

Comparison of Interleukin-1 β Levels in Gingival Crevicular Fluid and Peri-Implant Crevicular Fluid and Its Relationship with Clinical Indexes

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Abstract

Objective: Interleukin-1 β (IL-1 β) is one of the most important cytokines which seems to have an important role in the inflammatory process in gingival and peri-implant tissues. The aim of this split-mouth study was to investigate the relationship between the concentration of IL-1 β in gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF) and clinical parameters such as plaque index (PI), gingival index (GI), pocket depth (PD) and bone loss (BL).

Materials and Methods: In 32 patients, PICF and GCF samples of 41 implants and 41 contralateral teeth were collected and IL-1 β was determined by enzyme-linked immunosorbent assay (ELISA). PI, GI, PD and BL were recorded for each of the samples.

Result : The positive correlation between the level of IL-1 β and PI, GI, PD and BL in both groups was observed ($P < 0.0001$). In similar conditions, the level of IL-1 β was greatly higher in PICF than GCF (75.26 pg/ μ l and 45.71 pg/ μ l, respectively) ($P = 0.001$).

Conclusion: The findings of the present study indicated that the level of IL-1 β may be an important supplement to clinical findings in measuring the health status of gingival or peri-implant tissues.

Key Words: Interleukin-1beta, Gingival Crevicular Fluid, Peri-Implantitis

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INTRODUCTION

As a fact, periodontal diseases result from interactions between specific subgingival microbial species and the susceptible host, leading to the release of inflammatory mediators

which in turn mediate tissue destruction. The host immune responses to subgingival biofilms are conducted by cytokines. These molecular messengers are elements of effective innate and adaptive responses and cause fine

tuning of the interplay among the different components of the immune system. There are many cytokines, such as IL-1 β , IL-1 α and tumor necrosis factor α (TNF α), which have an important role in the inflammatory process [1-4]. IL-1 β is one the most important of these cytokines that stimulates bone resorption [5,6] and inhibits bone formation [7,8]. This mediator also stimulates the prostaglandin synthesis and protease production [9,10].

IL-1 β in co-operation with other inflammatory mediators has an important role in regulating and amplifying the inflammatory response in periodontal and peri-implant tissues [11,12]. A high level of IL-1 β in the gingival crevicular fluid (GCF) [13-15] and the gingival tissue [16-18] have been associated with chronic periodontitis.

Reduction of tissue destruction by inhibition of IL-1 β in experimental periodontitis has been shown in some studies [19-21]. On the other hand, in peri-implant crevicular fluid (PICF), higher levels of IL-1 β have been associated with peri-implantitis [22-25].

The aim of this split-mouth study was to investigate the correlation between clinical and radiographic parameters, such as plaque index (PI), probing depth (PD), gingival index (GI) and bone loss (BL), and the concentration of IL-1 β in the gingiva around the natural tooth and peri-implant tissue, and also comparison of IL-1 β level in GCF and PICF.

MATERIALS AND METHODS

Patient and site selection:

The present study included patients who had been treated with osseointegrated implant at a private clinic. Patients were excluded from the study if they were not medically healthy or had taken medication that could influence the peri-implant or periodontal status. No subjects had the habit of smoking at the implant installation and GCF or PICF sampling. A total of 41 solid implants (ITI, Straumann AG, Waldenburg, Switzerland) were selected from 32 patients. The mean age at the time of enrollment in the study was 53.3 years for 27 females (41-65 years) and 51.2 years for five males (43-62 years) and the mean period of 36.5 months (range, 12 to 42 months) had passed since the loading of implants. According to the split-mouth study protocol, a natural tooth at the same position of the located implant, but on the opposite site of the same jaw was considered as the control sample.

Written informed consent was obtained from each subject.

Clinical measurements:

Before starting crevicular fluid collection, supra gingival plaque was scored using modified plaque index (PI) and removed from each sample [26]. Gingival inflammation was scored using modified gingival index (GI) [27].

Table 1. Comparison of IL-1 β between GCF and PICF in Different Pocket Depths

Variable	IL-1 β in PICF	IL-1 β in GCF	Number	P. value
PD=3mm	41.31	67.33	14	0.3
PD=4mm	123.84	288.13	4	0.7
PD=5mm	162.72	537.98	5	0.3

Data are presented as mean

Probing depth (PD) measures were obtained from sample sites (mesial and distal mid-points) using a conventional periodontal probe (Hu-Fridy, Chicago, IL, USA).

The probe was directed parallel to the long axis of the implant or natural tooth. According to the probing depth, all the samples were categorized into three groups: $PD \leq 3$ mm, $PD = 4$ mm and $PD \geq 5$ mm. Radiographic bone loss was evaluated on the periapical radiograph. All clinical data were collected by one examiner.

