argyrophilic non-histonic proteins that are visualized with the use of a silver staining technique, the AgNOR protocol.[7,8] A greater number of studies have applied this technique as a useful method to evaluate cellular proliferative activity of the non neoplastic reactive lesions and benign or malignant neoplasms in surgical pathology practice.[9-22]

The proliferating cell nuclear antigen (PCNA) is a 36-kD acidic nonhistone nuclear protein that functions as an auxiliary protein for DNA delta polymerase and it is an absolute requirement for DNA synthesis.[23] Its distribution in the cell cycle increases through the G_1 phase, peaks at the G_1/S interphase and decreases, through the G_2 phase.[23,24] The current importance of PCNA as a marker of cellular proliferation activity relates to its ability to be reproducibly detected in routinely fixed and processed tissues. It

has been established that growth factors, technical factors, association of PCNA with repair processes, biological half-life about 20 h and cytokines released by the tumour or inflammatory cells may influence in PCNA immunohistochemistry expression.[25-29] The evaluation of PCNA expression also has been used as an auxiliary diagnostic tool for distinguishing non neoplastic reactive lesions and benign or malignant neoplasms.[28-31]

In accordance with the growth pattern of POF, COF, PEOF and CEOF, their distinct nature and classification, the study of their biological behavior through markers of cellular proliferative activity becomes important. There are not previous studies evaluating the comparative cellular proliferative activity between the odontogenic and ossifying fibromas. Therefore, the goal of this study is to evaluate and compare the cellular proliferative activity of POF, COF, PEOF and CEOF through AgNORs protocol and PCNA expression in an attempt to provide a scientific data for differences in biological behavior observed among these lesions.

Methods

Institutional ethical board

The protocol of the study was approved by the Committee of Bioethics in Research at the Federal University of Minas Gerais (UFMG, COEP – 124/07).

Specimens

Specimens with previous histological diagnosis of POF (9 cases), COF (4 cases), PEOF (8 cases) and CEOF (7 cases) were obtained from the files of the Oral Pathology Service of UFMG (Belo Horizonte, Brazil) and from Oral Pathology Service of University of Sao Paulo (São Paulo, Brazil). The histological criteria of POF, COF and CEOF were in accordance

with WHO-2005 Classification.[1] COF could present two patterns: the epithelium-poor type and the epithelium-rich type.[1] In this study, two cases of COF were considered epithelium-rich type and two cases epithelium-poor type. Histological criteria to identify the PEOF were in accordance with Buchner et al.[2]

AgNORs protocol

AgNORs protocol was performed according to the standardized method of Trerè.[32] Sections of 3 μm from routinely processed paraffin-embedded blocks were dewaxed and hydrated. The sections were immersed in sodium citrate buffer (0.01 M sodium-citrate monohydrate, pH 6.0) jars and boiled at 120 °C for 20 minutes in a pressure cooker. They cooled down to room temperature and were washed with distilled water. The sections were immersed in the solution of gelatin and silver nitrate in the dark at a room temperature for 25 minutes.

Immunohistochemistry

Immunohistochemistry was performed using streptavidin–biotin standard protocol. Sections of 3 μm from routinely processed paraffin-embedded blocks were dewaxed and hydrated. Specimens were immersed in a 10 mM citrate buffer (pH 6.0, 20 min at 98°C) for antigen retrieval. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide. Sections were incubated with primary antibody PCNA (PC10, Dako, Carpinteria, USA, MO879) for 18 hours at room temperature. Primary antibody was detected using a LSAB® +system, HRP Peroxidase Kit (KO675, Dako Corporation) and 3,3'-diaminobenzidine tetrahydrochloride chromogen (D5637, DAB; Sigma Chemical, St Louis, MO, USA). Sections of oral squamous cell carcinoma were used as positive control.

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Analysis of AgNOR and PCNA

Fibroblast-like cells (mesenchymal component) were evaluated in the four types of lesions. The epithelial cells (epithelial component) of the islands or strands of odontogenic epithelium in POF and COF were also selected for AgNOR and PCNA analysis. Inflammatory cells and the cells lining calcified material presented in POF and PEOF were not included in AgNOR and PCNA analysis.

