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ORIGINAL ARTICLE

Histological evaluation of peri-implant mucosal and gingival tissues in peri-implantitis, peri-implant mucositis and periodontitis patients: a cross-sectional clinical study

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ABSTRACT

Objective: Aim of present study was to evaluate gingival tissue samples obtained from healthy and diseased sites of teeth and dental implants in terms of hypoxia and collagenase activity.

Methods: Four study groups were created as Group-1; healthy individuals (H), Group-2; periodontitis patients with stage 3 grade B (P), Group-3; patients with peri-implant mucositis. Group-4; patients with peri-implantitis (P-IMP). Plaque index (PI), gingival index (GI) and probing pocket depth (PPD) were recorded. Gingival and peri-implant mucosal biopsies were obtained. Fibroblast and inflammatory cells were counted. Hypoxia-inducible factor (HIF)-1α, prolyl hydroxylase (PH), matrix metalloproteinase (MMP)-8, tissue inhibitor of MMPs (TIMP)-1, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) levels were determined via immunohistochemistry.

Results: Healthy controls had highest fibroblast cell counts and lowest inflammatory cell counts compared to other groups. Peri-implantitis and periodontitis samples had similar fibroblast and inflammatory cell counts, while peri-implant mucositis had higher fibroblast cells and lowered inflammatory cells compared to periodontitis and peri-implantitis samples. HIF-1α, COX-2 and iNOS levels were lowest in healthy controls and increased in other groups. Peri-implant mucositis samples had significantly lower expressions of HIF-1α, COX-2 and iNOS compared to peri-implantitis and periodontitis groups. PH expressions were lower in periodontitis and peri-implantitis groups compared to healthy controls and peri-implant mucositis groups. MMP-8 levels were lower in healthy group compared to other groups while levels were similar in periodontitis, peri-implant mucositis and peri-implantitis groups. TIMP levels were similar in groups.

Conclusion: Periodontitis, peri-implantitis, and peri-implant mucositis samples exhibited higher inflammation and lower fibroblast cell counts and tend to have increased tissue collagenase activity, hypoxia and inflammation compared to healthy samples.

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Hypoxia; inflammation; MMP-8; peri-implantitis; periodontitis

Introduction

Inflammation and hypoxia have a close cause-effect association since hypoxia can trigger inflammatory events and inflammatory lesions usually have hypoxic tissue alterations [1]. As for periodontium, all the hypoxia-associated tissue alterations indicate possible involvement of hypoxia in the development of periodontitis. Furthermore, tissue-related changes due to hypoxia are known to take place in gingivitis and periodontitis; however, little is known about these changes in peri-implant tissues. Hypoxia is the systemic or local depletion of the oxygen and triggers certain sets of events in the body in response to the oxygen deficiency [2–4]. The primary effect of hypoxia in periodontal tissues is the increase in inflammation, and inflammation, in return, increases hypoxia [2,5].

A major contributing factor in hypoxia-related inflammation was reported to be the nuclear factor κB (NF-κB) pathway and pro-inflammatory cytokines such as interleukin (IL)-1β, tumour necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS), which was driven by microorganisms such as *P. gingivalis* and *F. nucleatum* [2,6,7]. Therefore, inflamed gingival tissues were reported to have increased hypoxia while healthy tissues maintain normal oxygen levels [2]. Furthermore, hypoxia also decreases cell differentiation, proliferation and migration in the human periodontal ligament and gingival cells [4,8,9].

In hypoxic conditions, the response of the tissues against hypoxia is regulated by hypoxia-inducible factor (HIF)-1 α. In chronic inflammatory diseases such as periodontitis and diabetes, hypoxia, HIF-1α and its primary mediator, vascular endothelial growth factor (VEGF) are reported to be increased [5,10,11]. In normal oxygen levels, HIF-1α is rapidly degraded by prolyl hydroxylase (PH). PH is an oxygen-dependent enzyme that cannot function in oxygen depletion, and degradation of HIF is inhibited and HIF accumulates in the cytoplasm [10,12]. Increased hypoxia in chronic inflammation causes increased HIF-1α levels, and HIF-1α and hypoxia in periodontal tissues were reported to

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be associated with severe inflammation [5,13]. Vasconcelos et al. recently reported that healthy gingival tissues exhibited lower HIF-1α and VEGF levels while inflamed gingival tissues expressed increased levels [13]. Also, in another clinical study, the levels of HIF-1α, TNF-α and VEGF were found to be associated with the severity of periodontal inflammation [5]. Therefore, the existence of HIF-1α could be an indicator of inflammation in periodontal tissues [6,13,14].

The periodontal ligament is one of the most significant blood and oxygen supplies in the periodontium and has a crucial role in Defence against dysbiotic microbiota [15,16]. The lack of periodontal ligament, therefore, might lead to poor vascularisation and thus increased inflammatory status in the periodontium. In this regard, peri-implant tissues were suggested to have a higher risk of inflammation and collagenase activity compared to the periodontal tissues [17–21]. Involved immune cells also differ in peri-implantitis lesions compared to the periodontitis lesions [18]. Cell adhesion, complement activation and wound healing were also altered in peri-implantitis lesions with up-regulated pro-inflammatory cytokines and chemokine receptors [17,19,22]. Ghighi et al. recently reported that peri-implant connective tissues exhibited increased tissue inhibitor of MMPs (TIMP)-2, IL-10 and receptor activator of nuclear factor κB (RANKL) compared to the connective tissue samples with severe periodontitis [23]. Yu et al. also demonstrated elevated IL-1β, IL-10 and TNF-α in gingival tissues around peri-implantitis lesions [20].

