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BONE REMODELING BIOMARKERS RANKL, OPG AND CATHEPSIN K LEVELS IN PERIODONTAL DISEASE PATIENTS: A SYSTEMATIC REVIEW

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ABSTRACT

Objective: The aim of this systematic review was to evaluate biomarkers associated to the bone remodeling process in periodontal disease patients through the evaluation of oral fluids, assessing their validity as possible biomarkers in periodontal diseasediagnosis and progression. **Method and Materials:** A systematic search at the PubMed/Medline, CENTRAL (The Cochrane Library), EMBASE and Web of Science databases was performed: Studies involving the analysis of biomarkers associated to the bone resorption process in salivary fluid (SF) and gingival crevicular fluid (GCF) in patients diagnosed with periodontal disease evaluating their effectiveness as periodontal diseasebiomarkers were selected. **Results:** Twelve articles published between 2004 and 2017 met all the inclusion criteria and were selected for the systematic review. The selected studies demonstrated significantly higher RANKL and Cathepsin-K concentrations and decreased OPG levels in periodontal diseasepatients compared to controls, as well as in patients in more advanced periodontal diseasestages. **Conclusion:** In conclusion, the present study points out the potential use of biomarkers related to bone remodeling in patients diagnosed with periodontal diseasethrough GCF and SF analyses.

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INTRODUCTION

Periodontal disease is characterized as an inflammatory disorder resulting from the imbalance between the interaction process involving periodontal pathogens and host immune responses (Yakob, 2012). Periodontal disease affects the structural components of periodontal tissue at different levels, and an important aspect of tissue destruction in periodontal disease is alveolar bone loss (Bunaes, 2017; Ochanji, 2017; Tobón-Arroyave, 2012 and Tabari, 2013). Bone tissue is involved in continuous remodeling, necessary to maintain tissue homeostasis (Bunaes, 2017). However, pathological processes develop when this balance is altered (Baharuddin, 2015; Hienz, 2015; Sojod, 2017 and Costa, 2018). Alveolar bone resorption is a hallmark for periodontitis and, if not discontinued, may lead to dental mobility development. It is believed that the main mechanism involved in periodontal diseasebone tissue degradation process is RANKL up-regulation and osteoprotegerin down-regulation. The evaluation of biomarkers associated to periodontal disease through the analysis of biological samples, such as salivary fluid and gingival crevicular fluid, are promising means in the analysis of disease activity/prognosis, since they are easily collected materials obtained through non-invasive procedures (de Morais, 2018 and Ghallab, 2018). The analysis of possible biomarkers related to bone tissue reabsorption has been the subject of several studies (Chen, 2014 and Tang, 2016). Understanding the role of these biomarkers in biological periodontal diseasebehavior and identifying quantification means may represent a key aspect in periodontal disease evaluation, as well as in the development of a diagnostic panel assessing the patient's susceptibility toperiodontal disease development/progression by analyzing tissue responses to periodontal treatment (Dereka, 2010 and Belibasakis, 2012). In this context, the aim of this systematic review was to evaluate

biomarkers associated to the bone remodeling process in periodontal disease patients through the evaluation of oral fluids, assessing their validity as possible biomarkers in periodontal disease diagnosis and progression.

MATERIAL AND METHODS

This study was exempt from the Institutional Review Board as only information in the public domain was used. Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) guidelines were followed (Moher, 2009).

Research strategy, Selection, Inclusion and Exclusion criteria: A systematic review was conducted at the PubMed/Medline, CENTRAL (The Cochrane Library), EMBASE, and Web of Science databases, from initial records toOctober2019. A manual search of articles was also performed using the listed references of the selected studies displaying inclusion potential in the present systematic review.

The descriptors used in the systematic search were: Periodontal disease, gingivitis, periodontitis, bone remodeling, bone resorption, osteoclasts, receptor activator of nuclear factor-kB (RANK), RANKL, osteoprotegerin, biomarker and prognostic. Different combinations were used and the Boolean operators AND, OR, NOT were used. The following research strategy example was used for the EMBASE database and appropriately adapted to the other databases. The research strategies were elaborated according to PRESS guidelines recommendations[17] # 1: "Bone remodeling OR bone resorption*NEAR/6 periodontal disease". An initial screening was performed, analyzing the titles and abstracts of eachstudy. All studies considered relevant were obtained in their entirety and analyzed separately by three independent evaluators (EFM, AND, JCP). Subsequently, as inclusion criterion, papers involving the evaluation of biomarkers associated to the bone tissue resorption process in salivary fluid and gingival crevicular fluid in patients diagnosed with periodontal disease, while also evaluating their effectiveness as periodontal disease biomarkers, were selected. The periodontal disease diagnosis should be in agreement with that advocated by the American Periodontics Association (Caton, 2018 and Armitage, 1999). Review studies that did not present compatible methodologies for a systematic analysiswere excluded from this review, such as: reviews, editorial letters, opinions, book chapters, brief communications, conferences, abstracts, patents and studies with insufficient information related to periodontal and systemic health status. In vitro experiments, studies that interfered in the expression of the analyzed biomarkers through therapeutic methods and studies that evaluated pregnant patients were also excluded. Another exclusion factor was the absence of data regarding periodontal disease extension and absence of data related to the clinical analysis used for periodontal disease diagnosis, as well as lack of data related to the results of the biomarker analyses. A reference management software was used to control the analyzed articles and to remove duplicates (EndNote, Thomson Reuters, Philadelphia, PA, USA).

