ANALYSIS OF POTENTIAL FACTORS AFFECTING PERI-IMPLANT SULCUS FLUID (PISF) VOLUME

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ABSTRACT

Background and Aim: PISF analysis is receiving more importance among site-specific tests based on the understanding that it has the potential to reflect the actual status of peri-implant soft and hard tissues. Better understanding of the biodynamics of peri-implant sulcular fluid (PISF) may be an important concern both from a clinical and research aspect. As peri-implant sulcular fluid volume is known to be effected by an array of factors, the aim of the present study was to analyze the potential impact of various peri-implant site-related and dental implant-related factors on PISF volume.

Materials and Methods: PISF was obtained from a total of 128 implant sites. These sites were divided into different subgroups based on the presence/severity of peri-implant inflammation, probing depths (PD), implant features (diameter and length), location of implant (maxilla or mandible) and surgical preference (one-stage or two-stage surgery). Differences between these subgroups were statistically analyzed.

Results: PISF volume was higher at sites with peri-implant mucositis (0.19 µl) and peri-implantitis (0.21 µl) than clinically healthy sites (0.09 µl). Although peri-implantitis group had the highest volumetric value, the difference between peri-implantitis and mucositis groups was not significant. Higher PD values also lead to higher PISF volume. Despite the fact that higher PISF volume was detected at implant sites with one-stage surgery (0.198 µl) compared to two-stage surgery (0.147 µl), in mandible (0.166 µl) compared to maxilla (0.149 µl), in wide implants (0.165 µl) compared to narrow ones (0.141 µl), the difference was not significant (p>0.05). In long and medium implants compared to short ones, higher PISF volume was detected (p<0.05).

Conclusion: Findings of the present study suggest inflammatory peri-implant condition is the main factor that have an influence on PISF volume, while some other factors seemed to have less or limited impact. For reliable PISF analysis, all factors with the potential to influence the actual PISF volume are likely to need a particular concern.

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INTEGRATION

In order to maintain the clinical health of peri-implant tissues and to manage the possible etiological factors associated with peri-implant pathologies, periodical evaluation of dental implant sites is very critical. At this point, early detection of peri-implant inflammation deserves a particular interest. A number of methods that evaluate long-term success and survival rates of dental implants are available. Radiographic examination and determination of peri-implant clinical status by using an array of clinical indices of periodontal origin, such as indices which record gingival inflammation, plaque accumulation, bleeding on probing and probing depths are the most frequently used measures and can be considered as traditional methods of assessment. Since presence and extent of peri-implant inflammation or any pathological deepening in peri-implant sulcus can be detected by the use of such indices, they all provide us with valuable information regarding the clinical status of peri-implant soft tissues. However, most of these indices have certain limitations such as being subjective in nature and not being very sensitive and specific when the onset and progress of inflammatory process is concerned. Among their limitations, the fact that most measures are related to past tissue destruction and do not have the ability to reflect the present peri-implant status, deserves a particular interest. Unfortunately, rather than detecting early inflammatory response, a clinical index usually can only determine the well-established soft tissue inflammatory response. Therefore, with most clinical measures it is not possible to detect early signs of inflammation at dental implant sites. Some investigators have suggested that gingival bleeding is an indicator of periodontal disease activity, however its relationship to disease activity is still unclear. As a result scientists in the field of periodontology and implantology are increasingly focusing on developing site-specific tests of laboratory origin with higher specificity and sensitivity and which may overcome the well-known limitations of traditional clinical measures. It is clearly seen that these attempts are most likely to concentrate on local biological fluids such as gingival crevice fluid (GCF) and peri-implant sulcus fluid (PISF).

