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Evaluation of gingival crevicular fluid and peri-implant crevicular fluid levels of sclerostin, TWEAK, RANKL and OPG

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ABSTRACT

Background: The combination of local and systemic factors play role in the pathogenesis of periodontal and peri-implant diseases. Host-derived enzymes, cytokines and other proinflammatory mediators play an integral role in this destruction. The aim of this study is to evaluate gingival crevicular fluid (GCF) and peri-implant crevicular (PICF) fluid levels of sclerostin, TNF-related weak inducer of apoptosis (TWEAK), receptor activator of nuclear factor kappa-beta ligand (RANKL) and osteoprotegerin OPG in periodontal and peri-implant tissues in disease and health conditions and also to assess the potential for use as biomarkers.

Materials and methods: The study population was consisted of 50 women and 41 men, in the total of 91 individuals, with a mean age of 51.84 ± 14.05. Periodontitis (n = 22), periodontal health (n = 17), peri-implantitis (n = 27) and peri-implant health (n = 25) groups were established according to clinical and radiographic examination results of 39 teeth and 52 implants restored with fixed prosthetic restorations. In all groups, periodontal and peri-implant parameters (probing depth, gingival recession, gingival bleeding time index, gingival index, and plaque index) were recorded and GCF and PICF samples were also collected. Sclerostin, TWEAK, RANKL and OPG levels in GCF and PICF were measured with ELISA tests.

Results: Peri-implantitis group presented significantly higher levels of Sclerostin ($p = 0.002$), TWEAK ($p < 0.0001$), RANKL($p < 0.0001$), and OPG ($p = 0.037$) compared to peri-implant health group. Similarly, significantly higher levels of TWEAK ($p = 0.001$), RANKL($p < 0.0001$), and OPG($p = 0.025$) were detected in periodontitis group when compared to periodontal health group. Statistically significant correlations were also noted between biochemical parameters and clinical parameters.

Conclusion: Findings of this study evaluating four different bone metabolism related proteins at the same time, suggests levels of sclerostin may be a biomarker for peri-implant disease presenting significantly higher levels in the peri-implantitis group than in the peri-implant health group. Moreover, levels of TWEAK can be a good indicator for both periodontal and peri-implant disease, due to the correlations with periodontal clinical parameters and the higher levels of TWEAK in diseased sites compared to the healthy sites for both dental implants and teeth.

1. Introduction

Inflammatory lesions of periodontal tissues are mainly classified as gingivitis and periodontitis [1], similarly pathologies around dental implants are defined as peri-implant mucositis and peri-implantitis [2]. Gingivitis and peri-implant mucositis are the localized forms of inflammation in soft tissues, with no signs of loss of supporting bone. Periodontitis and peri-implantitis are more pronounced forms of the inflammatory lesions, featuring loss of attachment and supporting bone [1,2]. Similar composition of T and B cell populations, and accumulation of other cell infiltrates were demonstrated in the soft tissues of periodontal and peri-implant tissues [3]. Despite these similarities, the apical extension of inflammatory infiltrate in peri-implant mucosa was threefold higher than gingiva [4]. Also, animal studies revealed

Abbreviations: PICF, peri-implant crevicular fluid; GCF, gingival crevicular fluid; GI, gingival index; PI, plaque index; GBTI, gingival bleeding time index; PD, probing depth

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correlatively larger inflammatory infiltrate sites [5] and greater tissue breakdown in peri-implantitis [5,6]. Greater extention of peri-implantitis lesion to the apical direction compared to periodontitis and larger proportions of inflammatory cell infiltrates also noted in human biopsies [7].

