Archives of Oral Biology 136 (2022) 105385

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Immunohistochemical evaluation of IL-1β, IL-6, TNF-α and IL-17 cytokine expression in peripheral giant cell granuloma and peripheral ossifying fibroma of the jaws

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ARTICLE INFO

Keywords:
Peripheral giant cell granuloma
Peripheral ossifying fibroma
Inflammatory cytokines
Immunohistochemistry

ABSTRACT

Objective: To examine and compare the immunohistochemical expressions of IL-1β, IL-6, IL-17 and TNF-α in peripheral giant cell granuloma (PGCG) and peripheral ossifying fibroma (POF).
Design: The study included 20 POF and 20 PGCG cases diagnosed at the Pathology Department of Eskişehir Osmangazi University Medical Faculty. Hematoxylin & Eosin-stained slides obtained from each biopsy specimen were re-evaluated, and IL-1β, IL-6, IL-17 and TNF-α antibodies were investigated immunohistochemically. While staining in stromal cells was examined in POF cases, staining in both stromal spindle cells and multinucleated giant cells was evaluated in PGCG cases. An immunoreactivity score was established for each case by evaluating the staining percentage and intensity for each individual case. The significance level was set at 5% ($p < 0.05$).
Results: The level of IL-6 and TNF-α expressions in the multinucleated giant cells in PGCG lesions was found higher than that in stromal cells ($p < 0.005$ and $p < 0.000$, respectively). In PGCG lesions, there was no significant difference between giant cells and stromal cells in terms of IL-1β and IL-17 expression levels. There was no significant difference between PGCG and POF lesions in terms of IL-1β and IL-6 expression. TNF-α expression levels were significantly higher in spindle cells of PGCG lesions than that of POF lesions ($p < 0.00$). However, IL-17 expression levels were significantly lower in PGCG lesions than in POF lesions ($p < 0.05$).
Conclusion: The study results showed that TNF-α expression was significantly higher in PGCG lesions and IL-17 expression in POF lesions. IL-1β, IL-6, IL-17 and TNF-α are involved in the pathogenesis of both PGCG and POF lesions.

1. Introduction

Reactive lesions are characterized by excessive proliferation of connective tissue in response to chronic irritations (Verma et al., 2012). These lesions are exclusively seen in the oral cavity and include fibroepithelial hyperplasia(FH), pyogenic granuloma(PG), peripheral ossifying fibroma(POF), and peripheral giant cell granuloma(PGCG). Reactive lesions mostly show proliferation in stromal and parenchymal components and commonly occur on the gingiva.

PGCG is a lesion originating from the periosteum, connective tissue or periodontal ligament, and is the hyperplastic, reparative connective tissue response of the gingival tissue to injury. Giant Cell Granuloma (GCG) was first described by Jaffe (1953) as a giant cell reparative granuloma of the jawbones. PGCG tends to occur more frequently between the ages of 40 and 60, more frequently in women and in the mandible rather than the maxilla (Dojcinovic, Richter, & Lombardi, 2010). Although its etiology is not fully understood, it is thought to be associated with irritant and traumatic factors on the basis of poor oral

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https://doi.org/10.1016/j.archoralbio.2022.105385
Received 11 August 2021; Received in revised form 16 January 2022; Accepted 19 February 2022
Available online 21 February 2022

hygiene (Katsikeris, Kakarantza-Angelopoulou, & Angelopoulos, 1988). In the microscopic examination of PGCGs, fibroblastic spindle mesenchymal cells and osteoclast-like multinuclear giant cells are seen. Bleeding, hemosiderin pigment accumulation, dystrophic calcification and ossified tissue can be found within the lesion. Acute and chronic inflammatory cell accumulations are frequently observed. POF is a gingival growth composed of cellular fibroblastic tissue that may contain one or more mineralized tissue, such as bone, cement-like material, and dystrophic calcification (Sacks, Amrani, & Anderson, 2012). The first description of this pathology was made in 1844 by Shepherd et al. as "alveolar exostosis"; later in 1972, Eversol and Rovin proposed the term "peripheral ossifying fibroma"(Kfir, Buchner, & Hansen, 1980; Yadav & Gulati, 2009). Although the etiology of POF is not yet clear, it is thought to arise from inflammatory hyperplasia of periodontal ligament cells(Barot, Chandran, & Vishnoi, 2013). Chronic irritation of the periosteum and periodontal ligament causes metaplasia in connective tissue, resulting in bone formation and dystrophic calcification(Mishra, 2013). More than 60% of the POF cases are in the maxillary bone, and 50% of them are in the anterior region(Mishra, Bhishen, Mishra, & Mishra, 2011). This lesion often occurs in young adults, in the 2nd and 3rd decades, and more often in women(Bodner & Dayan, 1987). Histopathologically, the lesion manifests as a mass of excessive cellular connective tissue containing fibroblasts and fibrocytes and, in some cases, mineralized areas with multinucleated giant cells. Mineralization can consist of bone, cement-like material, and dystrophic calcification.

