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ORIGINAL ARTICLE

Effect of different localizations of microgap on clinical parameters and inflammatory cytokines in peri-implant crevicular fluid: a prospective comparative study

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Absract The purpose of this study was to evaluate the effect of microgap on clinical and biochemical parameters around dental implants for 1 year. All patients received four implants: group A—Standard Straumann® implants, group B—1 mm subcrestal placement of the polished surface of group A implants, group C—esthetic plus Straumann® implants, group D—subcrestal placement of the polished surface of group C implants. Clinical measurements and peri-implant crevicular fluid (PICF) were collected immediately before loading and at 3rd, 6th, and 12th months after loading, and interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) have been assessed in the crevicular fluid. No significant differences were found in plaque index, gingival index, and probing between the groups throughout the study. However, the PICF volumes of group D were significantly higher than that in the other groups, and group A were significantly lower than the other groups ($P<0.05$). With respect to bleeding on probing values, the percentage of BOP (+) sides in group A implants were fewer than group C and D implants ($P<0.05$). With regard to IL-1β, the levels of IL-1β in group A were lower than that in the other groups during the study ($P<0.05$). In point of TNF-α total amounts, the levels of TNF-α in group A implants were lower than those in group B and D implants ($P<0.05$). Moving microgap coronally from alveolar crest could be recommended for the health of periodontal tissues. Most coronal location of microgap can be suggested in order to maintain the peri-implant health status, particularly in implant sites without esthetic priority.

Keywords Dental implants · Microgap · Inflammatory cytokines · Peri-implant crevicular fluid

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Introduction

The replacement of missing teeth with implant-supported prosthesis has become a widely accepted treatment modality in dentistry, and so far many clinical studies have documented high success rates of endosseous dental implant therapy [1–4]. In implant dentistry, there are two basic implant systems, including submerged and non-submerged implants. In submerged implant systems, a microgap exists at or below the alveolar crest between the implant body and abutment, whereas in non-submerged implant systems because of the extending of implant body above the alveolar crest level, such a microgap does not exist at or below the alveolar crest level [5, 6]. The microflora colonizing the microgap or their products have been considered as a responsible factor for the occurrence of peri-implant bone loss [7, 8]. Several studies showed that the absence of microgap at or below the alveolar crest level in non-submerged implant systems will result in less peri-

implant marginal bone loss than submerged implant systems [8–10]. Implant countersinking below the bone crest, which was recommended in Branemark surgical procedure [1, 3], prevents implant exposure during bone remodeling. Well-documented long-term clinical studies with these systems have also revealed highly predictable outcomes [6, 11, 12].

Histometric studies revealed that the different implant designs influence the dimensions of biological width and the level of crestal bone around the implant [9, 13]. It has been showed that in non-submerged 1-piece implants, the level of first bone-to-implant contact (fBIC) depended on the location of rough/smooth border; however, in all 2-piece implants, the level of fBIC depended on the location of microgap, approximately 2 mm below the microgap [9]. In accordance with these finding, other studies confirmed that if the microgap was moved coronally away from alveolar crest, less bone loss would occur [14, 15].

The presence and importance of the microgap has been investigated in many studies, and some of these studies showed contradictory results to the studies stated above [6, 11, 16]. Thus, whether the location of microgap has an effect on the crestal bone resorption still remains unclear.

Interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are pro-inflammatory cytokines, which stimulate a number of events including alveolar bone loss. It has been considered that much of the damage that occurs during periodontal tissue destruction can be attributed to IL-1 and TNF activity [17–19]. Studies showed that higher levels of IL-1β both in gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF) have been associated with periodontitis and peri-implantitis [20–23]. It has also been showed that the higher levels of TNF-α are associated with periodontal disease and peri-implantitis [17, 18, 24], although there are also data available that its role in peri-implant mucositis and peri-implantitis is not yet clear [20–22].

There are several studies evaluating the importance of microgap; however, no data are available in the literature on the determination of two major inflammatory cytokine, IL-1β and TNF-α level, around dental implants, according to the microgap locations. The aim of this study was to investigate clinical parameters and IL-1β, TNF-α levels around dental implants with different microgap locations on the alveolar crest level for 1 year.

Materials and methods

Patient selection

Ten edentulous patients, 4 women and 6 men, with a mean age of 57 years (SD=10 years), referred to the Periodontal and Clinic at the Gazi University, Ankara. Patients were selected on the basis of the following inclusion criteria.

