

## RESEARCH ARTICLE

**Interleukin-1 beta, interleukin-6 and TGF-beta in follicular tissue of impacted third molars**

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**ABSTRACT.** The clinical evaluation and management of impacted third molars remain challenging. The aim of the present study was to investigate possible associations between follicular tissue cytokines and radiographic manifestations of impacted third molar. The population included 72 patients who underwent surgical extraction of impacted third molars. All these patients underwent a preliminary panoramic radiograph. Levels of interleukin-1 beta (IL-1 $\beta$ ), interleukin-6 (IL-6) and transforming growth factor beta (TGF- $\beta$ ) in tissue extracts were determined using ELISA. There were no significant differences between bony and tissue impaction as regards IL-1 $\beta$ , IL-6 and TGF- $\beta$  levels. Moreover, the same results were obtained as far as the amount of pericoronal space and the presence or absence of a history of pericoronitis are concerned. These results suggest that radiographic findings or a history of pericoronitis are not associated with levels of expression of pro-inflammatory cytokines in patients undergoing surgical removal of impacted third molars. However, further studies are needed to address the possibility of variability during disease progression.

**Key words:** pericoronitis, impacted third molars, cytokines

The clinical evaluation and management of impacted third molars remain challenging because of the unreliable correlation between radiographic measures and clinical behavior [1, 2]. Previous studies have suggested that normal pericoronal radiolucency is within the range of 2-3 mm, although this may not be an accurate indicator of pathosis [3-5].

Interleukins play different roles in the human body, including control of inflammation. Interleukin-1 (IL-1) is produced by macrophages, monocytes, fibroblasts and dendritic cells. It stimulates production of prostaglandin E2 and collagenase production by fibroblasts [6, 7]. Furthermore, IL-1 stimulates bone remodeling [8], bone osteoblast proliferation and osteoclast activation [9]. It has been shown that IL-1 affects pulp inflammatory processes in a dose-dependent manner, with a local inflammatory effect at low concentrations, and an endocrine effect at high concentrations [10]. Interleukin-6 (IL-6) is a cytokine that is involved in autoimmune phenomena; it amplifies acute inflammation, followed by a chronic inflammatory response process. It also mediates bone resorption and osteoclast formation in pathological conditions such as rheumatoid arthritis, multiple myeloma, and Paget's disease [11]. Transforming growth factor beta (TGF- $\beta$ ) is a

multifunctional protein that controls proliferation, cellular differentiation, and survival in diverse cell types [12, 13]. It exerts an antiproliferative effect on normal cells and cells in early stages of oncogenesis. Cancerous cells appeared to undergo functional inactivation of the TGF- $\beta$  signaling pathway in which the normal control of cell proliferation and apoptosis regulation is no longer operative [14].

Locating and determining the extent of pericoronal tissue might only reflect morphological changes, and the lack of need for medical intervention. Advances in the field of inflammatory biomarkers provide the chance to couple morphological datasets with inflammatory pathways in an attempt to better understand the properties of specific organs in normal and diseased conditions. Although the presence of inflammation in periodontitis and pericoronitis has been reported as a function of inflammatory markers [15, 16], the characterization of follicular tissue around impacted teeth and its relationship with panoramic radiography has not been undertaken. The aim of the present study was to investigate follicular tissue cytokines in the presence or absence of a history of pericoronitis, and associations between these measures and radiographic manifestations in patients undergoing surgical removal of impacted third molars.

## PATIENTS AND METHODS

Seventy-two patients (20 women and 52 men) were recruited from a patient population who had been referred to Tabriz Oral and Maxillofacial Department Clinic for surgical extraction of impacted third molars. All patients underwent a preliminary, panoramic radiograph (Promax, Planmega Oy, Helsinki, Finland) for determination of impaction type, pericoronal soft tissue width using a digital caliper, and impaction position and location in the jaw (figure 1). Exclusion criteria were age  $\geq 55$  years, cigarette-smoking, local acute infection, corticosteroid or antibiotic treatment during the previous three months, or diabetes. Exclusion of persons with local, acute infection was based on presenting clinical signs including swelling, hyperemia, purulent drainage, and local pain around the impacted area. The study protocol was approved by the Tabriz University of Medical Sciences committee for ethics and humanity.