Early and accurate diagnosis of periodontitis and peri-implantitis is important to prevent functional, aesthetic problems and loss of tooth or implant. Combination of clinical and radiographic parameters such as probing depth, bleeding on probing, suppuration and marginal bone loss are commonly used for diagnosis [8]. However, clinical measurements around teeth or implants may be challenged by subjective factors such as force applied and direction of probing or by implant geometry, prosthesis design, and soft tissue biotype [9]. As an adjunctive measurement to aid proper diagnosis, evaluation of levels of various biomarkers is one possible tool. A biomarker is an indicator of a biological situation, which can help to distinguish between health and disease [10]. In periodontology, one of the main challenges is to identify diagnostic and prognostic biomarkers for determination of disease activity and to differentiate between health and disease [11]. Sources of biomarkers for periodontal disease include saliva, serum, subgingival plaque and gingival crevicular fluid (GCF) [12]. GCF is a serum derivate transude in health or an exudate in disease which contains microorganisms, their products, and host derived substances (cytokines, enzymes, immunoglobulins, tissue degradation products, immune system cells) [13]. Similarly, peri-implant crevicular fluid (PICF), which is located in the peri-implant crevice contains valuable biological material that can be used for diagnostic purposes.

Both, periodontitis and peri-implantitis are characterized by bone loss. Thus, many studies examined bone resorption biomarkers such as RANKL and OPG in GCF or PICF in health and disease [13–17]. RANKL is a TNF family member protein, in health, its principal sources are osteoblasts, in disease, it is synthesized mostly from T and B lymphocytes [18]. Receptor activator of nuclear factor kappa-beta (RANK) is the receptor of RANKL, located on pre-osteoclasts. RANK-RANKL interaction activates transcription factors of osteoclastogenesis [19]. OPG is a soluble glycoprotein, the decoy receptor of RANKL, is synthesized from many different types of cells, Formation of RANKL-OPG complex inhibits RANK-RANKL interaction via blocking their binding sites [20]. Elevated concentrations of RANKL in GCF of periodontitis and PICF of peri-implantitis, when compared to healthy areas were demonstrated [14,15,17,21]. Moreover, OPG was also studied in GCF and PICF samples, but conflicting results were reported [17,21].

Sclerostin, another bone metabolism product, is an inhibitor of Wnt-B catenin signalization pathway, which is known to cooperate with bone morphogenic proteins in stimulating bone formation [22]. Sclerostin expression mainly occurs by osteocytes and its elevated levels are related with diminished bone formation by inhibition of osteoblast activity [23]. Napimoga et al. [24] outlined increased sclerostin levels in gingival biopsies from periodontitis subjects compared to biopsies from non-periodontitis subjects. GCF levels of sclerostin have also been studied by Balli et al. [25] and sclerostin total amounts and concentrations were found higher in patients with periodontitis. In PICF, Rakic et al. [26] detected higher sclerostin levels in peri-implantitis group, compared to peri-implant mucositis and peri-implant health groups.

TWEAK, a multi-functional cytokine, participating in inflammatory conditions, is another potential biomarker for periodontal disease. TWEAK has been shown to increase production of proinflammatory cytokines [27]. The potential of the use of levels of TWEAK as a biomarker has been studied for many times in medical sciences, especially in autoimmunology and osteoimmunology. Osteoimmunology is a banner of studies which investigates interactions between a skeletal system and immune system [28]. Rheumatoid arthritis, osteoporosis, multiple myeloma and periodontal diseases are frequently encountered examples of diseases to be subjects for osteoimmunology [28] Until now, TWEAK has been subject of only a few studies in periodontology. Studies by Hosokawa et al. [29] and Kataria et al. [30] presented increased levels of TWEAK in biopsy specimens from gingiva of periodontitis affected tissues. To our knowledge, no published study which investigates TWEAK levels in GCF or PICF is present. Therefore, the aim of this study was, to assess levels of sclerostin, TWEAK, RANKL and OPG in GCF and in PICF from healthy or diseased sites. It was also intended to evaluate the validity of these markers as disease determinants and to investigate correlations between clinical and biochemical parameters.

2. Materials and methods

2.1. Study population

The data and GCF/PICF samples for this study were received from total 91 individuals (41 males and 50 females, aged 22 to 78, mean age: 52.21 ± 14.02 years) who had at least one implant in function at least for 6 months and one contralateral natural tooth without prosthetic restoration. Patients with pregnancy, lactation, and systemic conditions related to peri-implant/periodontal status, diabetes or with a history of cardiovascular and metabolic bone diseases and smokers were excluded. The study procedure was explained in detail and written informed consent forms were obtained from the patients. The study protocol was approved by the Ethics Committee of Hacettepe University, Ankara, Turkey (GO 16/524-20).