GCF and PICF sample collection:

First, all clinically detectable supra gingival plaques was removed without touching the gingiva to minimize plaque contamination of the strips. Crevicular fluid was collected onto pre-weighted 2×8 mm filter paper strips of Whatman 3mm chromatography paper (Whatman industries /Dartford /kent, UK). Before inserting the papers, the individual implant or tooth was gently air-dried and the area was carefully isolated with cotton rolls. For avoiding salivary contamination of the sample, a saliva ejector was used.

Paper strips were consecutively inserted into the crevice until mild resistance was felt.

The strips were left in their position for 30s. Then the strips were placed in Eppendorf tubes which consisted of $400 \mu\text{l}$ phosphate-buffered saline (PBS), weighted and placed on ice. The tubes were then transported to the laboratory and frozen at -70°c until assayed.

GCF and PICF Elution from Strips

GCF and PICF were eluted from the filter paper strips with distilled water using the centrifugal method previously described [28]. Each sample strip was placed in capped micro-centrifuge tube containing a support cap inserted halfway into the tube. There was a drainage hole on the cap which was pierced with a 19-gauge needle. $20 \mu\text{l}$ of distilled water was applied to the strips and then at $3000g$ for 15min the tubes were centrifuged. $20 \mu\text{l}$ of distilled water was applied again and another cycle of centrifugation repeated. The paper strips were discarded and sample removed for protein estimation by a dye binding assay performed in 96-well microtitre plates using borine serum albumin as standard.

Table 2. Mean of PD, PI, GI and BL in the Implant and the Tooth

	Mean	Std. Deviation
PD_i	3.31	1.01
PD_t	3.08	1.31
PI_i	0.41	0.8
PI_t	0.34	0.61
GI_i	0.41	0.54
GI_t	0.31	0.47
BL_i	1.66	1.058
BL_t	1.48	1.055

i= Implant. t= tooth

Interleukin- 1 β assay

IL-1 β level was determined by using IL-1 β ELISA kit (Endogen, Inc, Woburn, MA, USA) according to the manufacturer's instruction using human recombinant standards. Briefly, 50 μ l of the test sample, IL-1 β standard solution, was applied to antibody precoated micro-titer plates. Then 50 μ l volume of biotinylated antibody was added and incubated for 3h at room temperature (RT). Following washing, 100 μ l of streptavidin-HRP solution was added to each well and incubated for 30min at room temperature. After washing, 100 μ l of substrate solution containing 3/3'/5/5'- tetramethyl benzene was added to each well. The plates were incubated for 30min at RT in the dark, followed by application of 100 μ l of INH2 SO4. The best absorbance at 450 nm was measured with a microplate reader.

Statistical analysis:

Data analysis was performed using the SPSS program. For comparison of the concentration of IL-1 β in GCF and PICF, paired sample test was used and the subject mean of each sample site variable was calculated and Pearson correlation coefficient between these subject means were calculated to examine the probability that the clinical measurement of PI, GI, PD and bone loss were associated with the concentration of IL-1 β in GCF and PICF.

RESULT

There was a significant difference in the IL-1 β level in GCF (45.71 pg/ μ l) and PICF (75.26 pg/ μ l) (P=0.001) (Table 1).

The mean clinical data, PD, PI, GI and BL were significantly higher in the case group in comparison with the control group (Table 2).

The correlation between the concentration of IL-1 β and clinical indexes (PD, PI, GI and BL) in both case and control groups were statistically significant (Table 3).

The correlation among different variables in the case and control groups are presented in Table 4 and 5.

In the case group, the range of pocket depth was from 2 to 5mm and a significant positive correlation between PD and other parameters, PI (P=0.01), GI (P=0.02), BL (P<0.0001) and IL-1 β level (P<0.0001) was observed. The correlation between PI and BL and IL-1 β was significant (P=0.02 and P<0.0001, respectively). There was no significant correlation between PI and GI (P=0.12).

A positive correlation was observed between GI and BL and IL-1 β (P=0.0. and P=0.01, respectively) and of course between BL and IL-1 β (P<0.001).

As mentioned former, a positive correlation between IL-1 β level and PD, PI and BL was detected (P<0.0001).

Table 3. Correlation between IL-1 β and Each of the Variables in the Implant and Tooth (Pearson Correlation Coefficient)

	IL-1 β (PICF)	IL-1 β (GCF)
PD	0.85 (P<0.0001)	0.9 (P<0.0001)
PI	0.52 (P<0.0001)	0.59 (P<0.0001)
GI	0.51 (P<0.001)	0.55 (P<0.0001)
BL	0.68 (P<0.0001)	0.72 (P<0.0001)

In the control group, the range of the pocket depth was from 1mm to 7mm and there was a significant correlation between PD and PI, GI and BL and IL-1 β level (P=0.001, P=0.02, P<0.0001 and P<0.0001, respectively).

A positive correlation between PI, and GI, BL and IL-1 β were found (P<0.0001, P=0.05 and P<0.0001, respectively).

On the contrary, the correlation between GI and BL was not significant (P=0.11), but there was a positive correlation between IL-1 β level and GI (P<0.0001) and BL (P<0.0001).

