

Assessment of Interleukin-1 β and Myeloperoxidase Levels around Implants versus Natural Teeth with the Impact of Aloe Vera

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Abstract: *Background:* It is essential that peri-implant and periodontal sites are studied to contribute comparison data for investigations that are dealing with diseased sites. The objective of this study was to compare the expression of host-derived markers interleukin-1 β (IL-1 β) and myeloperoxidase (MPO) in peri-implant sulcular fluid (PISF) with the gingival crevicular fluid (GCF) of contralateral natural teeth and to investigate the anti-inflammatory effect of Aloe vera gel on these biomarkers. *Materials and Methods:* 20 subjects aged 25- 45 year who have one clinically stable titanium implant carrying a full ceramic crown and one contralateral natural tooth were enrolled in a split-mouth study. Clinical parameters were measured including Plaque index (PI I), Gingival Index (GI), Probing depth (PD). PISF and GCF were collected and the levels of interleukin-1 β and myeloperoxidase were determined using ELISA technique at baseline and 2 weeks after application of Aloe vera gel. *Results:* IL-1 β level in PISF was higher than in GCF with no significant difference ($p > 0.05$) while MPO concentration was higher in PISF than in GCF with highly significant difference ($P < 0.01$). Positive correlations were found between the levels of IL-1 β and MPO with clinical parameters (PI I, GI and PD). After application of Aloe vera gel there was a reduction in the levels of both IL-1 β and MPO with no significant difference ($p > 0.05$) for IL-1 β and highly significant difference ($P < 0.01$) for MPO in implants group only. *Conclusions:* Differential expression of specific biomarkers in GCF versus their levels in PISF is important information prior to establishing PISF as a diagnostic fluid to monitor peri-implant health. Inflammatory mediators production were higher around implants than teeth and Aloe vera gel has anti-inflammatory effect on peri-implant tissue.

Keywords: Peri-implant sulcular fluid, interleukin-1 β , myeloperoxidase, Aloe vera.

1. Introduction

It is currently accepted to say that the interface between soft tissue and implant has similarities with that of natural teeth⁽¹⁾. The condition of the soft and hard peri-implant tissue is highly significant for implant longevity and function⁽²⁾. None of the clinical parameters typically recorded to assess peri-implant health status (i.e. probing depth, bleeding on probing, recession, etc.) have been validated as reliable diagnostic tools to monitor early changes in peri-implant tissues⁽³⁾, however, recent studies point to the utility of peri-implant sulcular fluid (PISF) as a valuable diagnostic aid for detecting early stages of peri-implant pathologies^(4,5). It is well established that cytokines, and biological mediators all play a crucial role in regulating healthy and pathological periodontal and peri-implant conditions⁽⁶⁾. One may assume that an inflammatory process starting at the peri-implant mucosa could involve a destructive reaction, leading to bone resorption in the peri-implant area and thereby endangering the integration achieved between the bone and the implant⁽⁷⁾. It has been shown that some of the constituents of PISF reflect the inflammatory status of tissues around dental implants accurately⁽⁸⁾. For example, levels of interleukin 1 β (IL-1 β), myeloperoxidase and other bone turnover markers correlate well with clinical findings of peri-implantitis^(3,5). Interleukin-1 β has an important role in regulating and in amplifying the inflammatory response in periodontal and peri-implant tissues⁽⁹⁾. An enzyme, myeloperoxidase (MPO), in gingival crevicular fluid (GCF)

is reported to increase in infectious periodontal diseases^(10,11).

The use of natural products in the prevention and treatment of oral conditions has increased recently⁽¹²⁾. Among the various currently available herbal agents, the most popular and currently receiving a lot of scientific attention is Aloe vera⁽¹³⁾ which is well known for its marvellous medicinal properties. These plants are one of the richest sources of health for human beings coming from nature⁽¹⁴⁾. The pharmacological actions of Aloe vera were studied *in-vitro* and *in-vivo* including anti-inflammatory, anti-arthritis, antibacterial and hypoglycemic effects⁽¹⁵⁾. The anti-inflammatory effects of Aloe vera is by inhibition of the cyclooxygenase pathway and reducing prostaglandin E2. In addition to the anti-inflammatory compound called C-glucosyl chromone which was isolated from gel extracts⁽¹⁶⁾.

