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Evaluation of peri-implant mucosa: Clinical, histopathological and immunological aspects

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ABSTRACT

Objective: The aim was to compare the inflammatory response in peri-implant mucosa between patients with peri-implantitis (PP-group) and patients with healthy peri-implant tissues (HP-group).

Materials and methods: Two fragments of peri-implant mucosa of 18 patients were collected and serial sections were performed for histological and immunohistochemical analysis.

Results: When compared with HP-group, PP-group showed higher immunostained cell density for TGF-β, IL-17 and CD31, beyond greater density of red cells, leukocytes, mast cells chymase (MCC) and mast cell tryptase (MCT). HP-group patients showed higher IL-13 expression and increased amount of collagen fibres when compared with PP-group. In PP-group there was significant positive correlation between MCT density and density of blood vessels immunostained, and between MCC density and density of blood vessels immunostained. There was significant negative correlation between the IL-17 density and collagen percentage.

Conclusions: This study demonstrated that in patients with peri-implantitis there was higher of TGF-β and IL-17, indicating that these cytokines are directly involved in the inflammatory process. Thus, understanding the influence of cytokines in the peri-implantitis installation, new therapies could be developed in order to inhibit the synthesis of IL-17 and induce synthesis of IL-13 in peri-implant tissue, contributing to increase the longevity of the implant.

1. Introduction

Peri-implantitis is an inflammatory lesion of bacterial aetiology characterized by mucosal inflammation and bone loss.[1]

The chronic inflammation is characterized by neovascularization and neoformation of collagen.[2] Although a higher percentage of collagen has been described in periodontal disease,[2,3] there are no reports in the literature describing the percentage of collagen in peri-implantitis. It is known that mast cells participate of the inflammatory process releasing

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mediators and proteases such as chymase and tryptase, important in the process of collagen neoformation.[4] Together with mast cells, the molecule of TGF-β is essential for the production of collagen. TGF-β is a cytokine well known for its potential effects on the repair, regeneration and regulation of matrix metalloproteinases.[5,6]

In inflammation there is increase in the production of various cytokines and adhesion molecules that promote the extravasation of leukocytes to the inflammatory site.[7] CD31 is an adhesion molecule present in the walls of capillaries that helps to regulate the traffic of leukocytes,[8] and can also be used to quantify the vessel density.[7] Although not described in peri-implant inflammation, an increase in the numbers of adhesion molecule CD31 has been shown in periapical lesions in Wistar rats.[7]

In peri-implant disease there are predominantly T cells, suggesting that the local immune response is also regulated by these cells.[9] They are divided into subgroups, among which are Th1, Th2 and Th17 which, according to their functions, are correlated with the production of distinct cytokines. Th1 cells principally secrete IL-2, IFN-γ and TNF-α, and are involved in cellular immunity, whereas Th2 cells secrete cytokines such as IL-4, IL-5 and IL-13 and are involved in humoral immunity. Whereas, Th17 cells produce pro-inflammatory cytokines such as IL-17 and play an important role in inflammation.[10]

Knowing that increasing numbers of dental implants have been placed in recent decades, and that peri-implantitis is an inflammatory disease, further studies are needed to better understand the pathogenesis of the inflammatory process in order to contribute to new therapies that will prevent the early loss of the implant. As peri-implantitis is an inflammatory disease that leads to tissue destruction, we hypothesized that in patients with peri-implantitis there are increased pro-inflammatory cytokine IL-17, decreased anti-inflammatory cytokine IL-13 and reduced collagen fibres.

2. Materials and methods

2.1. Study population

This study was previously approved by the Ethics Committee on Human Research of the Federal University of Triangulo Mineiro (CEP/UFTM) Uberaba/MG, Brazil, Protocol No. 1657). The subjects were selected during follow-up appointments at a private dental clinic in the city of Uberaba, Minas Gerais, Brazil, in the period from July 2010 to July 2011. Detailed medical and dental records were obtained, and subjects who fulfilled the inclusion/exclusion criteria and agreed to participate were included in the study. Demographic data such age, gender and ethnicity were assessed to obtain a homogeneous distribution between the two groups (Table 1). All eligible subjects were informed of the nature of study, potential risks and benefits of their participation in the study and signed a free informed term of consent. Eighteen implants were used, being nine patients from the group with peri-implantitis (PP) and nine from the group with healthy peri-implant tissues (HP).

The inclusion criteria for patient selection were: All patients had to have at least one implant in the oral cavity. The implants analyzed had to be in function for at least six months. Patients in the PP group should present probing depth >3 mm in at least one site of the implant, marginal bleeding in at least one implantation site and bone loss. The criteria for inclusion in the HP group were: patients who had implants with probing depth between 0 and 3 mm without marginal bleeding, suppuration or bone loss. Thus, a total of 18 implants from 18 patients were used, being nine patients from the group with peri-implantitis (PP) and nine from the group of healthy patients (HP). In PP group, no patient had periodontal disease.

Table 1 – Demographic characteristics, peri-implant clinical data of group with peri-implantitis (PP) and healthy patients (HP).

	PP ($n = 9$)	HP ($n = 9$)
Ethnicity[a] (C/NC)	7:2	7:2
Gender[b] (M/F)	4:5	4:5
Age[c] (years, mean ± SD)	64.00 ± 2.85	58.33± 3.02
Probing depth[d]	4.83 ± 0.09*	1.61 ± 0.11

C, Caucasian; NC, non-Caucasian; M, male; F, female; SD, standard deviation.
[a] Exact Fisher test, $p > 0.05$.
[b] Exact Fisher test, $p > 0.05$.
[c] Student's t-test, $p > 0.05$.
[d] Mann–Whitney test, $p < 0.001$.
* Statistically significant differences.

In patient selection, the exclusion criteria were as follows: previous periodontal or peri-implant therapy, presence of relevant systemic diseases, diabetes, intake of antibiotics or anti-inflammatory drugs in the previous six months, no current or former smokers and tobacco users, pregnant or lactating.

The following parameters were assessed at six sites in each implant (mesiobuccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) using a periodontal probe (Williams-Golgran, São Paulo, Brazil): (a) marginal bleeding: presence or absence of bleeding recorded by running a probe along the soft tissue margin; (b) suppuration: presence or absence of spontaneous or suppuration on probing; (c) probing depth: distance in millimetres between the mucosal margin and the bottom of the sulcus/pocket. Intraoral periapical radiographs were obtained for each implant using the paralleling technique with a radiographic positioner. Clinical examination and radiographs were performed by the same examiner (MLRM), who was previously trained and calibrated.

2.2. Collect of fragments

To collect the gum fragments, initially the patients rinsed the mouth with a 0.12% digluconate chlorhexidine solution. Subsequently a local anaesthetic block was performed and two fragments of marginal gingiva were collected. In the PP group the fragments were removed from the site with greatest probing depth, and in the HP group, they were removed from the lingual region. Each fragment measured approximately 4 mm × 4 mm[11,12]; one was used for histopathological analysis and the other for enzymatic immunosorbent assay (ELISA).

2.3. Histopathological analysis

The samples obtained were fixed in 3.7% formalin solution for 24 h, dehydrated, included in paraffin and processed for