Quantification of Osteoprotegerin Plasma Levels in Patients with Periodontitis

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Objective: Periodontitis (POD) is an infectious process directed at the structures supporting the teeth. Destruction of alveolar bone is considered one of the main causes of tooth loss in humans and is mediated by the host immune response. Osteoprotegerin (OPG), a protein that inhibits bone resorption by binding to the RANK ligand (RANKL), prevents osteoclastic differentiation. The aim of the study was to determine the plasma levels of OPG in patients with POD.

Methods: a case-control study with forty-nine patients with POD and 49 healthy controls were included in the study. OPG levels were determined by an ELISA test in plasma samples.

Results: OPG values (1.6203 ng/mL) were higher in the POD group compared with control group (1.2824 ng/mL). Among the studied groups, we detected significant differences in age, glycosylated haemoglobin (HbA1C), and plasma concentration of OPG ($p < 0.05$).

Conclusion: plasma OPG levels are associated with bone formation and destruction processes, suggesting that OPG acts in a protective manner.

Key words: Osteoprotegerin, Periodontitis, Bone resorption, Osteoclasts, ELISA

Periodontitis (POD) is a disease characterized by inflammation caused by microorganisms, which compromises the integrity of the supporting tissues of the teeth, including the gingiva, the periodontal ligament, and the alveolar bone, all known as periodontium (1, 2). Besides the loss of teeth, POD can also affect the systemic health of the patients, increasing the risk of arteriosclerosis, adverse pregnancy outcomes, rheumatoid arthritis, pneumonia, and cancer (1).

Osteoimmunology aims to understand the aetiology of POD, establishing a relationship between the immune system and the alveolar bone metabolism (3).

It has been proven that oral bacteria that have remained in the dental biofilm for a long time are the main cause of periodontal disease. Other variables, such as smoking, also aggravates the polymicrobial synergy of pathogenic bacteria and the subgingival dysbiosis that characterize chronic periodontal disease (4). The periodontopathogens activate a human immune response, which results in an inflammatory reaction that leads to progressive damage and resorption of the alveolar bone, with eventual loss of dental organs in susceptible individuals (3, 5-7). In an active and uncontrolled inflammatory process, there is an increase in the secretion of pro-inflammatory cytokines, such as interleukins and tumoral necrosis factors (TNF-α). Subsequently, neutrophils secrete large amounts of destructive enzymes, such as metalloproteinases (MMPs), inflammatory mediators, and the receptor activator NF-κB ligand (RANKL). Biochemical products that play an essential role in the extracellular matrix and the activation and differentiation of osteoclasts are responsible for the destruction of collagen and bone (5,8,9). Naturally, RANKL is located on the osteoblast's surface; by binding with its RANK receptor, found on the surface of osteoclasts and preosteoclast, it promotes the formation, proliferation, and differentiation of osteoclasts (3,8,9).

Osteoprotegerin (OPG) is a circulating soluble protein produced by a variety of cells, including osteoblasts, bone marrow stromal cells, periodontal ligament cells, fibroblasts, and epithelial cells. OPG is encoded by the TNFRSF11B gene. Also known as osteoclastogenesis inhibiting factor (OCIF), OPG is a member of the receptor superfamily of Tumor Necrosis Factor 11B (TNFRSF11B), which acts as a cytokine receptor, joining RANKL, avoiding the RANK/RANKL interaction, resulting in the inhibition of osteoclast differentiation, thereby blocking osteoclastogenesis (3, 8-10). The OPG/RANKL system is a critical factor in determining the activation degree of osteoblasts and bone destruction (11).

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In patients with diseases, such as Paget disease, where there is no bone turnover, there will be an even greater bone-weakening over time; high plasma levels of OPG have been demonstrated, unlike the controls, this, as a mechanism of protective action. Similarly, in patients with rheumatoid arthritis, where there is systemic and chronic autoimmune inflammation, higher plasma levels of OPG were reported than in controls. It has been shown that OPG levels increase in women after menopause. Additionally, in humans, there is still no established reference of ranges for OPG and RANKL (12). The destruction of the alveolar bone in the POD is mediated by the interaction of the TNF superfamily (13). However, the OPG/RANKL mechanism is complex; studies testing the plasma levels in patients with periodontitis report conflicting results. The aim of this study was to determine the plasma levels of OPG in patients with POD and healthy controls.

Materials and Methods

A case-control study was performed. Patients (25–65 years old) were selected from the Periodontics Department of the Autonomous University of Yucatan. Subjects who received previous periodontal treatment, active gingival disease, chemotherapy, antibiotics, and/or anti-inflammatory therapy in the six months prior to taking the sample were excluded, as well as patients with systemic diseases and pregnant women.

For determination of POD, the new (2017) parameters established by the American Academy of Periodontology and The European Federation of Periodontology (EFP) were used for the diagnosis of periodontal diseases. The new classification has four stages (severity and complexity of management) and three grades (evidence or risk). Patient inclusion criteria were targeted with stage 2, grade B POD by periodontal probing with a calibrated periodontal probe (UNC-15, Hu-Friedy, Chicago IL, USA); all teeth were examined except third molars.