Sampling and examination have been performed at the Department of Periodontology, Faculty of Dentistry, Hacettepe University from 2016 to 2017. Subjects divided into four groups: periodontal health, periodontitis, peri-implant health, and peri-implantitis. Clinical diagnosis of periodontitis was based on the American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions [31]. Periodontitis (Fig. 1a and b) was defined as one or more sites of at least one teeth, with inflammation (bleeding on probing), radiographic bone loss and probing depth more than 4 mm. Regarding this, periodontal health was defined as no sign of inflammation, no sites with more than 3 mm

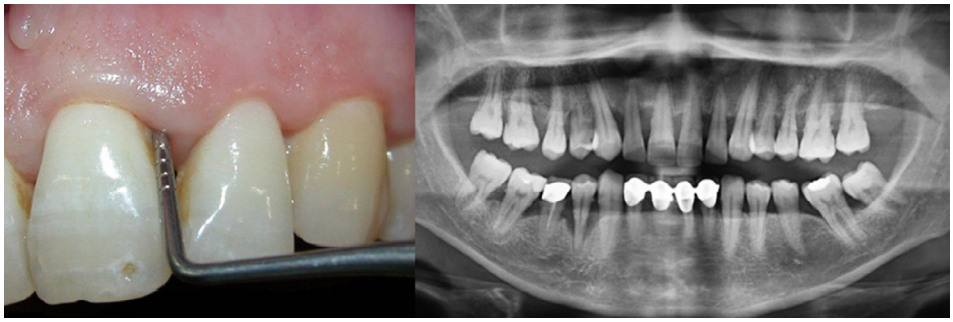

Fig. 1. (a) and (b): Clinical and radiographical presentation of a periodontitis patient. In Fig. 1a 6 mm probing depth and in Fig. 1b periodontal bone loss was presented.

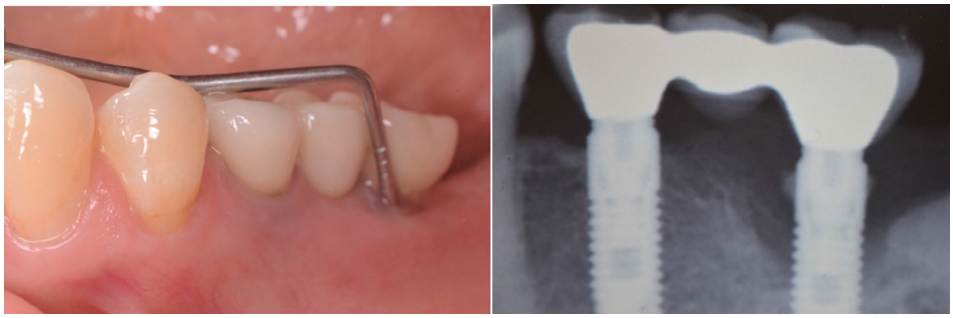

Fig. 2. (a) and (b): Clinical and radiographical presentation of a peri-implantitis patient. In Fig. 1a 6 mm probing depth and in Fig. 2b peri-implant bone loss was presented.

probing depth and no determined radiographic bone loss at a relevant tooth.

Peri-implantitis (Fig. 2a and b). was defined as the presence of at least one peri-implant site with a probing depth (PD) of ≥6 mm accompanied by at least one of the other signs as radiographic bone loss, purulent exudate or bleeding [32]. Peri-implant health was defined as no sign of inflammation, no sites with less than 4 mm probing depth and no evident radiographic bone loss at a relevant implant.

2.2. Clinical evaluation

All patients were treated with a protocol consisting of baseline periodontal treatment (scaling + oral hygiene instructions) and clinical evaluations, GCF and PICF samples were collected after one week period. Clinical parameters were recorded for each implant and tooth sites at four sites using a periodontal probe (Michigan O Color-Coded Probe, Hu-Friedy, Chicago, IL). Probing depth (PD), gingival recession (GR), gingival bleeding time index (GBTI) [33], plaque index (PI) [34] and gingival index (GI) [34] for both implants and teeth were recorded. All examinations performed by an experienced clinician (NY).