GCF is an inflammatory exudate originating from serum and can be harvested from the gingival sulcus surrounding natural teeth. The flow of this biologic fluid is an important determinant of the status of periodontal tissues as it reflects the cellular response in the periodontium by the constituents of serum and contributions from the gingival crevice. Likewise GCF, peri-implant sulcus fluid (PISF) is an osmotically mediated transudate/exudate, which consists of a large array of ingredients. Furthermore, upon the challenge with bacteria originating from the biofilm adherent on the tooth and implant surfaces, host defence mechanisms gingival inflammatory and immune responses are very similar to those of peri-implant mucosa. Adonogianaki et al. analyzed GCF and PISF to compare the inflammatory and immunological responses at implants and teeth and found no significant differences between PISF and GCF. Thus, it may be assumed that the characteristics of peri-implant sulcus are similar to those of periodontal sulcus regarding GCF and PISF. Currently PISF related measures are not applied routinely but focus on the diagnostic potential and validity of this biological fluid is gaining further interest each day. PISF analysis is receiving more importance among site specific tests based on the understanding that it has the potential to reflect the actual status of peri-implant soft and hard tissues. Thus, better understanding of the biodynamics of PISF may be an important concern both from a clinical perspective and research standpoint. Recent studies analyze both the volumetric features and content of this fluid. PISF volume can be affected by wide variety of factors including inflammation, loading protocols, prosthetic design and peri-implant health status. However, when compared to factors affecting GCF volume, factors affecting PISF seemed to be less studied. The aim of this cross-sectional study was to analyze the potential impact of various peri-implant site-related and dental implant-related factors on PISF volume. For this purpose, the potential impact of the presence/severity of peri-implant inflammation, probing depths (PD), dental implant features (diameter and length), location of dental implant (maxilla or mandible) and surgical preference (one-stage or two-stage surgery) were comparatively analyzed.
**MATERIALS AND METHODS**

*Inclusion Criteria and Selection of the Patients*

This study was conducted in Hacettepe University, Faculty of Dentistry, Department of Periodontology. A total of 59 patients were included. Each of these patients was advised of the experimental protocol and signed an informed consent form. Patients were required to have an unremarkable medical history, no known allergies or no metabolic diseases and all were non-smokers. They also had no history of any antibiotic treatment for the prior 3 months. All patients had undergone dental implant treatment. Five different brands, commercially available implants were used (i.e. Ti-Unite Branemark System, Nobel Biocare Goteborg, Sweden; Astra Tech AB, Mölndal, Sweden; ITI, Straumann, Basel, Switzerland; Biohorizons, Birmingham, AL, USA; TSV, Zimmer Dental Inc., Carlsbad, CA) and all patients had dental implant supported fixed porcelain restorations.

Since the study design did not consider the effects of race, gender or age, such variables were not among the exclusion/inclusion criteria. No attempt for any randomization was aimed. PISF was obtained from a total of 128 implant sites after conventionally loading and have been in function for at least six months.

*Determinat of the Peri-implant Health Status*

Clinical status of peri-implant tissues were evaluated by assessing the probing depth (PD), Plaque Index (PI) score, Gingival Index (GI) score, and presence of gingival bleeding on probing. PD measurements were performed around each implant using a Michigan ‘O’ probe and were rolled to the nearest mm to avoid the risk of any volumetric fluctuation due to mechanical irritation, all of the clinical measurements at a given site were record after PISF sampling. To reduce interexaminer variability, all clinical recordings and PISF samplings were performed by the same periodontist (SG).

*PISF Sampling*

PISF samples were obtained according to the method described by Rüdin and colleagues using standardized commercial paper strips (Periopaper®, OraFlow Inc., Amityville, NY, USA). Following the isolation of the sampling area with sterile cotton rolls, supragingival plaque was removed and experimental site was gently air dried to reduce any possible contamination with saliva. Extreme care was taken to minimize mechanical irritation during PISF sampling because this is known to affect the actual fluid volume. Therefore, paper strips were placed at a standardized depth of 1 mm at each sampling site independent from PD measurements and were lefted there for 30 seconds in each sampling. Papers strips contaminated by blood were excluded. To eliminate the risk of evaporation, paper strips with PISF were immediately transported to previously calibrated, switched on and allowed to warm up, Periotron 8000® (Ora Flow, Inc., Plainview, NY, USA) for volume quantification. Before volume measurement, a blank paper strip was placed in the device and the reading dial was set to zero. To increase reliability, the calibration of the device was checked periodically by triplicate readings, as previously described. The PISF was measured electronically in Periotron units, which were converted to microliters (µl) by MCCONVRT software (Ora Flow).