(i) Stage 2 periodontitis: clinical loss of attachment (CAL) by 3–4 mm interproximal attachment loss in the greater loss zone, coronal third (15-33%) radiographic loss, no tooth loss due to periodontitis, maximum probing depth ≤ 5 mm with mostly horizontal bone loss.

(ii) Grade B: direct evidence of progression of < 2 mm over 5 years and indirect evidence of progression 0.25–1.0 mm. Proportional destruction to biofilm waste, such as smoking < 10 cigarettes per day (14).

Blood sample collection

The study protocol was explained to all participants who provided informed consent. The study was approved by the Ethics Committee of CIR-Biomedics, Autonomous University of Yucatan and followed the ethical standards of the Declaration of Helsinki. Adopted in June 1964, it was modified by the World Medical Assembly of Korea in October 2008. Two groups were evaluated: Group 1 (Control, systemically healthy subjects) and Group 2 (subjects with POD). In the clinical analysis laboratory of the Medicine Faculty of the Autonomous University of Yucatan, peripheral venous blood samples were taken from each patient following the fasting parameters from 8 to 10 h. Later, the glycosylated haemoglobin (HbA1c) test was performed for the determination of the existence of diabetes mellitus, and the subjects who presented some type of glycemic control deficit were eliminated from the study. Subjects with HbA1c values of ≤ 5.6% were included, according to American Diabetes Association parameters (15). Samples were stored in tubes with ethylenediaminetetraacetic acid (EDTA) at -20ºC for further analysis. To obtain the plasma, the blood and EDTA tubes were centrifuged at 3000 g for five min; this process was carried out during the first three hours after the venous extraction.

To determine the plasma concentration of OPG in the studied subjects, an Enzyme-Linked Immunosorbent Assay (ELISA) was performed using the double sandwich technique (MyBiosource Catalog # MBS267842, California, San Diego, USA), following the manufacturer's instructions. The Mann-Whitney statistical test was used to compare the variables studied using the program SPSS (v. 22).

Results

Characteristics of the samples

Ninety-eight subjects were included in this study: in group 1 (control), 28 women and 21 men with a mean age of 35.59 ±13.06 years; and in group 2 (POD), 36 women and 13 men with a mean age of 47.34 ±12.54 years. Groups 1 and 2 had HbA1c values of 5.53 ± 0.57% and 5.45 ± 0.54%. The fasting blood sugar levels in groups 1 and 2 were 94.40 ± 13.81 mg/dL and 95.36 ± 9.94 mg/dL, respectively.

OPG plasma levels

The OPG plasma concentration was higher in the POD group (1.28 ± 0.59 ng/mL) than the control group (1.62 ± 0.58 ng/mL). Also, among the studied groups, we detected a significant differences for age ($p < 0.05$), HbA1c ($p < 0.05$), and OPG plasma levels ($p < 0.001$). Regarding loss of attachment, no significant difference was found between the groups, which suggests that the presence of OPG minimises the severity of POD (Table 1).

Table 1. Plasmatic levels of OPG in ng/mL and loss of attachment in mm between studied groups.

	Group 1: Control	Group 2: POD
Sex	28F/21M	36F/13M
Age*	35.59 + 13.06	47.34 + 12.54
OPG concentration (Mean) in ng/mL*	1.2824 + 0.59607 (.41-3.50)	1.6203 + 0.58599 (.88-3.88)
Loss of attachment (mm) (rank)	1.9224 + 0.28814 (1.50-2.70)	2.8265 + 0.75271 (1.70-5.20)

Mean + Standard Deviation (SD rank); *p < 0.05.

Discussion

OPG plays an important role in osteoimmunology, with some studies reporting that when OPG is higher, bone formation dominates. This is how OPG was named; because of its capacity to protect bone from immoderate resorption by offsetting the osteoclastic effects of RANKL (16).

The OPG/RANK/RANKL triad performs a significant role in osteoclastogenic regulation. While OPG and RANKL are important in the regulation of bone metabolism, the RANKL/OPG/RANKL ratio is thought to reflect bone resorption or replacement better than the level of any of these factors separately. Some clinical studies have explored the contribution of the RANKL/OPG system to alveolar bone loss in periodontal disease (13).

Saglam et al. reported that the intensity of both RANKL and OPG expression is chronic and aggressive in periodontitis tissues relative to healthy controls. These authors also reported higher levels in periapical active lesions than on inactive lesions (17). Others have found that on alveolar bone remodelling, periodontal ligament fibroblasts play a significant role under physiological conditions, synthesising higher OPG levels compared to RANKL, also concluding that OPG has an inhibitory effect on osteoclastogenesis and, when under the influence of bacterial challenge, this molecule risen (18). In periodontal surgery, it is possible that the participation of this protein is involved in periodontal tissue repair (8).

Furthermore, in systemic diseases (e.g., Paget, postmenopausal osteoporosis, rheumatoid arthritis, and cardiovascular disease), elevated OPG acts as a biomarker (12). In addition, an association between OPG plasma levels and arterial stenosis in type II diabetes has been reported; increased protein plasma levels (ng/mL) were noticed in arterial stenosis patients compared to healthy controls. As for POD, the hypothesis is that elevated plasma OPG levels might be a compensatory self-defensive response to the progression of this disease (19).