Taking into account these aforementioned factors, the objective of this study was to assess the clinical parameters and host response IL-1 β and MPO in PISF and compare them to the corresponding levels in GCF obtained from the contralateral natural tooth., as understanding inflammatory response in peri-implant tissue may help to develop treatment plan to prevent periimplant diseases and improve implant outcome.

2. Materials and Methods

Twenty subjects aged 25- 45 year who attended the dental Clinic at the Oral Surgery and Prosthodontic Departments at the Dentistry College / Teaching Hospital / University of Baghdad were selected to be enrolled in a split-mouth study after obtaining agreements to participate in the study by verbal consent. Sample collection was made between July 2015 and January 2016. The subjects were partially edentulous with at least one clinically stable titanium implant carrying a full ceramic crown and one contralateral natural tooth. All participants have to fulfill the following criteria: they have been treated with a two-piece implant system (DentiumCo., Ltd, Seoul Korea) which should be in function for at least 6 months. Additionally, they were free from any systematic diseases and receiving no medication for the three months prior to the study. On other hand, exclusion criteria for all subjects were as follows: smoking, pregnant females and parafunctional habits. Based on clinical assessments, both the 20 dental implants (study group) and 20 contralateral natural teeth (control group) were free from any signs of peri-implantitis and periodontitis. Clinically a stable implant characterized by absence of bleeding on probing, suppuration, and a peri-implant probing depth not exceeding 3 mm^(17,18). Clinical parameters were measured at four surfaces (mesial, buccal, distal and lingual) of each implant and tooth included an assessment of plaque index (PI I)⁽¹⁹⁾, gingival Index (GI)⁽²⁰⁾, probing depth (PD)⁽²¹⁾ and Bleeding on probing (BOP)⁽²²⁾. Clinical measurements were obtained at baseline and two weeks after uses of Aloe vera gel.

Samples were collected in the morning, 2–3 h after breakfast from 5 sites (buccal, mesio-buccal, , mesio-lingual, lingual and disto-lingual) at the selected implants and teeth after plaque index assessment and before registering any other clinical parameters⁽²³⁾. The sites to be sampled were isolated with cotton rolls and gently air-dried to prevent saliva contamination, supragingival plaque was carefully removed with curettes. Paper points (ISO size 30 Munchen, Germany) were gently placed into the sulcus until a mild resistance was felt and left in place for 30 seconds to obtain sulcular fluid samples as previously described^(24,25). This procedure was repeated at each site using a new paper point each time and any one contaminated with blood was discarded. Sample's measurements was immediately determined by using periotron (Harco 6000, U.S.A) which were converted into actual volumes using a calibration graph, then the paper points were placed in Eppendroffs tubes containing (500 µl) phosphate buffered saline (PH 7.4)⁽²⁶⁾. After being centrifuged at 400 rpm for 10 min, the paper points were removed and the samples stored at -80°C until further analysis⁽²⁷⁾ (Sigusch et al., 1992). Biochemical analysis was done by using ELISA kit (Human, Shanghai, China) to determine the level of IL-1B and MPO (in picogram per milliliter) for PISF and GCF before and after the application of Aloe vera gel which prepared by direct gel extraction⁽²⁸⁾ in which mature, healthy and fresh leaves of Aloe vera were washed in the running tap water for 5 min and rinsed with warm sterile distal water at a temperature of 40c° then dissected longitudinally and the gel was peeled from the parenchymal tissue using a sterile knife and blended in an electrical blender for 15 seconds to obtain Aloe vera gel

(100% concentration) which then collected in a sterile container. The gel had been applied by the patients directly on the gingival tissue of the implants and teeth using cotton applicator. The application was performed for 2 weeks, twice a day, in the evening before sleeping and in the morning after breakfast, and they have been instructed not to drink or eat for at least 30 minutes⁽²⁹⁾.

