INTERLEUKIN-1β AND INTERLEUKIN-6 IN PERI-IMPLANT CREVICULAR FLUID AND RELATIONSHIP WITH PERI-IMPLANTITIS

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ABSTRACT
Introduction. The aim of this study was to make a quantitative assessment of interleukin-1β and interleukin-6 in crevicular peri-implant fluid from patients with favorable evolution and patients with peri-implantitis, and to find out if there is a correlation between interleukin-1β and interleukin-6 values and the patient’s clinical status at 7, 30 and 90 days after the insertion of dental implants.

Methods. The study group comprised 32 patients: 5 patients with favorable evolution and 27 patients with different forms of peri-implantitis, selected from 220 patients with dental implants inserted during 1.01.2015-31.12.2016 in a dental private practice. Clinical oro-dental examination was performed at 7, 30 and 90 days after implants insertion. Also, at these moments, we harvested peri-implant crevicular fluid from which interleukin-1β and interleukin-6 were quantified by ELISA method (Salimetrics, USA).

Results. We have found statistically significant differences between interleukin-1β mean values in the two groups of patients in all 3 moments of evaluation (p<0.001). There have been also statistically significant differences regarding interleukin-6 mean values between the patients groups at the 3 evaluation moments.

RéSUMÉ
Introduction. Le but de cette étude est de faire une évaluation quantitative de l’interleukine-1β et de l’interleukine-6 dans le liquide péri-implantaire crémulaire et la relation avec l’état clinique du patient à 7, 30 et 90 jours après l’insertion des implants dentaires.

Méthodes. Le groupe d’étude comprenait 32 patients: 5 patients avec évolution favorable et 27 patients avec différentes formes de péri-implantite et de déterminer s’il existe une corrélation entre les valeurs de IL-1β et IL-6 et l’état clinique du patient à 7, 30 et 90 jours après l’insertion des implants dentaires.

Évaluation des résultats. Nous avons trouvé des différences statistiquement significatives entre les valeurs moyennes d’IL-1β dans les deux groupes de patients dans tous les 3 moments d’évaluation (p<0.001). Il y avait également des différences statistiquement significatives concernant les valeurs moyennes d’IL-6 entre les groupes de patients aux 3 moments d’évaluation.
The need of a correct diagnosis and efficient monitoring of the patient after the insertion of dental implants has initiated debates with the occasion of the 7th Workshop of Periodontology, where there has been reached a consensus on the importance of radiological examination associated with the clinical exam, as links to an early diagnosis in peri-implantitis8.

The variety of implants’ types used, the various implants prosthetics and the size of studied groups are elements that explain the existence of differences on prevalence values of this disease. Thus, Zitzmann et al report a 80% prevalence of mucositis, calculated on the total number of patients and 50% on the number of implants. Also, they found a 28-56% prevalence for peri-implantitis on the total number of patients and 50% on the number of implants, respectively 36.3%8.

Recent studies conducted in 2012 by Atieh et al on 1497 participants and 6283 implants reported a prevalence of between 30.7-63.3% for mucositis and between 9.6-18.8% for peri-implantitis, with a higher prevalence of between 30.7-63.3% for mucositis and 12-43% calculated on the number of implants2.

The aim of this study was to make a quantitative assessment of IL-1β and IL-6 in crevicular peri-implant fluid of patients with favorable evolution and patients with peri-implantitis and to find out if there is a correlation between interleukin-1β and interleukin-6 values and the patient’s clinical status at 7, 30 and 90 days after the insertion of dental implants.

**Abbreviations**
- PMN = polymorphonuclear
- IL-1β = interleukin-1β
- IL-6 = interleukin-6
- GI = gingival index
- BOP = bleeding on probing
- PI = plaque index
- PICF = peri-implant crevicular fluid

**Key words:** IL-1β, IL-6, peri-implantitis.

**INTRODUCTION**

The discovery in 1977 by Branemark of the potential of using titanium implant to treat toothless patients opened new perspectives regarding the possibilities of oral rehabilitation, completely revolutionizing dentistry1. The term “peri-implantitis”, as a complication occurred after insertion of dental implants, has been expressed in writing for the first time in the literature by Mombelli et al, who demonstrated that the presence of bacteria in the peri-implant fluid is an extremely important etiopathogenic factor for the disease and also that there are a lot of similarities with chronic periodontal disease2. Peri-implant tissue destruction is done directly, by bacterial enzymes, released by bacterial metabolites, which are toxic for human cells and also, by toxins releasing that activates macrophages, fibroblasts, keratinocytes present in the peri-implant space3,4.

This entire sequence of events will trigger an immune response, stimulating macrophages and lymphocytes to release a type of inflammatory mediators like cytokines, including interleukin-1β (IL-1β) and interleukin-6 (IL-6). These events will lead to further activation of polymorphonuclear neutrophil leukocytes (PMN), fibroblast, osteoblasts, and macrophages by various mechanisms, which will contribute to the destruction of tissue surrounding the implant3,4,5. Depending on the severity, peri-implantitis can lead to loss of the dental implant, so we can appreciate that this disease is a challenge, for which specialists in the field must find a solution.

