Analysis of IL-1β, CXCL8, and TNF Levels in the Crevicular Fluid of Patients With Periodontitis or Healthy Implants

Paweł Aleksandrowicz
Uniwersytet Medyczny w Lublinie
Ewa Brzezińska-Błaszczyk
Uniwersytet Medyczny w Lodzi
Elżbieta Kozłowska
Uniwersytet Medyczny w Lodzi
Paulina Żelechowska
Uniwersytet Medyczny w Lodzi
Andrea Enrico Borgonovo
Universita degli Studi di Milano Facolta di Medicina e Chirurgia
Justyna Agier (justyna.agier@umed.lodz.pl)
Uniwersytet Medyczny w Lodzi https://orcid.org/0000-0002-6759-5737

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Abstract

Purpose: The evaluation of periodontal and peri-implant tissue condition is mainly based on clinical examination and imaging diagnostics. Some data implies that an examination of cytokine level in peri-implant sulcular fluid (PISF) might prove useful when evaluating the condition of peri-implant tissues and monitoring the development of peri-implant inflammation, including both mucositis and peri-implantitis. Thus, in this paper, it has been decided to assess the level of TNF, CXCL8, and IL-1β in PISF collected from patients with no clinical symptoms of mucositis or peri-implantitis and compare them with cytokine concentration in gingival crevicular fluid (GCF) acquired from patients with healthy periodontium and those with varying severity of periodontitis.

Materials and Methods: A total of 189 subjects were included in the study, and GCF/PISF samples were checked for TNF, CXCL8, and IL-1β levels using an ELISA test.

Results: We documented that the IL-1β level, in PISF in patients with implants, was significantly lower than in GCF in patients with mild, moderate, or severe periodontitis. We also revealed that their CXCL8 level in PISF was considerably lower than in patients with moderate periodontitis. However, the TNF level in PISF in patients with implants was markedly higher compared to subjects with healthy periodontium or patients with mild periodontitis.

Conclusion: Our observation might imply that the monitoring of TNF, CXCL8, and IL-1β levels in PISF could help with the diagnosis of mucositis/peri-implantitis before any clinical manifestations, thus allowing a quicker appropriate therapy intervention at an early stage.

1. Introduction

Over the last decades, dental implants have rapidly become an indispensable therapy in dentistry to replace one (or more) missing tooth. Although they have a high success rate, some of these interventions can end in failure. The most frequent complication of dental implants is due to peri-implantitis, which occurs with a frequency ranging from 1% to 47% at the implant level [1, 2]. Peri-implantitis is an inflammatory response that affects the tissue surrounding the osseointegrated dental implant and results in excessive marginal bone loss [2]. The causes of the peri-implantitis development and progression of inflammation are very different. Several limiting factors, such as poor oral hygiene, untreated periodontitis, untreated endodontic lesions, unfavorable osseous density, alcohol drinking, smoking, diabetes, or others, contribute to the therapy failure. Without any doubt, anatomical factors and appropriate attachment of connective tissue and epithelium to the implant surface influence the maintenance of the implant and the development of possible inflammatory processes. Another critical contributing factor is the quality of the implant surface - its chemical, physical state, and mechanical features [3-5]. Peri-implantitis has been connected with a Gram-negative anaerobic microbiota, similar to that found in severe periodontitis around natural teeth [6, 7]. After the implantation of the implant, pathogenic bacteria migrate from periodontal pockets, tongue, tonsils, and inflamed gingival to colonize

the dental implant surface [8]. Bacterial dental plaque formation around dental implants leads to inflammatory reactions, which induce proliferation and an overgrowth of sulcular epithelium, the degeneration of connective tissue around the abutment, the loss of per mucosal seal, and an epithelial migration [9, 10].