Therefore, peri-implant tissues, which lack periodontal ligament and vascular plexus might be prone to inflammation and hypoxia more than natural teeth do. Hypoxia contributes to the inflammation thus etiopathogenesis of chronic destructive diseases; however, the hypoxic alterations in mucosal tissues around dental implants were not evaluated [2,4–8,14]. The present study aimed to determine hypoxia markers, inflammatory mediators and neutrophil collagenase activity in gingival tissues and peri-implant mucosal tissues.

Material and methods

The present study is a non-randomized cross-sectional clinical study. The ethical approval for the study protocol was achieved from the local Medical Ethics Committee of Tokat Gaziosmanpasa University. The study was performed at Tokat Gaziosmanpaşa University Faculty of Dentistry Department of Periodontology. Written informed consent was obtained from all participants. All patients and healthy volunteers had a detailed oral and radiographic examination and diagnosed by an experienced clinician (O.K.).

The study population consisted of systemically healthy sixty non-smoker participants as 15 healthy individuals, 15 periodontitis patients with stage 3 grade B, 15 patients with peri-implant mucositis and 15 patients with peri-implantitis.

Group-1; healthy individuals (H, mean age 42.41 ± 4.28, eight women, seven men)
Group-2; periodontitis patients with stage 3 grade B, (P, mean age 44.60 ± 3.56, eight women, seven men)
Group-3; patients with peri-implant mucositis, (PI-M, mean age 47.80 ± 3.90, eight women, seven men)
Group-4; patients with peri-implantitis, (P-IMP, mean age 46.36 ± 4.12, eight women, seven men)

Exclusion criteria were any systemic disease or condition, which might affect the inflammatory state, absence of multiple teeth (10 or over missing teeth), tobacco use, drug use, pregnancy or lactation and previous antibiotic or periodontal therapy within six months.

Periodontal and peri-implant tissue health were examined, and the diagnosis was made based on the criteria defined by the 2017 International World Workshop for a Classification of Periodontal Diseases and Conditions [24]. Peri-implant mucositis and peri-implantitis were diagnosed based on the criteria reported previously [25]. All healthy and periodontitis biopsy samples were obtained from maxillary premolar or molar region (from #4, #5, #6 and #7) by either gingivectomy or crown lengthening procedure in the routine treatment protocol or before orthodontically indicated tooth extraction. All biopsies were obtained from posterior maxillary teeth.

All patients with periodontitis had stage 3 grade B generalised disease involvement. Patients with peri-implant mucositis were untreated patients. Peri-implantitis and peri-implant mucositis patients were periodontitis patients who had previously generalised periodontitis involvement but had not received any treatment in the last six months. In peri-implantitis patients, the implant with peri-implantitis lesion had never been treated before. Peri-implant mucositis and peri-implantitis patients had two or more implants. Regardless of how many implants were affected in the patient's mouth, the sample was taken from the mucosa around one implant placed only in the maxillary posterior region. Peri-implant mucositis and peri-implantitis patients were partially edentulous patients, and patients who received full-mouth implant rehabilitation were not included. Patients had been using implant-supported fixed prostheses for a period of 2–7 years.

Periodontal clinical parameters

All patients were examined in detail. Full-mouth plaque index (PI), gingival index (GI) and probing pocket depth (PPD) were recorded as clinical periodontal measurements [26,27]. Clinical measurements were performed from six points around the teeth (mesial, middle and distal aspects of both buccal and lingual/palatal surfaces) and a mean value was recorded. PPD was measured as the distance from the gingival margin to the bottom of the pocket via a periodontal probe (Williams-type periodontal probe, Hu-Friedy Co., Chicago, IL, USA).

Collection of biopsy samples

The biopsy sampling was performed according to a report of a previous study [18]. One gingival biopsy from each participant was obtained by either gingivectomy/crown lengthening procedures from patients with impaired gingival

topography or before tooth extraction indicated for orthodontic treatment. Peri-implant mucosal samples were collected immediately before mechanical debridement. The diseased sites around dental implants consisted of inflamed tissue, and biopsies were obtained after the written consent of the patients was taken. All samples were taken from the maxillary posterior region of the individuals. Collected samples were stored in 10% neutral buffered formalin for 48 h and embedded in paraffin for histological analysis.

Histopathological evaluation

After fixation for 48 h, gingival and peri-implant mucosal tissues underwent histological tissue processing. Firstly, tissues were dehydrated with descending alcohol series and cleared with xylene then embedded in paraffin. An experienced blind examiner performed histological procedures (F.G.). Serial sections were obtained from paraffin blocks, and three sections from each block were chosen for each staining procedure. All selected slides underwent haematoxylin and eosin (H&E) staining and immunohistochemistry. Fibroblast and inflammatory cell infiltration were evaluated from H&E stained slides under 1000× magnification via a light microscope (Nikon Eclipse, E 600, Tokyo, Japan).