The morphometric analysis of AgNOR was established on 100 cells for each case using KS300 software coupled to a Carl Zeiss Image Analyser. The number, area and contour index of AgNORs were obtained from digital images through a micro camera JVC TK-1270/RGB, at x400 magnification. The cells were transmitted to a TV monitor where the AgNOR individualization was performed. The media numbers of AgNOR per nucleus, area and contour index for all cases were determined.

Clear brown nuclei, regardless of staining intensity, were considered PCNA-positive cells. The immunohistochemistry analysis for PCNA was performed by an index (IP). The IP was calculated considering the number of PCNA positive cells per 500 cells, counted at random in each case. This counting was performed at x 400 magnification in an optical microscopy (Carl Zeiss – Axiostar 1122-100, Carl Zeiss, Oberkochen, Baden-Wüttemberg, Germany). It was not always possible to count 500 cells, thus it was counted all the cells in the epithelial islands or strands in the epithelial component of POF and COF. IP was expressed as media values for all cases.

The Kruskal-Wallis and the Mann-Whitney tests were used to compare AgNOR and IP data in all lesions. The statistical analysis was performed in BioEstat® software and the alpha level was set at 0.05.[33]

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Results

AgNOR were visualized as black or brown, defined intranuclear homogeneous dots (Figure 1A). Nuclear immunoreactivity for PCNA was clearly and easily identified in sections of the lesions (Figure 1B). The values of contour index varied from 0.76 to 0.92. Values near 1 corresponded to a round structure and with a regular contour and a value far from 1 means an irregular structure. The number of AgNOR and IP in the mesenchymal component of the COF and CEOF was significantly higher than in the POF and PEOF, respectively. Otherwise, the AgNOR area of the central lesions is statistically smaller than those found in the peripheral lesions ($P \leq 0.05$, Table 1). The AgNOR media numbers, area and IP in the mesenchymal component between the peripheral lesions and between the central lesions did not presented statistically significant differences ($P > 0.05$, Table 2). The media number of AgNOR in the epithelial component was significantly higher in COF than in POF. However, the AgNOR area of the POF was statistically higher than those found in the COF ($P \leq 0.05$; Table 3). The epithelial component presented similar IP in the POF and COF ($P > 0.05$; Table 3). The comparison between epithelial and mesenchymal components of the peripheral odontogenic fibroma and of the central odontogenic fibroma was not statistically significant, except for the IP in POF in which the epithelial component presented a higher IP (Table 4).

Discussion

The POF, COF, PEOF and CEOF evaluated in this study presented distinct nature and classifications. However, they exhibit similar histomorphologic features. COF and its extraosseous counterpart, POF, are considered rare benign odontogenic tumours characterized by various amounts of inactive-looking odontogenic epithelium embedded in

a mature, fibrous stroma. The COF is more located in the mandible giving a maxilla:mandible ratio of 1:6.5 with most lesions. The tumour appears as a unilocular radiolucent area with well-defined often sclerotic borders. POF is most common in the attached gingiva, usually in the molar/ premolar area with equal distribution between the jaws.[1] POF is an uncommon, benign, focal unencapsulated exophytic gingival mass, pedunculated or sessile, red or pink, usually with a smooth surface and in some cases the overlying mucosa may be ulcerated. The lesion is usually firm to palpation and non-tender.[34-37] The CEOF, a fibro-osseous lesion, is a well-demarcated lesion composed of fibrocellular tissue and mineralized material of varying appearances, mostly seen in the posterior mandible. It causes expansion of the involved bone. Radiographs show a demarcated lesion that may have radiodense as well as radiolucent areas depending on the various contributions of soft and hard tissue components.[1] The PEOF, which occurs on gingiva, is often known as a “calcifying fibrous epulis”. It is usually composed of cellular fibroblastic tissue containing little or plenty of mineralized tissues – bone (woven and lamellar), cementum-like material, and dystrophic calcification. Some reports suggest that PEOF originates from the cells of the periodontal ligament. However, PEOF is not considered to be a neoplasm lesion but a hyperplastic reaction caused by chronic inflammation.[2,3] The present study demonstrated differences and similarity in the cellular proliferative activity in these four types of lesions giving data to emphasize their biological behaviors.