Because PGCG and POF share some histological features such as varying amounts of giant cells, focal bone formation, abnormal connective tissue proliferation, it can sometimes be difficult to distinguish these two lesions from each other both clinically and histologically. However, PGCG consists of relatively more immature and loose components compared to POF. POF appears likely to be a later stage lesion with more morphologically mature components(Prasad, Reddy, Patil, Kalburgi, & Puranik, 2008). POF has been reported to represent maturation of a pre-existing PG or PGCG (John, Kandasamy, & Achuthan, 2016). Histologically, lamellar or woven bone can be seen in PGCG, especially in older lesions. The bony trabeculae seen on PGCG are usually continuous with the underlying alveolar bone. However, the bone or cement-like material seen in POF is not usually lamellar and shows no connection with the alveolar bone. POF has a very specific mesenchymal component, with radiating fibroblast bundles and collagen fibers, according to PGCG. In addition, ulceration of the overlying epithelium and inflammatory cell infiltration within the lesion are less frequent in POF cases compared with PGCG (Dereci, Akgün, Celasun, Öztürk, & Günhan, 2017).

The histogenesis of PGCG and POF lesions is still not fully elucidated. The development of PGCG and POF lesions is considered to be inflammatory in nature. Inflammation is the body's defense mechanism and the organism tries to repair the damaged tissue by trying to remove the factor that causes inflammation. However, the increased reactive inflammatory response may cause failure, deterioration or excessive growth in organs and tissues as complications. Inflammation involves vascular and cellular responses, and many chemical mediators play a role in this response. One of these molecules that play a role in inflammation are cytokines, some of which act as pro-inflammatory and others as an anti-inflammatory (Baggiolini, Dewald, & Moser, 1997). Cytokines, which are polypeptides produced and secreted by various cell types, regulate immune and inflammatory events, including inflammation, cell growth, healing, and systemic response to injury. The stimulation of macrophages by diseases such as inflammatory infectious diseases, autoimmune diseases, neoplastic diseases, vascular diseases, and trauma causes secretion of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Tominaga et al., 2004). The roles of TNF-α, IL-6 and IL-1β in osteolytic lesions and pathological bone resorption have been proven (Steeve, Marc, Sandrine, Dominique, & Yannick, 2004). These osteoclastogenic cytokines have been investigated in giant cell tumors (GCTs) of long bones by several in-vivo and in-vitro studies (Liu, Yu, & Li, 2003). Previous studies have clinically investigated the relationship between the level of interleukin-17 (IL-17) family in serum, saliva, and gingival fluid and periodontitis, with a focus on IL-17A.IL-17B, IL-17 C, IL-17D, IL-17 F and IL-17A/F were all reported to have a proinflammatory function (Gu, Wu, & Li, 2013). In recent years, studies have been conducted to investigate the inflammatory cytokine expression in giant cell granulomas seen in the jaws (de Matos, de Moraes, Nonaka, de Souza, & de Almeida Freitas, 2012; Papanicolaou, Chrysomali, Stylogianni, Donta, & Vlachodimitropoulos, 2012; Souza et al., 2005). There are no studies in the literature regarding the expression of pro-inflammatory cytokines in POF lesions which is considered to have similar etiopathogenesis with PGCG. In this study, IL-1β, IL-6, TNF-α and IL-17 cytokine expressions will be investigated immunohistochemically in both PGCG and POF lesions. The aim of this study is to contribute to the elucidation of the histopathogenesis of both lesions by revealing the presence, role, and possible differences between proinflammatory cytokines in PGCG and POF lesions with similar etiopathogenesis.

2. Materials and methods

2.1. Ethical statement

The ethics approval was obtained from the "Non-Drug Clinical Research Ethics Committee of Eskişehir Osmangazi University Faculty of Medicine" (decision date and number: 28 May 2015-08) and was carried out in full compliance with the principles of the Declaration of Helsinki. The study was supported by Eskişehir Osmangazi University Scientific Research Projects Commission (project number: 2015-769).