Determination of fibroblasts and inflammatory cells

Fibroblast and inflammatory cells were counted from an area of 10.000 μm^2 at the connective tissue neighbouring gingival epithelium. First, the area was marked, and the total inflammatory cells (neutrophil, lymphocyte, eosinophil and macrophage cells) within the frame were counted. The fibroblasts were also counted likewise. The measurements were performed from three different points, and the mean of these three measurements was recorded [28].

HIF-1α, PH, matrix metalloproteinase (MMP-8), TIMP-1, cyclooxygenase (COX-2) and iNOS immunohistochemistry

Three different slides for each parameter were randomly selected from the sections obtained from each participant. Hypoxia in the samples was evaluated with HIF-1α and PH immunostaining. Collagenase activity was evaluated with MMP-8 and TIMP-1 immunostaining. COX-2 and iNOS were evaluated to determine inflammatory status. Firstly, all slides were deparaffinized with xylene and dehydrated with a descending alcohol series. After washing with distilled water, antigen retrieval was performed via sodium citrate buffer (pH 6.0) for two h at 70 °C, and then endogenous peroxidase activity was suppressed with 3% hydrogen peroxide treatment. After hydrogen peroxide treatment, all slides were incubated with normal rabbit serum for 30 min. After normal serum incubation, primary antibody diluents were prepared and applied to the samples overnight at a humidified chamber at 4 °C. The primary antibodies were goat polyclonal anti-HIF-1α antibody (Abcam plc, Cambridge, UK)(1:250), anti-PH antibody (Abcam plc, Cambridge, UK) (1:250), anti-MMP-8 antibody (Abcam plc, Cambridge, UK) (1:250), anti-TIMP-1 antibody (Abcam plc, Cambridge, UK) (1:250), anti-COX-2 antibody (Thermo Fisher Scientific, Waltham, Massachusetts, USA) (1:250) and anti-iNOS antibody (Abcam plc, Cambridge, UK) (1:250). After primary antibody incubation, all slides were washed with phosphate buffer solution (PBS) three times for five minutes (3 × 5), and biotinylated immunoglobulin G was applied for 30 min. Again a wash of 3 × 5 PBS, all samples were exposed to a streptavidin-horseradish peroxidase-conjugated reagent for another 30 min. After washed with 3 × 5 PBS, all sections were treated with 3-amino-9-ethylcarbazole chromogen to visualise staining. Counterstaining was performed with Meyer's haematoxylin and sections were mounted. After allowing dry for two days, samples were examined under 400× magnification using light microscopy (Nikon Eclipse, E 600, Tokyo, Japan) [14,28].

Immunohistochemical semi-quantitative H score analysis

An experienced blind examiner performed all histological evaluations. The region of interest was the connective tissue area below the epithelium. Three areas were selected from each section and analysed. All cells were marked according to their staining as no staining '0', low staining '1', mild staining '2' and dense staining '3'. Staining scores were converted to a numeric value as 'H score' through a formula ($\sum$Pi (i + l)), which is a frequently used value for immunohistochemistry [14,28]. In this formula, i shows the staining intensity score and Pi indicates the percentage of the stained cells.

Statistical analysis

Power for the study was calculated before the baseline based on a previous study with a similar study design [18]. Sixty participants, 15 individuals in each group, provided the power of over 80%. All data were tested for normality with One Sample K-S test. For the fibroblast cell counts and PPD, One Way ANOVA followed by Tukey test used. For other parameters, Mann Whitney-U and Kruskal Wallis tests were used. Data were presented as mean ± SD or percentage as appropriate. Results and statistical analyses were performed with digital software (IBM SPSS, IBM Corporation, New York USA). $p < .05$ were considered statistically significant.

Results

The age and gender of the participants were similar ($p > .05$) (Table 1). PI, GI and PPD values of healthy controls were lower than those of the other groups ($p < .05$) (Table 1). The PPD values of the PI-M group was also lower than those of the P and P-IMP groups ($p < .05$).

Fibroblast and inflammatory cell evaluation

Fibroblast cell density was significantly higher in the healthy group compared to the other groups ($p < .05$) (Figure 1 and

Table 1). Peri-implantitis and periodontitis groups had the lowest fibroblast cell density. PI-M also exhibited higher density compared to the periodontitis and peri-implantitis groups and lower than the healthy group ($p < .05$) (Figures 1, 2 and Table 1).

Inflammatory cell density was the lowest in the healthy group ($p < .05$). The periodontitis group exhibited the highest inflammatory cell density, and the difference between the control and PI-M groups were significantly different ($p < .05$). Periodontitis and peri-implantitis groups had similar inflammatory cell infiltration ($p > .05$) (Figure 1 and Table 1).