Focused question: A specific question was constructed according to the PICO/PECO guidelines (Participants, Interventions/Exposure, Control, Outcomes), (Maia, 2012) based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The addressed focused question was "Can the analysis of biomarkers associated to bone remodeling/destruction through the evaluation of oral fluids present themselves as valid mechanisms in the evaluation of periodontal disease diagnosis and progression/prognosis?"

(P) Participants: Participants in the research group were required to be diagnosed with periodontal disease.
(E) Types of exposure: Periodontal disease.
(C) Control intervention: Systemically and periodontally healthypatients were considered as controls.
(O) Outcome measures: bone remodelationbiomarkers according to periodontal disease stage.

Quality assessment and data extraction: The following information was collected from all included studies: authors; publication year; country; sample size (number of cases diagnosed with periodontal disease and controls); extension of the periodontal disease; diagnosis criteria; evaluated biomarkers associated to bone tissue resorption; applied biomarker evaluation method; relevant results and conclusion of each study. The methodologies applied in the selected studies (n=12) were analyzed by the reviewers through the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool, applied in assessing bias risk in systematic reviews.[21] Articles were classified as low bias risk, high bias risk or uncertain bias risk, according to the reviewers' critical analysis using the analysis tool.

Data synthesis/analysis: A meta-analysis could not be performed due to variability of the study groups of the included articles. Therefore, a narrative description was adopted herein.

RESULTS

Study selection: The search strategy elaborated in this systematic review resulted in a total of 3,441 studies located in the evaluated databases. After screening the titles and abstracts, 162 studies were considered potentially eligible and read in full by 3 independent evaluators (EFM, AND, JCP). At the end of the analyses, twelve articles published between 2004 and 2017 fulfilled all the inclusion criteria and were selected for this systematic review (Tobón-Arroyave, 2012; Tabari, 2013; Vernal, 2004; Mogi, 2007; Sakellari, 2008; Buduneli, 2009; Bostanci, 2011; Bandari, 2012; Al-Ghurabi, 2015; Hassan, 2015; Behfarnia, 2016 and Gabr, 2017). The flowchart of the screening and article selection process is displayed.

Study characteristics

Regarding methodological characteristics, the patient samples varied between 20[25] and 140[4], with an average of 60.8 participants per studyand total sample size of 730 patients, subdivided into different study groups and controls according to periodontal diseaseabsence/presence and stages (Tables 1 and 2). Among the selected studies, three developed longitudinal analyses, evaluating biomarker levels before and after periodontal treatment (Buduneli, 2009; Bostanci, 2011 and Hassan, 2015). while the other studies developed cross-sectional analyses (Tobón-Arroyave, 2012; Tabari, 2013; Vernal, 2004; Sakellari, 2008; Bandari, 2012; Al-Ghurabi, 2015; Behfarnia, 2016; Gabr, 2017). According to the studies evaluated herein, samples consisted of adult patients, between the 3rd and 6th decades of life.

Table 1. Summary of the descriptive characteristics of the included studies (n=12)