1. Patients suffering from reduced stability and insufficient retention of the mandibular denture on the severely resorbed mandible (class V–VI) [25].
2. Patients, who are systemically healthy, non-smokers, and not pregnant
3. Patients, who had an edentulous period of at least 2 years
4. Patients without any history of previously inserted oral implants and radiotherapy in the head or neck region

The study was approved by the institutional ethics board for human subjects and each patient has received a detailed description of the proposed treatment for informed consent. Each patient received four Straumann® implants; two standards, and two esthetic plus (ϕ 4.1–10 mm SLA coating, Straumann, AG, Waldenburg, Switzerland). Standard Straumann® implants have a coronal portion with a relatively smooth, machined surface with 2.8 mm; whereas esthetic plus implants have a coronal portion with a relatively smooth, machined surface with 1.8 mm allowing different microgap locations on the alveolar crest.

Treatment procedures

In each patient four implants with four different groups (group A, B, C, and D) were planned to insert in the intraforaminar region of mandible randomly: group A—Standard Straumann® implants, group B—1 mm subcrestal placement of the polished surface of group A implants, group C—esthetic plus Straumann® implants, group D—subcrestal placement of the polished surface of group C implants. Therefore, the microgap of group A implants were 2.8 mm above the alveolar crest; whereas the microgap of group D implants were at the level of alveolar crest. Following local anesthesia, crestal incisions were made, and a full-thickness flap was elevated. All patients received four implants in the intraforaminar region of mandible. Implants were inserted by an experienced surgeon, according to the surgical procedure described previously [26]. The surgical flaps were sutured with non-resorbable sutures. Unless contraindicated, all patients prescribed a non-steroidal anti-inflammatory (200 mg Flurbiprofen), chlorhexidine rinse. The sutures were removed 1 week after surgery. Patients were not allowed to wear the mandibular overdenture during the postoperative 2 weeks. After 2 weeks, the mandibular denture was adjusted by relining.

Three months after implant placement, new maxillary denture and mandibular overdenture were fabricated by one experienced prosthodontist. Implants were splinted with titanium Dolder bars and retentive clips were used to provide retention for mandibular overdentures.

Clinical measurements and PICF were collected immediately before the prosthetic phase (baseline-before loading) and at 3 rd month, 6th month, and 12th after the prosthetic phase (after loading). During the study, patients were instructed not to use systemic antibiotics at least 3 months prior to PICF sampling [27, 28].

Clinical measurements

All examinations were conducted by a single, experienced dental examiner. Clinical measurements were obtained before prosthetic phase, and 3 months, 6 months and 12 months after the surgery, which is described in the following section. Probing depth (PD) measurements were recorded at mesiobuccal, midbuccal, distubuccal, mesiolingual, midlingual, and distolingual surfaces using Williams probes. PD was assessed as the longest distance between the gingival margin and the base of the gingival sulcus. Full mouth gingival index (GI) [29] and plaque index (PI) [30] were also determined. Bleeding on probing (BOP) was recorded as positive if it occurred within 30 s of probing.

PICF sampling and processing

All PICF samples were collected from mesially and distally of each implant after removing all supragingival plaque. The sample site was gently air dried and the area was carefully isolated with cotton rolls in order to prevent samples from contamination. Standardized paper strips (Periopaper, Pro Flow, Amityville, NY, USA) were inserted into the sulcus until slight resistance was felt and left in place for 4 mins[31]. Strips contaminated by bleeding or exudates were discarded. PICF volumes were determined as described previously [32, 33]. Strips were placed into coded micro-centrifuge tubes and stored at −70°C until processing.

PICF enzyme-linked immunoabsorbent assay (ELISA) analysis for IL-1β and TNF-α

The levels of IL-1β and TNF-α in PICF were measured using a sandwich ELISA kit (Biosource, Invitrogen Corporation, Carlsbad, CA, USA). The ELISA procedures were carried out according to the manufacturer's instructions. Micro-centrifuge tubes, containing periopaper strips with absorbed PICF sample, were allowed to reach room temperature and eluted using a centrifugal method [34].

After centrifugation, the strips were removed and the fluid was assayed by ELISA for IL-1β and TNF-α. The ELISA plates were assessed spectrophotometrically at 450 nm. The concentrations of IL-1β and TNF-α in each sample were calculated by using the standards included with the kit. The results were expressed as pg/ml. Total amounts were also calculated by multiplying concentrations and PICF volumes [35].

