

CLINICAL RESEARCH

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The Effect of Periodontal and Peri-Implanter Health on IL-1 β and TNF- α Levels in Gingival Crevicular and Peri-Implanter Sulcus Fluid: a Cross-Sectional Study

El efecto de la salud periodontal y peri-implantar en niveles de IL-1 β y TNF- α en el fluido crevicular y sulcus peri-implantador: un estudio transversal

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ABSTRACT: It is stated that peri-implanter diseases have destructive effects similar to periodontal diseases. This study aims to compare IL-1 β and TNF- α levels in healthy and diseased areas. Forty non-smokers systemically health individuals (40 implants/40 teeth) (age:38-67) were included in the study. In clinical and radiographic examinations; plaque index (PI), gingival index (GI), periodontal pocket depth (PPD), clinical attachment level (CAL) and marginal bone loss (MBL) values were recorded. The gingival crevicular fluid (GCF) and peri-implanter sulcus fluids (PISF) of the patients were collected, and IL-1 β and TNF- α levels were measured by ELISA in the samples. The collected data were analyzed with the help of SPSS v.22 package program. Sample PPD score showed a statistically significant difference between the diseased and healthy groups and also sample CAL showed statistically significant higher in Periodontitis(P) compared to periodontally healthy(H) and Gingivitis(G) ($p>0.05$). IL-1beta and TNF- α levels in GCF/PISF; In the P and Peri-implantitis (PI) group, it was found statistically significantly higher than the H, Healthy-Implant (HI), besides P showed higher levels compared to G ($p<0.05$). Within the limitations of our study, it can be said that IL-1 β and TNF- α among inflammatory cytokines in GCF/PISF will increase in periodontal and peri-implanter diseases, it can also be said that this increase in cytokines may indicate that periodontal and peri-implanter diseases have similar immunological structure. Peri-implant mucositis without periodontitis history has similarity to peri-implantitis and periodontitis in terms of IL-1 β and TNF- α levels in GCF/PISF.

KEYWORDS: Periodontal disease; Peri-implantitis; Peri-implanter disease; Gingival crevicular fluid; Peri-implanter sulcus fluid.

RESUMEN: Se afirma que las enfermedades peri-implantarias tienen efectos destructivos similares a los de las enfermedades periodontales. Este estudio tiene como objetivo comparar los niveles de IL-1 β y TNF- α en zonas sanas y enfermas. Cuarenta individuos no fumadores con salud sistémica (40 implantes/40 dientes) (edad:38-67) fueron incluidos en el estudio. En los exámenes clínicos y radiográficos se registraron los valores de índice de placa (Pln), índice gingival (GI), profundidad de la bolsa periodontal (PPD), nivel de fijación clínica (CAL) y pérdida de hueso marginal (MBL). Se recogieron los fluidos creviculares gingivales (GCF) y los fluidos del surco peri-implantario (PISF) de los pacientes, y se midieron los niveles de IL-1 β y TNF- α mediante ELISA en las muestras. Los datos recogidos fueron analizados con la ayuda del programa de paquete SPSS v.22. La puntuación PPD de la muestra mostró una diferencia estadísticamente significativa entre los grupos enfermos y sanos y también la muestra CAL mostró estadísticamente significativa más alta en la Periodontitis (P) en comparación con la salud periodontal (H) y la Gingivitis (G) ($p > 0.05$). Los niveles de IL-1beta y TNF- α en GCF/PISF. En el grupo de P y Peri-implantitis (PI), se encontró estadísticamente significativo más alto que el H, implante sano (HI), además P mostró niveles más altos en comparación con G ($p < 0.05$). Dentro de las limitaciones de nuestro estudio, se puede decir que IL-1 β y TNF- α entre las citoquinas inflamatorias en GCF/PISF aumentarán en las enfermedades periodontales y peri-implantarias. También se puede decir que este aumento de citoquinas puede indicar que las enfermedades periodontales y peri-implantarias tienen una estructura inmunológica similar. La mucositis peri-implantaria sin antecedentes de periodontitis tiene similitudes con la peri-implantitis y la periodontitis en cuanto a los niveles de IL-1 β y TNF- α en la GCF/PISF.