Morphometric and quantitative analysis of AgNOR can imply the degree of cellular nucleolar activity in non neoplastic reactive and neoplastic lesions.[38] Benign neoplasms and non neoplastic reactive lesions are characterized by small numbers of AgNOR per nucleus, large size (area) and regular shape (contour index).[9,39,40] These AgNOR features

were observed in all the four types of lesions evaluated in this study. This aspect demonstrated that these lesions presented a profile compatible with a benign neoplasm or a non neoplastic reactive lesion. Investigations of cellular proliferative activity have used PCNA and others markers in oral and other regions tumours since they give different information on cell cycle.[23]

Mesquita et al.[9] and Ono et al.[41] demonstrated, through of the AgNOR and IP comparative analysis, that the cellular proliferative activity in the CEOF was higher than in PEOF. These results were also showed in the current study and they suggested that the smaller cellular proliferation activity of PEOF when compared to benign neoplasm lesions may be due to its reactive nature. This fact is in accordance with the different clinical behavior of the CEOF and PEOF.[42-44] It was also verified through the AgNOR and the IP analysis in the mesenchymal component and with the AgNOR analysis in the epithelial component. Moreover, the cellular proliferative activity was similar in the peripheral lesions (POF and PEOF). It could be also observed when the central fibromas were compared (COF and CEOF). So, these results seem to demonstrate that POF may represent a non neoplastic reactive lesion in resemblance to the PEOF. This observation is also in accordance with the different clinical behavior of the POF and COF.[37]

AgNOR analysis and IP have been performed in many odontogenic cysts and tumours.[10,11,27] Martins et al.[11] evaluated the AgNOR features in the amelobastic fibroma, a mixed epithelial and mesenchymal odontogenic tumor, and it was demonstrated that the epithelial and the mesenchymal components have similar cellular proliferative activity. This observation is in accordance with the mixed epithelial and mesenchymal nature of the ameloblastic fibroma in which both, the epithelial and the mesenchymal components, are considered to be neoplastic.[45] In the current study, it was verified that the epithelial and the

mesenchymal components presented similar AgNOR and IP values in the COF and similar AgNOR media in the POF. It demonstrates that the epithelial and the mesenchymal components in both, POF and COF, presented similar cellular proliferative activity. Thus, these findings reflect that both, the epithelial and the mesenchymal components, may be considered parts of the non neoplastic reactive process in POF and of the neoplasm process in COF. So, it can be suggested that the COF, to the resemblance of the ameloblastic fibroma, may represent an odontogenic epithelium with odontogenic ectomesenchyme tumour, with or without hard tissue formation (mixed epithelial and mesenchymal odontogenic tumour) instead of a mesenchyme and/or odontogenic ectomesenchyme with or without odontogenic epithelium tumor (mesenchymal odontogenic tumor).[1] However, it is important to emphasize that other studies using different makers and distinct technologies to confirm the last suggestion are necessary.

An uncommon result, in the current study, was the analysis of IP in the epithelial component of the POF. The value of the IP was higher in contrast to the AgNOR media (tables 3 and 4). Cytokines released by inflammatory cells present in POF may be responsible by this observation.[46] So, it would be important to evaluate the real role of the cytokines in this lesion.

In conclusion, AgNOR and IP date found in the current study demonstrated that 1) all the four types of lesions showed a profile of a benign neoplasm or of a non neoplastic reactive lesion, and 2) these findings reflected a similar cellular proliferative activity in the peripheral odontogenic and ossifying fibromas and in the epithelial and in the mesenchymal components of peripheral odontogenic fibroma and central odontogenic fibroma.

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Tables

Table 1- Comparative analysis of data concerning to the AgNOR number, area and PCNA index (IP) in the mesenchymal component between peripheral (POF) and central (COF) odontogenic fibromas and peripheral (PEOF) and central (CEOF) ossifying fibromas.