Statistical analysis

Differences between implant groups were evaluated by repeated measurement two factors ANOVA. ANOVA detected significant main effects for groups of implants and the time period as well as the interaction between these two factors. If ANOVA tests were significant ($P<0.01$), Duncan tests were performed in order to identify differences across implant groups. The values of clinical and biochemical parameters are expressed mean ± standard error of mean (SEM).

Results

Clinical findings

Plaque index (PI) The results of ANOVA indicated that the differences between the implant groups and the interaction between implant and time were non-significant after 12 months of evaluation ($P>0.01$) (Table 1).

Gingival index (GI) The results of ANOVA indicated that the differences between the implant groups and the interaction between implant and time were non-significant after 12 months of evaluation ($P>0.01$) (Table 2).

Probing depths (PD) The results of ANOVA indicated that the differences between the implant groups and the interaction between implant and time were non-significant after 12 months of evaluation ($P>0.01$) (Table 3).

Bleeding on probing (BOP) The results of ANOVA indicated that the differences between the implant groups were significant ($P<0.01$), whereas the interaction between implant and time were non-significant ($P>0.01$). The percentages of BOP (+) sites of group A implants were lower than those from the other groups and the results of Duncan test showed that these difference were significantly lower than those from implants of group C and D ($P<0.05$) (Fig. 1).

Peri-implant crevicular fluid volume (PICF) The results of ANOVA indicated that the differences between the implant groups were significant ($P<0.01$), whereas the interaction between implant and time were non-significant ($P>0.01$). The results of Duncan test showed that the PICF volume of group A implants were significantly lower than the other groups ($P<0.05$) and the PICF volume of group D implants were significantly higher than the other groups ($P<0.05$) (Fig. 2).

Table 1 Plaque index scores between implant groups after 12 months of evaluation

Implant	Sample size	Mean	Standard error of mean	Standard deviation	Minimum	Maximum
A	10	0,4440	0,1030	0,6490	0,000	2,500
B	10	0,2750	0,0681	0,4304	0,000	1,750
C	10	0,3250	0,0647	0,4090	0,000	1,500
D	10	0,3375	0,0661	0,4181	0,000	1,250

Biochemical parameters

IL-1β concentration (pg/ml) The results of ANOVA indicated that the differences between the implant groups were significant ($P<0.01$), whereas the interaction between implant and time were non-significant ($P>0.01$). The results of Duncan test showed that the concentration of IL-1β in group A implants were significantly lower than those from the other groups throughout the study ($P<0.05$).

IL-1β total amount (pg) The results of ANOVA indicated that the differences between the implant groups were significant ($P<0.01$), whereas the interaction between implant and time were non-significant ($P>0.01$). The results of Duncan test showed that the total amount of IL-1β in group A implants were significantly lower than those from the other groups during the study ($P<0.05$) (Fig. 3).

TNF-α concentration (pg/ml) The results of ANOVA indicated that the differences between the implant groups were significant ($P<0.01$), whereas the interaction between implant and time were non-significant ($P>0.01$). The results of Duncan test showed that the concentration of TNF-α in group A implants were significantly lower than those from the implants in group B ($P<0.05$).

TNF-α total amount (pg) The results of ANOVA indicated that the differences between the implant groups were significant ($P<0.01$), whereas the interaction between implant and time were non-significant ($P>0.01$). The total amount of TNF-α in group A implants were lower than those from the other groups during the study, and these difference was statistical different from the implants in group B and D ($P<0.05$) (Fig. 4).

Discussion

The purpose of this study was to determine the effect of microgap on clinical and biochemical parameters. In order to achieve maximum standardization, all implants were placed in the intraforaminar region of mandible; the implants, used in this study, were of the same size and diameter and in order to prevent the possible diverse effects of microflora of remaining teeth on dental implants, the patients recruited to this study had an edentulous period of at least 2 years in upper and lower jaws.

In the past decades investigating the biochemical parameters in gingival or PICF has became very popular because of giving possibility of determining the current activity of the disease, the patient's susceptibility and the possible destruction in the future. These biochemical methods provide the early diagnosis and treatment of the disease [36]; therefore, we analyzed IL-1β and TNF-α levels in the PICF in addition to the clinical parameters. IL-1β and TNF-α are both pro-enflamatuar mediators and have a direct effect on the bone metabolism [18, 37].

It has been demonstrated that the total amount of the cytokines seem to be a better indicator to reflect the disease activity than the concentrations [35, 38, 39]; therefore, we based our discussion mainly on the total amount of data, although both total amounts and concentrations were calculated.