As in periodontitis, pathogens and their virulence stimulate the release of several immunoinflammatory biomarkers in peri-implant cells. The most meaningful mediators of inflammation are cytokines, which play an essential role in the pathogenesis of periodontal diseases and act as an intermediary in peri-implantitis [11]. The inflammatory process in regards to bacterial infection is mediated by the release of pro-inflammatory cytokines, such as interleukins (IL)-1β, IL-6, IL-12, and IL-17, tumor necrosis factor-alpha (TNF-α), chemokines CXCL8 and macrophage inflammatory protein (MIP)-1α, and neutrophil lysosomal enzymes, reactive oxygen species (ROS) or eicosanoids (prostaglandins, leukotrienes). Those mediators elicit tissue destruction and bone resorption by the stimulation of collagenase and also through the receptor activator of nuclear factor-kappa B ligand (RANKL), which induces osteoclast differentiation [11-12]. Therefore, the evaluation of such cytokines level in the peri-implant sulcus fluid (PISF) has been suggested as a non-invasive method of monitoring the healthy or diseased states of the peri-implant tissues as well as the local response of peri-implant treatments [13, 14]. Using the cytokine assay in the analysis of PISF may help describe the pathogenesis stage more efficiently and predict an early diagnosis of peri-implantitis and prevision in high-risk patients. Despite investigative exertions to identify the levels of several cytokines in the PISF, the efficacy of these parameters to predict or to contribute to the diagnosis of peri-implantitis is still undetermined.

Evaluation of the state of periodontal and peri-implant tissues is primarily based on clinical revision and imaging diagnostics. The measurement of the concentration of humoral factors after inflammation in gingival crevicular fluid (GCF) and PISF may be advantageous in assessing the severity of the inflammatory responses within the periodontal tissues, especially in the early stage of periodontitis and/or peri-implantitis. The literature in this field, particularly in the case of peri-implantitis, is not sufficient. Thus, this study aimed to investigate the IL-1β, CXCL8, and TNF levels in GCF in patients with different degrees of periodontitis and PISF in patients with healthy implants or displaying no signs of mucositis or peri-implantitis.

2. Materials And Methods

2.1. Patients Study

The study group comprised of 189 adult European Caucasian patients: 85 men and 104 women between the age of 20 and 71 years old. Patients were selected and recruited from the Department of Periodontology at the Medical University of Lublin. After a full explanation of what the aim of this study was, written informed consent forms were obtained from all participants in accordance with the Helsinki Declaration. Medical and dental histories of each patient were gathered: no one had any systemic disease, nor had they taken any antibiotics and/or anti-inflammatory drugs within the last 3 months prior

to taking part in the study, neither have they undergone any periodontal therapy in the previous 6 months period. In the study were taken part only non-smoker patients.

The diagnosis of patients was based on clinical and radiographic criteria. Clinical parameters recorded included gingival index (GI), probing pocket depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP). Both PD and CAL measurements were performed using a conventional periodontal probe. One examiner recorded the clinical data. The baseline characteristics of each study group are presented in Table 1. Based on the clinical data, patients were later divided into five clinical groups as follow: (1) a control group of periodontally healthy patients (13 men and 23 women) with no clinical evidence of gingival inflammation, no radiographic evidence of alveolar bone loss, and PD < 3 mm; (2) patients with mild periodontitis (18 men and 30 women) with PD of 3-4 mm; (3) patients with moderate periodontitis (21 men and 22 women) with PD of 4-6 mm; (4) patients with severe periodontitis (18 men and 12 women) with PD > 6 mm; (5) periodontally healthy subjects (15 men and 17 women) who received an implant treatment (implants with a new alternative hydrophilic surface SPI ELEMENT INICELL, Thommen Medical AG, Grenchen, Switzerland and Brånemark system implant, Nobel Biocare, Gothenburg, Sweden). All patients who had undergone a maxillary implant surgery were subjected to a laryngological examination to exclude, from the study, those with paranasal sinuses disorders and potential complications related to the mentioned conditions. The bone density grade was determined according to a scale of D1-D4 defined by Misch [15]. The examination of implants, including assessment of oral hygiene, PD, BOP, and mobility of implant, was performed. Dental implant survival lasted from 36 to 147 months. In the study group of patients that have received dental implants, none of them showed any symptoms of peri-implantitis. On the other hand, patients that had undergone multiple restorative works presented low clinical mucositis as a result of the prosthetic type used. The characteristics of patients with implants are shown in Table 2.

Periodic clinical examinations were performed for each patient, at least once a year, over 6 to 18 months to assess their bone levels, radiographic images were taken by qualified technicians with the use of the parallel method during each follow-up visit. The bone level (radiographic image analyses) was measured separately by two experienced dentists in a blind test manner who estimated the distance between the alveolar bone crest and the respective tooth cusp.