2.2. Study design and groups

This study includes POF and PGCG patients, who were treated in the Department of Oral and Maxillofacial Surgery of the Faculty of Dentistry in Eskişehir Osmangazi University and whose pathological examinations were performed at the Department of Pathology of the Faculty of Medicine in Eskişehir Osmangazi University. Based on data from a previous study (de Matos et al., 2012), the sample size was calculated as a total of 40 samples at least 20 samples from each group using the G*Power version 3.1.9.2 program(Heinrich-Heine-Universitat, Dusseldorf, Germany; power 0.85, a = 0.05). The clinical data and paraffin blocks of 40 patients with PGCG and POF that had been treated with surgical excision and curettage were randomly selected from the laboratory archive. Study patients were allocated into two groups. Group 1 included 20 cases of POF and Group 2 comprised 20 cases of PGCG. Hematoxylin & Eosin-stained slides obtained from each biopsy specimen were re-evaluated, diagnoses have been confirmed and the paraffin blocks best representing the morphology were selected for immunohistochemical analyses.

2.3. Immunohistochemical staining

3μ thick sections were taken from the tissues.Immunohistochemical staining was performed with anti-TNF-α (polyclonal, dilution: 1/100, Abcam, USA), anti-IL-6 (monoclonal, dilution: 1/150, Abcam, USA), anti-IL-17 (polyclonal, dilution: 1/100, Abcam, USA) and anti-IL-1β (polyclonal, dilution: 1/100, Abcam, USA) antibodies.Positive controls used for these antibodies were as follows: human gastric carcinoma for anti-TNF-α, prostate tumor tissue for anti-IL-6, human small intestine tissue for anti-IL-17, and human lymph node for anti-IL-1β. After the sections were taken, the treatment was performed with distilled water for 1 min, with 3% hydrogen peroxidase for 10 min, with distilled water for 1 min, with phosphate-buffered saline (pH 7.4) for 2 × 3 min, and with blocking solution for 5 min. An antigen retrieval procedure was applied with citrate buffer (pH 6). Then, the incubation process was applied with primary antibodies. Afterwards, the treatment was

performed with 2 × 3 min with phosphate-buffered saline, 20 min with link solution, 2 × 3 min with phosphate- buffered saline, 20 min with streptavidin, 2 × 3 min with phosphate-buffered saline, 3 min with AEC chromogen, 2 × 1 min with distilled water, 30 min with hematoxylin dye, 3 × 1 min with distilled water.

2.4. Evaluation of immunoreactivity

The evaluation of immunoreactivity was done in a semi-quantitative manner as in the study by Papanicolaou et al. (2012). Two pathologists (MFA and OP), blinded to the original diagnoses, evaluated the stained slides. The percentage of positively stained cells and the staining intensity were evaluated at 400x magnification by randomly selected 4 areas on each section. In POF cases, the staining of stromal spindle cells, and in PGCG cases, staining of both stromal spindle cells and multinucleated giant cells were examined.

The following scoring was used for the staining percentage assessment: Score 0: < 10% staining; Score 1: 10–24% staining; Score 2: 25–49% staining; Score 3: 50–74% staining and Score 4: 75–100% staining.

The following scoring was used to evaluate the staining intensity: Score 0: No staining; Score 1: Weak staining; Score 2: Moderate staining and Score 3: Strong staining.

The immunoreactivity score (IRS) value was found by multiplying the staining percentage score and the staining intensity score for each field (IRS = Staining percentage x Staining intensity). The average of four randomly selected fields was accepted as the immunoreactivity score of the respective sample.

2.5. Statistical analysis

Data were analyzed in the Statistical package for social sciences, version 22.0 (SPSS Inc. Chicago, Ill. USA). If the data showed a parametric distribution, the Independent variables t-test was used, if they showed a non-parametric distribution, the Mann-Whitney U test was used. While mean and standard deviation values are given for normal distributions, median and percentage values are given for non-normal distributions.A level of $p < 0.05$ was accepted as the limit of significance.

3. Results

3.1. Clinical features of the cases

PGCG cases were in the 8–85 age range (mean age 44.2 years). Seventy percent of the cases were women, 30% were men, and the female to male ratio was about 2:1. In seventy-five percent of the cases, the lesions were in the mandible and 25% in the maxilla. Sixty percent of the cases in the maxilla were seen in the anterior region, 40% in the posterior region, while 46% of the cases in the mandible were in the anterior region and 54% in the posterior region. In addition, two cases seen in the maxilla occurred in the anterior edentulous alveolar crest.