HIF-1α, PH, MMP-8, TIMP-1, COX-2 and iNOS immunohistochemistry results

The immunohistochemistry results were presented in Table 2 and Figures 3 and 4. Hypoxia tends to decrease in the healthy participants, and HIF-1α levels of the healthy individuals were lower than those of the other groups ($p < .05$) (Figures 3, 4 and Table 2). Peri-implant mucositis group had higher HIF-1α levels compared to the control group and lower HIF-1α levels compared to the periodontitis and peri-implantitis groups ($p < .05$) (Table 2).

PH immunohistochemistry exhibited a reverse pattern to the HIF-1α and was higher in the healthy and peri-implant mucositis groups compared to the periodontitis and peri-implantitis groups ($p < .05$). Periodontitis group had the lowest PH expressions, but the difference between periodontitis and peri-implantitis was not significant ($p > .05$). The healthy groups also had similar PH levels with peri-implant mucositis group ($p > .05$) (Figures 3, 4 and Table 2).

Regarding MMP-8 expressions, periodontitis, peri-implant mucositis and peri-implantitis groups had significantly higher MMP-8 levels compared to the healthy controls ($p < .05$) but similar levels among each other ($p > .05$) (Figure 4 and Table 2).

TIMP-1 levels were lower in the healthy controls; however, the differences among the groups did not reach significance ($p > .05$) (Figure 4 and Table 2).

COX-2 and iNOS expressions were lower in the healthy controls compared to the periodontitis and peri-implantitis groups ($p < .05$). Peri-implant mucositis also had increased levels of COX-2 and iNOS than healthy controls ($p < .05$) and lower levels compared to the periodontitis and peri-implantitis groups ($p < .05$). Peri-implantitis and periodontitis groups had similar iNOS expressions ($p > .05$). Even though peri-implantitis group exhibited slightly increased COX-2 levels, the difference was not found significant ($p > .05$) (Figures 3, 4 and Table 2).

Table 1. Mean age, plaque index, GI, clinical attachment levels, fibroblast and inflammatory cell counts in the study groups.

Groups/ parameters	Healthy controls	Periodontitis	Peri-implant mucositis	Peri-implantitis
Age	42.41 ± 4.28	44.60 ± 3.56	47.80 ± 3.90	46.36 ± 4.12
Plaque index	0.5 ± 0.5	2.5 ± 0.5[a]	2.0 ± 0.5[a]	2.5 ± 0.5[a]
GI	0.5 ± 0.5	2.5 ± 0.5[a]	2.0 ± 0.5[a]	2.5 ± 0.5[a]
PPD (mm)	2.0 ± 1.0	7.8 ± 1.0[a]	4.00 ± 0.5[a,b]	7.5 ± 0.5[a,c]
Fibroblast cell counts (*n*)	38.00 ± 7.40	7.25 ± 1.28[a]	16.12 ± 1.80[a,b]	5.12 ± 2.53[a,c]
Inflammatory cell counts (*n*)	2.00 ± 1.85	40.25 ± 6.88[a]	23.75 ± 4.83[a,b]	36.00 ± 5.55[a,c]

[a]$p < .05$ vs. Control group; [b]$p < .05$ vs. Periodontitis group; [c]$p < .05$ vs. Peri-implant mucositis group.

Discussion

This is the first study to investigate hypoxia-related tissue changes in peri-implant mucositis and peri-implantitis. A comparison of the findings with periodontitis and healthy controls showed a similarity between peri-implantitis and periodontitis. The hypoxia markers, inflammatory mediators,

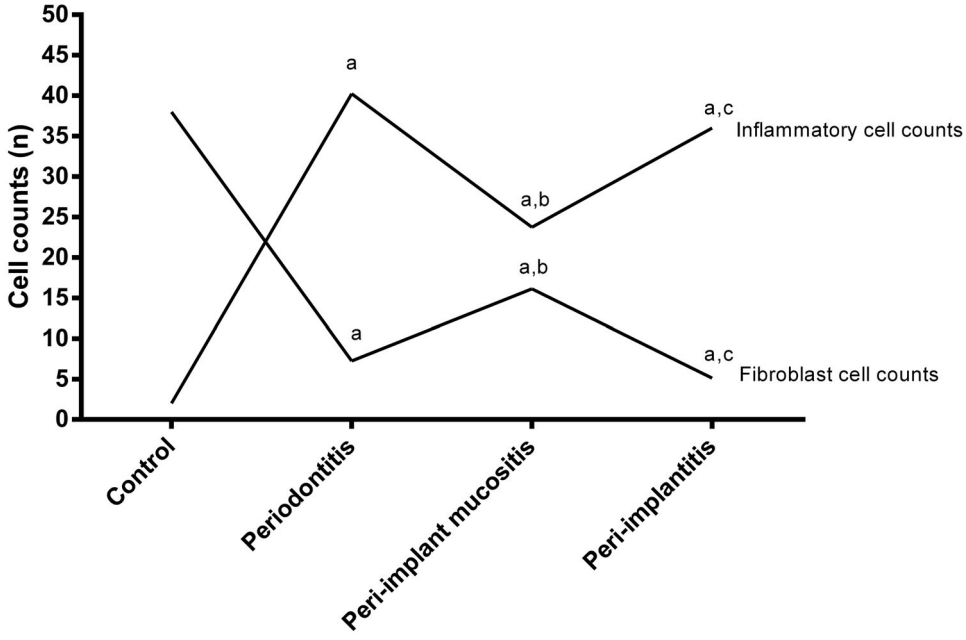

Figure 1. Graphic demonstration of fibroblast and inflammatory cell counts in the study groups. [a]$p < .05$ vs. Control group, [b]$p < .05$ vs. Periodontitis group, [c]$p < .05$ vs. Peri-implant mucositis group.