2.3. GCF or PICF sampling

GCF and PICF samples were obtained by the method described by Rüdin et al. [35] Following isolation of implant or tooth sites from saliva with cotton rolls, the supragingival plaque was removed by sterilized cotton pellets. Standardized paper strips (PerioPaper Strips, Oraflow Inc., Hewlett, NY) were inserted 1 mm depth at the entrance of gingival or peri-implant sulcus regardless of the PD for 30 s. The samples were obtained from mesiobuccal, midbuccal, distobuccal and palatinal/lingual sites (at least 4 sites) of each implant or tooth. After sampling, paper strips were immediately transferred to a previously calibrated device (Periotron 8000, Oraflow Inc., Hewlett, NY). Care was taken to minimize the time between sampling and the transfer of the paper strips to the device to eliminate the risk of evaporation. GCF or PICF volume was electronically measured in the proprietary units of the measuring device and then converted to microliters using a software program (MLCONVERT.EXE, Oraflow, Hewlett, NY). Paper strips from each implant or tooth were put in a single Eppendorf tube and carefully wrapped to be stored at -20 °C until the day of laboratory analysis.

2.4. Quantification of Sclerostin, TWEAK, RANKL and OPG in GCF or PICF

To determine the levels of sclerostin, TWEAK, RANKL, and OPG in GCF or PICF, paper strips were placed in sterile tubes and stored at -20 °C. Paper strips were cut and their content was extracted by adding 400 µl of sterilized PBS. Samples were studied using commercially available ELISA kits (Human Sclerostin, TWEAK, RANKL, OPG ELISA Kits, Elabscience, Texas) by following the instructions of the manufacturer. Minimum detection level or lower level of detection (LLD) values for ELISA kits were as follows: For sclerostin 62.50 pg/mL; for TWEAK 78.13 pg/mL; for RANKL 15.63 pg/mL and for OPG 0.16 ng/mL. Biochemical data were expressed as concentrations (pg/mL).

2.5. Statistical analysis

Kruskal Wallis Analysis has been performed for the comparison of study groups. Dunn Test has been used in pairwise for the parameters which demonstrate significance with Kruskal Wallis Analysis. The correlations have been investigated by using Spearman's correlation coefficient (*rho*). p values less than 0.05 were considered statistically significant for all parameters. The software was used for all statistical analyses and power calculations (SPSS for Windows, SPSS Inc., Chicago, IL). A difference can be detected at an alpha level of 0.05 between health and disease groups, with a statistical power of 98%, 100%, 98% and 79% for sclerostin, TWEAK, RANKL, and OPG, respectively. However, a statistical power for RANKL/OPG is 65%.

3. Results

Data regarding age, gender, and sampling areas are presented in Table 1. No statistical differences were noted between groups in terms of age and gender indicating the homogeneity of the study population.

3.1. Clinical parameters

Comparison of clinical parameters was represented in Table 2. As expected, PD, GI, GBTI, and GCF/PICF volumes were significantly higher in peri-implantitis group compared to peri-implant health group ($p < 0.05$); and in periodontitis group compared to periodontal health group ($p < 0.05$) (Table 2). When GR values evaluated, no statistically significant difference was detected within teeth and implant groups. However, when implant and teeth groups compared, GR was higher in teeth groups when compared to implant groups in disease and health conditions (periodontitis to peri-implantitis and periodontal health to peri-implant health). For PI values, significantly higher values were detected in periodontitis group when compared to peri-implantitis group ($p = 0.02$)(Table 2).

3.2. Biochemical parameters

Comparison of biochemical parameters for all study groups was summarized in Table 3. When diseased and healthy groups (peri-implantitis to peri-implant health; periodontitis to periodontal health) were compared statistically significant differences were detected in all biochemical parameters, except sclerostin levels when compared between periodontitis and periodontal health (Table 3).

The mean values of PICF levels of sclerostin in peri-implant health group (33.39 ± 22.94 pg/ml) was found significantly lower than peri-implantitis group (63.05 ± 23.62) ($p = 0.002$). For comparison of periodontitis group (66.63 ± 30.20 pg/ml) and periodontal health

Table 1
Descriptive values of groups. (F: Female, M: Male).