The ages of patients with POF lesions were between 12 and 68 years (mean age 42.9 years). Similar to PGCG cases, 70% of the cases were female and 30% male.Sixty percent of the lesions were observed in the maxilla and 40% in the mandible.It was observed that 66.7% of the maxillary cases were located in the anterior region and 33.3% in the posterior region. Sixty-two point five percent of the cases in the mandible were located in the anterior region and 37.5% in the posterior region. It was observed that a case seen in the posterior mandible appeared in the edentulous alveolar crest.

3.2. Immunohistochemical findings

While the expressions of inflammatory cytokines in PGCG lesions were examined in both multinucleated giant cells and spindle stromal cells, inflammatory cytokine expressions in POF lesions were examined only in spindle stromal cells.

The expressions of inflammatory cytokines IL-1β, IL-6, IL-17 and TNF-α were compared between spindle stromal cells and multinucleated giant cells in PGCG cases (Table 1). IL-1β expression showed widespread and strong positivity in both giant cells and spindle stromal cells (Fig. 1A and B). With IL-17, some giant cells showed weak to moderate staining, while spindle stromal cells showed weak staining (Fig. 1C). There was no statistically significant difference in terms of IL-1β and IL-17 expressions between multinucleated giant cells and stromal cells ($p = 0.506$ and $p = 0.201$, respectively). However, a significant difference was found between the multinucleated giant cells and stromal cells in terms of IL-6 and TNF-α expressions ($p < 0.05$ and $p < 0.00$, respectively). With IL-6, some giant cells showed weak to moderate staining, while a few spindle stromal cells showed weak staining (Fig. 1D). Similarly, most giant cells showed weak to moderate staining with TNF-α, while scattered spindle stromal cells showed weak staining (Fig. 1E and F). As a result, In PGCG cases, it is observed that IL-6 and TNF-α expressions were significantly higher in multinucleated giant cells compared to stromal cells.

The comparison of cytokine expressions values in spindle stromal cells of PGCG and POF cases was shown in Table 2. In PGCG cases, the highest IRS value was observed in IL-1β and the lowest IRS value was seen in IL-6, whereas in POF cases, the highest IRS value was seen in IL-1 and the lowest IRS value was seen in TNF-α. When cytokine expressions in POF and PGCG spindle cells were compared, diffuse positivity for IL-1β was seen in both lesions (Fig. 2A and B). With this, a small number of IL-6 expressions were observed in spindle cells in both lesions (Fig. 2C and D). There was no significant difference between PGCG and POF cases in terms of IL-1β and IL-6 cytokine expressions in spindle stromal cells ($p = 0.799$ and $p = 0.157$, respectively). However, there was a significant difference in spindle stromal cells of both lesions in terms of IL-17 and TNF-α. IL-17 expression was found to be significantly higher in spindle stromal cells of POF cases compared to PGCG cases ($p < 0.05$) (Fig. 2E and F), while TNF-α expression was found to be significantly higher in spindle stromal cells of PGCG lesions ($p < 0.00$) (Fig. 2G and H).

4. Discussion

The histogenesis of PGCG and POF lesions is still not fully elucidated. Innovative studies in lesions with giant cells have focused on multinucleated giant cells with properties similar to osteoclasts, bone resorption, responses to calcitonin, specific monoclonal antibodies attached to osteoclasts, and tartar-resistant acid phosphatases (Flanagan, Tinkler, Horton, Williams, & Chambers, 1988). In the last 10 years, studies have been conducted to investigate the inflammatory cytokine expression in giant cell granulomas seen in the jaws. In this study, firstly, the expressions of pro-inflammatory cytokines (IL-1β, IL-6, IL-17 and TNF-α) in PGCG lesions were examined immunohistochemically in both multinucleated giant cells and spindle-shaped stromal cells. IL-17, whose existence and role has been proven in periodontal infections, was demonstrated in PGCG and POF lesions in this study.

Table 1
Comparison of Immunoreactivity Score (IRS) values of IL-1β, IL-6, IL-17, and TNF-α in multi-nucleated giant cells and stromal spindle-shaped cells of PGCG.

Cytokines	Multi-nucleated giant cells Median ± SD	Spindle-shaped cells Median ± SD	P value
IL-1β	9.37 ± 3.20	8.25 ± 3.16	0.506
IL-6	2.12 ± 1.45	0.75 ± 0.80	0.014*
IL-17	2.87 ± 1.88	2.00 ± 2.17	0.201
TNF-α	5.12 ± 2.04	1.00 ± 1.01	0.000**

IL: Interleukin; PGCG: peripheral giant cell granuloma; TNF: Tumor necrosis factor.
* $p < 0.05$; ** $p < 0.001$.