	POF	COF	P value	PEOF	CEOF	P value
AgNOR number	1.26	1.49	0.0136*	1.25	1.48	0.0428*
AgNOR area (μm^2)	1.46	1.21	0.0308*	1.62	1.25	0.0128*
IP	43.6	61.2	0.0087*	47.6	57.1	0.0038*

* Values significantly statistically (P≤0.05)

Table 2- Comparative analysis of data concerning to the AgNOR number, area and PCNA index (IP) in the mesenchymal component between peripheral odontogenic (POF) and peripheral ossifying (PEOF) fibromas and central odontogenic (COF) and central ossifying (CEOF) fibromas.

	POF	PEOF	P value	COF	CEOF	P value
AgNOR number	1.26	1.25	0.9233	1.49	1.48	0.5708
AgNOR area (μm^2)	1.46	1.62	0.0922	1.21	1.25	0.8501
PCNA IP	43,6	47.6	0.8099	61.2	57.1	0.2193

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Table 3- Comparative analysis of data concerning to the AgNOR number, area and PCNA index (IP) in the epithelial component between peripheral (POF) and central (COF) odontogenic fibromas.

	POF	COF	P value
AgNOR number	1.25	1.45	0.0372*
AgNOR area (μm^2)	1.47	1.23	0.0449*
PCNA IP	78.5	78.45	0.7576

* Values significantly statistically (P≤0.05)

Table 4- Comparative analysis of data concerning to the AgNOR number, area and PCNA index (IP) between mesenchymal (m) and epithelial (e) components in the peripheral (POF) and central (COF) odontogenic fibromas.

	mPOF	ePOF	P value	mCOF	eCOF	P value
AgNOR number	1.26	1.25	0.8946	1.49	1.45	0.5637
AgNOR area (μm^2)	1.46	1.47	0.7573	1.21	1.23	0.5637
PCNA IP	43,6	78.5	0.0071*	61.2	78.45	0.2482

* Values significantly statistically (P≤0.05)

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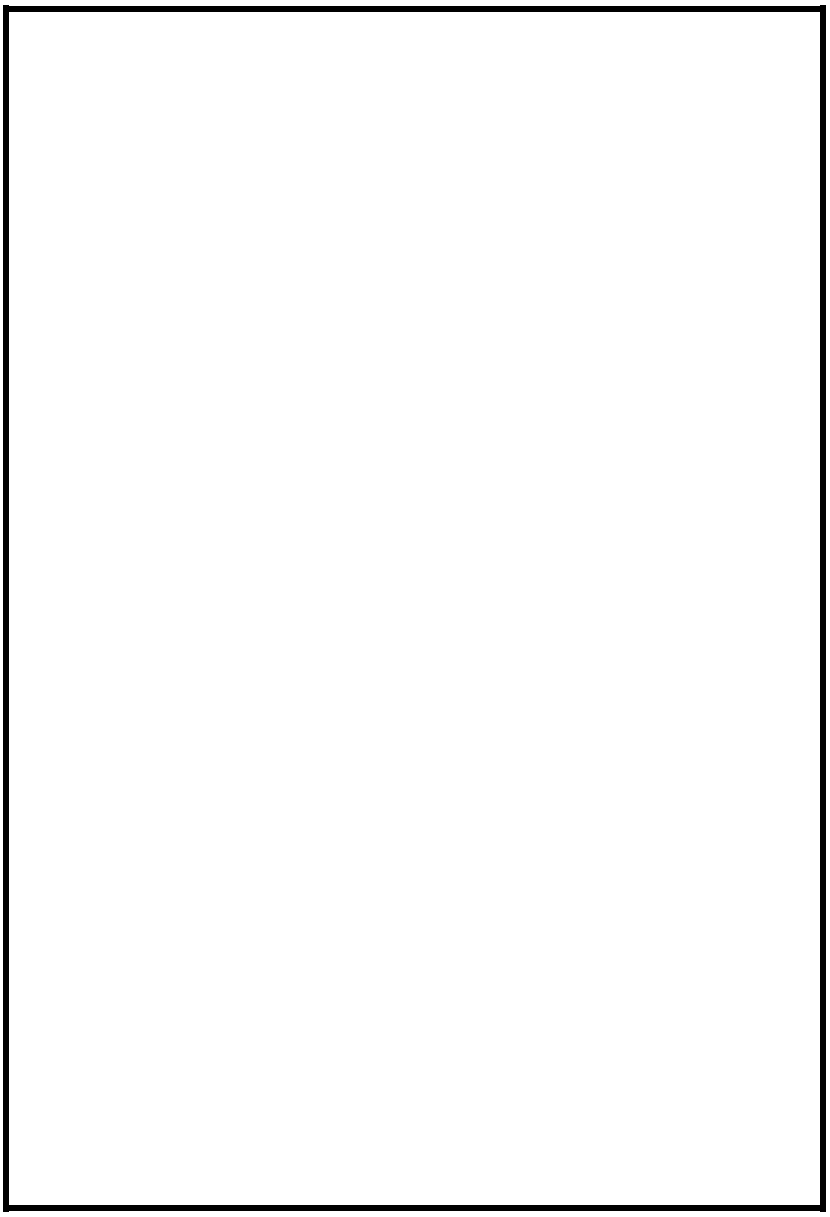
Figure