*Determinat of Subgroups*

Implant sites were divided into 3 groups according to the presence/severity of peri-implant inflammation. GI score of 0 with PD ≤3, was considered to represent the state of clinical health (noninflamed); a GI score >0 with PD ≤3 was considered to reflect the status of peri-implant mucositis, and GI score >0 with PD >3 and evident bone loss in standardized radiographic evaluation was considered to represent the pathologic state of peri-implantitis. Because of the lack of any consensus on classification of implants according to the implant diameter and length, we preferred to divide implants into two main groups as wide dental implants (implant diameter >3.8 mm), and narrow dental implants (implant diameter ≤3.8 mm). When length of dental implants were considered, implants were assigned to one of three experimental study groups as; long implants (implant length ≥12mm) short implants (implant length ≤9mm) and the implant lengths between these limitations were considered as medium implants. To analyze the potential effect of gravity, dental implants were also analyzed based on their location (maxilla or mandible). The type of surgical procedure preferred (one-stage or two-stage surgery), was also considered.

*Statistical Analysis*

SPSS 11.5.0 software for Windows (SPSS, Chicago, IL) was used for statistical analyses. Chi-square test was used for gingival bleeding. Among the data that were distributed normally, ANOVA test was used. Multiple comparisons were done by Tukey HSD test. For the data which were not normally distributed, Kruskal-Wallis and Mann-Whitney tests were used. After the Kruskal-Wallis...
test. Pairwise comparisons were done by Mann Whitney test with Bonferroni correction. For all parameters p values <0.05 were considered to be statistically significant.

RESULTS

A total of 128 implant sites in 59 subjects were included in the present cross-sectional study. All patients had been treated with fixed implant supported prosthesis, and experimental measurements were carried out after conventional loading of dental implants. The number of the sites presented with healthy peri-implant clinical status was 50, peri-implant mucositis was 48, and with peri-implantitis was 30.

Analysis of Clinical Parameters

Table I shows the data regarding clinical parameters. The differences in GI, PD, bleeding on probing (BOP) between all groups were significant (p<0.0001) (Peri-implantitis>peri-implant mucositis>healthy). PI scores were significantly higher in peri-implantitis (p<0.01) and peri-implant mucositis (p<0.0001) than clinically healthy group but the difference between peri-implantitis and peri-implant mucositis group was not significant (peri-implantitis= peri-implant mucositis > healthy).

PISF Volume Based on Inflammation and Peri-implant Pocket Formation

PISF volumes were significantly higher at sites with peri-implant mucositis (0.190 µl) and peri-implantitis (0.213 µl) compared to clinically healthy sites (0.096 µl) (p<0.0001). Analyses demonstrated a trend of increase in PISF volume with the severity of peri-implant inflammation (healthy<peri-implant mucositis<peri-implantitis). Although peri-implantitis group had the highest volumetric value, difference between the inflammed groups was not significant (p > 0.05) (Table 1).

Higher probing depths were detected around 30 implant sites (PD>3mm), and 98 had probing depths ≤3 mm. Higher PD also lead to apparently higher PISF volume. At dental implant sites with pocket formation higher PISF volume (0.212 µl) was detected when compared to dental implant sites without any pathological pocket formation (0.14 µl) (p<0.05). Further statistical analyses were carried out for the presence of inflammation and pocket formation. In the absence of pocket formation (peri-implant mucositis and peri-implant health status groups) significantly higher PISF volumetric values in inflamed peri-implant tissue were detected (p<0.0001) (Table 2).

PISF Volume Based on the Gender of the Patient

Sixty-four implant were studied in both gender to analyze the possible impact of gender on PISF volume. For male patients slightly higher PISF volume was detected (0.161 µl) than female patients (0.155 µl) however the difference was not significant (p>0.05). Pocket formation increase PISF volume in both gender (male 0.183 µl, female 0.222 µl) compared to sites without pocket formation (male 0.154 µl, female 0.13 µl). In addition, inflammation also increases PISF volume compared to healthy sites. However the difference did not reach to a significant level (Table 3).

PISF Volume Based on the Type of Surgical Procedure and Location of Dental Implants

The impact of dental implant site location and the type of surgical implant placement procedure were also analyzed. Of all dental implant sites, 35 dental implants were placed by the use of one-stage surgery, while 93 of them were placed with two-stage surgery. Despite the fact that higher PISF volume was detected at implant sites with one-stage surgery (0.198 µl) compared to two-stage surgery (0.147 µl) the difference was not significant (p>0.05) (Table 4). When the location of dental implants were considered, seventy one of the implants were located in the mandible and 57 of them in the maxilla. The difference between PISF volumetric values measured in mandible (0.166 µl) and maxilla (0.149 µl) also was not significant (p>0.05) (Table 5).