Author	Year	Country	Patients included in the analysis	Sex	Age	Groupsofstudy
Vernal et al. (22)	2004	Chile	32	11 ♂ 21 ♀	46.6*	Group I – 12healthy patients (control); Group II – 20 patients with CP.
Mogi and Otogoto (23)	2007	Japan	85	NI	43.9*	Group I – 19 healthy patients (control); Group II – 20 patients (mild periodontitis); Group III – 24 patients (moderate periodontitis); Group IV – 22 patients (severe periodontitis group).
Sakellari et al. (24)	2008	Greece	73	NI	47.09*	Group I – 38 healthy patients (control); Group II – 35 patients with CP.
Buduneli et al. (25)	2009	Turkey	20	NI	48*	Group I – 10 smoking patients with chronic periodontitis; Group II – 10 non-smoking systemically healthy patients with chronic periodontitis.
Bostanci et al. (26)	2011	Turkey	27	13 ♂ 14 ♀	28.8 (Group I) 44.7 (Group II)	Group I – 13patients with aggressive periodontitis; Group II – 14 patients with CP.
Bandari et al. (27)	2012	India	64	32 ♂ 32 ♀	30-39*	Group I – 16 healthy patients (control); Group II– 16 patients with gingivitis; Group III – 16 patients (mild periodontitis); Group IV – 16 patients (moderate periodontitis); Group V – 16 patients (severe periodontitis group).
Tobón-Arroyave et al. (4)	2012	Colombia	140	52 ♂ 88 ♀	30 (Group I)* 49 (Group II)*	Group I – 43 healthy patients (control); Group II – 97patients with CP.
Tabari et al. (5)	2013	Iran	50	22 ♂ 28 ♀	22-62	Group I – 25 healthy patients (control); Group II – 25patients with CP.
Al-Ghurabi and Mohssen (28)	2015	Iraq	80	57 ♂ 23 ♀	24-64	Group I – 25 healthy patients (control); Group II – 55patients with CP.
Hassan et al. (29)	2015	Egypt	30	12 ♂ 18 ♀	41.2	Group I – 10 healthy patients (control); Group II – 20patients with CP.
Behfarnia et al. (30)	2016	Iran	39	39 ♂	28-57	Group I – 10 healthy patients (control); Group II – 15 smoking patients with chronic periodontitis; Group III – 14 non-smoking systemically healthy patients with chronic periodontitis.
Gabr et al. (31)	2017	Egypt	90	NI	30-55	Group I – 45 healthy patients (control); Group II – 45patients with CP.

Table 2. Results of systematic literature review

Author (year)	Year	Sample analysis	Biomarkers analyzed	Levels of biomarkers (mean ± SD)*	Detectionmethod	Mainconclusion
Vernal et al. (22)	2004	GCF	RANKL	Group I – 63.08±55.08 pg/site Group II –115,53±78.18 pg/site	ELISA	GCF total amount of RANK-L is significantly increased in periodontal disease, supporting its role in the alveolar bone loss developed in this disease.
Mogi and Otogoto (23)	2007	GCF	Cathepsin-K RANKL	*Cathepsin-K* Group I – N. D. Group II – 3.67±0.48 pg/µl Group III: 1.94±0.42 pg/µl Group IV - 1.16±0.26 pg/µl *RANKL* Group I – 10.9±2.8 Group II – 87.7±16.2 pg/µl Group III: 48.9±8.4 pg/µl Group IV - 37.8±10.2 pg/µl	ELISA	There was a positive correlation between cathepsin-K and RANKL levels, suggesting that both of them contribute to osteoclastic bone destruction in periodontal disease.
Sakellari et al. (24)	2008	GCF	sRANKL	Group I – 0.07±0.17 pg/site Group II – 0.19±0.04 pg/site	ELISA	Findings from the present study suggest a correlation of levels of sRANKL with important pathogens in periodontitis patients.
Buduneli et al. (25)	2009	GCF	sRANKL Osteoprotegerin	*sRANKL* Group I – 0.44±0.27 pg/ml Group II – 0.60±0.46 pg/ml Osteoprotegerin Group I – 1.78±1.9 pg/ml Group II – 2.40±4.26 pg/ml	ELISA	Neither smoking nor periodontal inflammation seemed to influence GCF RANKL levels in systemically healthy patients with chronic periodontitis. Smoking and non-smoking patients with chronic periodontitis were not affected differently by the initial periodontal treatment with regard to GCF osteoprotegerin concentrations.