Legend

Figure 1. A- AgNOR in the peripheral odontogenic fibroma are visualized as black or brown, defined intranuclear homogeneous dots in epithelial (arrows) and mesenchymal components (AgNOR protocol, X400). B- PCNA-positive cells are identified as clear brown nucleus in the epithelial and mesenchymal components of the central odontogenic fibroma (Streptavidin–biotin standard protocol, X400).

3. CONSIDERAÇÕES FINAIS

As lesões de FOP, FOC, FOPE e FOCE, analisadas nesse estudo, apresentaram natureza e classificações distintas. No entanto, exibiram características histopatológicas similares. O FOC e sua variante periférica, FOP, são considerados tumores odontogênicos benignos compostos por quantidades variáveis de epitélio odontogênico inativo em meio a um tecido conjuntivo fibroso (OMS, 2005). O FOC é mais prevalente na mandíbula, apresentando uma relação de incidência em relação à maxila de 1:6.5. O tumor apresenta-se como uma área radiolúcida unilocular com bordas escleróticas bem definidas. O FOP prevalece na região de gengiva inserida, geralmente na área de pré-molar e molar, com distribuição equivalente entre os maxilares (OMS, 2005). O FOP é uma lesão incomum, benigna, pediculada ou séssil, vermelha ou rósea, que pode apresentar-se ulcerada (Buchner et al., 1987; Buchner, 1989; Buchner e Sctubba, 1987; Garcia et al., 2007). O FOCE é uma lesão fibro-óssea composta por tecido conjuntivo celularizado e quantidades variadas de tecido mineralizado, mais prevalente na região posterior de mandíbula. Radiograficamente, caracteriza-se por uma área bem demarcada apresentando regiões radiopacas e radiolúcidas, dependendo da quantidade de tecido duro e mole presentes na lesão (OMS, 2005). O FOPE é uma lesão proliferativa não-neoplásica composta por tecido conjuntivo fibroso entremeado por material mineralizado-osso, cemento, calcificações displásicas-, mais prevalente na reião de gengiva. O presente estudo demosntrou semelhanças e diferenças quanto à atividade de proliferação celular desses quatro tipos de lesões, fornecendo dados para o conhecimento de seus comportamentos biológicos (Buchner e Hansen, 1987; Eversole et al., 1985).

A análise quantitativa e morfométrica das AgNOR vem sendo muito utilizada para a distinção entre células malignas e benignas e, consequentemente, como auxiliar de diagnóstico (Allison e Spencer, 1993). A base dessa avaliação está fundamentada no achado de que em células malignas, com maior atividade proliferativa, as NOR são mais

numerosas, menores e apresentam-se de forma irregular, quando comparadas com células benignas, com menor atividade proliferativa, nas quais as NOR estão presentes em pequeno número, são maiores e de forma regular (Deferenzi e Trère, 1991; Cabrini et al., 1992; Mesquita et al., 1998). Nas lesões avaliadas, observaram-se características indicativas de um grupo de lesões benignas, classificadas como neoplasias benignas ou lesões proliferativas não-neoplásicas. Investigações sobre atividade de proliferação celular também utilizam PCNA e diversos outros marcadores em doenças da boca e de outras regiões, uma vez que eles oferecem informações distintas sobre o ciclo celular (Bravo et al., 1987).