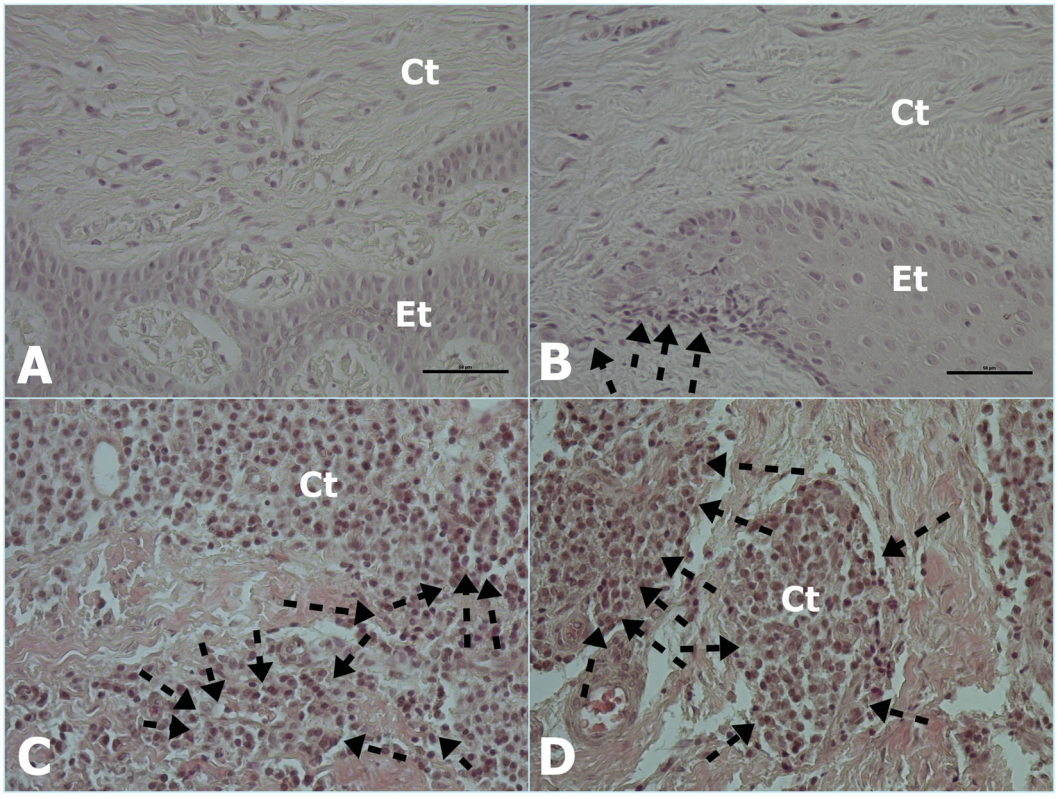

Figure 2. Representative histological images of the study groups. Interrupted black arrows indicate inflammatory cells. Ct: connective tissue; Et: Epithelial tissue.

Table 2. Immunohistochemistry results of the HIF-1α, PH, TIMP-1, MMP-8, COX-2 and iNOS.

Groups/ H scores	Healthy controls	Periodontitis	Peri-implant mucositis	Peri-implantitis
HIF-1α	15.40 ± 5.03	37.07 ± 3.37[a]	28.74 ± 3.99[a,b]	40.72 ± 8.48[a,c]
PH	25.19 ± 4.49	9.35 ± 6.10[a]	22.15 ± 3.47[b]	12.37 ± 6.07[a,c]
TIMP-1	33.63 ± 20.34	40.82 ± 5.08	42.95 ± 1.94	41.97 ± 8.90
MMP-8	16.85 ± 4.90	37.91 ± 8.84[a]	30.95 ± 6.85[a]	40.60 ± 11.40[a]
COX-2	26.08 ± 11.82	63.30 ± 6.32[a]	49.09 ± 13.87[a,b]	74.60 ± 5.08[a,c]
iNOS	38.61 ± 13.78	63.92 ± 8.45[a]	46.74 ± 5.30[a,b]	63.21 ± 12.44[a,c]

[a] $p < .05$ vs. Control group; [b] $p < .05$ vs. Periodontitis group; [c] $p < .05$ vs. Peri-implant mucositis group.

and neutrophil collagenase activity in gingival tissues and peri-implant mucosal tissues were evaluated in the present study. Fibroblast cells were lower and inflammatory cells were higher in peri-implantitis and periodontitis patients. Hypoxia, COX-2 and iNOS expressions increased in periodontitis, peri-implant mucositis and peri-implantitis patients while PH levels decreased. MMP-8 also increased in periodontitis, peri-implant mucositis and peri-implantitis patients while TIMP-1 levels remained similar in all groups. Peri-implantitis samples exhibited similar levels compared to the periodontitis samples. Peri-implant mucositis patients had increased inflammation compared to the control group but lower than those of the periodontitis and peri-implantitis groups.