			Dental implants		Natural teeth			
			Peri-implantitis	Peri-implant health	Periodontitis	Periodontal health	Total	p
Age mean ± SD (min–max)			55.85 ± 14.22 (22–76)	50.64 ± 13.36 (22–78)	52.43 ± 12.49 (29–76)	48.47 ± 16.13 (22–68)	52.21 ± 14.02 (22–78)	0.345
Gender			17F/10M	12F/13M	11F/11M	10F/7M	50F/41M	0.549
Implant/tooth region	Maxillary	Incisor	1	–	6	1	8	
		Canine	–	–	2	1	3	
		Premolar	2	8	–	4	14	
		Molar	7	4	6	3	20	
	Mandibular	Incisor	–	1	–	–	1	
		Canine	–	0	1	–	1	
		Premolar	4	4	1	4	13	
		Molar	13	8	6	4	31	
Total			27	25	22	17	91	

group (55.38 ± 20.99 pg/ml) the difference did not reach to a statistically significant point ($p > 0.05$). Mean levels of TWEAK in peri-implantitis, peri-implant health, periodontitis, and periodontal health were 250.35 ± 122.95, 80.02 ± 89.24, 269.09 ± 134.75 and 100.58 ± 64.73 as pg/ml respectively. According to these results, when implant groups compared and when teeth groups compared, differences between disease and health were found statistically significant ($p < 0.0001$) (Table 3). Mean levels of RANKL in peri-implantitis group (1.84 ± 0.93 pg/ml) was found significantly higher than peri-implant health group (0.64 ± 0.43 pg/ml) ($p < 0.0001$) and similarly, level of RANKL in periodontitis (2.54 ± 2.43 pg/ml) was significantly higher than periodontal health (0.46 ± 0.42 pg/ml) ($p < 0.0001$). Likewise, TWEAK and RANKL, mean levels of OPG in peri-implantitis were significantly higher than peri-implant health ($p = 0.037$) and the value of periodontitis was significantly higher than periodontal health ($p = 0.025$) (Table 3).

When diseased teeth or implant groups and healthy teeth or implant groups were compared among themselves, no statistically significant differences were detected for sclerostin, TWEAK, RANKL and OPG levels. The only difference of investigated biochemical findings between implants and teeth was seen for RANKL/OPG ratio. Periodontal health revealed a statistically significant decrease in RANKL/OPG ratio compared to peri-implant health ($p = 0.033$).

3.3. Correlations

When correlations between biochemical and clinical parameters were evaluated, positive correlations were found between PD and sclerostin ($p = 0.0014$, $r = 0.257$); PD and TWEAK ($p < 0.0001$, $r = 0.495$); PD and RANKL ($p < 0.0001$, $r = 0.551$), PD and OPG ($p = 0.036$, $r = 0.227$). Other positive correlations were observed between GBTI and TWEAK ($p = 0.043$, $r = 0.228$); GBTI and RANKL ($p = 0.005$, $r = 0.289$); GI and TWEAK ($p = 0.008$, $r = 0.297$); GI and RANKL ($p < 0.0001$, $r = 0.386$).

Positive correlations between biochemical parameters are as: sclerostin and TWEAK ($p < 0.0001$, $r = 0.697$), sclerostin and RANKL ($p < 0.0001$, $r = 0.686$), TWEAK and OPG ($p = 0.001$, $r = 0.366$) and RANKL and OPG ($p = 0.04$, $r = 0.219$).

Table 2
Comparison of clinical parameters.