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Bostanci et al. (26)	2011	GCF	RANKL Osteoprotegerin	*RANKL* Group I – 468±580 pg/µL Group II – 433± Osteoprotegerin Group I – 71±74 pg/µL Group II – 85±70 pg/µL	ELISA	The RANKL/osteoprotegerin ratio may not be a helpful molecular predictor of clinically successful treatment. As conventional therapy does not negatively modulate this ratio, the host could still be susceptible to further bone loss. Adjunctive treatments targeting RANK/Osteoprotegerin may be useful in this respect.
Bandari et al. (27)	2012	GCF	Osteoprotegerin	Group I – 162.4±51.1 pg/µL Group II – 40.2±16.6 pg/µL Group III – 23.4±1.9 pg/µL Group IV – 10.9±1.9 pg/µL Group V – 15.6±4.6 pg/µL	ELISA	Osteoprotegerin concentration in GCF was inversely proportional to CAL and not an active progression factor for periodontal disease. Further, after the treatment of moderate-to-severe periodontitis subjects, osteoprotegerin concentrations increased. Hence, it can be concluded that osteoprotegerin could be considered as a 'novel bone marker' the host modulation of periodontal disease.
Tobón-Arroyave et al. (4)	2012	SF	sRANKL Osteoprotegerin	*sRANKL* Group I – 4.00±2.6 pg/ml Group II – 6.00±5.1 pg/ml *Osteoprotegerin* Group I - 131.60±71.4 pg/ml Group II - 95.20±50.0 pg/ml	ELISA	Although salivary concentrations of sRANKL, osteoprotegerin and its ratio may act as indicators of the amount/extent of periodontal breakdown, the mutual confounding and synergistic biological interactive effects related to ageing and smoking habit of the susceptible host may also promote the tissuedestruction in CP.
Tabari et al. (5)	2013	SF	sRANKL Osteoprotegerin	*sRANKL* Group I – 207±83 pg/ml Group II – 266±48 pg/ml Osteoprotegerin Group I - 2.1±1.0 pg/ml Group II - 2.20±0.78 pg/ml	ELISA	Salivary sRANKL/osteoprotegerin ratio may be helpful in the screening and diagnosis of periodontitis. However, longitudinal studies with larger populations are needed to confirm these results.
Al-Ghurabi and Mohssen (28)	2015	SF	RANKL Osteoprotegerin	*RANKL* Group I – 2.21 pg/ml Group II – 56.8 pg/ml Osteoprotegerin Group I - 17.99 pg/ml Group II - 15 pg/ml	ELISA	This study demonstrates that salivary levels of RANKL and osteoprotegerin play a crucial role in pathogenesis of periodontitis, and the relative RANKL/osteoprotegerin ratio appears to be indicative of disease occurrence.
Hassan et al. (29)	2015	GCF SF	Osteoprotegerin	Group I – 198.9 ± 31.5 pg/ml (GCF) 121.8 ± 21.4 pg/ml (SF) Group II – 61 ± 11.9 pg/ml (GCF) 64.5 ± 20.9 pg/ml (SF)	ELISA	Osteoprotegerin might be considered as a diagnostic and prognostic biomarker of periodontal bone destruction.
Behfarnia et al. (30)	2016	GCF SF	RANKL Osteoprotegerin	*RANKL* Group I – 13.33±6.02 pg/ml (SF) 8.61±5.44 pg/ml (GCF) Group II – 12.61±6.49 pg/ml (SF) 8±4.2 pg/ml (GCF) Group III – 20.04±8.8 (SF) 8.08±4.68 pg/ml (GCF) Osteoprotegerin Group I – 80.25±26.75 pg/ml (SF) 75.9±20.92 pg/ml (GCF) Group II – 71.86±21.83 pg/ml (SF) 69.55±23.6 pg/ml (GCF) Group III – 64.31±19.16 (SF) 72.5±20.9 pg/ml (GCF)	ELISA	The salivary RANKL/Osteoprotegerin ratio was higher in nonsmokers with periodontitis in comparison with smoker periodontitis patients.
Gabr et al. (31)	2017	GCF SF	RANKL Osteoprotegerin	*RANKL* Group I – 0.21±0.03 ng/ml (SF) 0.35±0.08 ng/ml (GCF) Group II – 0.35±0.08 ng/ml (SF) 0.33±0.06 ng/ml (GCF) *Osteoprotegerin* Group I – 0.39±0.09 ng/ml (SF) 0.28±0.04 ng/ml (GCF) Group II – 0.22±0.03 ng/ml (SF) 0.20±0.03 ng/ml (GCF)	ELISA	Both GCF and saliva collection are a noninvasive approaches as a diagnostic markers for RANKL, osteoprotegerin and RANKL/osteoprotegerin ratio.

Abbreviators: ELISA, enzyme-linked immunosorbent assays; CP, chronic periodontitis; SF, salivary fluid; GCF, gingival crevicular fluid; RANKL, Receptor activator of nuclear factor-kappa B ligand; sRANKL, Soluble receptor activator of nuclear factor- kappaB Ligand; *, baseline.

Among the selected articles, four did not mention male/female relationship among the participants (Mogi, 2007; Sakellari, 2008 and Buduneli, 2009). The study carried out by Behfarnia *et al*. (Behfarnia, 2016) included only male participants, whilethe studies carried out byTobón-Arroyave*et al*. (Tobón-Arroyave, 2012) and Al-Ghurabi& Mohssen[28] displayed a predominance of male patients. Regarding the other studiesdescribing male/female relationships, no significant difference between the samples according to genderwas detected (Tabari, 2013; Vernal, 2004; Bostanci, 2011; Bandari, 2012; Hassan, 2015).