Data analysis for the present study was carried out using Statistical Package for the Social Sciences (SPSS)-21. Normality test used as Shapiro-Wilk test, so for normally distributed variables, the statistical tests are Independent Sample T-test, Paired T-test while for not normally distributed ones, Wilcoxon sum rank test, Wilcoxon Sign rank test. Spearman correlations coefficient were used to assess the relationships between the biomarkers and clinical parameters. P-values ≤ 0.05 were considered as statistically significant and P-values >0.05 were regarded as not significant, while P-values <0.01 were considered as a highly significant.

3. Results

Clinical parameters of dental implants and natural teeth were summarized in Table (1), PI was not significantly differ between implants and teeth (P> 0.05) while GI and PD were higher in implants than in teeth with significant difference (P≤ 0.05) and highly significant difference (P< 0.01) respectively. Table (2) shows IL-1β and MPO expression for both implants and teeth. The concentration of IL-1β around implants although it was higher than that of teeth but the difference was not significant (P> 0.05), conversely, MPO concentration was significantly higher around implants than that of teeth GCF (P< 0.01).

Spearman rank correlation analysis revealed positive correlations between the levels of IL-1β and MPO and clinical parameters (PI, GI and PD) although these correlations were not statistically significant (P> 0.05) as illustrated in Table (3).

After application of Aloe vera gel there was a reduction in the level of IL-1β with no significant difference (P> 0.05) for both implants and teeth as shown in Table (4). Concerning MPO, its level was decreased after application of Aloe vera gel in both implants and teeth groups with highly significant difference (P< 0.01) for implants compared to non significant difference (P> 0.05) for the teeth (Table 5).

Table 1: Clinical parameters of implants and teeth

Variables	Implants (N=20)		Teeth (N=20)		P-value
	Median	Mean rank	Median	Mean rank	
PI I	1.00	21	1.00	20	0.74
GI	2.00	23.50	1.00	17.50	0.05*
PD	2.25	25	1.50	16	0.01**

DF= 38, *Significant difference (P≤ 0.05), **Highly significant difference (P< 0.01)

Table 2: IL-1B and MPO levels around implants and teeth

Biomarker (pg/ml)	Implants (N=20)		Teeth (N=20)		P-value
	Mean ± SD		Mean ± SD		
IL-1B [†]	83.019 ± 16.880		81.552 ± 9.577		0.73
MPO	Median	Mean rank	Median	Mean rank	0.00**
	6.43	29.05	5.13	11.95	

[†]Df= 38 , **Highly significant difference (P< 0.01)

Table 3: Correlations coefficient between the levels of IL-1β and MPO with clinical parameters

Variables	PLI		GI		PD	
	r	P-value	r	P-value	r	P-value
IL1-B	0.069	0.671	0.024	0.882	0.181	0.264
MPO	0.087	0.592	0.040	0.807	0.128	0.431

Table 4: IL-1B level before and after application of Aloe vera gel

Group		Mean	±SD	P-value
Implants	IL-1B bef.	83.019	16.880	0.40
	IL-1B Aft.	78.673	15.703	
Teeth	IL-1B bef.	81.552	9.577	0.48
	IL-1B Aft.	78.146	20.768	

Df=19

Table 5: MPO level before and after application of Aloe vera gel

Group	Before		After		P-value
	Median	Mean rank	Median	Mean rank	
Implants	6.43	29.05	4.94	21.13	0.00 **
Teeth	5.13	11.95	4.56	19.88	0.76

Df=19, **Highly significant difference (P< 0.01)

4. Discussion

This split-mouth study permitted an intraindividual comparison for evaluation of the trend of interleukin 1β (IL-1β) and myeloperoxidase (MPO) in GCF and PISF around the teeth and implants, respectively before and after the application of Aloe vera gel .

PISF may have the potential to provide important information about the events taking place at peri-implant sites. Certain biomarkers have been proposed as potentially valid diagnostic or prognostic markers of periodontal or peri-implant tissue pathology⁽³⁰⁾. To explore the effect of early changes on the levels of molecular marker of inflammation, the basal levels of these markers in PISF must be established.