Clinical characteristics such as pericoronal space width and history of pre-surgical pericoronitis, based on previous localized pain, discharge or swelling, were recorded by the same surgeon. Surgical operations were performed in accordance with routine techniques. All operations were performed by the same surgeon, under local anesthesia and under similar conditions. Dental follicles around impacted third molars were collected, immediately rinsed with phosphate-buffered saline, placed in liquid nitrogen and taken to the laboratory. The frozen specimens were sealed in screw-top vials and preserved at  $-70^{\circ}\text{C}$  until the time of assay.

For protein extraction, 20 mg of tissue specimens were homogenized in ice-cold lysis buffer containing protease inhibitors. The suspension was centrifuged at 14,000xg for 30 seconds at  $4^{\circ}\text{C}$ . Protein concentration in the resultant supernatant was measured using the method of Lowry, with bovine serum albumin as a standard [17]. The concentrations of IL-1 $\beta$ , IL-6 and TGF- $\beta$  were determined in each extract using ELISA (ALPCO Diagnostics, Windham, NH, USA) and an Immunoscan model 310 microplate reader (Labsystems, Helsinki, Finland). Values were normalized to the corresponding total protein and expressed as units of cytokine/mg protein.

## RESULTS

The clinical details of the study subjects are presented in table 1. The majority of patients studied had the bony impaction (76%) type. Radiographic investigation showed 32% of patients had more than 3mm of pericoronal space. The majority of patients (82%) had no history of pericoronitis before surgery. Crowding (39%) and temporomandibular joint problems (32%) were the most frequent complaints reported by patients. The mesioangular position was evident in nearly half (42%) of the cases. The lowest frequency (1.4%) was seen for the inverted and linguoversion types.

Table 2 illustrates the levels of tissue inflammatory markers according to the preoperative radiographs and history of precoronitis. Apart from a non-significant trend towards a higher level of IL-6 ( $p=0.08$ ) in the tissue impaction group, there was no significant difference in IL-1 $\beta$ , IL-6 and TGF- $\beta$  levels between the bony and tissue impaction types. Moreover, the same results were obtained regarding the extent of pericoronal space, jaw location and the presence or absence of a history of pericoronitis. No significant differences were found as regards gender or angulation of teeth.

## DISCUSSION

Although cytokines play extremely important roles in both innate and adaptive immune responses, their adverse effects on growth and bone metabolism have been observed in humans [18]. Recent studies have shown a link between periodontal disease and the production of pro-inflammatory cytokines similar to those seen in osteoclastic bone resorption [9]. The aim of this study was to characterize the relationship between expression of key cytokines by follicular tissue, and the history of pericoronitis and radiographic observations of impacted third molars. Our data demonstrate the lack of any significant association between the levels of cytokines measured and the radiographic space serving as the main diagnostic reference standard [19]. In addition, the association between cytokine expression and history of pericoronitis, which is known to be an important clinical indicator of increased

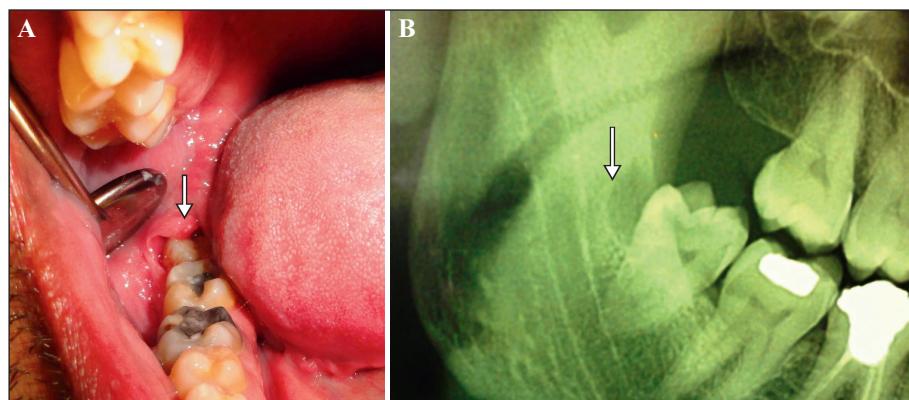


Figure 1

Pericoronitis associated with an impacted mandibular third molar.  
**A)** Photograph showing erythema of the gingiva surrounding the third molar.  
**B)** Radiograph showing a pericoronal radiolucency area distal to the affected tooth.