Chronic inflammatory destructive diseases such as periodontitis and peri-implantitis cause a dense inflammatory cell infiltration and a significant decrease in fibroblast cell proliferation, migration and other functions [15,18,22,29,30]. Peri-implant tissues exhibit a higher risk of inflammation as they lack major two components of the periodontium, junctional epithelium and periodontal ligament and cellularity and vascular structures [21]. Irshad et al. reported that fibroblast obtained from peri-implantitis and periodontitis lesions expressed higher MMP-8, IL-1β, IL-8 and monocyte chemotactic protein compared to the fibroblast obtained from healthy controls [31]. Verardi et al. demonstrated that peri-implantitis fibroblast showed increased IL-6, IL-8, VEGF and transforming growth factor-β expressions compared to the fibroblast from healthy individuals [32]. They also reported altered fibroblast function in peri-implantitis compared to the periodontitis [32]. Bordin et al. also found increased VEGF and MMP-1 expressions along with decreased transforming growth factor-β and TIMP-1 expressions in fibroblast obtained from peri-implantitis lesions compared to the fibroblasts of periodontitis and healthy controls [33]. In another study, slight increase, which did not reach statistical significance, was also reported [34].

Furthermore, Ghighi et al. showed elevated IL-10, RANKL and TIMP-2 levels in peri-implantitis [23]. Yu et al. demonstrated increased inflammatory cell infiltration around dental implants compared to periodontitis [20]. Diseased gingival tissues around dental implants have higher neutrophil and macrophage cells with giant cells than periodontitis [35]. As for the present results, the connective tissues around dental implants and teeth exhibited significantly different compositions. Fibroblast cells in peri-implant mucositis were found to be lower than healthy controls and higher than periodontitis and peri-implantitis patients. The periodontitis and peri-implantitis caused a similar decrease in fibroblast cells. As for the inflammatory cell infiltration, a reverse pattern was

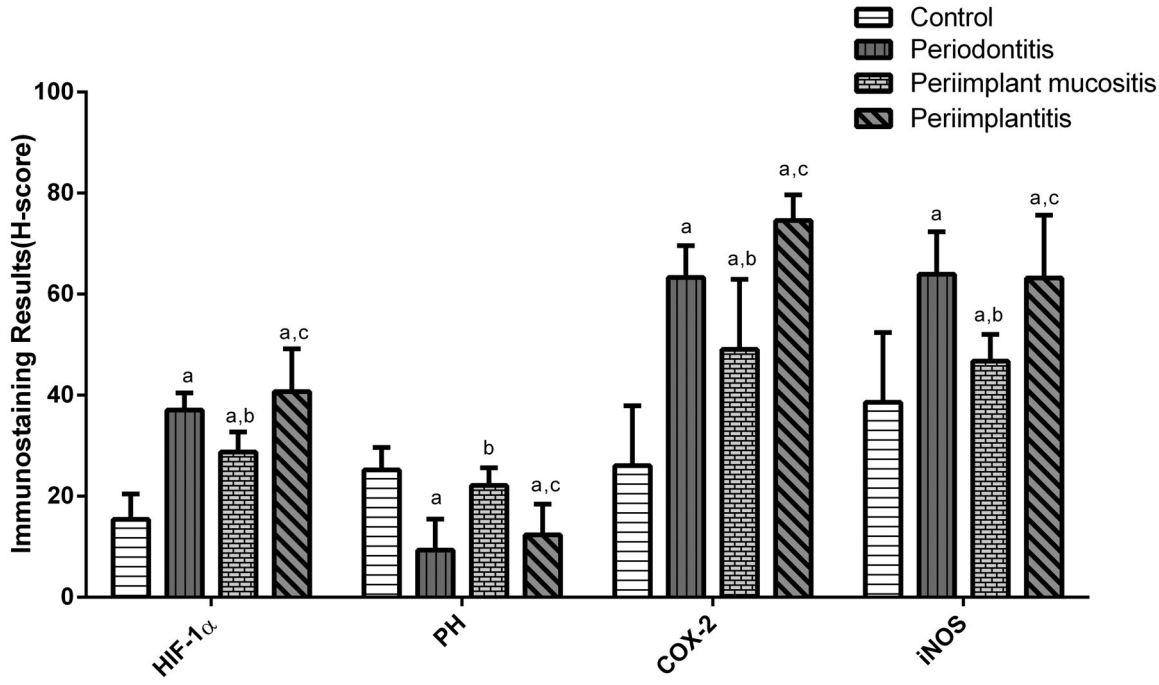

Figure 3. Graphic demonstrations of immunohistochemistry results of the HIF-1α, PH, COX-2 and iNOS. [a]$p < .05$ vs. Control group, [b]$p < .05$ vs. Periodontitis group, [c]$p < .05$ vs. Peri-implant mucositis group. HIF-1α: hypoxia-inducible factor-1α; PH: prolyl hydroxylase; COX-2: cyclooxygenase-2; iNOS: inducible nitric oxide synthase.