**Conclusion.** Correlations between interleukin-6, interleukin-1β and peri-implant sulcus depth demonstrate the usefulness of quantifying these interleukins in monitoring patients with peri-implantitis and, in the same time, the opportunity to be included in the peri-implantitis diagnostic scheme.

**Résultats.** Il existe des différences statistiquement significatives entre les valeurs moyennes d’IL-1β aux deux groupes de patients dans les 3 moments d’évaluation (p <0,001). Il existe également des différences statistiquement significatives en ce qui concerne les valeurs moyennes d’IL-6 entre les patients des deux groupes aux 3 moments d’évaluation (p <0,001). Les résultats montrent qu’il existe une forte corrélation entre l’IL-1β et la profondeur du sillon péri-implantaire chez les patients présentant une péri-implantite avancée (p = 0,0008, r = 0,904) et une corrélation entre l’IL-6 et la profondeur des sillons péri-implantaires chez les patients avec péri-implantite avancée (p = 0,029, r = 0,717).

**Conclusion.** Les corrélation entre IL-6, IL-1β et la profondeur du sillon péri-implantaire démontrent l’utilité de la quantification de ces interleukines dans le suivi des patients avec péri-implantite et, en même temps, la possibilité d’être inclus dans le schéma diagnostique de péri-implantite.

**Mots-clés: IL-1β, IL-6, péri-implantite.**
Material and Methods

Study group: The 32 patients who constituted the study group were selected from a group of 220 patients presented during 1.01.2015-31.12.2016 in a dental private practice. Sex distribution in the study group was: 19 women (59.4%) and 13 men (40.6%). Patients from the study group were aged between 26-63 years; within this broad range we delimited the following patient groups: 26-35 years = 9 patients; 36-49 years = 20 patients; 50-63 years = 3 patients.

Clinical evaluations included the assessment of gingival index (GI), bleeding on probing (BOP), plaque index (PI) and radiographic analyses. Clinical measurements of GI, PI and BOP were taken at four sites (mesial, buccal, distal and lingual).

Peri-implant crevicular fluid (PICF) sampling and markers analyzing: Peri-implant crevicular fluid sampling was done after orodontal clinical examination, seven days by the moment of dental implant insertion. PICF was sampled using a filter paper technique. PICF samples were taken from peri-implant sulcus. The gingiva was dried by air and cotton pellets for 1 min before sampling and the area was isolated by using cotton rolls. A paper strip (Periopaper, USA) was inserted into the peri-implant sulcus for 30 seconds. The sample strip was inserted into Eppendorf tubes and diluted in 200 μL phosphate buffer. The samples were transported to the laboratory where they were centrifuged at 1000 rpm/min for 5 minutes. The supernatant was subsequently separated and stored in a freezer at -80°C until the quantitative determination. In order to quantify IL-1β and IL-6, we used ELISA competitive technique, as described by the manufacturer (Salimetrics, USA). This assay is based on reaction between enzyme labeled reagent, consisting of Ag (or Ab) conjugated to an enzyme that is active and reacts with either Ag (or Ab) from the sample, immobilized on the solid support, and also with the appropriate substrate of the enzyme. We chose these kits because they allow the evaluation of a wide range of values, also having a high detection sensitivity, respectively 0.6 pg/mL for IL-1.

Statistical analyses: Data were analyzed using SPSS 19.0 for Windows and MedCalc 11.0. In this study, we used descriptive statistics (means, standard deviation). Independent sample t-test was used to compare the results for IL-1β between study groups (p<0.05 was considered to have a statistic significance). Pearson test was used to test the correlations between the IL-1β values and the peri-implant probing depth.

Ethical permission: We obtained the agreement from the Ethics Committee of „Ovidius” University from Constanta to comply with the ethical principles for medical research involving human subjects, under the auspices of the International Medical Association Declaration of Helsinki. Subjects included in the study groups were informed about the purpose of investigations and they signed the informed consent.

Results

Of the 220 patients who have dental implants inserted, 193 had a favorable evolution and 27 had an unfavorable evolution (Figure 1).

The 27 patients with poor outcome were included in the study group. We added to these, five patients, in the random selection from the 193 patients with favorable evolution, which constituted the control group. Thus, the study group consists of a total of 32 patients from the initial group of 220 patients (Figure 2).
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**Figure 3.** Distribution of patients after the first clinical and paraclinical evaluation

**Figure 4.** Graphical representation of the lots of patients with peri-implantitis

**Figure 5.** Mean and standard deviation IL1-\(\beta\) values in patients from the group with favorable evolution and those with peri-implantitis

**Figure 6.** Correlation between IL-1\(\beta\) and peri-implant sulcus depth in patients with severe form peri-implantitis at 90 days
After the insertion of dental implants, at 7 days it was conducted the first clinical and radiological examination. By corroborating clinical parameters with the data from the radiological examination, it was possible to separate them into two categories, as follows: 5 patients with a favorable evolution and 27 patients with peri-implantitis (Figure 3).