At sites with pocket formation generally higher PISF volumes were found for implant sites with one stage surgery (0.248 µl), two stage surgery (0.204 µl) and implants which were placed in mandibula (0.292 µl) and maxilla (0.19 µl) than sites without pocket formation. However the values did not differ significantly among the groups (p>0.05).

When the presence/absence of inflammation was considered in addition to pocket formation, increased PISF volumes were found at inflammed sites without pocket formation (p>0.05).

PISF Volume Based on Width of Dental Implants

Implants were divided into two groups as wide dental implants (width >3.8 mm), and narrow dental implants (width ≤3.8 mm). Analyses demonstrated a trend of increase in PISF volume with increased width of dental implant (wide>narrow) (p>0.05).

Pocket formation increases the PISF volume in both narrow (0.217 µl) and wide (0.21 µl) implants compared to sites without pocket formation (narrow 0.12 µl, wide 0.152 µl).
Table 1. Data regarding clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>Health (n=50)</th>
<th>Peri-implant Mucositis (n=48)</th>
<th>Peri-implantitis (n=30)</th>
<th>Multiple Comparisons</th>
</tr>
</thead>
</table>
| GI               | 0             | 0.7±0.44 (0.25 - 2.25)        | 1.28±0.41 (0.5 - 2.25) | Health vs mucositis, *  
Health vs peri-implantitis, *  
Mucositis vs peri-implantitis, * |
| PI               | 0.08±0.18 (0 - 1) | 0.36±0.46 (0 - 2) | 0.33±0.42 (0 - 1) | Health vs mucositis, *  
Health vs peri-implantitis, **  
Mucositis vs peri-implantitis |
| BOP              | 0             | %57.4                         | %85.7                  | Health vs mucositis, *  
Health vs peri-implantitis, *  
Mucositis vs peri-implantitis, * |
| PD (mm)          | 1.46±0.53 (1 - 2.75) | 2.09±0.52 (1 - 3) | 3.29±0.72 (1.83 - 4.83) | Health vs mucositis, *  
Health vs peri-implantitis, *  
Mucositis vs peri-implantitis, * |

Chi-square test was used for bleeding on probing. ANOVA test was used for PD. Multiple comparisons were made by Tukey HSD test. For GI and PI values, after the Kruskal Wallis test, pairwise comparisons were done by Mann Whitney test with Bonferroni correction.

*: Significant (p<0.0001), **: Statistically significant difference (p<0.01)
Mean±SD, Minimum-maximum values are provided in parantheses

Table 2. Data of subgroups based on the peri-implant pocket formation and presence of inflammation around dental implants

<table>
<thead>
<tr>
<th>PERI-IMPLANT POCKET FORMATION (-) (n=98)</th>
<th>0.141±0.130 µl (0-0.54 µl)</th>
<th>PERI-IMPLANT POCKET FORMATION (+) (n=30)</th>
<th>0.212±0.163 µl ** (0-0.63 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Inflammation (-) (n=50)</td>
<td>0.09±0.08 µl (0-0.35 µl)</td>
<td>Peri-implant mucositis Inflammation (+) (n=48)</td>
<td>0.19±0.147 µl * (0.01-0.54 µl)</td>
</tr>
<tr>
<td>Peri-implantitis Inflammation (-) (n=0)</td>
<td></td>
<td>Inflammation (-) (n=0)</td>
<td></td>
</tr>
<tr>
<td>Peri-implantitis Inflammation (+) (n=30)</td>
<td>0.213±0.163 µl * (0-0.63 µl)</td>
<td>Peri-implantitis Inflammation (+) (n=30)</td>
<td>0.213±0.163 µl * (0-0.63 µl)</td>
</tr>
</tbody>
</table>

Kruskal-Wallis and Mann-Whitney tests were performed.
*: Statistically significant difference (p<0.0001), **: Statistically significant difference (p<0.01)
Mean±SD, Minimum-maximum values are provided in parantheses