There are two basic implant systems stand out Branemark's and Schroeder's original research reports: (a) submerged, two staged implant systems; (b) non-submerged, one stage implant systems. In submerged, two-stage implant systems, a microgap exists at or below alveolar crest between the implant and abutment; however, in non-submerged systems the implant body extends above the alveolar crest, so the microgap does not exist at the level of bone [5]. It has been suggested that the microflora

Table 2 Gingival index scores between implant groups after 12 months of evaluation

Implant	Sample size	Mean	Standard error of mean	Standard deviation	Minimum	Maximum
A	10	0,2437	0,0661	0,4179	0,000	1,500
B	10	0,2938	0,0780	0,4932	0,000	2,000
C	10	0,2938	0,0754	0,4767	0,000	1,500
D	10	0,3062	0,0773	0,4886	0,000	1,500

Table 3 Probing depths measurements between implant groups after 12 months of evaluation (mm)

İmplant	Sample size	Mean	Standard error of mean	Standard deviation	Minimum	Maximum
4	10	1,8640	0,1020	0,646	1,000	3,830
B	10	1,9640	0,1130	0,717	1,000	4,500
C	10	2,0425	0,0997	0,6308	1,000	3,660
D	10	2,1760	0,1170	0,740	1,000	4,660

colonizing microgap or their products are responsible for peri-implant bone loss [14, 40], which is more extended around two staged implants than one-stage implants [7, 41, 42].

Hermann et al. [9] investigated the influence of microgap on the peri-implant tissue formation. They used six different types of implants designs: two types of 1-part implants and four types of 2-part implants. After 3 months of implant placement abutment connections were carried out and animals were sacrificed after 3 months of additional healing. The results of CADIA (computer-assisted densitometric image analysis) showed that the location of microgap influences crestal bone loss and the first bone to implant contact. These findings were supported by a histometric analysis of the same group [10]. Similar to these findings, in our study the levels of inflammatory cytokines associated with bone loss and the percentage of BOP (+) sites in group A implants, which had the most coronal location of microgap, were lower than group D implants, which had the apical location of microgap (at alveolar crest level) ($P<0.05$).

Studies showed that the presence of plaque induces to develop a zone of inflammatory cells in the connective tissue of submerged implants [43, 44]. Even if under normal oral hygiene conditions, these inflammatory cells remain to be established. Broggini et al. [8] investigated the influence of microgap on peri-implant soft tissues histomorphometrically. The results of this study demonstrated that the absence of microgap at the bone crest was associated with the presence of reduced inflammatory cell infiltrate and minimal bone loss. Piattelli et al. [14] evaluated the bone response to the different locations of microgap on the alveolar crest histologically: implants inserted 1 to 2 mm above the alveolar crest, implants inserted at the level of alveolar crest, and implants inserted 1 to 1.5 mm below the alveolar crest. In accordance with previous findings, the results of this study confirmed data published previously that if the microgap was moved coronally away from the alveolar crest, minimum bone loss and minimum inflammatory infiltrate would occur. Similar to these findings, the most coronal location of microgap was related with less numbers of inflammatory cytokines and reduced percentages of BOP (+) sites both before and after abutment connection in our study ($P<0.05$). In addition to these findings, the differences in cytokine levels in our study seem to be occurred under normal oral hygiene conditions like in the previous study [8], as there were no significant differences in plaque indexes between the groups ($P>0.01$), and the scores of plaque indexes were mostly at the minimum level throughout the study.

The cytokines IL-1β and TNF-α were shown to be potent stimulators of bone resorption [21, 45, 46], and several studies have been conducted on the presence and levels of these cytokines in patients with adult periodontitis. [17, 47, 48]. Because of the similar nature of periodontitis and peri-implantitis, several inflammatory markers were

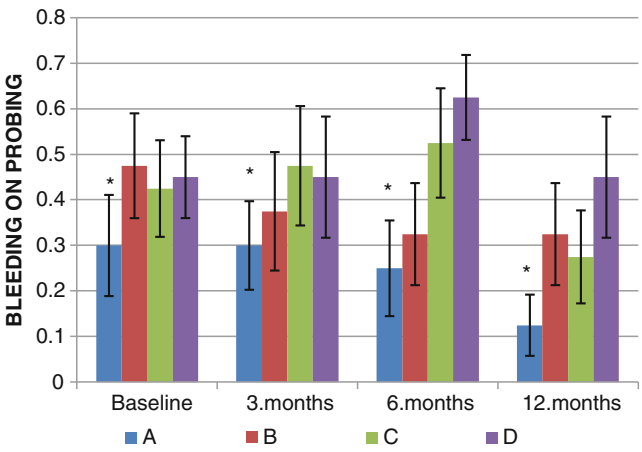

Fig. 1 Bleeding on probing percentages during 12 months of study period (*different from C and D ($P<0.05$)