Table 3. Quality assessment according to QUADAS-2: level of risk

Author	Patients selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Vernal et al. (22)	Low	Low	Low	Low	Low	Low	Low
Mogi and Otogoto (23)	Unclear	Low	Low	Low	Unclear	Low	Low
Sakellari et al. (24)	Unclear	Low	Low	Low	Unclear	Low	Low
Buduneli et al. (25)	Unclear	Low	Low	Low	Unclear	Low	Low
Bostanci et al. (26)	Low	Low	Low	Low	High	Low	Low
Bandari et al. (27)	Low	Low	Low	Low	Low	Low	Low
Tobón-Arroyave et al. (4)	Low	Low	Low	Low	Low	Low	Low
Tabari et al. (5)	Low	Low	Low	Low	Low	Low	Low
Al-Ghurabi and Mohssen (28)	Low	Low	Low	Low	Low	Low	Low
Hassan et al. (29)	Low	Low	Low	Low	Low	Low	Low
Behfarnia et al. (30)	Low	Low	Low	Low	Low	Low	Low
Gabr et al. (31)	Low	Low	Low	Low	Low	Low	Low

Regarding periodontal health, all studies evaluated chronic periodontitis (Tobón-Arroyave, 2012; Tabari, 2013; Vernal, 2004; Mogi, 2007; Sakellari, 2008; Buduneli, 2009; Bostanci, 2011; Bandari, 2012; Al-Ghurabi, 2015; Hassan, 2015; Behfarnia, 2016 and Gabr, 2017). Mogi and Otogoto (Mogi, 2007) and Bandari *et al*. (Bandari, 2012), in their respective clinical analyses, classified chronic periodontitis into mild, moderate and severe, according to the severity and extent of the disease. Bostanci *et al*. (Bostanci, 2011) included a group of patients with aggressive periodontitis in their sample, while Bandari *et al*. (Bandari, 2012) also analyzed a group of patients diagnosed with gingivitis. Two of the selected studies evaluated the influence of smoking habits on the biomarker levels analyzed in periodontal diseasepatients (Buduneli, 2009 and Behfarnia, 2016). Only the study by Bostanci*et al*. (Bostanci, 2011) did not evaluate a control group.

Risk of bias within and across studies: The evaluation of the methodological quality of included studies was performed using the QUADAS-2 tool. No double-blind study was observed (high risk of bias) among the selected articles. Patient selection and material collection was performed specifically for the development of the selected studies (low risk of bias). The bias risk analysis of the selected studies is displayed in Table 3.

Bone remodeling biomarker analyses: The enzyme immunoabsorption technique (ELISA) was used in the selected studies to analyze the evaluated biomarkers. Six of the included studies assessed gingival crevicular fluid exclusively (Vernal, 2004; Mogi, 2007; Sakellari, 2008; Buduneli, 2009; Bostanci, 2011 and Bandari, 2012), three performed analyses through salivary fluid collection (Hassan, 2015; Behfarnia, 2016 and Gabr, 2017) and three studies analyzed both biological materials (Hassan, 2015; Behfarnia, 2016; Gabr, 2017). RANKL, osteoprotegerinandCathepsin-K were the most analyzed biomarkers in the selected studies. Gabr *et al*. (Gabr, 2017) reported a mean RANKL value in gingival crevicular fluidof 0.21 ± 0.02 ng/ml in control patients and 0.33 ± 0.06 in patients presenting chronic periodontitis, with a statistically significant difference between both groups ($p < 0.001$). These results corroborate the findings of the other studies selected for this systematic review (Tobón-Arroyave, 2012; Tabari, 2013; Vernal, 2004; Mogi, 2007 Sakellari, 2008and Behfarnia, 2016). Bostanci *et al*. (2011) observed higher gingival crevicular fluidRANKL levels in patients presenting aggressive periodontitis compared to patients presenting chronic periodontitis. In the study carried out by Bandari *et al* (Bandari, 2012), a means of 162 pg/μL in gingival crevicular fluid was observed in the control group, significantly higher compared to patients diagnosed with gingivitis (40.2 pg/μL), mild chronic periodontitis (23.4 pg/μL) moderate chronic periodontitis (10.9 pg/μL) and severe chronic periodontitis (15.6 pg/μL). In the analysis performed by Gabr *et al*. (Behfarnia, 2016), the mean osteoprotegerin value in salivary fluid was of 0.39 ng/ml in the control group, while the chronic periodontitis group presents means of 0.22 ng/ml, with a significant difference between both groups ($p = 0.001$). Mogi andOtogoto (Mogi, 2007) evaluated Cathepsin-K levels in gingival crevicular fluid and reported Cathepsin-K levels in the control group below detection levels and its presence in patients presenting mild chronic (1.94 pg/μl) and severe (1.16 pg/μl) periodontitis. A significant difference between the control and study groups ($p < 0.05$) was detected. These authors were the only ones to perform Cathepsin-K level analyses among the selected studies.