PALABRAS CLAVE: Enfermedad periodontal; Peri-implantitis; Enfermedad peri-implantaria; Fluido crevicular gingival; Fluido de surco peri-implantario.

INTRODUCTION

Periodontal and peri-implant diseases are infectious diseases that develop after specific and complex interactions between pathogenic bacteria and host response (1,2). Although the primary etiological factor in periodontal diseases is microbial dental plaque, local and systemic factors can change the severity and tissue destruction speed of periodontal disease via affecting the host response balance; such as diabetes, stress, smoking, etc. (3).

In the last periodontal diseases classification, peri-implanter diseases are included in the

classification of periodontal diseases (1), even gingivitis is considered to be the precursor of periodontitis (4), peri-implantation mucositis is also considered to be the precursor of peri-implantitis (5). The mediators secreted by the host defense system against bacteria may be sources of tissue destruction (6), furthermore these mediators such as cytokines can affect the shift from gingivitis or mucositis to periodontitis or peri-implantitis (7).

Cytokines are the generic name of a large family of mediators consisting of growth factors interleukins, colony-stimulating factors. They are involved in many important biological

events, including inflammation, proliferation, regeneration, differentiation and hemostasis. They can be produced by many cells, particularly T cells and macrophages (5). Cytokines are produced temporarily; they may show autocrine or paracrine effect at very low concentrations. The term of inflammatory cytokine is the general name given to cytokines that are associated with the onset, progression, slowdown and stopping of inflammation. Pro-inflammatory cytokines act to increase tissue destruction and inflammation, while anti-inflammatory cytokines act to reduce tissue destruction (8). IL-1 α and IL-1 β are cytokines belonging to the IL-1 family and are vital mediators in immunity, inflammation and tissue destruction and hemostasis (9). IL-1 can be synthesized from a variety of immune cells and is associated with periodontal diseases. TNF- α is another important mediator in the pathogenesis of periodontal and peri-implant diseases (10). TNF- α plays a key role in periodontal disease. It has similar immunological effects compared with IL-1 β . One of these effects is increased neutrophil activation and tissue turn-over rate in periodontal tissue. TNF- α ; in particular, they are secreted from macrophages that encounter lipopolysaccharides of pathogenic bacteria (11). Individual differences in inflammatory and immunological responses to bacterial infection in the periodontium may affect host susceptibility to periodontal diseases (12). Genetic factors determine the cascades of the inflammatory mediators of the host in response to bacterial infection that may destroy connective tissue and alveolar bone (13). Although different cytokines have been evaluated in past studies(14-16), a holistic study investigating the difference between healthy and periodontal and peri-implant diseased regions was not found (17).

In the diagnosis of periodontal and peri-implanter diseases; the clinical and radiological examination is still a valid and gold standard method. Nevertheless; in order to determine the severity and prognosis of periodontal and peri-implanter

diseases, researches focused on determining biochemical markers. Understand the role of IL-1 and TNF- α in the pathophysiology of peri-implanter and periodontal diseases, and also the relationship of these cytokines with bleeding on probing, gingival index and probing pocket depth has been frequently investigated (18). However, the majority of studies have evaluated peri-implant diseases among themselves and have not compared them with periodontal disease such as periodontitis and gingivitis (19). In this context, this cross-sectional study aimed to evaluate the IL-1 β and TNF- α levels in GCF and PISF and their relationship with clinical parameters in patient with periodontally and peri-implanter healthy and diseased.

MATERIALS AND METHODS

STUDY POPULATION

Forty non-smoker individuals (40 implants/40 teeth), 20 females and 20 males who were admitted to the Department of Periodontology of the Faculty of Dentistry of Gaziantep University were included. The study was conducted by the Helsinki Declaration and study was approved by Gaziantep University Clinical Studies Ethics Committee (date: 27.11.2017/number:393) All participants were given verbal / written information about our study, and informed consent was obtained.