2.2. GCF/PISF Sampling and Processing

Before a GCF sampling, the supragingival plaque was carefully removed. The collection sites were isolated using cotton rolls and dried with air jets. The GCF samples were subsequently obtained from the mesiobuccal root using sterile Periopaper strips (Oraflow Inc., Plainview, NY, USA) that were overlaid and placed at the gingival crevice region until mild resistance was felt. The strips were left in place for 30 seconds to prevent any mechanical irritation. The strips contaminated with blood were discarded. Following the GCF collection, the strips were kept in sterile test tubes and stored in aliquots at -80°C until needed for analysis.

Clinical examinations in the group of patients with implants were performed after removal of the supra-constructions. PISF samples have been drawn, at least 18 months after the surgery, in a similar way using sterile Periopaper strips that were inserted into the gingival crevice until mild resistance was felt. For 30 seconds the strips were left in place. After that the paper points were transferred to sterile test tube and then immediately stored in aliquots at a temperature of -80°C.

2.3. Cytokine measurements

For GCF/PISF extraction, paper strips were put in tubes containing 500 µL of phosphate-buffered saline (PBS) (pH 7.2) and next gently shaken and incubated at room temperature for 1 hour. After that, the strips were pulled, and the fluids were analysed. Commercially available enzyme-linked immunosorbent assays (ELISA) were used to measure concentration of IL-1β, CXCL8, and TNF (Quantikine R&D Systems Inc., Minneapolis, MN, USA). All ELISA procedures were performed according to the manufacturer's instruction. All tests were repeated. The GCF/PISF IL-1β, CXCL8, and TNF concentrations were equated to a standard calibration curve.

2.4. Statistical analysis

The statistical analysis for this study was conducted using Statistica 13.1 (Statsoft Inc., USA). Shapiro-Wilk test was used to analyze the normality of distribution, while Mann-Whitney U test was performed to analysed differences in the levels of IL-1β, CXCL8, and TNF in GCF, and differences in the levels of IL-1β, CXCL8, and TNF between G1 and G2 bone density groups, as well. The Spearman's rank correlation coefficient was used to test correlations between IL-1β, CXCL8, and TNF concentrations in G1 and G2 bone density groups. Statistical significance was set at P = 0.05.

3. Results

IL-1β levels in GCF in patients with healthy periodontium and patients with varying severity of periodontitis are presented in Figure 1A. As expected, we found that the mean concentration of IL-1β was the highest in patients with severe periodontitis (61.04 ± 41.41 pg/mL). IL-1β level in PISF in patients with implants reached 23.73 ± 27.07 pg/mL and was significantly lower than in GCF in patients with mild (p = 0.029), moderate (p = 0.0005), and severe (p = 0.000014) periodontitis (Table 3). The statistical analysis also showed that the GCF concentration of IL-1β was greater in patients with mild (p = 0.0008), moderate (p = 0.000003), and severe (p = 0.0000001) periodontitis compared to patients with healthy periodontium, and higher in patients with severe than mild periodontitis (p = 0.003).

It was observed that the concentration of CXCL8 in each group of patients with periodontitis varied significantly according to the stage of the disease (Figure 1B). The CXCL8 level in PISF was similar to the CXCL8 level in GCF in healthy patients and ranged from 3.1 to 296.3 pg/mL with a mean of 40.90 ± 56.63 pg/mL (Table 4). Statistical analysis revealed that the CXCL8 level in PISF was significantly lower than in patients with moderate periodontitis (P = 0.011). Furthermore, CXCL8 levels in GCF in patients with moderate periodontitis were statistically higher than in periodontally healthy subjects (p = 0.046).

TNF levels in GCF in patients with healthy periodontium and patients with periodontitis are shown in Figure 1C. The mean concentration of TNF was the highest in patients with moderate periodontitis (5.41 ± 2.12 pg/mL) and significantly greater than in GCF of healthy subjects ($p = 0.025$). TNF level in PISF in patients with implants reached 5.71 ± 1.94 pg/mL and was markedly higher compared to subjects with healthy periodontium ($p = 0.003$) and patients with mild periodontitis ($p = 0.010$) (Table 5).