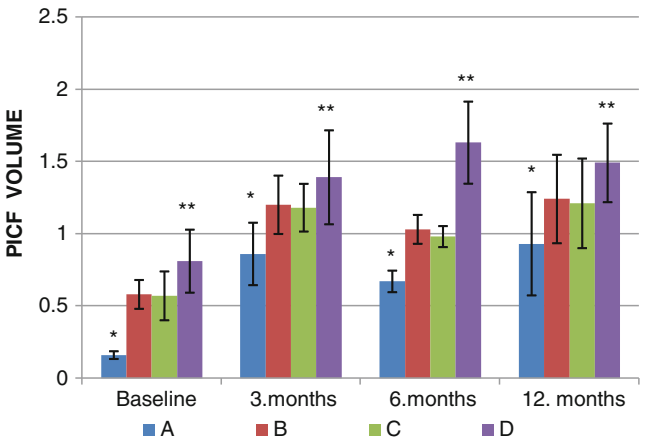

Fig. 2 Peri-implant crevicular fluid (PICF) volume values during 12 months of study period (*different from B, C, D ($P<0.05$), **different from A, B, C ($P<0.05$))

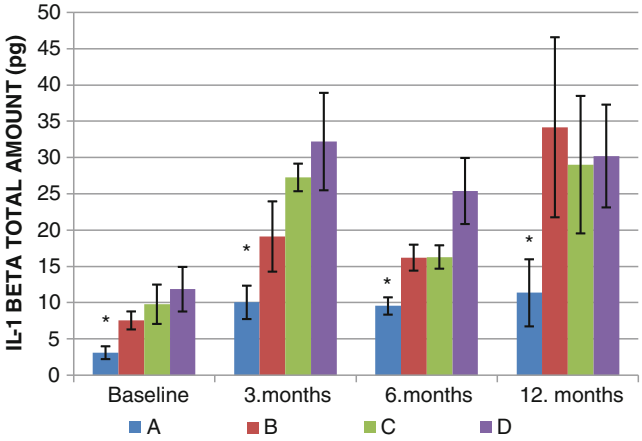

Fig. 3 IL-1 BETA total amounts during 12 months of study period (*different from B, C, D ($P<0.05$))

also investigated in PICF in order to monitor peri-implant health [20–22, 49]. IL-1 composite genotypes has been proposed to modulate the host response to the bacterial challenge and influence susceptibility to peri-implantitis [50]. An elevated level of IL-1β has been found to be associated with peri-implantitis or peri-implant mucositis [19, 21, 22]. Panagakos et al.. [21] investigated the IL-1β levels around healthy implants and implants with peri-implantitis. Implants were categorized clinically as healthy, early peri-implantitis, or advanced peri-implantitis. IL-1β was detected in the crevicular fluid of implants in all three groups and diseased sites showed higher IL-1β levels than healthy sites. Interestingly implants with early peri-implantitis had higher levels of than those from implants with advanced peri-implantitis indicating that this cytokine might be a useful marker especially in the early stages of peri-implant attachment loss. In our study the levels of IL-1β of group A implants were lower than the other groups during the study. Similar to IL-1β levels, BOP (+) sites as well as PICF volumes of group A implants were lower than those the

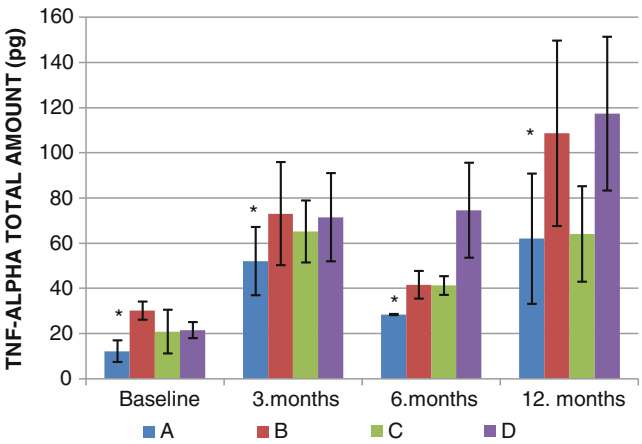

Fig. 4 TNF-alpha total amounts during 12 months of study period (*different from B, D ($P<0.05$))

other groups. These findings supports previous data of Broggini et al.. [8] and Piattelli et al. [14] which showed that the more coronal location of microgap was associated with less inflammatory cell infiltrate.