Depending on the value of clinical parameters, the 27 patients with peri-implantitis were classified into 3 categories as follows: peri-implantitis with easy form – 6 patients, peri-implantitis with moderate form – 12 patients and peri-implantitis with severe form – 9 patients (Figure 4).

Quantitative evaluation of IL-1β in PICF was performed in patients with favorable evolution of the implant and in patients with severe form of peri-implantitis in three periods, respectively at 7, 30 and 90 days after insertion of dental implants.

As the results show in the Figure 5, there are statistically significant differences between IL-1β mean values in patients with favorable evolution and in patients with peri-implantitis, in all 3 moments of evaluation.

There is also a high correlation between the value of peri-implant sulcus depth and value IL-1β, as shown in Figure 6.

Comparing the values of IL-6 quantified in PICF in patients with favorable evolution of implant and patients with peri-implantitis in the 3 moments assessment, we obtained results that show that there are significant statistically differences or highly significant statistically differences ($p <0.0001$) after 7 days post dental-implant insertion (Figure 7).

Also, there is a high correlation between the values of peri-implant sulcus depth and IL-6 value, as shown in Figure 8.
DISCUSSION

The idea of identifying biomarkers that allow early diagnosis of diseases and their monitoring was also used in dentistry. The specialists consider that an ideal biomarker should fulfill the condition to have sensitivity and specificity related to that condition, reasons why it is very difficult to define such a perfect instrument. Concerning peri-implantitis, there is clearly no biomarker that meets the two conditions mentioned9,10.

In this context, IL-1β and IL-6 are proinflammatory cytokines quantifiable in peri-implant fluid and saliva, with a demonstrated contribution to the onset of many diseases, among which peri-implantitis11,12.

It was very difficult to compare the results of this study to similar literature, given that there is a great variability of techniques used to quantify IL-1β or IL-6 and also a wide variety of expression volumes of the two interleukins13.

The results obtained in this study show a clear involvement of IL-1β in the pathophysiology of peri-implantitis, as demonstrated by the significantly statistical differences between the high values of IL-1β in patients with favorable evolution and those with peri-implantitis and highly significant statistical correlations between IL-1β and peri-implant sulcus depth in patients with severe peri-implantitis.

Similar results to those from this study were obtained by Siamak Y. et al, who quantified IL-1β in crevicular fluid around the healthy teeth, the peri-implant crevicular fluid in patients with favorable development and in patients with peri-implantitis14.

Very similar results to those obtained in this study are cited by Panagakos F. et al and Javier Ata-Ali et al, studies which present IL-1β values quantified in patients with favorable evolution and peri-implantitis15.

It is shown that IL-1β correlates best with peri-implant sulcus depth and bleeding index, and the results are similar to those of other studies published by Siamak Y. et al17.

In the same direction, we consider that the results obtained in this study regarding the correlation between IL-1β and peri-implant sulcus depth, a high correlation for the patients with advanced form of peri-implantitis, strengthen the assessment made by Duarte PM. et al, according to whom, the peri-implant sulcus depth is the most important parameter for assessing the severity of peri-implantitis, being directly influenced by the level of pro-inflammatory cytokines, among which IL-1β plays an important role11.

Starting with the similarities between periodontitis and peri-implantitis, namely from the fact that the crevicular fluid comes from the periodontal tissue around the teeth in periodontitis and from around the implant in peri-implantitis, Liu Han et al highlight that the IL-1β value in the inflamed periodontal tissue can reach up to 511.12 pg/site and it is identified in 112 of 115 patients from the study; going on the idea of inflammatory process similarity between periodontitis and peri-implantitis, the results presented by Liu Han et al are similar to those obtained in our study18,19.

The results of this study show that there are significant statistically differences between the IL-6 values quantified from the peri-implant fluid in patients with favorable evolution and patients with different forms of peri-implantitis. Also, the results demonstrate the existence of a correlation between IL-6 values and clinical parameters, particularly a very high correlation between IL-6 and peri-implant sulcus depth in patients with advanced form of peri-implantitis. The results of this study are similar to other studies from the specialty literature20,21.

CONCLUSIONS

The differences in the amount of IL-1β and IL-6 in patients with favorable evolution compared to those with peri-implantitis demonstrate that IL-1β and IL-6 might be useful markers delineating the two evolutionary clinical possibilities: patients with favorable evolution and patients with peri-implantitis.

High correlations between IL-1β, IL-6 and peri-implant sulcus depth demonstrate the usefulness of quantifying these interleukins in monitoring the peri-implantitis patient and in the same time, the opportunity to be included in the peri-implantitis diagnostic scheme.

Dynamic tracking of IL-1β and IL-6 in diagnostic and monitoring supports indirectly the correct choice of optimal treatment scheme.

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