We have also evaluated whether there was any relationship between cytokine levels in PISF and the bone density grade (patients with bone density D1 and D2 formed group G1, n = 12; patients with bone density D3 and D4 formed group G2, n = 20) (Table 6). Statistical analysis showed no significant differences between G1 and G2 groups for any of the cytokine/chemokine levels (IL-1β $p > 0.05$; CXCL8 $p > 0.05$; TNF $p > 0.05$). Besides, a statistically significant positive correlation between IL-1β and CXCL8 levels in PISF was observed in the G1 group ($r = 0.6827$; $p = 0.14$).

4. Discussion

Due to the dynamic progression of modern dental implantology, which has been going on for over 50 years, various implantological systems have been developed. Depending on the longitudinal study, and the geographic region of the patients' origin, the success implant rates of 90%–95% has been reported [16-18]. Despite this, there are treatment failures, and one of the problems causing implant loss is an inflammation of the tissue surrounding the implant, i.e., mucositis and peri-implantitis. The last decades have confirmed the thesis that, as in the case of periodontal disease, the occurrence of peri-implantitis is a symptom of an imbalance between the pathogenic microorganisms and the host immunity. The bacterial agents are probably the most crucial cause of the existence and progression of peri-implantitis; however, the picture of this condition is the sum of many factors that affect the course of the inflammatory response [6, 7]. Although different inflammatory mediators have been evaluated, the cytokine concentrations that differentiate among healthy and stable sites and the onset of a pathological periodontal and peri-implant processes are not known. Some data imply that inflammatory mediators' levels in GCF and PISF, including cytokines, chemokines, and matrix metalloproteinases (MMPs), might be useful for evaluating the condition of periodontal and peri-implant tissues and monitoring development of inflammation. However, there is only little information available on this issue, and the results are ambiguous. Hence, in this study, we have decided to evaluate the levels of the IL-1β, CXCL8, and TNF in PISF obtained from patients with no clinical symptoms of mucositis or peri-implantitis and compared them with the level of mediators in GCF obtained from patients with healthy periodontium and those with varying degrees of periodontitis.

IL-1β is a multifunctional cytokine with diverse biologic activities implicated in the pathophysiology of not only periodontitis but also peri-implantitis. IL-1β is the primary cytokine that stimulates alveolar bone resorption. It increases the expression of collagenolytic enzymes, MMPs, which contribute to the extracellular matrix degradation and, in turn, lead to bone resorption and tissue destruction [19, 20]. Moreover, IL-1β strongly initiates inflammatory processes. In this study, we have found that the mean concentration of IL-1β was the highest in patients with severe periodontitis. Similar observations were

made by Ramseier et al. [21]. They observed that levels of IL-1β are different in GCF according to the periodontal conditions with the highest concentration found in mild-to-moderate periodontitis. Similar results were presented by Gonzales et al. [22], who found that IL-1β concentration in GCF increases with the intensification of clinical symptoms of gingivitis. Heasman et al. [23] found that IL-1β levels in GCF during the development of experimental gingivitis increased 8-times, compared to baseline, one week after the first measurement. Rawlinson et al. [24] also showed a strong relationship between the severity of inflammation in the periodontium and IL-1β in GCF. Interesting studies were conducted by Engebretson et al. [25]. Authors found that the level of IL-1β in GCF increases with the advancement of periodontitis, and in patients with the most severe form of the disease is almost 8-times higher than in patients from the control group. In turn, we stated that IL-1β level in PISF in patients with healthy implants was significantly lower than in GCF in patients with all stages of periodontitis. Yaghobee et al. [26] observed a significant difference between the level of IL-1β in GCF from the gingiva around the natural tooth (45.71 pg/μL) and PISF from the peri-implant tissue (75.26 pg/μL). In turn, Nowzari et al. [27], Recker et al. [28], and Teixeira et al. [29] stated a comparable level of this cytokine between GCF and PISF from healthy patients. Guncu et al. [30] observed a significantly higher level of IL-1β in patients with gingivitis/inflamed dental implants than in healthy patients without inflammation around the implant. Interestingly Abduljabbar et al. [31] research showed that the level of IL-1β in PISF is statistically significantly higher among individuals smoking waterpipe compared with non-smokers.