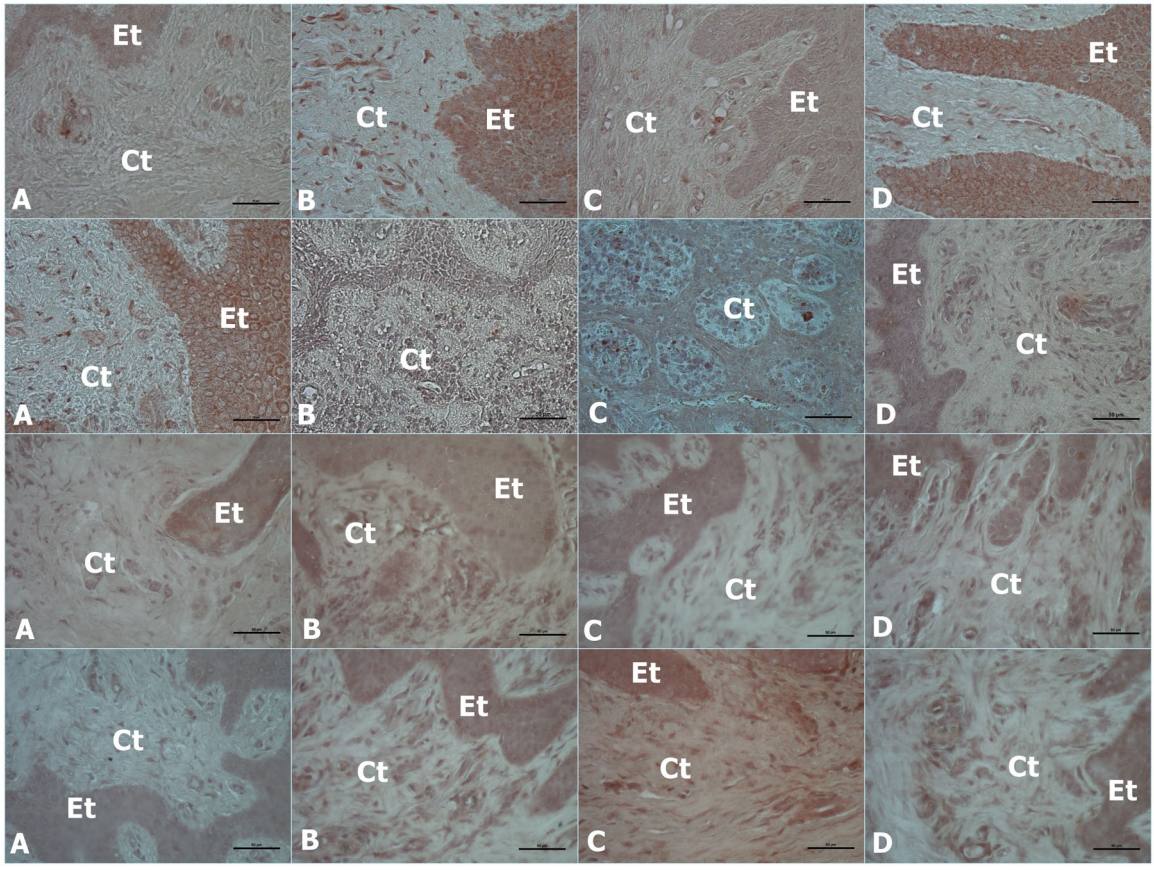

Figure 4. Representative immunohistochemistry images of the HIF-1α, PH, COX-2 and iNOS in the study groups. First row presents HIF-1α staining, second row presents PH staining, third row presents COX-2 staining and fourth row presents iNOS staining. A: Control group; B: Periodontitis group; C: Peri-implant mucositis group; D: Peri-implantitis group. HIF-1α: hypoxia-inducible factor-1α; PH: prolyl hydroxylase; COX-2: cyclooxygenase-2; iNOS: inducible nitric oxide synthase. Ct: connective tissue; Et: Epithelial tissue

observed. Higher inflammatory cell counts were observed in periodontitis and peri-implantitis groups compared to the peri-implant mucositis and control groups. The control group also had lower inflammation than peri-implant mucositis.