INCLUSION AND EXCLUSION CRITERIA

The criteria for inclusion in the study were older than 18 years, without any systemic disease, not using antibiotics and anti-inflammatory drugs in the last six months and not receiving dental treatment in the last six months. Also, there was no filling or prosthetic restoration in the teeth, and the implants to be sampled with peri-implanter sulcus fluid should be functional for at least 24 months after loading. There should be no overdenture support or bridge support, only single implant restorations were included. The determination of

healthy tooth (H), healthy implant (HI), gingivitis (G), Periodontitis (P), Peri-implantation mucositis (PM) and peri-implantitis (PI) was also considered with the 2017 EFP and AAP workshop criteria (1,2). Besides, tissue level and hexagonal connected implants were not included in the study for standardization purposes. Additionally, in implant groups there was no history of periodontitis in all patient, according to clinical anamnesis and radiographic examination via panoramic radiograph. In all groups, especially periodontitis, patients have at least 18 teeth.

CLINICAL EXAMINATION

Periodontal pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), plaque index (Pln) scores were recorded. Clinical periodontal and peri-implant examinations were performed by an initially calibrated periodontist (HG). Intra-examiner k values were 0.94 (PPD) and 0.79 (CAL). BOP was considered positive if bleeding occurred 15 seconds after probing. Except for the third molars, four sides of teeth (mesio-buccal, mid-buccal, disto-lingual, mid-lingual) were examined with a periodontal probe for the measurement of PPD and CAL (Williams Probe, Hu-Friedy, USA). All clinical parameters measured as sampled tooth/implant (exp., sPPD) and full-mouth (exp, fmPPD). Periapical or/and panoramic radiographs were taken from all participants to determine the presence or absence of bone loss around dental implants. Additionally, marginal bone loss (MBL) was measured by ImageJ [National Institutes of Health (NIH), Bethesda, MD, USA].

GCF AND PISF SAMPLING AND ANALYSIS

GCF/PISF samples were collected based on radiographic data before clinical measurements from single-rooted teeth or implant. But after clinical examination if BOP was positive, sample

changed from healthy (teeth/implant) to gingivitis and peri-implant mucositis, only 5 samples were changed (2 samples in gingivitis, 3 samples in peri-implant mucositis group) During sample collection, the tooth/implant was isolated with cotton rolls, gently dried by air/water spray, and then the paper strips were placed in the pocket until slight pressure was felt. After waiting for 30 seconds, the paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA) were placed in an eppendorf tube. Samples were stored at -80 degrees until analysis day. After the paper strips in eppendorf tubes were kept at room temperature for at least 30 minutes, 100 μ l assay buffer included in the kit was added to each eppendorf tube and put into the shaker device for 45 minutes (12). Then the tubes were centrifuged at 11,200 rpm for 15 minutes. After the GCF/PISF in the paper strips were transferred to the assay buffer, the assay buffer in the eppendorf was taken by means of a clean polypropylene pipettor, and the levels of IL-1 β and TNF- α in GCF/PISF samples were measured according to the manufacturer's instructions (Cloud-Clone Corp. IL-1 β AND TNF- α ELISA kit) using Enzyme-Linked Immunosorbent Assay. Also, detection ranges were 15.6-1000pg/ml, 3.12-200pg/ml respectively for IL-1 β and TNF- α .

STATISTICAL ANALYSIS

Two-sided two-sample t-test with 5% significance level was used for sample size calculation, a sample size of 12 subjects was determined for each group in order to detect 1 mm (SD 0.85) difference to obtain 80% power in terms of mean MBL between PM and PI.

The suitability of the numerical data to the normal distribution was tested with the Shapiro Wilk test. Mann Whitney U test was used to compare the standard non-distributed variables in two groups. ANOVA and LSD multiple comparison tests were used for comparison of normally distributed

numerical data in 6 groups. For descriptive statistics, mean \pm std. deviation is given, and for categorical variables, it is given as a number (%). SPSS v22.0 package program was used in the analyzes. $P < 0.05$ was considered significant.

RESULTS

In our study, 80 samples from 20 male and 20 female patients between the ages of 28-60 were included (H; $n=13$, HI; $n=13$, P; $n=14$, G; $n=13$, PM $n=14$, PI $n=14$). The mean age \pm standard deviation value of the participants was 49 ± 13.36 , 53.54 ± 11.95 , 52.43 ± 11.12 , 45.36 ± 13 , 56.5 ± 12.63 , 53.54 ± 13.33 for the HI, PM, PI, H, G and P groups, respectively.

