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Involvement of mast cells and microvessels density in reactive lesions of oral cavity: A comparative immunohistochemical study

Stephany Vasco Ferreira[a], Flávia Caló Aquino Xavier[a], Maria da Conceição Andrade de Freitas[b], Fábio Daumas Nunes[c], Clarissa Araújo Gurgel[a], Maria Cristina Teixeira Cangussu[d], Manoela Domingues Martins[e], Valéria Souza Freitas[f], Jean Nunes dos Santos[a,*]

[a] Laboratory of Oral Surgical Pathology, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Avenida Araújo Pinho, 62, Canela, Salvador, Bahia 40110-150, Brazil
[b] School of Dentistry, State University of Southwest Bahia, Jequié, Bahia, Brazil
[c] Laboratory of Molecular Pathology, School of Dentistry, University of São Paulo, Avenida Prof Lineu Prestes, 2227, Butantã, São Paulo, SP 05508-000, Brazil
[d] Department of Dental Public Health, School of Dentistry, Federal University of Bahia, Avenida Araújo Pinho, 62, Canela, Salvador, Bahia 40110-150, Brazil
[e] Department of Oral Pahology, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil
[f] Department of Health, School of Dentistry, State Universitiy of Feira de Santana, Feira de Santana, Bahia, Brazil

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ABSTRACT

In view of the similarity of clinicopathological features between reactive lesions of the oral cavity, the objective of the present study was to investigate the density of MCs (mast cells) and microvessels in a series of these lesions. Thirty-seven cases of reactive lesions including fibrous hyperplasia (FH, n = 10), inflammatory fibrous hyperplasia (IFH, n = 10), peripheral giant cell lesion (PGCL, n = 10) and lobular capillary hemangioma (LCH, n = 7) were investigated using immunohistochemistry for mast cell tryptase and CD34. For comparative purposes, central giant cell lesions (CGCL, n = 5) were included. A higher MC density was observed in LCH (37.01), while CGCL exhibited the lowest density (n = 8.14). There was a significant difference in MC density when all reactive lesions were compared to CGCL (p = 0.001). The largest mean density of microvessels was observed in LCH (n = 21.69). The smallest number was observed in CGCL (n = 6.24). There was a significant difference in microvessel density when the reactive lesions were compared to CGCL (p = 0.003). There was a significant and direct correlation between the density of MCs and microvessels only for IFH (p = 0.048) and CGCL (p = 0.005). A significant and direct correlation between the mean density of MCs and microvessels was observed when the reactive lesions were analyzed as a whole (p = 0.005). Our results suggest that mast cells contribute to the connective tissue framework and angiogenic function, as well as the development, of reactive lesions of the oral cavity, including FH, IFH, LCH and PGCL.

1. Introduction

Mast cells (MCs) are mobile cells derived from bone narrow that circulate in the blood. These cells are found in all mucosa milieu and connective tissue, especially in perivascular areas [1]. Several authors have suggested MCs to be potentially fibrogenic since they secrete powerful mediators of fibrosis, such as histamine, cytokines, proteoglycans, heparin, hyaluronic acid, proteases, and growth factor [1–7]. Studies have shown the interaction of MCs with fibroblasts and their contribution to the synthesis of collagen in many diseases and pathological conditions, such as oral submucous fibrosis [3], scleroderma [7], skin fibrosis [8], gingival fibromatosis [9], and fibrotic changes in the minor salivary glands of patients with Sjögren's syndrome [10]. Mast cells have also been reported to be involved in tissue repair [11] and tumor growth [12,13].

MCs are particularly prominent around capillaries [1,11–16]. These cells are known to release a variety of factors that increase

* Corresponding author at: Laboratório de Patologia Cirúrgica – UFBA, Avenida Araújo Pinho, 62, Canela, Salvador, Bahia, 40110-150, Brazil.
E-mail addresses: stephanyferreira3@gmail.com (S.V. Ferreira), f.calo@uol.com.br (F.C.A. Xavier), fadnunes@usp.br (F.D. Nunes), gurgel.clarissa@gmail.com (C.A. Gurgel), cangussu@ufba.br (M.C.T. Cangussu), manomartins@gmail.com (M.D. Martins), valeria.souza.freitas@gmail.com (V.S. Freitas), jeanunes@ufba.br, jeanpatol@gmail.com (J.N. dos Santos).

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angiogenesis, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGFs), transforming growth factor alpha (TGF-α), transforming growth factor beta (TGF-β), and interleukin 8 (IL-8) [6]. Angiogenesis is a highly organized multistep process that involves not only the formation of blood vessels, but also the proliferation and migration of endothelial cells [6,17]. Hence, the dysregulation of blood vessel formation has an impact on our health and can contribute to the development of different disorders [17].

The oral mucosa is constantly subjected to external and internal stimuli. Therefore, it can manifest a variety of diseases, including inflammatory, neoplastic or reactive lesions. Reactive lesions of the oral cavity are non-neoplastic lesions characterized by similar clinical features [18–21]. Although clinical features contribute to the diagnosis of each lesion, microscopic analysis is required for the definite diagnosis [19,23].

Reactive lesions frequently found in the oral cavity are fibrous hyperplasia (FH), inflammatory fibrous hyperplasia (IFH), lobular capillary hemangioma (LCH), and peripheral giant cell lesion (PGCL). Histologically, FH and IFH are characterized by hyperplasia in the epithelial lining and abundant collagen in addition to a variable amount of blood vessels, and the presence or absence of a chronic inflammatory infiltrate [20,22]. Lobular capillary hemangioma is a common reactive angiomatous proliferation that occurs on the skin and mucocutaneous membranes [24]. In contrast, PGCL consists of a proliferation of multinucleated giant cells amidst oval or spindle-shaped stromal cells, associated with blood vessels and extravasated red blood cells [20,25]. Although the reactive lesions described here have common etiological features, their growth vary and can often be exuberant [19].