It is also advocated that smoking may lead to a shift in the composition of the subgingival biofilm with an increase in the prevalence of periodontal pathogens, because this has suggested to shift the balance of neutrophil activities to a more destructive nature aggression initiating the inflammatory cascade on the periodontal tissue (20).

Hideaki Hayashida et al. analysed different states of health and periodontal disease and reported a positive correlation between the levels of HbA1c in non-diabetics and the severity of periodontal disease (although without reaching statistical significance) (21). Like our results, the case group had a higher proportion of HbA1c than the subjects without POD with values of ($p < 0.05$); which confirms that periodontitis and the levels of glycosylated haemoglobin are closely related.

In-situ data indicates that the RANKL/OPG proportion in gingival crevicular fluid (GCF) is higher in POD patients and that this increase suggests the existence of periodontitis (13). Others have reported that OPG was detected in diseased and healthy sites in patients with periodontitis, but was not found in healthy subjects' sites, indicating that OPG is practically undetectable in any of the control sites (22). Others have reported the opposite: where the OPG mean value was lower in POD patients than controls (23). This decrease in GCF could indicate that the in-situ levels are decreased but high in plasma. Baeza et al. used GCF samples of patients with chronic periodontitis, asymptomatic apical periodontitis (AAP), and healthy controls, reporting that the OPG levels in patients with CP, with high sensitivity but low specificity, whereas in AAP and healthy patients, OPG activity could not be found (24).

In other biological fluids, OPG has been studied, such as saliva, where longitudinal investigations showed at baseline, OPG high salivary levels, whereas after the recovery phase those values decreased (25). Gomes et al. evaluated the levels of salivary biomarkers in patients with dental implants. These patients were divided into two groups, those with periodontal/peri-implant maintenance and those without. Even though there was no statistical relevance, OPG levels in patients without periodontal maintenance were higher than in those with periodontal maintenance (26).

Recent research has quantified OPG levels in the blood. Baltacioglu et al., detected higher serum levels of OPG in control patients than in POD patients (0.285 ± 0.118 vs. 0.224 ± 0.051 pg/mL) (3). Similarly, Xu et al. reported the OPG concentrations 232.60 ± 70.85 pg/mL in patients with periodontitis and 244.96 ± 85.13 pg/mL in healthy patients (27). In plasma, Ozçaka et al. reported OPG concentrations of 39.07 ± 9.01 in POD subjects vs. 40.37 ± 6.87 pg/mL in controls (28). In contrast, our study shows OPG concentrations in plasma of 1.2824 ± 0.59607 ng/mL in Group 1 and 1.6203 ± 0.58599 ng/mL for Group 2 ($p < 0.05$).

Consistent with our findings, Behfarnia et al. have reported increased OPG serum levels in POD patients relative to controls; saliva and GCF samples were the opposite, whereas OPG was higher in healthy participants compared to POD patients (29). Increased OPG serum levels can act as a protective mechanism, counteracting disease progression (22); if OPG is higher, bone formation dominates (16).

Our results suggest that peripheral increase of this protein could be part of a protective mechanism against alveolar bone destruction in POD. Likewise, Wanby et al. suggest that the increased OPG levels is a homeostatic mechanism that limits bone loss in patients with high bone turnover (30). OPG levels in plasma are associated with bone formation and destruction processes, suggesting that OPG is protective. A higher quantity of OPG was found in subjects with POD, suggesting that there is an active process inhibiting osteoclastogenesis that results in a decrease in the severity of POD.

In this manner, OPG levels could be used for diagnosis, treatment, and monitoring of POD. However, future studies are necessary.

Resumen

Objetivo: La Periodontitis (POD) es un proceso destructivo infeccioso dirigido a las estructuras de soporte de los dientes. La destrucción del hueso alveolar es considerada como una de las principales causas de pérdida de dientes en humanos y esta medida por la respuesta inmune del huésped. La osteoprotegerina (OPG), proteína que inhibe la resorción ósea uniéndose al ligando RANK (RANKL), evita la diferenciación osteoclástica. El objetivo de este estudio es determinar los niveles plasmáticos de OPG en pacientes con POD. Métodos: estudio de casos y controles. Se seleccionaron cuarenta y nueve pacientes con POD y cuarenta y nueve sujetos controles. Ambos grupos sistémicamente sanos. Los niveles de OPG en plasma fueron determinados mediante pruebas de ELISA de las muestras de estudiadas. Resultados: los valores de OPG (1.6203 ng/mL) fueron mayores en el grupo con POD en comparación con el grupo control (1.2824 ng/mL). Se detectó diferencia estadísticamente significativa en edad, hemoglobina glucosilada (HbA1C) y concentración de plasma al comparar ambos grupos. Conclusión: Los niveles plasmáticos de OPG se encuentran asociados en procesos de formación y destrucción ósea, sugiriendo que OPG actúa de manera protectora.

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