To the best of our knowledge the present study is the first study in Iraq that investigate some biomarkers around dental implant with the effect of Aloe vera gel. Results of this study showed deeper probing depths around dental implants due to the positioning of implant platform relative to the crestal bone. This may favor the colonization of microorganisms, which may be involved in triggering inflammatory response and increases gingival index around dental implants⁽³¹⁾.

Results of this study showed that the level of IL-1β was higher around implants than that around teeth although the difference was not significant, this finding in accordance with other studies that reported a higher level of IL-1β in the crevicular fluid around implants than around teeth^(32,33) this

may be due to that ions released from dental implants can stimulate peripheral blood mononuclear cells to produce IL-1 β⁽³⁴⁾. On the other hand, previous investigation not find difference in the level of IL-1β in the crevicular fluid around healthy teeth and implants⁽³⁵⁾ which may be due to differences in sample size or methodology of the study.

In regard to the MPO level, it was significantly higher in PISF than in GCF, this finding could be due to that the inflammatory response of PISF and GCF at the molecular level does not seem to be identical in terms of their MPO content. As an indicator of leukocyte migration presence/absence of MPO in either GCF or PISF samples seems to be a good marker of clinical periodontal or peri-implant health⁽³⁶⁾. MPO in the GCF is reported to increase in periodontal inflammation⁽³⁷⁾ initiated by bacteria that colonize the supra- and subgingival environments. On the other hand, differences in the anatomy, histology and function between periodontal and peri-implant tissues are cited as plausible reasons for the differential expression of certain biomarkers⁽³¹⁾. Although epithelium is similar around implant and tooth, many fundamental differences were present between peri-implant connective tissue and its counterpart around natural teeth. In implants, which surface is lack cementum, collagen fibers run a course more or less parallel to the abutment surface and more importantly, they are not inserted into the implant/abutment surface⁽³⁸⁾. As a consequence, the connective tissue adhesion at implant has a poor mechanical resistance as compared to that of natural teeth⁽³⁹⁾ which may lead to differences in inflammatory and immunological responses to bacterial infection⁽²⁾.

Other important result in this study was the positive correlations between the levels of these markers and clinical variables (PI I, GI, PD). It is well established that cytokines, and biological mediators all play a crucial role in regulating healthy and pathological periodontal and peri-implant conditions⁽⁴⁰⁾, therefore the constituents of PISF may reflect the inflammatory status of tissues around dental implants accurately⁽⁴¹⁾. The results of the present study confirm the previous findings which reported that the IL-1β and MPO levels correlate well with clinical findings of peri-implant diseases^(3,5).

The anti-inflammatory effect of Aloe vera gel was reported in this study by using direct gel extraction (100% concentration). Findings of this study revealed that the level of IL-1β has been decreased after the application of Aloe vera gel, although the difference not reach the statistical significance, while the MPO level was decreased in a highly significant differences. This reduction in the level of these biomarkers after the application of Aloe vera gel can be attributed to that Aloe vera could inhibit the inflammatory process as characterized by the reduction of leukocyte adhesion, as well as proinflammatory cytokines⁽⁴²⁾ which may be due to the presence of sterols as anti-inflammatory agents and lupeol as a potential antiseptic ingredient⁽⁴³⁾. Other explanation to this result is that Aloe vera decreases the number of neutrophils and prevents migration of polymorphonuclear leucocytes⁽⁴⁴⁾, as well it diminish the chemical and alternative pathways of complement activity to inhibit the production of free oxygen radicals⁽⁴⁵⁾.

Data of the present study provide a basis for further developing and testing inflammatory and immunological mediators in PISF which appears to have a diagnostic potential for the differentiation between peri-implant health and disease and for a better understanding of the peri-implant biological mechanisms on a molecular level for the early detection or prevention of any peri-implant pathology including peri-implant soft tissue inflammation which is vital for proper functioning of dental implants in the long term. Additionally, based on the present data, the use of Aloe vera gel at optimum concentrations in toothpastes or mouthwashes could be useful for prevention of peri-implant and periodontal diseases.

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