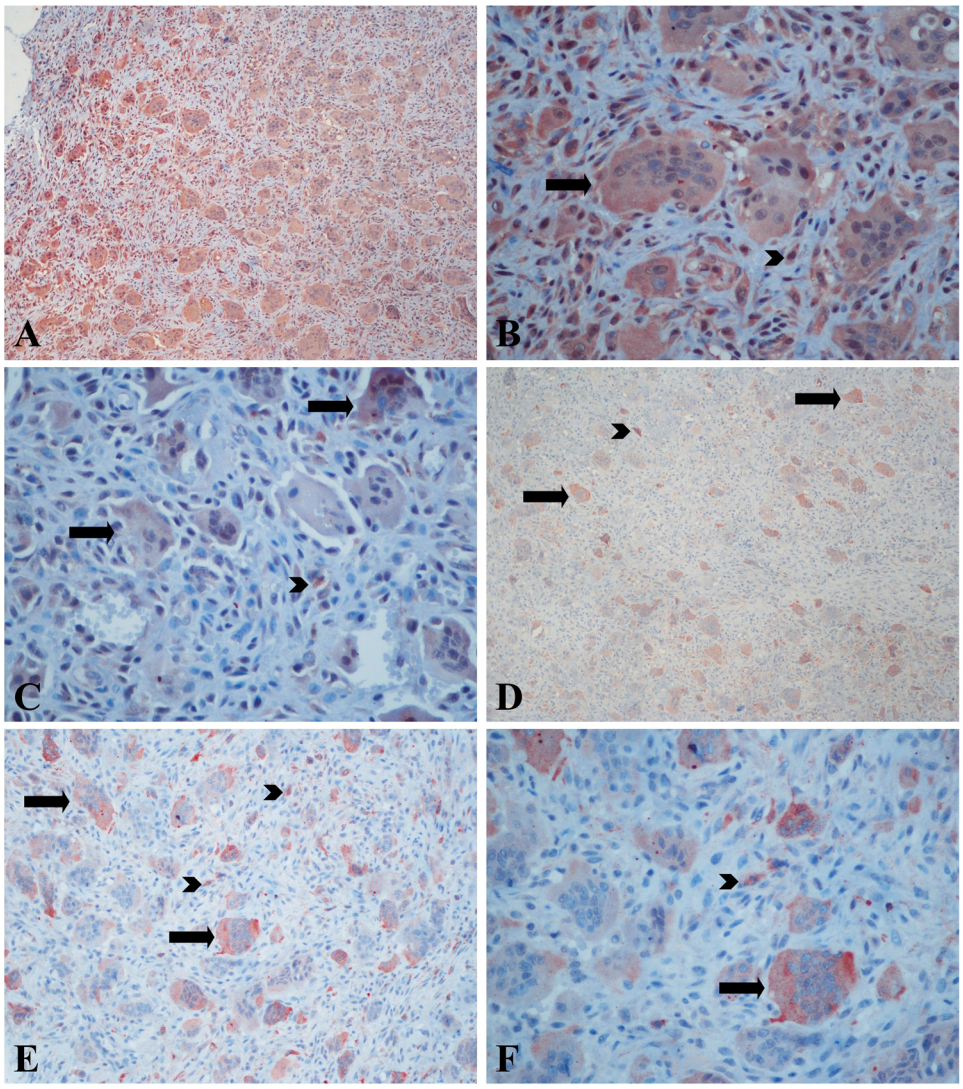

Fig. 1. Immunohistochemistry of PGCG. A. Widespread and strong positivity in both giant cells and spindle stromal cells for IL-1β (x100). B. High power view showing widespread positivity for IL-1β in both giant cells (arrow) and spindle stromal cells (arrowhead) (x400). C. Weak to moderate staining in some of the giant cells(arrow) and weak staining in scattered spindle stromal cells (arrowhead)for IL-17 (x400). D. Weak to moderate staining in some of the giant cells (arrow) and weak staining in a few spindle stromal cells (arrow head) for IL-6 (x400). E and F. Weak to moderate staining in most of the giant cells (arrow) and weak staining in scattered spindle stromal cells (arrowhead) for TNF-α (x200andx400, respectively).

Table 2
Comparison of IRS values in spindle stromal cells of PGCG and POF.