DISCUSSION

Based on the data resulted from the present study, there was a significant difference between the level of IL-1 β in GCF and PICF. This observation may be supported by the result of previous studies, Machtei et al. in 2006 and Scheirano et al. in 2008 [24,30].

It could be due to the different character of the tissue surrounding natural teeth and the peri-implant tissue. In the present study, the correlation between the level of IL-1 β in PICF and PD, PI, GI and also BL have been evaluated and with respect to the achieved data, it has been suggested that there was a positive correlation between IL-1 β level and these variables.

These findings are in agreement with previous studies, such as Murata et al. in 2002, Ataoglu et al. in 2002 and Machtei et al. in 2006 [23,24,29].

The results of the present study confirm previous findings reported by Stashenko et al. (1991), Preiss et al. (1994), Zhong et al. (2007) that have clearly demonstrated a significant correlation between the level of IL-1 β in GCF and clinical parameters such as PI, GI, and PD and also BL [16,31-33].

Table 4. Correlation between Different Variables in the Implant

			PD	PI	GI	BL	IL-1 β
Pearson	PD	Correlation coefficient	1	0.48	0.35	0.87	0.85
		P. Value	0	0.01	0.02	<0.0001	<0.0001
		N.	41	41	41	38	41
	PI	Correlation coefficient	0.48	1	0.24	0.36	0.52
		P. Value	0.01	0	0.12	0.024	<0.0001
		N.	41	41	41	38	41
	GI	Correlation coefficient	0.35	0.24	1	0.36	0.51
		P. Value	0.021	0.12	0	0.02	0.001
		N.	41	41	41	38	41
	BL	Correlation coefficient	0.87	0.36	0.36	1	0.68
		P. Value	<0.0001	0.02	0.02	0	<0.0001
		N.	38	38	38	38	38
	IL-1 β	Correlation coefficient	0.85	0.51	0.51	0.68	1
		P. Value	<0.0001	<0.0001	<0.0001	<0.0001	0
		N.	41	41	41	38	41

N= number

Teles et al. showed that aggressive periodontitis subjects were characterized by a higher IL-1 β /IL-10 ratio than periodontally healthy subjects, suggesting an imbalance between pro- and anti-inflammatory cytokines in aggressive periodontitis [34]. Chaudhari et al. evaluated simplified oral hygiene index (OHI-S), gingival index (GI), periodontal disease index (PDI), probing depth (PD), tooth mobility and bleeding on probing (BOP) between healthy and chronic periodontitis subjects. Gingival crevicular fluid (GCF) was collected and subjected for ELISA for estimation of IL-1 β . They concluded that the amount of GCF IL-1 β is closely associated with the periodontal status [35]. Petković et al. concluded that patients from the control group (healthy patients) had significantly lower concentrations of IL-1 β , TNF-alpha, IL-8 and MIP-1alpha in PICF compared with the group with mucositis [36].

There are some controversies in the literature regarding the significant difference between the level of IL-1 β in GCF and PICF. Masashi et al. (2002) and Adonogisaki et al. (1995) showed no significant difference of IL-1 β level when they compared components of PICF and GCF [37,38].

In another study, by Melo et al. (2011), no meaningful differences of the level of IL-1 β were found in PICF and GCF in healthy teeth and healthy peri implant and peri implantitis when they compared these three groups [39]. It is suggested that these discrepancies could be mainly due to the different methodology of the researches, for example the number of samples, difference in the loading period of the implant and different times of sampling due to the periodic activity of the inflammatory process (active or silence period) after analyzing the findings.

Table 5. Correlation between Different Variables in the Tooth

		PD	PI	GI	BL	IL-1 β	
Pearson	PD	Correlation coefficient	1	0.5	0.34	0.86	0.9
		P. Value	0	0.001	0.02	0.0001	0.0001
		N.	41	41	41	33	41
	PI	Correlation coefficient	0.5	1	0.52	0.33	0.59
		P. Value	0.001	0	<0.0001	0.05	<0.0001
		N.	41	41	41	33	41
	GI	Correlation coefficient	0.34	0.52	1	0.27	0.55
		P. Value	0.02	<0.0001	0	0.11	<0.0001
		N.	41	41	41	33	41
	BL	Correlation coefficient	0.86	0.33	0.27	1	0.72
		P. Value	<0.0001	0.05	0.11	0	<0.0001
		N.	33	33	33	33	33
	IL-1β	Correlation coefficient	0.9	0.59	0.55	0.72	1
		P. Value	<0.0001	0.001	<0.0001	<0.0001	0
		N.	41	41	41	33	41

N = number

CONCLUSION

It seems that there is a positive correlation between IL-1 β level and variables such as PI, GI, PD and BL, in both GCF and PICF. In the same situation, the level of IL-1 β in PICF is greater than in GCF. Although our findings are in line with many previous studies, there are a lot of controversies in this issue. So maybe it is still soon to consider the markers of bone resorption and inflammatory mediators as predictable markers for determination of the peri-implant health status. It seems that further longitudinal clinical studies are necessary in this issue.

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