DISCUSSION

To date, several possible biomarkers present in oral fluids have been proposed, aiming at evaluatingperiodontal disease activity and the possible biological course of the disease, its prognosis and the most appropriate therapeutic procedure for such cases (de Morais, 2018; Ghallab, 2018; Novakovic, 2014). Although many advances related to the identification of such biomarkers have transpired, most reflect the inflammatory process itself, with the development of studies regarding biomarkers involved in theperiodontal tissue process degradation being necessary. Recent systematic reviews have been developed to further identify the role of type 8 matrix metalloproteinases (MMP-8) in systemically healthy patients presenting periodontal disease diagnosed with type II diabetes mellitus (de Morais, 2018 and de Morais, 2018). However, the present study is the first to perform a systematic review evaluating markers associated to bone remodeling in oral fluids in order to identify their possible role in the identification of periodontal disease activity, as well as in the analysis of the clinical course of the disease and its prognosis. Among the selected studies, high RANKL levels were

observed in patients presenting periodontal disease (Tobón-Arroyave, 2012; Tabari, 2013; Vernal, 2004; Mogi, 2007; Sakellari, 2008; Buduneli, 2009; Al-Ghurabi, 2015; Gabr, 2017). Increased RANKL levels are stimulated by inflammatory cytokines found in oral fluids, such as salivary fluid and gingival crevicular fluid. Its presence mediates alveolar bone destruction by stimulating osteoclast activation.[3,12]Consistent with the data presented in the present systematic review, other studies have demonstrated that RANKL levels are increased in periodontal disease patients, where a continuous increase in RANKL is verified according to the stage of the disease and the involvement of modulating factors, such as smoking (Belibasakis, 2012; Sakellari, 2008; Buduneli, 2009 Salminen, 2014; Tang, 2009). Tobón-Arroyave*et al* (Tobón-Arroyave, 2012), Demonstrated a strong correlation between RANKL and osteoprotegerin levels, corroborating the findings of other analyzed studies (Tabari, 2013; Bostanci, 2011; Al-Ghurabi, 2015; Gabr, 2017). The link between RANKL and RANK expressed in osteoclast precursors is the main stimulatory event for their differentiation and subsequent activation (Tang, 2016 and Xu, 2016). RANKL activities are regulated by osteoprotegerin, which inhibits bone resorption by preventing the interaction between RANKL and RANK (Hienz, 2015; Jianru, 2015; Lappin, 2007; Beklen, 2015; Wen, 2016). In the present study, periodontal treatment was a modifying factor regarding the analyzed biomarkers (Buduneli, 2009; Bostanci, 2011 and Hassan, 2015). Bostanci *et al*. (Bandari, 2012) reported no relationship between non-surgical periodontal treatment and alterations in RANKL and osteoprotegerin levels in patients with chronic and aggressive periodontitis, corroborating the results reported by Buduneli *et al*. (Buduneli, 2009) Other studies also reported no relationship between periodontal treatment and alteration of bone remodeling markers RANKL/osteoprotegerin levels (Dereka, 2010; Santos, 2010). These findings suggest that, despite the potential use of RANKL/osteoprotegerin as biomarkers related to periodontal disease activity and biological behavior, their role in the analysis of the host response to periodontal treatment remains unclear. Studies have been developed aiming at inhibiting the bone loss process occurring inperiodontal disease by blocking RANKL activity (El-Sharkawy, 2010 and Rizzoli, 2010). Li *et al* (Li, 2015), evaluated the in vitro effect of Astragaloside IV (AS-IV), a natural plant extract associated to activation of the osteoblastic response, and reported an inhibitory effect on RANKL activity, thus suggesting the potential use of AS-IV as a natural agent for the treatment of osteoclast related diseases, such as periodontal disease.

Conclusion

In summary, the present study points to the potential use of biomarkers related to bone remodeling in patients diagnosed with periodontal disease through salivary fluid and gingival crevicular fluid analyses, allowing for identification of periodontal disease activity, its biological behavior, as well as the disease stage. However, it is important to develop more in-depth studies evaluating the effectiveness of these biomarkers in hostresponses to periodontal treatment. The development of auxiliary therapeutical means to model periodontal treatment is also suggested, aiming at minimizing the effect of the deregulation of such biomarkers associated to the destruction of periodontal tissue.