**Table 1**  
Clinical characteristics of the 72 patients studied<sup>†</sup>.

Age, y	24.6±6.1
Sex, women %	28
Impaction type, % bone/tissue	76/24
Pericoronal space >3 mm, %	32
History of pericoronitis, %	18
Jaw, % maxilla/mandible	23/77
Cytokines in follicular tissue of impacted third molars <sup>‡</sup>	n=72
- Interleukin-1 $\beta$	1.00±0.49
- Interleukin-6	1.87±0.52
- TGF- $\beta$ 1	4.09±2.48

<sup>†</sup> Values are means±SD or percentage with condition. <sup>‡</sup> Expressed as units of cytokine/mg protein.

**Table 2**  
Tissue levels of inflammatory markers according to the pre-operative radiographic findings<sup>†</sup>.

n	Interleukin-1 $\beta$	Interleukin-6	TGF- $\beta$ 1
<b>Pericoronal space width</b>			
<3mm	49	0.98±0.48	1.87±0.56
>3mm	23	1.04±0.51	1.86±0.42
p-value		0.61	0.94
			4.02±2.53
			4.24±2.25
			0.72
<b>History of pericoronitis</b>			
No	59	1.01±0.45	1.92±0.49
Yes	13	0.95±0.62	1.69±0.60
p-value		0.70	0.15
			3.90±2.40
			4.95±2.80
			0.17
<b>Impaction type</b>			
Bone	55	0.99±0.46	1.82±0.52
Tissue	17	1.04±0.56	2.10±0.49
p-value		0.68	0.08
			4.11±2.61
			4.04±2.07
			0.92

<sup>†</sup> Values are means±SD and expressed as units of cytokine/mg protein, p< 0.05 (t-test).

risk of periodontal disease [20], was non-significant. Furthermore, levels of cytokine expression could not be attributed to the impaction position.

The present study confirms observations made by others, of a lack of association between radiographic findings and inflammation in the dental follicle of the third molars [21], suggesting that the relationship between pericoronal space and pathological changes cannot be explained through a link with the cytokine expression profile *per se*, whereas both are independent risk factors for pericoronal lesions. Accordingly, Miller and Bean have shown that pericoronal radiolucency could not be reliably associated with histological findings [22]. However, Beklen *et al.* reported intense staining in a high number of TNF- $\alpha$ - and IL-1 $\beta$ -positive cells in the gingival tissue of patients with pericoronitis compared with healthy controls. The authors concluded that TNF- $\alpha$  and IL-1 $\beta$  may play an important and active role in pericoronitis [15]. There may be differences between follicular and gingival tissues as regards their role in the process of dental development [23]. Levels of IL-6 tended to be higher in the pericoronal soft tissues of bony-impacted as compared to tissue-impacted third molars. These findings might be related to the well-known role of IL-6 in bone pathophysiology [24]. Our data suggest that radiographic evidence of pericoronal space

might not be a sensitive enough indicator of the extent of tissue inflammation around the impacted teeth. These data are similar to those previously recorded in Brazilian subjects [21]. However, this does not exclude the possibility that the radiographic findings for pericoronal tissues may be a consequence of an effect of inflammation on pericoronal soft tissue during early stages. This hypothesis is motivated by the idea that the role of pro-inflammatory cytokines in the earlier stages of pericoronitis, *i.e.* *in situ* macrophage activation [25], is probably more important. To our knowledge, this study is the first clinical study to examine pericoronal soft tissue cytokine levels with regard to the radiographic characteristics of impacted molars. This study focused only on patients who were candidates for surgical removal of impacted teeth, who had no interfering health conditions, and in which the variables of interest (three major cytokines that are involved in inflammation and that are characteristic of pericoronal tissue) were simultaneously included.

In conclusion, our results did not support the hypothesis that radiographic findings or a history of pericoronitis are associated with levels of expression of pro-inflammatory cytokines in patients undergoing surgical removal of impacted third molars. Further studies are needed to address the effect of pericoronal pathology during disease progression, on the relationship between radiographic evaluation and the cytokine expression profile.

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