Table 3. Data of implant sites based on gender of patients

<table>
<thead>
<tr>
<th></th>
<th>MALE (n=64)</th>
<th>0.161±0.134 µl (0-0.54 µl)</th>
<th>FEMALE (n=64)</th>
<th>0.155±0.146 µl (0-0.52 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pocket (-) (n=49)</td>
<td>0.154±0.133 µl (0-0.54 µl)</td>
<td>Pocket (+) (n=15)</td>
<td>0.183±0.135 µl (0.06-0.63 µl)</td>
<td></td>
</tr>
<tr>
<td>Pocket (-) (n=49)</td>
<td>0.13±0.123 µl (0-0.5 µl)</td>
<td>Pocket (+) (n=15)</td>
<td>0.222±0.184 µl (0-0.52 µl)</td>
<td></td>
</tr>
</tbody>
</table>

Mann-Whitney test was performed (p>0.05).
Pocket: Peri-implant pocket formation >3mm
Mean±SD, Minimum-maximum values are provided in parantheses
Table 4. Data of subgroups based on the type of surgical procedure of dental implants

<table>
<thead>
<tr>
<th>Procedure</th>
<th>PISF Volume (Mean±SD)</th>
<th>Minimum-maximum Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Stage Surgery</td>
<td>0.198±0.168 µl</td>
<td>(0.01-0.83 µl)</td>
</tr>
<tr>
<td>Pocket (-) (n=29)</td>
<td>0.188±0.154 µl</td>
<td>(0.01-0.5 µl)</td>
</tr>
<tr>
<td>Pocket (+) (n=6)</td>
<td>0.248±0.236 µl</td>
<td>(0.06-0.63 µl)</td>
</tr>
<tr>
<td>Two Stage Surgery</td>
<td>0.147±0.127 µl</td>
<td>(0-0.54 µl)</td>
</tr>
<tr>
<td>Pocket (-) (n=69)</td>
<td>0.121±0.113 µl</td>
<td>(0-0.54 µl)</td>
</tr>
<tr>
<td>Pocket (+) (n=24)</td>
<td>0.204±0.146 µl</td>
<td>(0-0.52 µl)</td>
</tr>
</tbody>
</table>

Mann-Whitney test was performed (p>0.05).
Pocket: Peri-implant pocket formation >3mm
Mean±SD, Minimum-maximum values are provided in parantheses

Table 5. Data of subgroups based on location of dental implants

<table>
<thead>
<tr>
<th>Location</th>
<th>PISF Volume (Mean±SD)</th>
<th>Minimum-maximum Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandibular Implants</td>
<td>0.166±0.151 µl</td>
<td>(0.01-0.63 µl)</td>
</tr>
<tr>
<td>Pocket (-) (n=66)</td>
<td>0.157±0.141 µl</td>
<td>(0.01-0.54 µl)</td>
</tr>
<tr>
<td>Pocket (+) (n=5)</td>
<td>0.292±0.236 µl</td>
<td>(0.09-0.63 µl)</td>
</tr>
<tr>
<td>Maxillary Implants</td>
<td>0.149±0.127 µl</td>
<td>(0-0.52 µl)</td>
</tr>
<tr>
<td>Pocket (-) (n=32)</td>
<td>0.11±0.09 µl</td>
<td>(0-0.04 µl)</td>
</tr>
<tr>
<td>Pocket (+) (n=25)</td>
<td>0.19±0.14 µl</td>
<td>(0-0.04 µl)</td>
</tr>
</tbody>
</table>

Mann-Whitney test was performed (p>0.05).
Pocket: Peri-implant pocket formation >3mm
Mean±SD, Minimum-maximum values are provided in parantheses

However, the differences did not reach to a significant level. When the presence/absence of inflammation was considered in addition to pocket formation, inflammed sites generally presented with higher PISF values (Table 6).

PISF Volume Based on Length of Dental Implants
Dental implants were assigned to one of the three study groups according to their length as; long implants (with ≥12 mm length) and short implants (with ≤9 mm) while implants between these limits were considered as medium implants. There were 13 short, 76 medium and 39 long dental implants. A trend of increase in PISF volume was detected with the increased length of dental implants (long>medium>short). PISF volume differences between short (0.061 µl) and medium (0.161 µl) (p<0.05) and short and long (0.182 µl) implants (p<0.05) were statistically significant, however the difference between medium and long implants was not found to be significant (p>0.05).

At sites with pocket formation generally higher PISF volumes were found for short, medium and long implants than sites without pocket formation (p>0.05).