Compliance with Ethical Standards

Conflict of Interest: The authors declare no conflicts of interests.

Ethics approval: Not applicable.

Figure legends: Flow diagram of the literature search and PRISMA-adapted selection criteria.

REFERENCES

Al-Ghurabi BH, Mohssen SM. Salivary level of RANKL and OPG in chronic periodontitis. *J bagh College Dentistry* 2015; 27:189-194.

Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999; 4:1– 6.

Baharuddin NA, Coates DE, Cullinan M, Seymour G, Duncan W. Localization of RANK, RANKL and osteoprotegerin during healing of surgically created periodontal defects in sheep. *J Periodontal Res*., 2015;50:211-219.

Bandari P, Prasad MV, Maradi A, Pradeep AR, Mallika A, Sharma D. Gingival crevicularfluidosteoprotegerin levels in Indian population. *Dent Res J*., 2012;9:774-82.

Behfarnia, P, Saied-Moallemi Z, Javanmard SH, Naseri R. Serum, saliva, and GCF concentration of RANKL and osteoprotegerin in smokers versus nonsmokers with chronic periodontitis. *Adv Biomed Res*., 2016;5:80.

Beklen A, Al-Samadi A, Konttinen YT. Expression of cathepsin K in periodontitis and in gingival fibroblasts. *Oral Dis* 2015; 21:163-169.

Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. *J Clin Periodontol* 2012; 39:239–248.

Bostanci N, Saygan B, Emingil G, Atilla G, Belibasakis GN. Effect of periodontal treatment on receptor activator of NF-κB ligand and osteoprotegerin levels and relative ratio in gingival crevicular fluid. *J Clin Periodontol* 2011;38:428-433.

Buduneli N, Buduneli E, Kütükçüler N. Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and non-smoking patients with chronic periodontitis during initial periodontal treatment. *J Periodontol* 2009;80:1274-1280.

Bunaes DF, Mustafa M, Mohamed HG, Lie SA, Leknes KN. The effect of smoking on inflammatory and bone remodeling markers in gingival crevicular fluid and subgingival microbiota following periodontal therapy. *J Periodontal Res* 2017;52:713-724.

Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J Clin Periodontol* 2018; 45:S1-S8.

Chen B, Wu W, Sun W, Zhang Q, Yan F, Xiao Y. RANKL expression in periodontal disease: where does RANKL come from? *Biomed Res Int* 2014;2014:731039.

Costa LC, Fonseca MAD, Pinheiro ADR, et al. Chronic Periodontitis and RANKL/OPG Ratio in Peri-Implant Mucosae Inflammation. *Braz Dent J* 2018;29:14-22.

de Morais EF, Dantas AN, Pinheiro JC, et al. Matrix metalloproteinase-8 analysis in patients with periodontal disease with prediabetes or type 2 diabetes mellitus: A systematic review. *Arch Oral Biol* 2018;87:43-51.

de Morais EF, Pinheiro JC, Leite RB, Santos PPA, Barboza CAG, Freitas RA. Matrix metalloproteinase-8 levels in

periodontal disease patients: A systematic review. *J Periodontal Res* 2018;53:156-163.

Dereka XE, Markopoulou CE, Fanourakis G, Tseleni-Balafouta S, Vrotsos IA. RANKL and OPG mRNA level after non-surgical periodontal treatment. *Inflammation* 2010; 33:200–206.

El-Sharkawy H, Aboelsaad N, Eliwa M, Darweesh M, Alshahat M, Kantarci A, Hasturk H, Van Dyke TE. Adjunctive treatment of chronic periodontitis with daily dietary supplementation with omega-3 Fatty acids and low-dose aspirin. *J Periodontol* 2010; 81:1635–1643.

Gabr AM, E-Guindy HM, Saudi HM, Morad AM. Evaluation of receptor activator of nuclear factor-κB ligand and osteoprotegerin levels in saliva and gingival crevicular fluid in patients with chronic periodontitis. *Tanta Dental Journal* 2017; 14: 83-88.

Ghallab NA. Diagnostic potential and future directions of biomarkers in gingival crevicular fluid and saliva of periodontal diseases: Review of the current evidence. *Arch Oral Biol* 2018; 87:115-124.

Hassan SH, El-Refai MI, Ghallab NA, Kasem RF, Shaker OG. Effect of periodontal surgery on osteoprotegerin levels in gingival crevicular fluid, saliva, and gingival tissues of chronic periodontitis patients. *Dis Markers* 2015;2015: 341259.

Hienz SA, Paliwal S, Ivanovski S. Mechanisms of Bone Resorption in Periodontitis. *J Immunol Res* 2015; 2015:615486.