All clinical data showed in Table 1. In terms of clinical parameters, it was found that the sPPD score showed a statistically significant difference between the diseased and healthy groups and also sCAL showed statistically significant higher in P compared to H and G ($p > 0.05$). Additionally; only

P groups showed higher fmPPD level compared to PM, H and HI, the others did not show statistically significant differences compared with each other. Besides, the fmPI_n value was found statistically significantly higher only in the P group compared to the other groups. ($p < 0.05$) (Table 1). When analyzed in terms of sMBL parameter, PI group was found statistically significantly higher, but PM and HI groups did not show a significant difference in each other ($p < 0.05$) (Table 1).

Regarding biochemical parameters, statistically significant differences were detected between the groups ($p > 0.05$) (Table 2). IL-1 β and TNF- α levels in GCF/PISF; In the P and PI group, it was found statistically significantly higher than the H, HI, besides P showed higher levels compared to G ($p < 0.05$). Interestingly, there was no statistically significant differences between PM and PI and P groups ($p > 0.05$). In terms of GCF/PISF volume only H groups showed statistically significant lower value compared with G, PI and P ($p < 0.05$) (Table 2).

Table 1. Comparison of clinical parameters.

Clinical Parameters	Implants (n=40)			Teeth (n=40)		
	HI (n=13)	PM (n=14)	PI (n=13)	H (n=13)	G (n=13)	P (n=14)
Age	49 \pm 13.36	53.54 \pm 11.95	52.43 \pm 11.12	45.36 \pm 13	56.5 \pm 12.63	53.54 \pm 13.33
sPPD(mm)	1,87 \pm 0,34 *, †	2,38 \pm 0,54 †	4,71 \pm 1,6	1,41 \pm 0,31 *, †, ж	2,12 \pm 0,52 *, †	4,12 \pm 0,84
sCAL(mm)	NA	NA	NA	0 \pm 0 *	0 \pm 0 *	4,13 \pm 1,2
sPI _n	0,95 \pm 0,33	1,17 \pm 0,37	1,25 \pm 0,41	1,25 \pm 0,63	1,23 \pm 0,66	1,48 \pm 0,84
sGI	1,04 \pm 0,14*	1,32 \pm 0,38	1,37 \pm 0,43	1,08 \pm 0,18*	1,24 \pm 0,28	1,5 \pm 0,33
sMBL(mm)	0,21 \pm 0,12 †	0,61 \pm 0,31†	3,23 \pm 1,47	NA	NA	NA
fmPPD(mm)	1,68 \pm 0,58 *	1,76 \pm 0,44 *	2,28 \pm 0,52	1,76 \pm 0,39 *	2,28 \pm 0,62	3,18 \pm 0,74
fmCAL(mm)	0,13 \pm 0,17 *	0,15 \pm 0,16 *	0,16 \pm 0,19 *	0,11 \pm 0,12*	0,65 \pm 0,35*	4,51 \pm 0,94
fmPI _n	1,02 \pm 0,43 *	1,28 \pm 0,3 *	1,41 \pm 0,31	1,15 \pm 0,44	1,22 \pm 0,36	1,70 \pm 0,71
fmGI	1,16 \pm 0,1 *	1,19 \pm 0,12	1,27 \pm 0,18	1,12 \pm 0,1*	1,23 \pm 0,12	1,45 \pm 0,26

sPPD: sampled teeth/tooth periodontal pocket depth, sCAL: sampled teeth/tooth clinical attachment level, sPI_n: sampled teeth/tooth plaque index, sGI: sampled teeth/tooth gingival index, fmPPD: full-mouth periodontal pocket depth, fmCAL: –full-mouth clinical attachment level, fmPI_n: full-mouth plaque index, fmGI: gingival index, P: Periodontitis, G: Gingivitis, H: Periodontally Healthy, PI: Peri-implantitis, PM: Peri-implant mucositis, HI: Healthy implant.