Mesquita et al. (1998) e Ono et al. (2007) demonstraram, através da análise das AgNOR e do índice de marcação do PCNA, que a atividade de proliferação celular no FOCE foi maior que no FOPE. Esses resultados foram compatíveis com os achados do presente estudo, o que sugere uma proliferação celular menor no FOPE quando comparado a lesões neoplásicas, em decorrência da sua natureza reativa. Esse achado, desta forma, está de acordo com os distintos comportamentos biológicos observados no FOPE e no FOCE (Eversole et al., 1985; Buchner e Sctubba, 1987; Waldron, 1993). Observou-se também que a atividade proliferativa foi semelhante entre as lesões periféricas (FOP e FOPE) e entre as lesões centrais (FOC e FOCE). Assim, esses resultados demonstram que o FOP pode representar uma lesão proliferativa não-neoplásica, em semelhança ao FOPE.

A análise das AgNOR e do índice de marcação do PCNA vêm sendo realizados em muitos estudos sobre cistos e tumores odontogênicos (Do Carmo e Silva, 1998; Martins et al., 2001; Meer et al., 2003). Martins et al. (2001) avaliou as características das AgNOR em fibromas ameloblásticos, um tumor odontogênico de origem mista. Os resultados demonstraram que os componentes epitelial e mesenquimal possuíam atividade de proliferação celular semelhante. Essa observação está de acordo com a

natureza mista da lesão, na qual ambos os componentes são considerados neoplásicos (Sano et al., 1998). No presente estudo, verificou-se que os componentes epitelial e mesenquimal apresentaram média do número de AgNOR/núcleo e valores de índice de marcação do PCNA semelhantes no FOC e média de AgNOR semelhante no FOP. Isso demonstra que os componentes epitelial e mesenquimal presentes nas duas lesões apresentam atividade proliferativa celular semelhante. Desta forma, esses achados refletem que ambos, epitélio e mesênquima, podem ser considerados parte de um processo proliferativo não- neoplásico no FOP e parte de um processo neoplásico no FOC. Assim, sugere-se que o FOC, em semelhança ao fibroma ameloblástico, poderia representar um tumor misto. Entretanto, é importante enfatizar que outros estudos utilizando diferentes marcadores e distintas técnicas são necessárias para confirmar essa última observação.

Um resultado inesperado, no presente estudo, foi observado na análise do índice de marcação do PCNA no tecido epitelial do FOP. O valor do índice de marcação do PCNA foi maior em contraste com a média do número de AgNOR/núcleo. Citocinas liberadas por células inflamatórias presentes no FOP podem ser responsáveis por essa observação (Harrison et al., 1993). Desta forma, seria importante avaliar o real papel dessas citocinas na referida lesão.

Os dados das AgNOR e o índice de marcação do PCNA observados no presente estudo demonstraram que as lesões avaliadas mostraram perfis de neoplasias benignas ou de lesões proliferativas não-neoplásicas, e que os achados sugerem uma atividade de proliferação celular semelhante nas lesões periféricas (FOP e FOPE), assim como, nos componentes epitelial e mesenquimal dos fibromas odontogênicos central e periférico.

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5. ANEXOS

5.1. Confirmação de envio- Oral Sugery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology

De: TripleO (tripleo@sod.umsmed.edu)
Enviada: sexta-feira, 30 de maio de 2008
Para: bgg104@hotmail.com

Dear Dr. Garcia,

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Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 124/07

Interessado(a): Prof. Ricardo Alves de Mesquita
D.C.P.C.O
Faculdade de Odontologia -UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 25 de abril de 2007, o projeto de pesquisa intitulado **"AgNOR e imunomarcação de PCNA e Ki-67 nos fibromas odontogênicos central e periférico e fibromas ossificante periférico e central"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Dra. Maria Elena de Lima Perez Garcia
Presidente do COEP-UFMG

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