	Dental implants		Natural teeth		
	Peri-implantitis	Peri-implant health	Periodontitis	Periodontal health	p
	(n = 27)	(n = 25)	(n = 22)	(n = 17)	
	mean ± SD (min–max)	mean ± SD (min–max)	mean ± SD (min–max)	mean ± SD (min–max)	
PD(mm)	5.52 ± 1.64[a] (3.50–10.00)	3.40 ± 0.50 (2.00–4.50)	6.36 ± 2.18[b] (3.75–11.25)	2.19 ± 0.71 (1.00–3.00)	< 0.0001
GR (mm)	0.36 ± 0.62[c] (0.00–2.75)	0.07 ± 0.19[d] (0.00–0.75)	1.67 ± 1.15 (0.00–4.25)	0.69 ± 0.95 (0.00–3.25)	< 0.0001
GBTI	2.37 ± 0.63[a] (1.00–3.75)	1.27 ± 0.76 (0.00–3.25)	2.00 ± 0.79[e] (0.25–3.00)	1.00 ± 1.06 (0.00–3.00)	< 0.0001
GI	1.95 ± 0.26[f] (1.00–2.75)	0.00 ± 0.00 (0.00–0.00)	1.82 ± 0.44[b] (0.00–1.75)	0.00 ± 0.00 (0.00–0.00)	< 0.0001
PI	0.56 ± 0.71 (0.00–2.00)	0.26 ± 0.58 (0.00–2.00)	1.35 ± 0.69[b,g] (0.00–3.00)	0.63 ± 0.54 (0.00–1.75)	< 0.0001
GCF or PICF Volume (μl)	0.40 ± 0.18[h] (0.10–0.68)	0.28 ± 0.12 (0.09–0.52)	0.47 ± 0.13[i] (0.16–0.64)	0.30 ± 0.14 (0.02–0.59)	< 0.0001

[a] : Statistically significant difference between peri-implantitis and peri-implant health ($p < 0.0001$).
[b] : Statistically significant difference between periodontitis and periodontal health ($p < 0.0001$).
[c] : Statistically significant difference between peri-implantitis and periodontitis ($p < 0.0001$).
[d] : Statistically significant difference between peri-implant health and periodontal health ($p = 0.026$).
[e] : Statistically significant difference between periodontitis and periodontal health ($p = 0.014$).
[f] : Statistically significant difference between peri-implantitis and peri-implant health ($p = 0.015$).
[g] : Statistically significant difference between peri-implantitis and periodontitis ($p = 0.02$).
[h] : Statistically significant difference between peri-implantitis and peri-implant health ($p = 0.048$).
[i] : Statistically significant difference between periodontitis and periodontal health ($p = 0.011$).

Table 3
Comparison of biochemical parameters.

	Dental implants		Natural teeth		
	Peri-implantitis	Peri-implant health	Periodontitis	Periodontal health	p
	(n = 27)	(n = 25)	(n = 22)	(n = 17)	
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	
	(min–max)	(min–max)	(min–max)	(min–max)	
Sclerostin (pg/ml)	63.05 ± 23.62[a] (19.95–124.27)	33.39 ± 22.94 (2.90–85.02)	66.63 ± 30.20 (11.44–147.76)	55.38 ± 20.99 (19.99–97.38)	< 0.0001
TWEAK (pg/ml)	250.35 ± 122.95[b] (19.95–124.27)	80.02 ± 89.24 (3.64–295.76)	269.09 ± 134.75[c] (89.65–521.43)	100.58 ± 64.73 (10.72–265.29)	< 0.0001
RANKL (pg/ml)	1.84 ± 0.93[b] (0.27–3.60)	0.64 ± 0.43 (0.14–2.21)	2.54 ± 2.43[d] (0.28–10.52)	0.46 ± 0.42 (0.01–1.42)	< 0.0001
OPG (pg/ml)	190.70 ± 180.02[e] (13.27–724.00)	107.87 ± 103.41 (10.18–499.28)	260.93 ± 196.71[f] (24.13–767.64)	126.61 ± 87.40 (25.68–346.62)	< 0.0001
RANKL/OPG	0.0234 ± 0.0244 (0.0003–0.1084)	0.0153 ± 0.0171[h] (0.0010–0.0729)	0.0278 ± 0.0548[g] (0.0009–0.2483)	0.0073 ± 0.009 (0.0289–1.00)	0.014

[a] :Statistically significant difference between peri-implantitis and peri-implant health (p = 0.002).
[b] :Statistically significant difference between peri-implantitis and peri-implant health (p < 0.0001).
[c] :Statistically significant difference between periodontitis and periodontal health (p = 0.001).
[d] :Statistically significant difference between periodontitis and periodontal health (p < 0.0001).
[e] :Statistically significant difference between peri-implantitis and peri-implant health (p = 0.037).
[f] :Statistically significant difference between periodontitis and periodontal health (p = 0.025).
[g] :Statistically significant difference between periodontitis and periodontal health (p = 0.022).
[h] :Statistically significant difference between peri-implant health and periodontal health (p = 0.033).