Because CXCL8 is a potent chemoattractant cytokine and activator of neutrophils in inflammatory regions, which is released from gingival fibroblasts in the gingival crevice, we have also assessed the level of this cytokine. Literature data on the level of CXCL8 in GCF regarding the clinical condition are ambiguous and often even contradictory. Jin et al. [32] found lower CXCL8 levels in GCF in patients with periodontitis compared to healthy people. In turn, Chung et al. [33], when comparing CXCL8 concentration in GCF in periodontologically healthy people and patients with periodontitis, observed a significantly higher level of chemokine in people with a healthy periodontium. In our studies, the CXCL8 level in PISF was similar to the CXCL8 level in GCF in healthy patients. Furthermore, we have found that the CXCL8 level in PISF was significantly lower than in GCF from patients with moderate periodontitis. Our results confirmed the observations of Recker et al. [28], Severino et al. [34], and Ata-Ali et al. [35]. In turn, Hall et al. [36] and Lagdive at el. [37] observed that the level of CXCL8 was significantly upregulated in the peri-implantitis probes. It seems that the assessment of CXCL8 levels in GCF or PISF cannot be a measurable indicator of the dynamics of the inflammatory process in periodontal tissues. This is probably due to the fact that IL-8 is present in GCF, even in people without any inflammatory changes within the periodontal tissues [38-40]. It is perhaps related to the defense process of "physiological" inflammation in the gingival gap, where neutrophils play a significant role.

TNF is considered a key cytokine involved with the innate response against the periodontopathogenic bacteria. It is believed that TNF is a promising biomarker for periodontal disease diagnosis, prognosis, and therapeutics. Although no correlation could be found between the levels of TNF in GCF in different degrees of periodontitis, this molecule showed a strong correlation with the severity of periodontal destruction, and it could be used to compare the various stages of periodontal disease [41]. In our study,

we have proved the presence of TNF in GCF of patients with periodontitis and PISF obtained from individuals without any clinical symptoms of developing mucositis or peri-implantitis. What is more, we have also established that the concentration of this mediator was remarkable higher in patients with implants compared to subjects with healthy periodontium and patients with mild periodontitis. Significantly higher levels of TNF were also noted in PISF when compared with their levels in GCF by Recker et al. [28].

It is well established that the initial stage of peri-implantitis is asymptomatic. Thus, our observation might imply that the monitoring of cytokine levels in PISF could help with the diagnosis of peri-implantitis in an early stage, before clinical manifestations, which may allow for a quick start of appropriate therapy. In our previous study, we have documented that scaling and root planing (SRP) in patients with chronic periodontitis resulted in a significant decrease in MMP-8 concentration in GCF [42]. Moreover, we have indicated that the levels of MMP-8 in the GCF in patients with various severity of periodontitis were significantly higher than in patients with a healthy periodontium [43]. Additionally, we have documented that MMP-8 level in PISF obtained from the patients without any symptoms of mucositis or peri-implantitis was significantly higher not only than in GCF of periodontally healthy patients but also, which seems to be very interesting, in GCF of patients with varying severity of periodontitis. There are only a handful of studies in the dental literature that examined the levels of critical biological mediators between GCF and PISF samples.

Declarations

Authors' contributions

PA was principal investigator, performing all dental procedures; EBB contributed to data interpretation; EK collaborated to data collection and analysis; PŻ was involved in writing the original draft; AEB contributed to paper writing and editing; finally, JA contributed to data interpretation and to supervision and validation of the manuscript. All authors read and approved the final manuscript.

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Data availability statement

The data used to support the findings of this study are included within the article.

Ethics approval and consent to participate

Ethics approval and consent to participate The present study was approved by the Ethics Committee of the Medical University of Lublin. All patients had previously signed a generic informed consent to treatment, and a condition for inclusion in this study, on the nature of which all patients were properly informed, was signing a further specific consent.

Consent for publication

Not applicable.

Competing interests

None conflicts of interest to declare. The authors do not work for, consult, own shares in or receive funding from any company or organisation that would benefit from this study, and have disclosed no relevant affiliations beyond their academic appointment.

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Tables

Table 1. Clinical and demographic characteristics of study participants.