Cytokines	PGCG (n = 20) Median ± SD	POF (n = 20) Median ± SD	P value
IL-1β	8.25 ± 3.16	8.25 ± 4.13	0.799
IL-6	0.75 ± 0.80	1.25 ± 2.54	0.157
IL-17	2.00 ± 2.17	4.37 ± 3.35	0.026*
TNF-α	1.00 ± 1.01	0.37 ± 0.42	0.002*

IL: Interleukin; PGCG: peripheral giant cell granuloma; POF: peripheral ossifying fibroma;
IRS: immunoreactivity score; TNF: Tumor necrosis factor. * $p < 0.05$.

The most important pro-inflammatory cytokines are IL-1β, IL-6 and TNF-α. The role of TNF-α, IL-6 and IL-1β in osteolytic lesions and pathological bone resorption has been proven (Steeve et al., 2004). These osteoclastogenic cytokines have been investigated in giant cell tumors of long bones by several in-vivo and in-vitro studies (Liu et al., 2003). TNF-α expression has also been studied in patients with giant cell tumors (GCTs)of the jaws (Amaral et al., 2010). In a study evaluating TNF-α expression in circulating lymphocytes and monocytes of patients with central giant cell lesions in their jaws, it was observed that TNF-α expression increased in CD4 (+) T cells and decreased in CD68 monocytes (Souza et al., 2005). Atkins et al. (2000) observed that TNF-α, IL-1 and IL-6 mRNA expression increased in GCTs. The expression of TNF-α, IL-6 and IL-1β in giant cells and stromal cells in PGCG lesions was first demonstrated in the study of Papanicolaou et al. (2012). In their study, TNF-α, IL-6, and IL-1β expression in both peripheral and central giant cell granulomas were significantly higher in giant cells compared to stromal cells. In the present study, the expression of IL-1β, IL-6, IL-17 and TNF-α in PGCG cases was observed to be higher in giant cells compared to spindle stromal cells. This increase was found to be significantly higher in IL-6 and TNF-α.

TNF-α and IL-6 may play a critical role in the regulation of bone resorption in multinucleated giant cells. Although the main source of IL-6 in bone is osteoblastic cells and stromal cells, IL-6 is effective on osteoclastogenesis in the bone (Steeve et al., 2004). IL-6 activates osteoclasts and causes bone resorption (Sun, Shu, Zhang, & Wu, 2008). IL-6 can also indirectly increase osteoclastogenesis by increasing the release of receptor activator of NF-κB ligand (RANKL) (Taga, 1997). Gamberi et al. (2004) found that IL-6 expression was increased in GCTs with high biological aggressiveness without significant difference in giant cells and stromal cells. Amaral et al. (2010) reported increased transcription of the nuclear factor of activated T cells (NFAT-c1), which is required for differentiation of osteoclasts in PGCG and SGCG lesions, and the authors claimed that the development of giant cell lesions in the jaws was regulated by overexpression of NFAT-c1. de Matos et al. (2012) showed that the interactions between transforming growth factor-β (TGF-β) and TNF-α in giant cell lesions may be important in