Jianru YI, MeiLe L, Yang Y, Zheng W, Yu L, Zhao Z. Static compression regulates OPG expression in periodontal ligament cells via the CAMK II pathway. *J Appl Oral Sci* 2015;23:549-554.

Lappin DF, Sherrabeh S, Jenkins WM, Macpherson LM. Effect of smoking on serum RANKL and OPG in sex, age and clinically matched supportive-therapy periodontitis patients. *J Clin Periodontol.,* 2007; 34:271-277.

Li M, Wang W, Geng L, et al. Inhibition of RANKL-induced osteoclastogenesis through the suppression of the ERK signaling pathway by astragaloside IV and attenuation of titanium-particle-induced osteolysis. *Int J Mol Med* 2015; 36:1335-1344.

Maia LC, Antonio AG. Systematic reviews in dental research. A guideline. *J Clin Pediatr Dent*., 2012;37:117–124.

McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement. *J Clin Epidemiol* 2016; 75:40-46.

Mogi M, Otogoto J. Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. *Arch Oral Biol* , 2007; 52: 894-898.

Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6: e1000097.

Novakovic N, Todorovic T, Rakic M, et al. Salivary antioxidants as periodontal biomarkers in evaluation of tissue status and treatment outcome. *J Periodontal Res* 2014; 49:129-136.

Ochanji AA, Matu NK, Mulli TK. Association of salivary RANKL and osteoprotegerin levels with periodontal health. *Clin Exp Dent Res*. 2017; 3:45-50.

Rizzoli R, Yasothan U, Kirkpatrick P. Denosumab. *Nat Rev Drug Discov*., 2010;9: 591–592.

Sakellari D, Menti S, Konstantinidis A. Free soluble receptor activator of nuclear factor-kappab ligand in gingival crevicular fluid correlates with distinct pathogens in periodontitis patients. J Clin Periodontol2008;35:938-943.

Salminen A, Gursoy UK, Paju S, et al. Salivary biomarkers of bacterial burden, inflammatory response, and tissue destruction in periodontitis. *J Clin Periodontol* 2014; 41:442-450.

Santos VR, Lima JA, Goncalves TE.Receptor activator of NF-(capital KA,Cyrillic)B ligand/osteoprotegerin ratio in sites of chronic periodontitis of poorly-controlled and wellcontrolled type 2 diabetic subjects. *J Periodontol* 2010; 81:1455-1465.

Sojod B, Chateau D, Mueller CG, *et al.* RANK/RANKL/OPG Signalization Implication in Periodontitis: New Evidence from a RANK Transgenic Mouse Model. *Front Physiol* 2017; 8:338.

Tabari ZA, Azadmehr A, Tabrizi MA, Hamissi J, Ghaedi FB. Salivary soluble receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio in periodontal disease and health. *J Periodontal Implant Sci* 2013;43:227-232.

Tang TH, Fitzsimmons TR, Bartold PM. Effect of smoking on concentrations of receptor activator of nuclear factor kappa B ligand and osteoprotegerin in human gingival crevicular fluid. *J Clin Periodontol* 2009;36:713–718.

Tang X, Han J, Meng H, et al. Downregulation of RANKL and RANKL/osteoprotegerin ratio in human periodontal ligament cells during their osteogenic differentiation. *J Periodontal Res*., 2016; 51:125-132.

Tobón-Arroyave SI, Isaza-Guzmán DM, Restrepo-Cadavid EM, Zapata-Molina SM, Martínez-Pabón MC. Association of salivary levels of the bone remodelling regulators sRANKL and OPG with periodontal clinical status. *J Clin Periodontol.,* 2012; 39:1132-1140.

Vernal R, Chaparro A, Graumann R, Puente J, Valenzuela MA, Gamonal J. Levels of cytokine receptor activator of nuclear factor kappaB ligand in gingival crevicular fluid in untreated chronic periodontitis patients. *J Periodontol* 2004; 75: 1586-91.

Wen X, Yi LZ, Liu F, Wei JH, Xue Y. The role of cathepsin K in oral and maxillofacial disorders. *Oral Dis* 2016; 22:109-115.

Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–536.

Xu JL, Meng HX, He L, Wang XE, Zhang L. The Effects of Initial Periodontal Therapy on the Serum Receptor Activator of Nuclear Factor-κβ Ligand/Osteoprotegerin System in Patients With Type 2 Diabetes Mellitus and Periodontitis. *J Periodontol* 2016; 87:303-311.

Yakob M, Kari K, Tervahartiala T, et al. Associations of periodontal microorganisms with salivary proteins and MMP-8 in gingival crevicular fluid. *Clin Periodontol* 2012; 39:256-263.

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