	Control group (I)	Group of mild periodontitis (II)	Group of moderate periodontitis (III)	Group of severe periodontitis (IV)	Implant group (V)
N	36	48	43	30	32
Age of patients (years) mean ± SD range	35 ± 8 20-51	38 ± 9 27-58	40 ± 9 27-60	42 ± 10 28-59	52 ± 16 20-71
Gender Men Women	13 23	 18 30	 21 22	 18 12	15 17
Total natural teeth mean ± SD range	29 ± 3 24-32	28 ± 3 20-32	27 ± 3 22-32	26 ± 3 22-32	14 ± 11 0-29
Total implants mean ± SD range	0 -	0 -	0 -	0 -	6 ± 3 1-12
PD (mm) mean ± SD range	1.64 ± 0.61 0.2-2.5	3.34 ± 0.39 3-4	4.49 ± 0.23 4-5	5.55 ± 0.42 4-6	2.84 ± 0.57 1.9-4
CAL (mm) mean ± SD	0.71 ± 1.20	1.14 ± 1.32	1.80 ± 1.60 0-6	6.48 ± 1.65 1.5-10	-

range	0-4.5	0-4.5			
GI (mm)					
mean ± SD	0.81 ± 0.98	1.27 ± 0.74	1.30 ± 0.6	1.67 ± 0.71	0.34 ± 0.55
range	0-3	0-2	0-2	0-3	0-2

SD, standard deviation.

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Table 3. The comparison of IL-1b levels in different patients' groups.

	control (I)	mild periodontitis (II)	moderate periodontitis (III)	severe periodontitis (IV)	Implant (V)
IL-1b (pg/mL)					
Range	0.10-77.90	0.10-156.80	0.10-181.90	6.40-153.80	0.10-125.70
mean ± SD	16.90 ± 18.65	36.16 ± 33.98	46.76 ± 38.62	61.04 ± 41.41	23.73 ± 27.07
I *vs* II $P = 0.0008$		II *vs* III $P = 0.071$		III *vs* IV $P = 0.135$	
I *vs* III $P = 0.000003$		II *vs* IV $P = 0.003$		III *vs* V $P = 0.0005$	
I *vs* IV $P = 0.0000001$		II *vs* V $P = 0.029$		IV *vs* V $P = 0.000014$	
I *vs* V $P = 0.355$					

SD, standard deviation.

Table 4. The comparison of CXCL8 levels in different patients' groups.

	control (I)	mild periodontitis (II)	moderate periodontitis (III)	severe periodontitis (IV)	Implant (V)
CXCL8 (pg/mL)					
Range	3.20-132.20	0.90-205.20	4.90-274.30	2.50-185.20	3.10-296.30
mean ± SD	37.91 ± 34.06	44.10 ± 38.05	58.16 ± 55.26	51.94 ± 45.87	40.90 ± 56.63
I *vs* II *P* = 0.273		II *vs* III *P* = 0.181		III *vs* IV *P* = 0.560	
I *vs* III *P* = 0.046		II *vs* IV *P* = 0.664		III *vs* V *P* = 0.011	
I *vs* IV *P* = 0.284		II *vs* V *P* = 0.092		IV *vs* V *P* = 0.074	
I *vs* V *P* = 0.388					

SD, standard deviation.

Table 5. The comparison of TNF levels in different patients' groups.

	control (I)	mild periodontitis (II)	moderate periodontitis (III)	Severe periodontitis (IV)	implant (V)
TNF (pg/mL)					
Range	0.10-9.10	0.10-10.20	1.30-9.50	1.20-11.20	1.50-9.40
mean ± SD	4.31 ± 2.02	4.56 ± 2.15	5.41 ± 2.12	5.34 ± 2.39	5.71 ± 1.94
I *vs* II *P* = 0.491		II *vs* III *P* = 0.088		III *vs* IV *P* = 0.725	
I *vs* III *P* = 0.025		II *vs* IV *P* = 0.233		III *vs* V *P* = 0.650	
I *vs* IV *P* = 0.055		II *vs* V *P* = 0.010		IV *vs* V *P* = 0.166	
I *vs* V *P* = 0.003					

SD, standard deviation.