Contradictory results exist about the role of TNF-α in peri-implant health status. [20, 21, 24]. Schierano et al. [22] showed no significant change in the amount of TNF-α levels after the establishment of plaque-associated mucosal inflammation. Similar to these results, Ataoglu et al. [20] found no correlation between the severity of peri-implant inflammation and the presence of TNF-α. However, recent study of Konttinen et al. [24] showed increased levels of TNF-α both in peri-implantitis and chronic periodontitis. In our study, the levels of TNF-α in group A implants were lower than the other groups during the study and these differences were statistically significant ($P<0.05$) except the differences between the group C implants. These differences in TNF-α levels of group A implant were also in accordance with IL-1β levels and clinical findings proposing that TNF-α could be a useful marker in assessing peri-implant health status.

The placement of polished surface of Straumann implants was investigated by Hämmerle et al. [51]. They found additional marginal bone loss at implants placed deeper and discussed several factors, such as compression of the marginal bone and the biological width concept. In contrast to radiographical findings, the authors reported no significant differences in clinical parameters between test and control implants at any time, except for the modified GI at 4 months (mean difference 0.21, SD 0.19, $P<0.05$). Group B and group C implants used in our study had the same microgap level to the alveolar crest, but the polished surface of group B implants was placed 1 mm deeper into the jaw bone. During 12 months of evaluation, no clinical and inflammatory parameters yielded significant differences between the implant groups at any time, suggesting that from a clinical point of view the microgap level to the alveolar crest might have more effective role than deeper placment.

In spite of many studies reporting that alveolar bone loss around 2-part implants depends on the location of microgap, there are also other studies conflicts with them. Heijdenrijk et al. [11] researched the feasibility of using a 2-piece implant system in a non-submerged procedure and the impact of the microgap. After 5 years of functioning, the results of this study showed no significant differences in clinical, radiological, and microbiological data. The authors concluded that the microgap at the crestal level in 2-piece implants does not appear to have an adverse effect on the peri-implant bone loss.

Todescan et al. [16] evaluated osseous remodeling by placing implants in three different positions in an animal study and after 3 months of abutment connection the results

of this study showed that when the microgap between abutment and implant was placed deeper the bone, additional bone loss did not occur. However, the authors also reported that the deeper the implants, there was a clear tendency to be longer the epithelium and connective tissue, although those differences were not significant.

BOP has been used an objective inflammatory parameter for the evaluation of periodontal conditions. A BOP prevalence of 25% has been considered the cut-off point between patients with maintained periodontal stability and patients with recurrent disease [52]. Studies of Claffey et al. [53] and Badersten et al. [54] revealed further evidence of BOP percentages between 20% and 30% determining a higher risk for disease progression. Individuals with low-BOP percentages (<10%) have been regarded as patients with a low risk for recurrent disease [55]. In our study, the percentage of BOP (+) sides in group A implants (% 12) was fewer than BOP (+) sides in group D implants (45%) ($P<0.05$) suggesting that microgap at alveolar crest might have a tendency to further attachment loss.

It has been showed that the volume of GCF does not differ between implant sites, and natural teeth and the features of inflammation are similar around natural teeth and implants [56]. Researches on peri-implant soft tissues also revealed that the histological arrangement of soft tissues around implants is resemble around natural teeth [57, 58]. Based upon these findings, PICF have been analyzed in many studies in order to determine the implant health status. Several studies showed that the PICF volume increases significantly after the plaque accumulation [23, 59]. The results of our study showed that the PICF volume of group A implants was significantly lower than the other groups ($P<0.05$), and the PICF volume of group D implants was significantly higher than the other groups ($P<0.05$).

The aim of this study was to evaluate the different locations of microgap on alveolar crest both clinically and biochemically. After 12 months of evaluation, the results of our study showed that moving microgap coronally from the alveolar crest would be related with less inflammatory markers and can be suggested in order to maintain the peri-implant health status, particularly in implant sites without esthetic priority. Many conflicting data are available in the literature on the effect of microgap on peri-implant bone loss. Up to the author's knowledge, this was the first study evaluating the effects of in PICF. Further studies are needed to determine the effect of microgap both clinically and biochemically.

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Disclosure None.

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