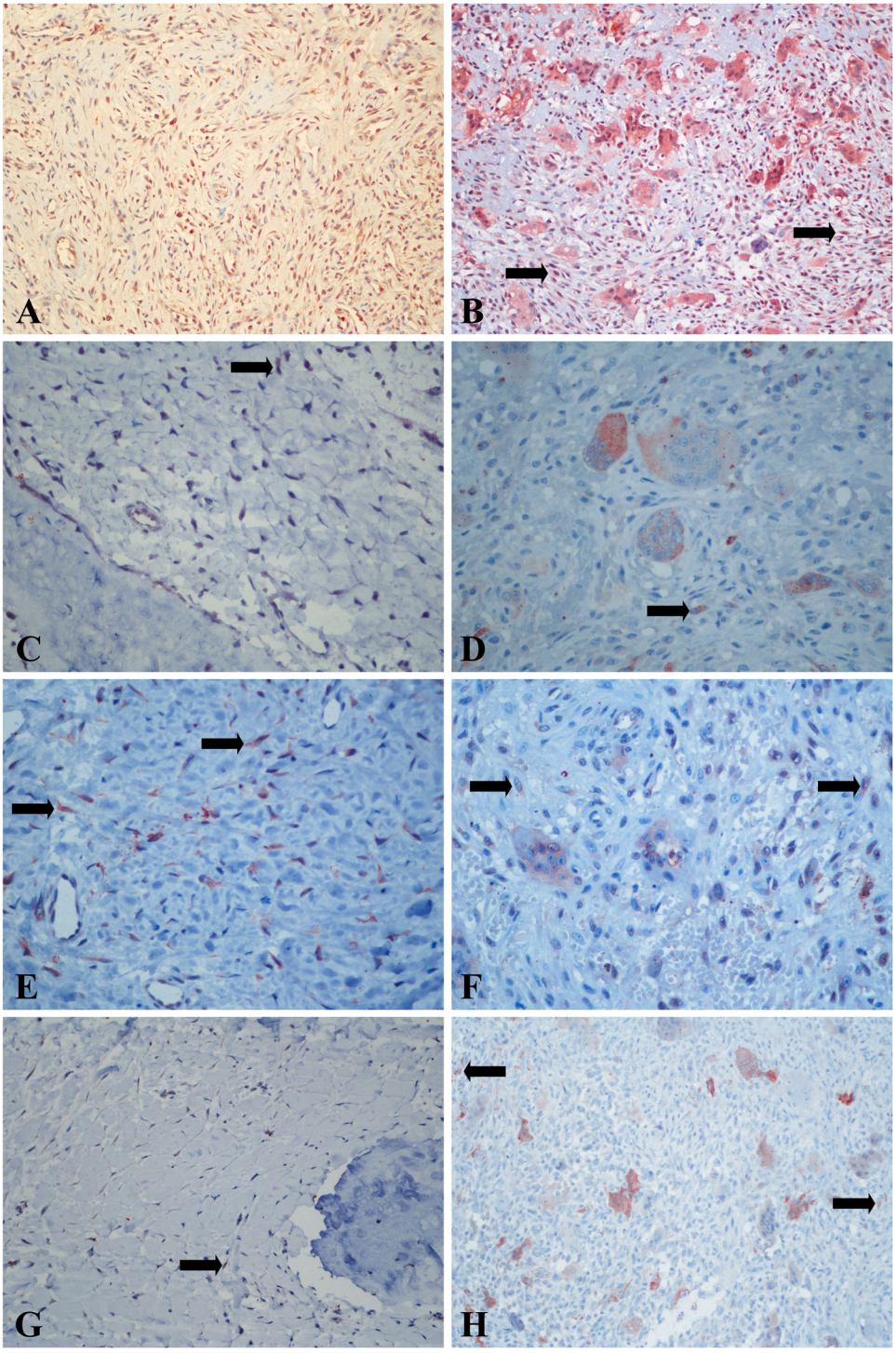

Fig. 2. Comparison of cytokine expressions in spindle-cells of POF and PGCG. A. Widespread positivity for IL-1β in spindle cells of POF (x200), B. Widespread positivity for IL-1β in spindle-cells of PGCG (x200). C.Expressions of IL-6 in a small number of spindle cells of POF (arrow) (x400), D. Expressions of IL-6 in rare spindle-cell of PGCG (arrow) (x400). E.Expressions of IL-17 in the spindle cells of POF (arrow) (200), F. Expressions of IL-17 in a small number of spindle-cells of PGCG (arrow) (x200), G.Expressions of TNF-α in rare spindle-cell of POF (arrow)(x100), H. Expressions of TNF-α in a small number of spindle cells of PGCG (arrow) (x200).

osteoclastogenesis and bone resorption. Because it was seen that TNF-α did not induce the increase of NFAT expression in the absence of TGF-β.

In present study, PGCG and POF lesions were compared for the level of inflammatory cytokine expression in stromal cells. While IL-17 expression increased significantly in POF lesions, TNF-α expression increased significantly in PGCG lesions. TNF-α is a multifunctional cytokine released by activated monocytes, macrophages and T lymphocytes and regulates immune responses, growth, differentiation and further production of other cytokines, inflammatory mediators and enzymes. TNF-α is a potent inducer of bone resorption that stimulates osteoclast differentiation and activation. The high level of TNF-α in PGCG cases is consistent with the results of similar studies in the literatüre (Amaral et al., 2010; de Matos et al., 2012). de Matos et al. (2012) investigated TNF-α expression in peripheral and central giant cell lesions of the jaws. In their study, the immunohistochemical expression intensity of TNF-α was observed as score 4, the expression rate was found to be 40% in SGCGs and 55% in PGCGs. Amaral et al. (2010) investigated the TNF-α mRNA expression level in SGCG and PGCG lesions, but no significant difference was found between the two lesions. In PGCG, TNF-α, IL-6, and IL-1β associations can control the cellular activities of different cell populations (multinucleated cells, monocytes/macrophages, spindle fibroblasts/osteoblasts), possibly mainly

contributing to the mechanisms of lesion growth (Papanicolaou et al., 2012). Inflammatory cell infiltration within the lesion is observed more frequently in PGCG cases compared to POF (Dereci et al., 2017). Higher expression of TNF-a in PGCG lesions compared to POF lesions may be explained by the more inflammatory character of PGCG lesions.