When, the presence/absence of inflammation was considered in addition to pocket formation, inflammation increased PISF volumes at sites without pocket formation at medium (p<0.05) and long (p= 0.001) implant sites (Table 7). Other bilateral comparisons could not be performed due to limited sample size.

DISCUSSION
Dental implant supported prosthesis is an important component of modern dentistry, and one of the important reasons for failure of dental implants is peri-implantitis which...
CLINICAL DENTISTRY AND RESEARCH

Table 6. Data of subgroups based on the width of dental implants

<table>
<thead>
<tr>
<th></th>
<th>NARROW IMPLANTS (≤3.8mm) (n=50)</th>
<th>WIDE IMPLANTS (&gt;3.8mm) (n=78)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Pocket (-)</td>
<td>0.141±0.103 µl (0.01-0.42 µl)</td>
<td>0.165±0.161 µl (0.01-0.63 µl)</td>
</tr>
<tr>
<td>Pocket (+)</td>
<td>0.12±0.095 µl (0.01-0.42 µl)</td>
<td>0.21±0.149 µl (0.09-0.54 µl)</td>
</tr>
<tr>
<td>Inflammation (-)</td>
<td>0.151±0.10 µl (0.01-0.42 µl)</td>
<td>0.217±0.1 µl (0.09-0.42 µl)</td>
</tr>
<tr>
<td>Inflammation (+)</td>
<td>0.217±0.1 µl (0.09-0.42 µl)</td>
<td>0.22±0.167 µl (0.01-0.54 µl)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mann-Whitney test was performed (p>0.05).
Pocket: Peri-implant pocket formation >3mm
Mean±SD, Minimum-maximum values are provided in parantheses

is responsible for soft and hard tissue break-down. Early detection of this inflammatory condition is critical in order to prevent the irreversible effects of inflammatory condition on soft and hard tissues that support implants. Clinical indices provide us with valuable information regarding the clinical status of peri-implant soft tissues. However, most of these indices have certain limitations such as being subjective in nature and not being very sensitive and specific when the onset and progress of inflammatory process is concerned. Rather than detecting early inflammatory response, a clinical index usually can only determine the well-established soft tissue inflammatory response. Therefore, with most clinical measures it is not possible to detect early signs of inflammation at dental implant sites. However, early signs of soft tissue inflammation includes increase in sulcular fluid and bleeding on probing. In most cases evaluating the clinical parameters alone cannot be used as disease predictor. When the limitations of most clinical measures are concerned i.e. show past tissue destruction and do not have the ability to reflect the present peri-implant status, PISF, as an analog of GCF, is considered to have a potential diagnostic value for the early identification of metabolic and destructive processes around dental implants. Despite the potential similarities between volumetric and inflammatory characteristics of GCF and PISF, and the significant amount of research conducted on GCF, PISF volume and various biological components of this fluid have gained further interest recently. Various host-derived enzymes and their inhibitors, inflammatory mediators, host-response modifiers and byproducts of soft and hard tissue breakdown have been studied in PISF to evaluate clinical health and different clinical stages of peri-implant disease. Duarte et al. assessed the effects of mechanical anti-infective therapies for mucositis and peri-implantitis and compared the levels of cytokines in untreated and treated peri-implant diseased sites to healthy ones. The results of this study showed that significant improvements in all clinical parameters for mucositis and peri-implantitis and a significant reduction in TNF-alpha levels for both diseased groups occurred, achieving the same level as the healthy group at 3 months after therapies. In another study, Schierano et al. analyzed levels of tumour necrosis factor alpha (TNF-α), transforming growth factor beta 2 (TGF-β2) and IL-1β in GCF and PISF after a 21-day-period of de novo plaque accumulation. They noted that TNF-α and TGF-β2 did not change significantly among the three different samples. A significant increase of IL-1β was observed after plaque accumulation around the teeth GCF, whereas in the PISF the increase was not statistically significant. Tözüm et al. studied nitrite and myeloperoxidase (MPO) metabolism in both GCF and PISF and, demonstrated a tendency to increase in these ingredients with the presence of gingival peri-implant inflammation. In studies analyzing the volumetric features of PISF, increased PISF volume with the presence/extent of peri-implant inflammation is generally reported.
Table 7. Data of subgroups based on the length of dental implants