* significant difference from P ($p < 0.05$). † significant difference from PI ($p < 0.05$). ж significant difference from PM ($p < 0.05$).

Table 2. Comparison of biochemical parameters.

Biochemical Parameters pg/ml	Implants (n=40)			Teeth (n=40)		
	HI (n=13)	PM (n=14)	PI (n=13)	H (n=13)	G (n=13)	P (n=14)
IL-1 β	20,66 \pm 2,9 *,†, ж	46,06 \pm 11,47	49,36 \pm 10,02	23,62 \pm 7,23 *,†, ж	36,76 \pm 9,7 *	65,79 \pm 18,58
TNF- α	15,19 \pm 2,92*,†, ж	31,45 \pm 7,31	37,05 \pm 8,07	14,17 \pm 1,65*,†, ж	21,46 \pm 4,76*	52,07 \pm 12,02
GCF/PISF vol	2,17 \pm 1,04	2,32 \pm 1,38	6,08 \pm 4,22	1,43 \pm 1,06*,†, Ω	3,15 \pm 1,02	5,33 \pm 3,01

IL-1 β : Interleukin 1 beta, TNF- α : Tumor Necrosis Factor Alpha, GCF/PISF vol: Gingival crevicular fluid/Peri-implanter sulcus fluid volume, P: Periodontitis, G: Gingivitis, H: Periodontally Healthy, PI: Peri-implantitis, PM: Peri-implant mucositis, HI: Healthy implant.

* significant difference from P ($p < 0.05$). † significant difference from PI ($p < 0.05$). Ω significant difference from G ($p < 0.05$). ж significant difference from PM ($p < 0.05$).

DISCUSSION

Peri-implanter diseases challenge clinicians as one of the common complications of dental implant treatment (20). Besides, although non-invasive diagnostic tools are needed, although radiographs are helpful in the diagnosis of peri-implanter diseases (21). Peri-implanter diseases are similar to periodontal diseases in terms of their inflammatory characteristics (22). In this context, we aimed to investigate healthy periodontal and peri-implanter conditions and diseased conditions clinically and biochemically. TNF- α and IL-1 β are pro-inflammatory cytokines that appear to play a central role in peri-implant tissue destruction, as in periodontal tissues (19). Studies are showing that the biological effects of IL-1 β depend on tissue concentration (5). The properties of these cytokines related to tissue destruction include stimulation of bone resorption and stimulation of tissue destructive proteinases. According to the results of our study, it can be said that IL-1 β and TNF- α levels increase in peri-implanter diseases similar to periodontal diseases. And also, it can be said according to present study peri-implantitis, peri-implant mucositis and periodontitis have similar IL-1 β and TNF- α levels in GCF/PISF. These results may explain why the PM is not very close to reversible characteristic like from gingivitis to healthy tooth.

Interleukin-1 beta is a pro-inflammatory cytokine that plays a key role in the immunoinflammatory response and also provides increased bone destruction (11). It has been reported that the level of IL-1 β is lower in healthy individuals compared to individuals with periodontitis and that there is a decrease in the level of IL-1 β after treatment in individuals with periodontitis (23). In our study, the high doses of IL-1 β in diseased groups in the inter-group evaluations coincide with the literature. Also, there are studies showing that IL-1 β levels are higher in peri-implanted areas compared to healthy implant regions (24,25), similarly, it has been emphasized in some studies that IL-1 β level is higher in peri-implanter mucositis compared to healthy regions (17,26). TNF- α is a cytokine that has many functions, from stimulating fibroblasts and releasing collagenase to releasing pro-inflammatory cytokines. It induces osteoclast differentiation and increases vascular permeability (27). It has been observed in some studies that the areas with periodontitis increased compared to healthy areas; there are similar studies showing that it also increases in peri-implanter disease (24,28). However, there is no study evaluating both peri-implanter and periodontal diseases. In this context, we can say that present study was the first study to compare both IL-1 β and TNF- α levels in peri-implanter and periodontal diseases with healthy groups.