4. Discussion

Peri-implant diseases occur with a mechanism similar to periodontal diseases. Although microbial and immunological factors of diseases around implants and teeth are mostly similar, greater amount of bone loss and larger proportions of inflammatory cells are present in peri-implant lesions [5,6]. Since peri-implantitis is a disease difficult to the treat, early detection of inflammatory changes around dental implant is essential for the prevention and the treatment of peri-implant diseases at earlier stages [36].

GCF is a biological fluid which was described as 'a window to periodontium' by Uitto [37]. GCF and PICF include the information about the physiological situation of periodontal or peri-implant tissues. Therefore, their use in advanced diagnostic methods was studied widespread [9,13]. Numerous inflammatory mediators, enzymes, and tissue degradation products are released to GCF in periodontitis [38]. More than 90 different components in GCF have been evaluated [39]. Among these biochemical markers that contribute to bone homeostasis, RANKL and OPG are some of the most studied ones [38]. To the knowledge of authors, this is the first study evaluating TWEAK in GCF and PICF; comparatively assessing GCF or PICF sclerostin levels between teeth and implants; and investigating their correlations with previously studied biomarkers such as RANKL and OPG.

Sclerostin is the inhibitor of canonical Wnt signalization, which contributes to osteoblast differentiation. Its expression is regulated by cytokines, mechanosensors and endocrine factors [40]. Studies investigating levels of sclerostin in periodontitis patients revealed elevated levels in GCF and PICF samples [25,26]. In the present study, increased levels of sclerostin in peri-implantitis compared to peri-implant health is consistent with the study of Rakic et al [26]. Balli et al. [25] reported increased levels of GCF sclerostin in periodontitis patients and also found a decrease in sclerostin concentrations after non-surgical periodontal treatment. In the present study, although slightly higher sclerostin levels were detected in periodontitis group when compared to periodontal health group; the difference was not statistically significant. This may be due to limited participant number. In future studies with higher participant numbers, statistically significant differences could be detected between health and disease groups in implants. When four groups were evaluated, the minimum sclerostin levels were detected in peri-implant health group. These differences in levels of sclerostin may be explained by a study performed by Jager et al. [41] demonstrating sclerostin mRNA expression and protein translation from periodontal ligament cell culture. Therefore, lack of periodontal ligament around dental implants may explain lower levels of sclerostin in absence of periodontal inflammation.

Features of an ideal biomarker include high sensitivity, specifity and owning positive or negative predictive values [42]. Studies investigating TWEAK as a biomarker, revealed its potential via presenting elevated levels in serum or other biological fluids in chronic inflammatory and autoimmune diseases such as rheumatoid arthritis, multiple sclerosis or systemic lupus erythematosus [42]. Since mice with TWEAK deficiency did not present any skeletal anomalies, it gathers attention that catabolic effects of TWEAK on bone occurring via inflammatory conditions [43]. The study performed on TWEAK knockout mice suggested that TWEAK controls transition from innate to adaptive immunity [43].

Hosokawa et al. [29] investigated TWEAK in periodontal tissues. Meanwhile, they determined a low quantity of TWEAK from only one of four biopsies harvested from healthy gingiva, seven of nine gingival specimens from periodontitis affected sites revealed high TWEAK levels. Similarly, a later study performed by Kataria et al. [30] with gingival biopsies found out that gingiva specimens with higher inflammatory scores in immunohistochemical staining revealed higher TWEAK concentrations. In our study, statistically significant increase was observed in GCF or PICF from periodontitis or peri-implantitis compared to periodontal health or peri-implant health. Besides, levels of TWEAK were considerably higher than RANKL, indicating that TWEAK is easier to detect. Another important finding of the present study is the important correlations between clinical gingival inflammation markers and TWEAK. According to these correlations present results, TWEAK is found to be a better indicator for both periodontal and peri-implant disease, due to the correlations with periodontal clinical parameters and the higher levels of TWEAK in disease conditions compared to the health in both dental implants and teeth. Further studies including gingivitis and peri-implant mucositis groups are required to evaluate the potential role of TWEAK in the transition from

gingivitis/mucositis to periodontitis/peri-implantitis.