<table>
<thead>
<tr>
<th>SHORT IMPLANTS (n=13) (≤9mm)</th>
<th>Pocket (+) (n=1)</th>
<th>Inflammation (-) (n=0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.07 µl</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammation (+) (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.07 µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pocket (-) (n=12)</td>
<td>Inflammation (-) (n=8)</td>
</tr>
<tr>
<td></td>
<td>0.06±0.044 µl</td>
<td>0.061±0.45 µL (0.01-0.14 µl)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammation (+) (n=4)</td>
</tr>
<tr>
<td></td>
<td>0.057±0.05 µL</td>
<td>0.01-0.13 µl</td>
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<td></td>
<td>Pocket (-) (n=64)</td>
<td>Inflammation (-) (n=34)</td>
</tr>
<tr>
<td></td>
<td>0.152±0.131 µl</td>
<td>0.109±0.99 µL (0.035 µl)</td>
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<td>Inflammation (+) (n=30)</td>
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<tr>
<td></td>
<td>0.199±0.15 µL</td>
<td>0.01-0.5 µl</td>
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<tr>
<td>MEDIUM IMPLANTS (n=76) (9&lt;x&lt;12)</td>
<td>Pocket (+) (n=12)</td>
<td>Inflammation (-) (n=0)</td>
</tr>
<tr>
<td></td>
<td>0.205±0.172 µl</td>
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<td></td>
<td></td>
<td>Inflammation (+) (n=12)</td>
</tr>
<tr>
<td></td>
<td>0.205±0.172 µl</td>
<td>0.06-0.63 µl</td>
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<td></td>
<td>Pocket (-) (n=22)</td>
<td>Inflammation (-) (n=8)</td>
</tr>
<tr>
<td></td>
<td>0.147±0.145 µl</td>
<td>0.035±0.26 µL (0.01-0.09 µL)</td>
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<td>Inflammation (+) (n=14)</td>
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<tr>
<td></td>
<td>0.212±0.143 µL</td>
<td>0.01-0.54 µL</td>
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<tr>
<td></td>
<td>Pocket (+) (n=17)</td>
<td>Inflammation (-) (n=0)</td>
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<tr>
<td></td>
<td>0.227±0.163 µl</td>
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<tr>
<td></td>
<td></td>
<td>Inflammation (+) (n=17)</td>
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<tr>
<td></td>
<td>0.227±0.163 µl</td>
<td>0.052 µL</td>
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<tr>
<td>LONG IMPLANTS (n=39) (≥12mm)</td>
<td>Pocket (+) (n=17)</td>
<td>Inflammation (-) (n=0)</td>
</tr>
<tr>
<td></td>
<td>0.227±0.163 µl</td>
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</table>

*: Statistically significant difference (p<0.05), **: Statistically significant difference (p<0.01)
Pocket: Peri-implant pocket formation >3mm
Mean±SD, Minimum-maximum values are provided in parantheses

C-telopeptide pyridinoline cross-links of type I collagen (ICTP) and osteocalcin (OC) levels in the PISF for oral implants with and without peri-implant bone destruction were analyzed and a significantly higher PISF volume at bone destruction sites compared with noninflamed sites demonstrated. Schierano et al.49 revealed that both GCF and PISF could be useful markers of early inflammation in both gingival and peri-implant tissues. There are other studies confirming the volumetric increase of PISF with existence of inflammation.52,53 Schierano et al.49 showed a significant increase in the volumes of GCF and PISF for a period of 21 days of plaque accumulation around teeth and implants and a significant volumetric decrease in both fluids by 69 days. When the possible impact of clinical status, such as presence and severity of inflammation and loading, on
nitric oxide (NO) metabolism around mandibular dental implants was analyzed, Tözüm et al.\textsuperscript{25} found higher mean total nitrite levels and higher PISF volume in inflamed sites compared to noninflamed sites and, a tendency to increase in PISF volume with the severity of inflammation at both early loading and delayed loading implants. Strbac et al.\textsuperscript{17} investigated PISF cathepsin K levels and demonstrated a positive correlation between PD, modified PI, modified bleeding index and increased PISF volume. In the present study, it was noticed that volume of PISF harvested from inflamed dental implant sites was greater than clinically healthy dental implant sites. This finding, suggesting a clear relationship between the presence of inflammation and PISF volume, is consistent with the findings of all previous studies concerning volumetric features of PISF.\textsuperscript{54-56}