In recent years, studies have been conducted on the role of IL-17 in periodontal inflammation. It has been reported that IL-17 stimulates the expression of IL-23 in human periodontal ligaments (Zhu et al., 2011). It has been reported that IL-17 regulates bone resorption by increasing RANKL expression and decreasing osteoprotegerin (OPG) expression in human periodontal ligaments (Lin et al., 2015). It has also been shown that IL-17 stimulates osteo/odontoclastogenesis by inducing IL-6 (Hayashi et al., 2012). Shibata, Shintaku, Matsuzaki, and Uematsu (2014) reported that IL-17 stimulates the production of matrix metalloproteinase-1 (MMP-1) in human periodontal ligaments by stimulating IL-6. Recently, several studies (Beklen et al., 2007; Shibata et al., 2014; Wu, Zhu, Wei, & Peng, 2013)have shown that IL-17 increases IL-6 and MMP-1 production in human periodontal ligaments. Beklen et al. (2007) reported that IL-17 may play a role in tissue destruction in the periodontitis by stimulating the production of MMP-1 and MMP-3 in gingival fibroblasts.

Since POF and PGCG lesions exhibit similar histopathological features, differential diagnosis of these two lesions is important. Hybrid lesions with histopathological features similar to both PGCG and POF lesions have been described in the literature (Katsikeris et al., 1988). The diagnosis of these hybrid lesions is made according to the dominant morphological features. While there is an increase in the accumulation of type 3 collagen in the early and immature stages of wound healing, type 1 collagen, which has a great resistance to tensile forces, dominates in the late stages (Montes & Junqueira, 1991). In a study on hybrid lesions, it was observed that collagen type 1 was dominant in POF-like areas, while collagen type 3 was more common in PGCG areas (Dereci et al., 2017). These findings suggest that PGCG lesions have a looser stroma, while the POF stroma is more organized and robust. Therefore, the diagnosis of POsF is based on the presence of distinctive and characteristic stroma consisting of nested collagen bundles mixed with randomly arranged fibroblast-like cells along with bone and/or cement-like material (Ogbureke et al., 2015). In the present study, IL-17 expression was found to be significantly higher in POF cases than PGCG cases. POF lesions contain abundant fibroblasts compared to PGCG lesions. It was reported that IL-17 increases the expression and migration of human periodontal ligament (PDL) fibroblasts by increasing MMP-1. IL-17A regulates periodontal wound healing by controlling migration rather than the proliferation of PDL fibroblasts. IL-17 directly affects the migration of human PDL fibroblasts, and migration of PDL fibroblasts is significantly increased in the presence of IL-17 (Wu et al., 2013). Animal studies have revealed that IL-17A significantly increases pulmonary fibroblast proliferation and type I collagen. Thus, it has been reported that IL 17 participates in the pathogenesis of skin and lung fibrosis by increasing fibroblast proliferation (Lei et al., 2016). It has been stated that IL-17 has critical importance in the proliferation of fibroblastic reticular cells that it metabolically reprograms activated fibroblastic reticular cells for proliferation and survival (Majumder et al., 2019). The findings of this study indicate that IL-17 may contribute more to the histopathogenesis of POF lesions, especially through its effects on periodontal ligament fibroblasts, among other inflammatory events. IL-17's effects on both proliferation and migration of fibroblasts and enhancing type 1 collagen synthesis may cause POF lesions to have a firmer stroma than PGCG lesions and may contribute to proliferative character of POF lesions.

According to the results of the present study, IL-6 and TNF-α cytokine expressions were found to be higher in giant cells compared to stromal cells in PGCG lesions. When PGCG and POF cases were compared, there was no significant difference in both groups in terms of IL-1β and IL-6 expressions. It was observed that, in spindle stromal cells, TNF-α expression was higher in PGCG cases and IL-17 expression was higher in POF cases. In addition, our study demonstrates the positive synergistic role of proinflammatory cytokines in the development of PGCG and POF lesions. Explaining the functional role of cytokines in the development of PGCG and POF lesions may enable strategies for medical treatment of these lesions in the future. More studies are needed to explain the presence, role and mechanism of action of cytokines in reactive lesions of the jaws.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Eskişehir Osmangazi University Scientific Research Projects Commission for financial support (project number: 2015-769). Authors also thank Assoc. Prof. Dr. Ömür DERECİ for his contribution to this study.

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