Table 6. IL-1b, CXCL8, and TNF levels in PISF depending on bone density

	G1 (bone density D1+D2)	G2 (bone density D3+D4)
IL-1b (pg/mL)		
Range	1.00 – 125.70	0.10 – 63.20
mean ± SD	35.44 ± 36.11	16.70 ± 17.40
CXCL8 (pg/mL)		
Range	4.90 – 107.70	3.10 – 296.30
mean ± SD	33.99 ± 30.94	45.06 ± 68.04
TNF (pg/mL)		
Range	1.50 – 9.40	3.10 – 9.40
mean ± SD	5.39 ± 2.41	5.91 ± 1.64

SD, standard deviation.

Figures

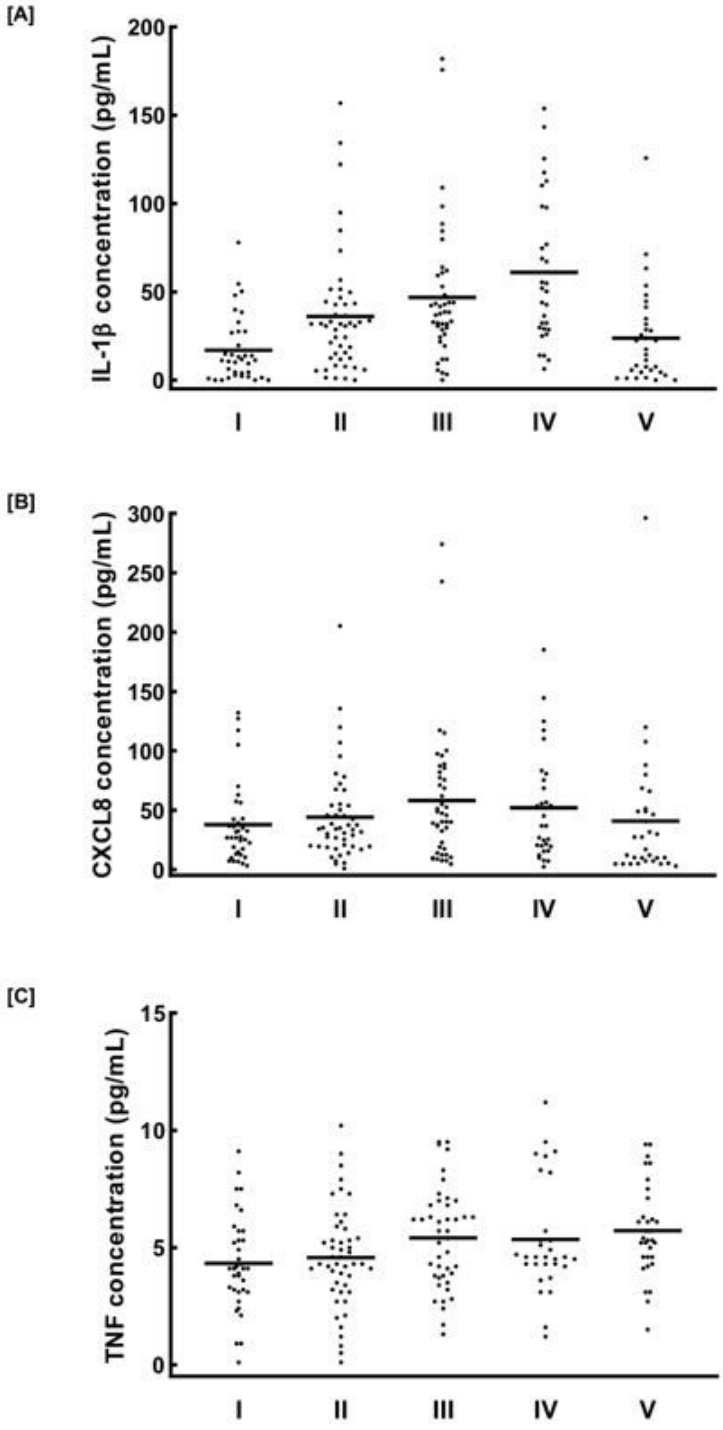

Figure 1

Comparison of (A) IL-1β, (B) CXCL8, and (C) TNF levels in GCF and PISF in different patients' groups. Black lines represent means. I – healthy control; II – mild periodontitis; III – moderate periodontitis; IV – severe periodontitis; V – implant

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.JPG