We hypothesized that tryptase released by MCs stimulates the synthesis of collagen and angiogenic potential. This release could exert influence on the development of FH, IFH, PGCL and LCH. Therefore, the aim of this study was to perform an immunohistochemical analysis of the density of MCs and microvessels in these lesions. Although being considered a reparative or reactive process [26,27], central giant cell lesions (CGCL) exhibit aggressive growth and can cause massive destruction of the jawbones [28]. So, CGCL were included for comparative purposes.

2. Material and methods

The study was approved by the Ethics Committee of the School of Dentistry, Universidade Estadual do Sudoeste da Bahia, Jequié, Brazil (Protocol No. 128/2011-CAAE: 0107.0.454.000-11). Thirty seven cases of oral lesions were studied and included 10 cases of IFH, 10 of FH, 10 of PGCL and 7 of LCH. For comparative purposes, five cases of CGCL were included.

The histological diagnosis was revised and classified by an experienced oral pathologist (J.N.S), and those cases with histopathological resemblance to either CGCL or PGCL were distinguished using clinical and image data. The specimens were obtained from the archive of the Pathological Anatomy Service of the School of Dentistry, Universidade Federal da Bahia. Clinical data (gender, age, and location) were collected from the biopsy records of the service.

The paraffin-embedded specimens were deparaffinized in xylene (twice for 10 min), rehydrated in absolute alcohol (twice for 5 min) at room temperature, and cut into 3-μm sections. For antigen retrieval, the sections were incubated in 1% trypsin at 37 °C for 30 min (three cycles of 10 min each) for mast cell tryptase antibody (1:50, clone AA1, Dako Corporation, Carpinteria, CA, USA), and in citrate, pH 6.0, at 98 °C for 40 min for CD34 antibody (1:50, clone QBEnd-10, Dako Corporation). Then, the sections were incubated with the primary antibody diluted in background-reducing solution (Dako Corporation) at 4 °C for 18 h. The EnVision polymer (Dako Corporation) was applied for 30 min at room temperature and the reaction was developed with 3,3-diaminobenzidine (Dako Corporation) as chromogen for 5 min in a dark chamber. The sections were counterstained with Harris hematoxylin. Actinic cheilitis specimens with known reactivity to the antibodies were used as positive controls. Specimens in which the primary antibody was replaced with normal serum of the same isotype as the primary antibody served as negative control.

The density of MCs in the different oral lesions was expressed as the mean number of positive cells in an area of 56 $\mu m/mm^2$. Brown staining of the cells indicated positive cases. The shape of the cells and their degranulation were also evaluated [12,13,15,16]. The density of microvessels in the lesions was expressed as the mean number of vessels per area (56 $\mu m/mm^2$) at 200× magnification [16]. Positive cases were identified by brown staining of endothelial cells in hot spot areas.

Two trained observers (S.V.F. and J.N.S.) performed the histomorphometric analysis under a high-definition light microscope (Axiostar Plus, Zeiss, Germany) at 400× magnification. Up to 10 fields were examined and the images were captured with a camera (Axiocam Icc3, Zeiss). Blood vessel density and MC counts per square millimeter were obtained by using a specific software (Axiovision Rel 4.8, Zeiss, 2008) and the arithmetic mean was determined for each case. Microvessels were counted and those with a lumen >50 μm were excluded [29]. Both observers were unaware of the microscopic diagnosis and immunohistochemical analysis was performed in a blind manner.

Differences between groups were evaluated by ANOVA, followed by the Tukey test. Correlations were determined by Pearson's test. All statistical analyses were performed using the Minitab 14 program (Pennsylvania, USA). A p value < 0.05 was considered statistically significant.

3. Results

All of reactive lesions (FH, IFH, PGCL) showed a predilection for females (68.86%) and the age ranged from 15 to 80 years (mean 46.93; DP± 18.44). There was a wide variation in the site affected including buccal mucosa (16%), gingiva (16%) and alveolar edge (8%) and lower lip (5%). With respect to CGCL, there was a male predominance (60%) with age ranging from 8 to 19 years (mean 13 years, DP± 18.44). The most affected anatomical location was the mandible. Some cases were not included due to lack of information.

Mast cells were detected in all lesions studied and exhibited varied morphology (oval, elongated or round). In general, MCs were found near blood vessels and in areas of fibrosis. In cases of FH, MCs were located around blood vessels (Fig. 1A). In the presence of inflammation, MCs were also detected in these areas (Fig. 1B, C). In LCH, the MC population was found in stromal areas, more specifically in fibrous areas (Fig. 1D). Some areas showed MCs entrapped inside the lobes, but always near blood vessels (Fig. 1E, F). In PGCL, MCs were clearly seen concentrated in the band of lamina propria, which separated the lesion (Fig. 1G), and around areas of ulceration. Sometimes, this cell population was located in bands of fibrous stroma and amidst multinucleated giant cells, but this finding was rare (Fig. 1H). In CGCL, MCs were more concentrated in fibrous areas that separated groups of multinucleated giant cells (Fig. 1I) rather than close to stromal proliferations of giant cells (Fig. 1J). Mast cells were often seen in the epithelial lining of some lesions (Fig. 1C, D).

Degranulation of MCs was observed mainly in IFH cases (n = 9; Fig. 1E) and was rare in FH (n = 4). Degranulation was also present in cases of LCH (n = 3) and CGCL (n = 3), and was observed near bone tissue in CGCL.

In cases of PGCL, degranulation was not seen in areas inside the lesion. However, degranulation was present around the lesions.