Pro-inflammatory cytokines cause bone resorption by inducing RANKL and inhibiting OPG expression from osteoblasts and stromal cells [44]. Vernal et al. [15] reported higher RANKL levels in GCF of periodontitis patients compared to healthy control group. Moreover, Monov et al. [16] investigated RANKL and OPG in PICF and they didn't find any relationships between clinical parameters and RANKL or OPG levels. A study by Rakic et al. [17] presented higher sRANKL levels in PICF from peri-implantitis patients compared to peri-implant health. Gürlek et al. [21] who designed a split-mouth study, also found increased sRANKL levels in GCF and PICF from patients with periodontal or peri-implant disease. This study also compared GCF and PICF from implant and teeth, higher sRANKL concentrations in gingivitis than peri-implant mucositis was found. The finding of this study presenting higher RANKL levels in peri-implantitis than peri-implant health and periodontitis than periodontal health are consistent with results of previous studies [14,15,17,21].

OPG, an inhibitor of RANKL, was detected to be higher both in GCF and PICF from healthy control groups [21] and conversely higher levels of OPG in periodontitis compared to periodontal health and in peri-implantitis compared to peri-implant health was also reported [17]. In the present study, OPG levels of disease groups found higher than healthy groups, in accordance with Rakic et al. [17] Increased levels of OPG in inflammatory conditions may be related its functions as low affinity-receptor of TRAIL. TRAIL and its receptors are responsible for apoptosis in endotel cells and inflammatory gene expression [45]. An in vitro study by Kobayashi-Sakamato et al. [46] revealed that P. gingivalis induces OPG expression in endothelial cell lines. Another mechanism may be the anti-inflammatory activity of IL-10 by the increment of OPG for limiting the destruction.

Increased RANKL and decreased OPG levels in periodontitis, and increased RANKL/OPG ratio found in GCF from patients with periodontitis were common findings of previous studies [14,21,25]. Similarly, in the present study, RANKL/OPG ratio in periodontitis was higher than periodontal health. Still, there are some discrepancies reported between studies for RANKL/OPG ratio as an indicator of disease severity or activity [47]. Concerning the studies by Gürlek et al. [21] and Rakic et al. [17], which investigated RANKL/OPG ratio in PICF different results were reported. While higher RANKL/OPG ratio in peri-implantitis was detected in the former study [21], no statistically significant differences were reported between peri-implant health and disease in the latter study [17]. Rakic et al.[17] reported higher RANKL/OPG ratio in peri-implantitis than periodontitis and discussed that this may be due to the spreading of inflammatory lesion directly into bone around dental implants. The present study evaluated the potential differences between teeth and implants regarding investigated biomarkers and the only difference between teeth and implants was found for RANKL/OPG ratio. Peri-implant health group revealed significantly higher RANKL/OPG ratio compared to periodontal health. The result of the present study with a higher ratio in the clinically healthy situation in implants compared to teeth may also refer to higher progression rate of a subclinical lesion in implants.

One limitation of this study was the cross-sectional design, thus, interpretation of the dynamic changes in cytokine profile wasn't possible. A longitudinal design, evaluating different stages of the loading of the dental implants would be better to detect changes in cytokine profile as a response to mechanical loading. As well known, osteocytes respond to mechanical stress and regulation of sclerostin expression from osteocytes depends on skeletal loading. In decreased mechanical loading, upregulation of sclerostin occurs [40]. A recently published study in rabbits by Diao et al. [48] investigated changes of sclerostin and RANKL levels during microdamage caused by impact forces applied to osseointegrated dental implants revealed that sclerostin and RANKL levels increase during resorption. Sclerostin levels should be evaluated in future long-term studies to evaluate the effect of mechanical stress around dental implants.

5. Conclusions

According to present results, it can be suggested that while TWEAK is a valuable biomarker for peri-implant and periodontal disease; sclerostin is a biomarker for peri-implant diseases. However, present interpretations require caution due to the limited number of participants in groups and due to the wide range of ages in groups. Further, long-term studies are needed to clarify the role of TWEAK and sclerostin in the pathogenesis of periodontal and peri-implant diseases.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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