Although it is emphasized that periodontal diseases and peri-implanter diseases have similar histopathologic background; In many studies on peri-implant mucositis and peri-implantitis, they have often been compared within each other or with their healthy implant groups. Peri-implant mucositis without periodontitis history has similarity to peri-implantitis and periodontitis in terms of IL-1 β and TNF- α levels in GCF/PISF. Accordingly, in the present study significantly higher levels of IL-1 β and TNF- α in GCF/PISF were found in regions of peri-implantitis and peri-implant mucositis compared to healthy implants, as well as in gingivitis and periodontitis regions compared to healthy tooth regions. These findings provide further support for previous studies showing that peri-implant and periodontal disease have similar patterns in terms of host response and bone destruction. However, when compared to natural teeth and implants, there were no statistically significant differences between the healthy implant group than the healthy tooth regions. This partially contradicts the findings of a longitudinal study that showed that IL-1 levels increased in healthy tooth regions compared to healthy implant sites(17). It has been reported that IL-1 β level is lower in healthy individuals compared to individuals with periodontitis, and there is a decrease in IL-1 β level after treatment in individuals with periodontitis, and also IL-1 β was increased in patient with experimental mucositis(26) A systematic review conducted by Ghassib *et al.*(5) showed only IL-1 β and IL-6 can be used an external tool to adjunct clinical examination. While there is agreement with the literature in terms of IL-1 β levels in the current study, there are differences in terms of TNF-alpha. Considering the immunological characteristics and irreversible pathophysiology status of PM, its clinical diagnosis appears to be of critical importance. Moreover; maintenance of dental implant treatment and routine periodontal examination is so crucial to early detect of not only peri-implant mucositis but also periodontitis and peri-implantitis.

The destructive characteristics of periodontal and peri-implanter diseases are known. Among these diseases, tooth or implant losses can be observed after peri-implantitis and periodontitis, and alveolar bone loss occurs around the tooth/implant (19). Considering the clinical parameters in our study, there was a statistically significant difference between the gingivitis and periodontitis groups in terms of CAL levels, and the statistically significant differences between the mucositis and peri-implantitis groups in terms of MBL scores are both consistent with the previous studies and are an expected result (29,30). Implants with a conical implant-abutment connection have less micro-gap than other types of connections. Therefore, there is less risk of peri-implantitis with less bacterial invasion (31). Although studies are showing that the implants with hexagonal connections have a similar biological and biomechanical effect compared with implants with conical connections, however; conical connection implants were preferred for the standardization of our study.

Among the limitations of this study, It can say that present study is cross-sectional. Because in cross-sectional studies, only the parameters of individuals with instant disease diagnosis are examined, not following the formation of the factors related to the disease (32). Another limitation is the low sampling regions available for the periodontitis category to match the peri-implantitis regions in the same individuals. The approach of matching different disease categories in the same individuals severely limits the number of patients eligible for the study. This posed a significant challenge in our study as it is not possible to match diseased areas with different disease categories, but to overcome this limitation, so to maximize host tissue standardization as much as possible, samples were taken from the same individual's teeth or implants belonging to the same disease group (17). Also; In our study, systemic healthy non-smoking of all participants can be shown as a limitation because it has been reported in the studies that

smoking affects mediators, mainly involved in the inflammatory process (33). Similarly, it changes the inflammatory response in diseases such as diabetes mellitus (8). In this context, the presence of only non-smokers without any systemic disease in the groups prevents the results from spreading to the broad base. And also, in implants groups all implants were examined after 24 months after loading, but host response are unique thus, some individuals may showed peri-implanter mucositis due to function time. Depending on the time in some patient, we think that peri-implant mucositis may turn into peri-implantitis.

CONCLUSION

Within the limitations of our study; A statistically significant increase in IL-1 β and TNF- α levels was detected in periodontitis and peri-implanter disease (PI,PM) groups compared to healthy groups. In this context, it can be said that periodontal and peri-implant diseases have similar inflammatory properties. And also, PM has similarity to P and PI in terms of immunologic marker. However; microbiological and immunological studies at not only in GCF but also in periodontal tissue (especially gingiva) are needed, and also cytokines levels in GCF are also examined after non-surgical periodontal treatment of periodontal and peri-implanter disease.

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