Hypoxia is the oxygen deficiency that triggers a set of events causing inflammation and collagenase activity and contributes to the pathogenesis of several chronic inflammatory diseases being one as periodontitis through its main regulatory protein, HIF-1α [4,6,10–13,36–39]. Furthermore, studies reported that hypoxia could affect cell densities, tissue composition and homeostasis in connective tissue [2,4,5,39–42]. Inflammation has a significant role in hypoxia-related tissue alterations since IL-1β, RANKL and other pro-inflammatory cytokines induce hypoxia and hypoxia also up-regulates inflammatory cytokines [4,9,38,39]. NF-κB is also a significant participant in hypoxia-related inflammation and collagenase activity [7,9,43]. Apart from the inflammation, another possible reason for the decreased cell density might be the apoptosis or autophagy of periodontal and gingival cells, which was caused by hypoxia [44,45]. Increased apoptosis and autophagy were also shown by Song et al. accompanied an increase in MMP-8 and IL-1β levels [45]. Gölz et al. demonstrated that COX-2, MMP-1, -2, -9, IL-1β and NF-KB levels increased along with increased HIF-1α and VEGF expressions in hypoxia [7]. In terms of inflammation, the present results showed that both periodontitis and peri-implantitis groups had iNOS and COX-2 expressions with a similar pattern as HIF-1α. Peri-implant mucositis group had higher inflammatory marker levels than control but lower than periodontitis and peri-implantitis groups, which are similar to the inflammatory cell counts. Both iNOS and COX-2 levels increased in periodontitis and peri-implantitis lesions as well as fibroblast and inflammatory cell counts and HIF-1α expressions. On the other hand, neutrophil collagenase, MMP-8 was lower in the healthy group and similarly higher in the periodontitis, peri-implant mucositis and peri-implantitis groups. As for TIMP-1 levels, unlike MMP-8, all groups exhibited similar levels. Periodontitis and peri-implantitis samples had similar levels of inflammatory markers; however, the peri-implant mucositis samples as the precursor of peri-implantitis had lower inflammation compared to the peri-implantitis samples.

All gingival tissues including healthy tissues, gingival tissues with gingivitis, or periodontitis can exhibit hypoxia and HIF-1α [2,5,28,39,41]. Afacan et al. recently reported that pro-inflammatory cytokine TNF-α and hypoxia markers HIF-1α and VEGF levels significantly increased in patients with periodontitis, and these markers were found to be associated with the site-specific clinical periodontal parameters [5]. Contrarily, another clinical study demonstrated no significant change in HIF-1α levels either in healthy controls or in periodontitis patients while VEGF levels increased in periodontitis patients [28]. Ng et al. also reported elevated HIF-1α, VEGF and TNF-α expressions in periodontitis compared to controls [39]. De Araujo et al. recently found that HIF-1α and neutrophil marker CD15 were significantly higher in peri-implantitis tissues compared to healthy peri-implant tissues suggesting a possible role in the pathogenesis of peri-implantitis [46]. Existence of bacteria and lipopolysaccharides in periodontal tissues directly induce HIF-1α via Toll-like receptors and cause hypoxic alterations even in the normoxic environments and inhibition of HIF-1α recovers inflammation [6,7,40,42,43]. Therefore, PH has great importance in suppressing HIF-1α activity and regaining tissue homeostasis [47]. Similar to Afacan et al. and de Araujo et al., the present results also revealed that HIF-1α levels increased in periodontitis, peri-implant mucositis and peri-implantitis patients indicating an association to inflammatory status [5,46]. On the other hand, PH, the inhibitor of HIF-1α was higher in the control and peri-implant mucositis groups than periodontitis and peri-implantitis groups. HIF-1α was found to be elevated in periodontitis, peri-implantitis and peri-implant mucositis samples, while PH elevated in only periodontitis and peri-implantitis patients.

The results of the present study showed that peri-implantitis and periodontitis samples exhibited similar histological results, which were mainly composed of the detection of protein levels of gingival tissues. This finding is compatible with the report of Berglund et al. (1992), who have stated gingiva and peri-implant mucosa had similar inflammatory cell infiltration levels in an experimental model [48]. Nonetheless, evaluation of the parameters in the expression levels and detecting the related pathways might have revealed a difference between periodontitis and peri-implantitis lesions. In this respect, examination of different signal molecules and pathways such as RANKL/OPG, NF-κB, Wnt or Nrf2 signalling could provide a better understanding of the mechanism involved in hypoxia and inflammation in periodontitis and peri-implantitis lesions. The present results also revealed that peri-implant mucositis, as the precursor of the peri-implantitis, showed lower HIF-1α levels, inflammatory cell counts, inflammatory mediators COX-2 and iNOS and higher fibroblast cell counts compared to the peri-implantitis samples. However, all evaluated parameters in peri-implant mucositis group, except for the TIMP levels, were higher than the healthy controls. Furthermore, the results would be more comparable with the involvement of a group with reversible inflammatory status in gingiva, a gingivitis group.

The lack of a gingivitis group is one of the major limitations of the present study. And also, these results should be interpreted considering that this is a histological study, which is another significant limitation of the current study design since not using a stereological evaluation in the histological analysis might have caused more subjective results.

Conclusions

In conclusion, the present study found that periodontitis and peri-implantitis had similar connective tissue compositions in terms of fibroblast and inflammatory cells while it is quite different from peri-implant mucositis. Inflammatory mediators' iNOS, COX-2 and neutrophil collagenase MMP-8 expressions also exhibited a similar pattern. However, the present study is a histological study, which is a significant limitation of the current study design. Examination of different signal

molecules and pathways such as RANKL/OPG, NF-κB, Wnt or Nrf2 signalling could provide a better explanation of the mechanism involved in hypoxia and inflammation in gingival tissues around teeth and dental implants.

Disclosure statement

The authors declare that there are no conflicts of interest in this study.

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