In the present study, although PISF volume increased with the presence of peri-implant inflammation, it did not differ significantly between peri-implantitis and peri-mucositis groups. In a similar manner, Tözüm et al. also analyzed volumetric changes of GCF and PISF in healthy and inflamed sites and, demonstrated increased GCF and PISF volume with inflammation but reported no volumetric differences between these two fluids at inflamed sites.\textsuperscript{50} The findings of both studies suggest the obvious impact of peri-implant inflammation on PISF volume, but do not support any additional impact of the severity of inflammation on the volumetric features of this biologic fluid. Sole presence of inflammation seems to be adequate for a clear increase in PISF volume.

In earlier studies the peri-implant condition was determined based on the presence and extent of inflammation. However, in the present study besides peri-implant inflammation, peri-implant condition was also analyzed based on the presence and extent of pocket formation. Although the inflammatory status of soft tissues around dental implants seemed to be the major determinant of PISF volume in a given site, presence of peri-implant pockets also had the potential to have an impact on PISF volume. Wider implants may be considered to have wider peri-implant sulcular area and than more PISF volume may be expected to found within the peri-implant sulcus of wide implants. When the impact of sulcus dimensions on PISF volume was concerned, a trend of increase with implant width was observed, as the highest PISF volumes were noticed at sites with the widest implant diameters. Although a trend of increase was also observed with implant length, differences did not reach to a level of significance when only the presence of pocket formation was considered. Yet, the potential impact of peri-implant soft tissue inflammation was also observed in the presence of inflammation, although there was no pocket formation in medium and long implants. For short implants, it is likely that the absence of definite distinction for implant length (and also width), limited sample size and irregular measures were the interrupted results of the study.

When the relationship between PISF volume and various peri-implant site-related and dental implant-related factors was considered, the type of implant surgery did not seem to have any impact on PISF volume. Although was not significant, higher PISF volume was measured around dental implants in the mandibula when compared to maxilla, suggesting a limited impact of the gravity on PISF volume. However, it was noted that PISF volumes presented with a wide range of distribution. For example the minimum and maximum PISF volumes in “narrow” dental implants group was 0.01-0.42 µl, while in “wide” dental implants group PISF volume range was 0-0.63 µl. Even this was noticed at sites with and without peri-implant inflammation the distribution range of volume was widespread, with prominent overlaps between the different clinical peri-implant conditions (Table2). This feature of PISF is also likely to resemble GCF, as similar wide range of volumetric distribution of GCF was noted in various studies.\textsuperscript{27,57,58} In addition, sample number was not high enough to have definite conclusion. Thus, for both biological fluids the wide range of volumetric distribution, limited sample size, effect of gender and the clear volumetric fluctuations among individual sampling sites need to be taken into account when the methodological design of GCF and PISF related studies or tests are concerned. Since different types of dental implants were used, implant-abutment connection type (like switching platform), implant surface properties and implant design may affect the crestal bone level.\textsuperscript{59,64}

In our knowledge, there is no study evaluating the effect of these parameters (gingival index, implant diameter, type of surgery) on peri-implant measurements in the literature. Therefore in future controlled studies it should be useful to detect the effect of implant design to peri-implant measurements in large comparative study samples.

**CONCLUSION**

When taken together, the findings of the present study suggest that inflammatory peri-implant condition is the main factor that have a clear influence on PISF volume,
while some other factors are likely to pay less or limited role. Similar to the fact that the increase in GCF is widely accepted as one of the earliest signs of inflammation and vascular response, higher PISF volume at inflamed sites may also be considered as one of the initial signs of presence of inflammation and vascular response.

PISF volume and its profile may have the potential to provide important information about the events taking place at peri-implant sites, especially within the peri-implant sulcus, which serves an anatomical region where the onset of inflammation takes place. Based on this understanding, further analysis of PISF volume and its dynamics, profile and ingredients and the potential associations among the parameters related to clinical peri-implant status of a given site and the specific similarities and discrepancies between PISF and GCF may have particular value. Further, PISF profile and its components may promise for a future diagnostic tool in order to develop chair-side tests, for use in daily practice.

REFERENCES


43. Daniel W. Non-parametric and distribution-free statistic. In:


