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Evaluation of Salivary Osteoprotegerin in Hypothyroidism and Periodontitis Patients Case-Control Study

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ABSTRACT

Introduction: The immune system has been used to mediate the link between periodontal disease and the endocrine system, particularly thyroid dysfunction. Osteoprotegerin, a dimeric glycoprotein, its structure similar to receptor activator of nuclear factor kappa-b is generated and secreted by osteoblasts and considered as a member of the TNF family. To prevent RANK from contacting RANKL, OPG functions as a "false target." OPG is necessary for preventing osteoclast activation and development; as a result, bone resorption will stop.

Objective: To demonstrate the salivary level of OPG in hypothyroidism and periodontitis patients in comparison to healthy controls.

Patients and Method: Saliva samples were taken from 90 subjects ranging in age from (22-65) years, 50 of whom were hypothyroid patients (25 participants with periodontitis and systemically healthy; 25 participants with hypothyroidism and a healthy periodontium, 25 participants with hypothyroidism and periodontitis, and 15 individuals systemically healthy with healthy periodontium, which serve as the control group. OPG in saliva was discovered using ELISA.

Result: According to the current findings, the levels of salivary OPG were significantly low in the disease group (periodontitis group, hypothyroid patient group with and without periodontitis) than in the control group. In addition, salivary levels of OPG were significantly lower in hypothyroidism with the periodontitis group in comparison to the periodontitis group.

Conclusion: OPG has a protective role in disease processes, in which the level of OPG decreased in all diseases group and increase in health. Hypothyroidism affects the level of salivary OPG.

Keywords: Periodontal disease, Osteoprotegerin, Hypothyroidism, Saliva.

INTRODUCTION

The potential link between periodontitis and hypothyroidism has only been investigated in a limited number of high-quality studies [(1)]**.** The metabolic activity of fibroblasts decreases as a result of hypothyroidism, delaying the healing of wounds [(2)]. Due to the long-term exposure of the injured tissue to pathogenic organisms, a delay in the healing process may be associated with a greater risk for infection, which shows clinically as higher gingival bleeding [(3)]. The cytokines produced by thyroid dysfunction increase inflammatoryn cascade easily [(4)].

Periodontitis defined as a severe, chronic disease of the periodontium brought on by an abnormal immune response in susceptible individuals and characterized by the predominance of Gram-negative bacteria in the subgingival niches [(5)]. Periodontitis considered as one of the most prevalent non-communicable chronic inflammatory illnesses, and affects 50% of adults and is the sixth most widespread disease worldwide. About 11% of individuals have periodontitis in its more aggressive form [(6)]. In people who are vulnerable, the dental plaque biofilm is thought to be the main etiological factor; however, smoking and diabetes also play a role in the progression of the disease [(7)]. thyroid hormones are two type Thyroxine (T4) and Triiodothyronine (T3) that secreted by the thyroid gland and needed by all metabolically active cells. As a result, a lack of or reduced activity of these hormones can cause hypothyroidism, a common endocrine disorder with a variety of adverse effects on the body [(8)]. Hypothyroidism is a chronic illness marked by decreased levels of T4 and T3 [(9)]. The primary function of thyroid follicular cells is the production of thyroid hormones, which are essential for many physiological processes, including regulation of the basal metabolic rate (BMR), growth, and development [(10)]. Cytokines are soluble proteins that initiate intracellular signaling cascades by binding to specific receptors on target cells, which modify how genes are controlled and result in changes in the phenotype of the cell [(11)].

Immune cell activation, death, proliferation, and inhibition are just a few of the basic processes that cytokines can regulate [(12)]. Osteoprotegerin (OPG), a dimeric glycoprotein produced and secreted by osteoblasts and consider as TNF family member, shares structural similarities with receptor activator of nuclear factor kappa-B [(13)]. OPG serves as a "false target" to keep RANK from making contact with RANKL. Formation and activation of the Osteoclast must be prevented by OPG to stop bone resorption [(14)].

A variety of tissues produce the OPG, including the heart, placenta, kidney, vasculature, lung, and bone [(15)]. Saliva is a complex, diluted, colorless, and opalescent aqueous solution that contains both inorganic and organic components [(16)]. Saliva collection lowers the likelihood of

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virus spread and is relatively risk-free [17]. In our study, the salivary level of OPG had a significant effect on hypothyroidism in periodontitis patients.

This study aims to demonstrate the salivary level of OPG in patients with hypothyroidism and periodontitis in comparison to healthy controls.

MATERIAL AND METHODS

Study design

Our research was a case-controlled study, at the Teaching Clinics of the Department of Periodontics, College of Dentistry, University of Baghdad and Educational Laboratories, Medical City during the period from January 2022 to June 2022. Ninety subjects were examined to collect salivary samples, and they were divided into the following four groups: systemically healthy with healthy periodontium as a control group (Group A) included 15 individuals who had BOP <10%, PPD ≤ 3mm, intact periodontium (no probing attachment loss) [18].

Systemically healthy with periodontitis group (Group B) included 25 individuals who had PPD ≥5mm or PPD at ≥4mm with BOP [19], Hypothyroidism without periodontitis group (Group C) included 25 individuals who had TSH: >4.5 mlU/L, T4: <12 pmol/L, T3: <3 pmol/L [20], also had BOP <10%, PPD ≤ 3mm, intact periodontium (no probing attachment loss) [18]. Group D: Hypothyroidism with periodontitis group included 25 subjects who had TSH**:** >4.5mlU/L, T4**:** <12 pmol/L, T3**:** <3 pmol/L [20], also had PPD ≥5mm or PPD at ≥4mm with BOP [19].

For this study, the cases will be represented by the last three groups. All cases of periodontitis were defined as [19]: Interdentally detectable CAL at ≥2 non-adjacent teeth or CAL present at ≥3mm on the buccal (facial) or lingual/palatal surfaces in association with pocketing >3mm at ≥2 teeth. The salivary samples were involved to demonstrate the salivary level of OPG in hypothyroidism with periodontitis patients in comparison to healthy controls. In addition, measuring clinical parameters and correlating them with the level of the selected biomarker.

Inclusion criteria:

The present study included patients with newly diagnosed hypothyroidism, with a minimum of 20 teeth and not under medications in the last three months.

Exclusion criteria:

Subjects that are alcohol drinkers or smokers, pregnant or nursing women, patients with systemic diseases other than hypothyroidism, patients who have undergone extensive periodontal treatment or receiving antibiotic/anti-inflammatory medication and any medication related to hypothyroidism in the last 3 months, patients with any oral disease of inflammatory nature (other than periodontitis) that could influence the biomarkers under investigation, women who take contraceptive pills.

Enrollment of participants

Subjects will be recruited from patients seeking periodontal therapy at the Teaching Clinics of the Department of Periodontics, College of Dentistry, University of Baghdad, and patients attending Educational Laboratories, Medical City.

Periodontal parameters and clinical examination

The periodontal examination will be done on all teeth except the third molar. This includes assessment of full mouth plaque score (FMPS) [21], gingival bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). Using a periodontal probe (Michigan O probe).

Collection of salivary samples

Unstimulated saliva was collected from all participants into sterile test tubes. An average of 3 ml of saliva per individual was the amount that was anticipated to be collected. The collected samples were immediately put on ice and centrifuged at 1000 rpm for 15 minutes using a centrifuge machine. Then aspirated the clear salivary supernatants into a plastic Eppendorf tube by using a micropipette. Finally frozen The Eppendorf tube at -20C until it was evaluated by the Enzyme-Linked Immunosorbent Assay.

Enzyme-linked immunosorbent assays Laboratory procedure

After the completion of the sampling procedure, we leave the saliva samples to melt at room temperature before the experimental procedures. Then saliva samples will be analyzed for protein levels and OPG using commercially available ELISA kits (MyBioSource in California, USA) after that, the concentrations were stored and transferred to spreadsheets for analysis.

Ethical Consideration

The study obtained ethical approval after being presented to the College of Dentistry Ethics Committee at the University of Baghdad. All subjects were freely enrolled in the study, after obtaining a thorough explanation of the study's goals and objectives and getting an informed consent form to sign following the declaration of Helsinki.

Statistical analysis

For the continuous data, descriptive statistics like mean, SD, median, and IQR were employed, whereas frequency and percentage were used for the categorial variables. To evaluate the distribution of parametric data,

the Shapiro-Wilk test was performed. The Kruskal-Wallis test was an option for non-parametric data. The Spearman's or Pearson's correlation test was used to determining whether clinical and biochemical parametric variables were correlated (depending on the distribution of the data).

RESULTS

In our study, the participants' ages ranged between (22-65) with significant female predominance with a total percentage of (68%) among study groups.

Figures 1, 2, 3, 4, 5, and **6** show the participant distribution by gender, age, and clinical periodontal characteristics in each group. According to biochemical tests, the concentration of salivary OPG in the disease groups (Group B, Group C, and Group D) was lower significantly in comparison to the control group. Also, the salivary OPG level was significant between group B and group D but there was a non-significant difference between group C and group D. The level of salivary OPG is illustrated in **Figure 7**.

The correlation test of salivary OPG with clinical periodontal parameters showed that there was a positive significant correlation between OPG and CAL in-group D, while in group A and group C, there was a positive non-significant correlation between OPG with PI and BOP. In-group B the OPG had a non-significant negative correlation with PI, PPD, and CAL and there was a positive correlation between OPG and BOP as shown in **Table 1.**

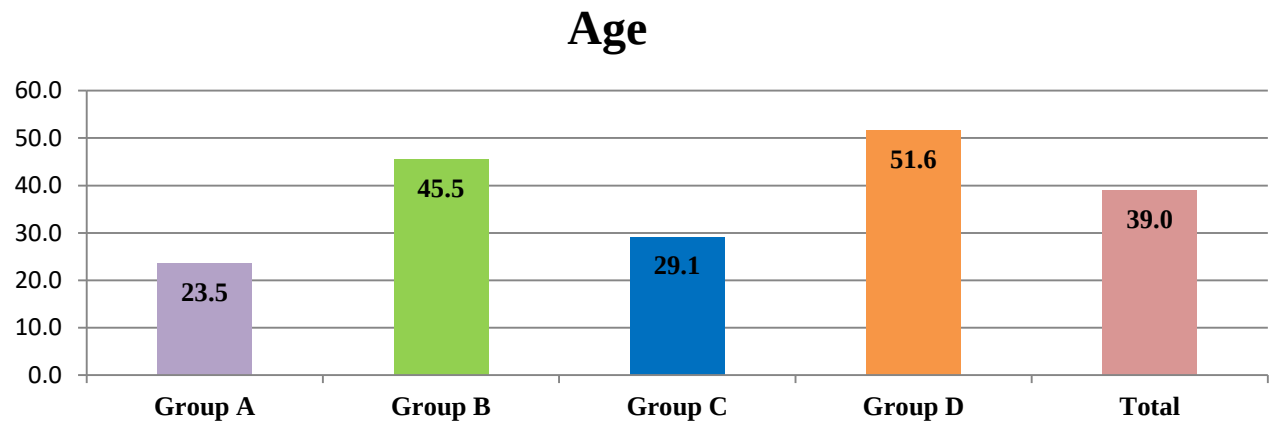

Figure (1): Distribution of age among the study groups.

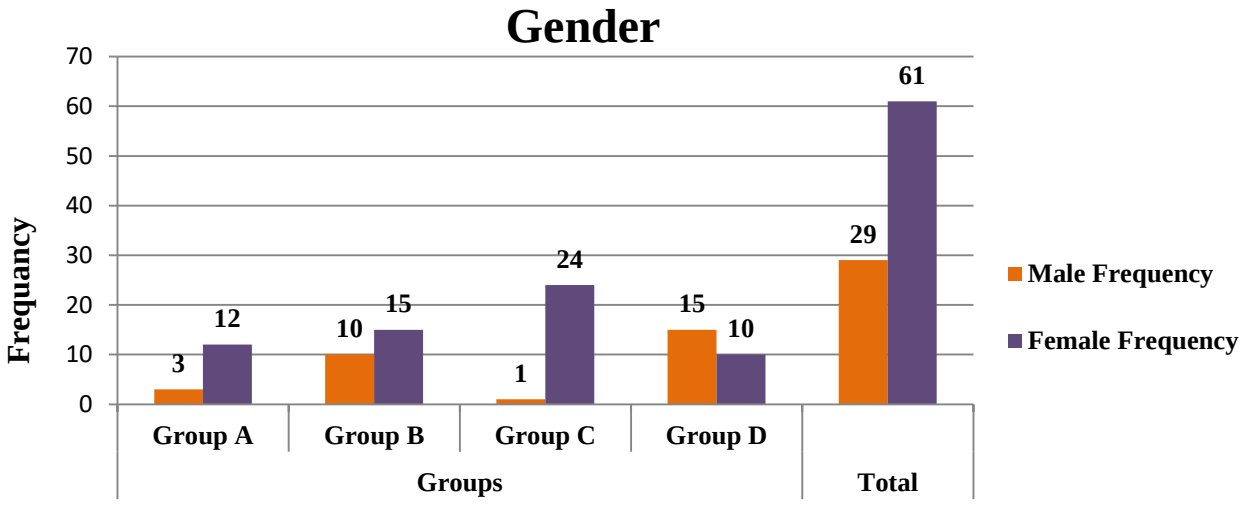

Figure (2): Distribution of gender among the study groups showed a significant female predominance except for group D.

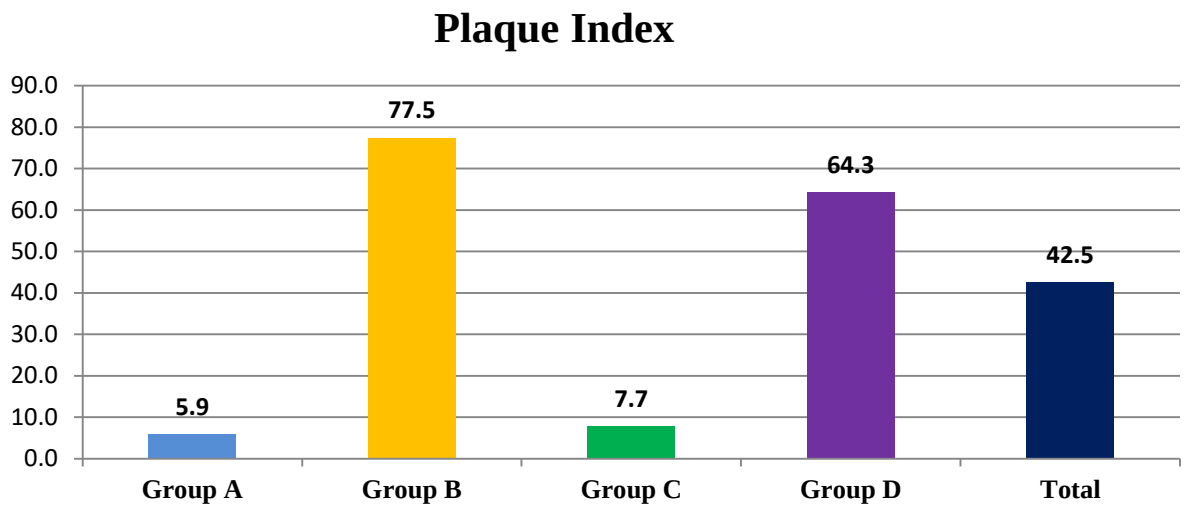

Figure (3): Distribution of PI among the study groups, the highest mean was found in group B.

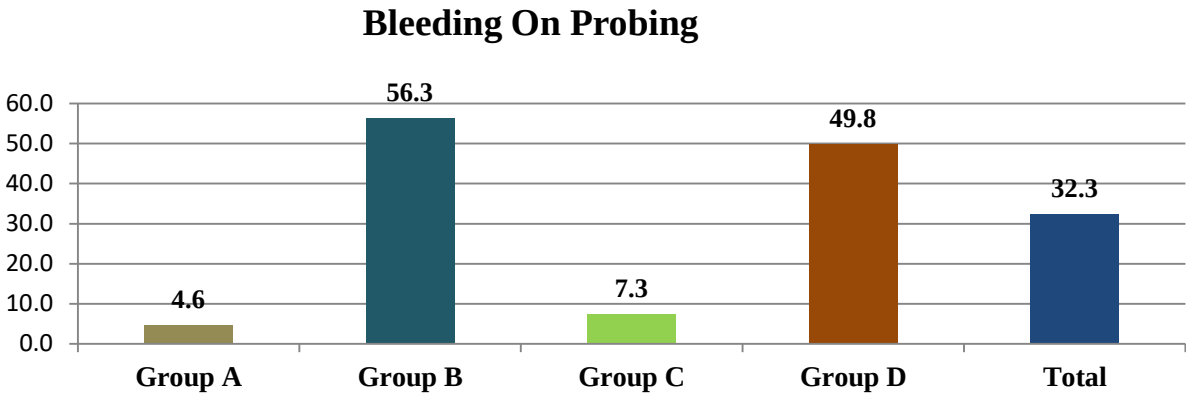

Figure (4): distribution of BOP among the study groups, the highest BOP mean was found in group B.

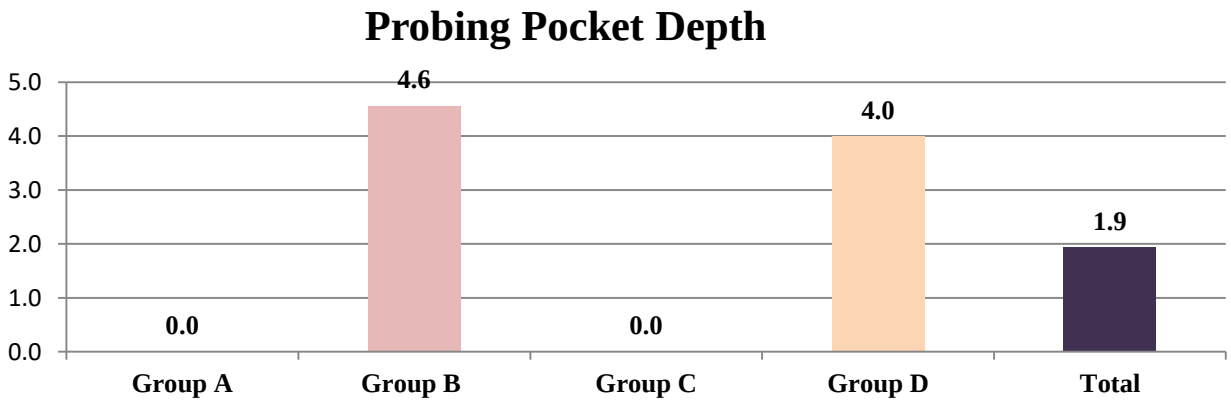

Figure (5): Distribution of PPD among the study group, the highest PPD mean was found in group B.

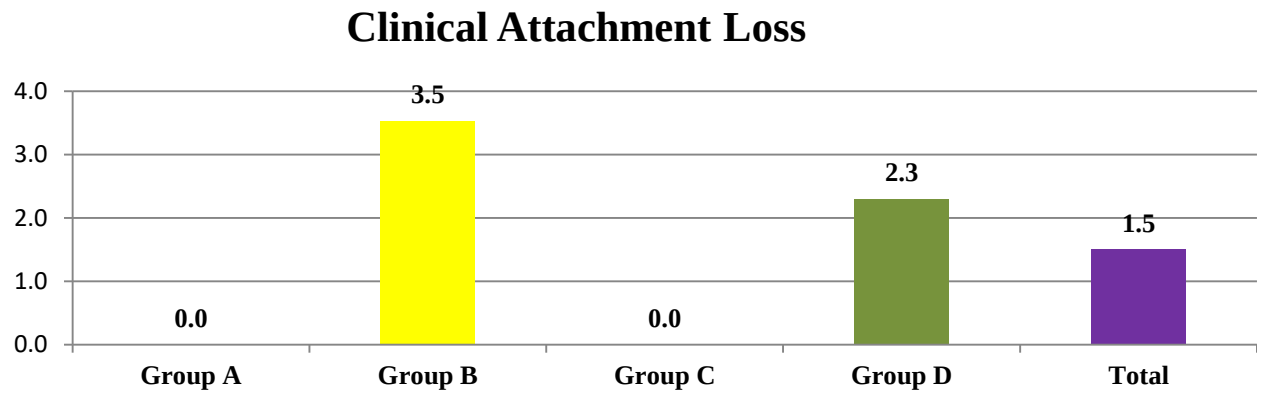

Figure (6): Distribution of CAL among the study group, the highest mean of CAL was found in group B.

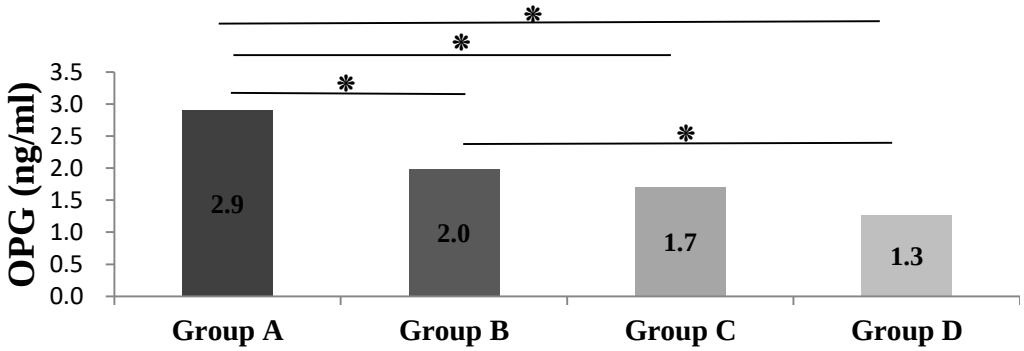

Figure (7): Salivary concentration of OPG among the study groups, the concentration of salivary OPG in the disease groups (Group B, Group C, and Group D) was lower significantly* in comparison to the control group. Also, the salivary OPG level was significant* between group B and group D.

Table (1): Correlation test between salivary OPG and clinical periodontal parameters

Groups	Markers	PI		BOP		PPD		CAL	
		r	P-value	r	P- value	r	P- value	R	P- value
Group A	OPG	0.156	0.578	0.388	0.153	0	0	0	0
Group B	OPG	-0.075	0.722	0.229	0.272	-0.011	0.959	-0.039	0.852
Group C	OPG	0.125	0.552	0.150	0.474	0	0	0	0
Group D	OPG	0.327	0.110	0.123	0.557	0.060	0.776	0.434*	0.030

*Correlation is significant.

DISCUSSION

Salivary OPG showed low level in periodontitis and hypothyroidism groups in comparison to healthy control group, while salivary OPG did not show significant level in hypothyroidism when compare to periodontitis. So the aim of the study was to evalute the level of OPG in hypothyroidism and periodontitis patients in comparison to healthy control.

Periodontitis is characterized by inflammation and bone loss, a lot of studies indicate that periodontitis involves substances produced by bacteria and antigens that cause a localized inflammatory response and activate the innate immune system. In this mechanism, cytokine networks and proinflammatory chemicals are crucial [(22)]. We used Saliva in this study because saliva considered one of the most significant bodily saliva, which has a significant correlation with the inflammatory, connective tissue destruction, and bone remodeling phases of periodontal disease. Saliva contains many easily accessible proteins and peptides that may be used as a source to measure biomarkers released during disease initiation and progression [(23)].

Participants ages in this study ranged from (22-65), particularly in the disease groups (periodontitis group and hypothyroidism with periodontitis group) with an increase in groups with healthy periodontium. Other studies support the present study's finding that periodontitis severity increases with age [(24)].

The logical theory is that age-related immune dysregulation is the cause of the rising periodontitis found in aging populations [(25)].

Nearly every body tissue, at every stage of development, depends on thyroid hormones to function properly, which is also true for hypothyroidism. So the people's age changes in thyroid function were more obvious at both ends of the life span [(26)].

Also, other studies linked this to an increase in the synthesis of antithyroglobulin antibodies, antiperoxidase antibodies, and TSH hormone levels [(27)].

The results of this study indicate that the level of salivary OPG decreased from health to disease, the salivary level of OPG was lower significantly in the periodontitis group when compared to the control group. These results agree with the results of other studies which indicate that the level of OPG decreases in periodontitis in comparison to the controls [(28) (29)].

OPG, a dimeric glycoprotein, it's structure similar to RANK that is generated and secreted by osteoblasts [(14)], OPG is produced by, gingival fibroblasts, human periodontal ligament (PDL) cells, and epithelial cells [(30)].

There will be fewer bone-associated cells and less bone-surrounding tissue found as periodontitis severity increases due to bone resorption. So, there will also be fewer cells supplying OPG, which will decrease the amount of these modulators released into the GCF [(28)]. Also, Pro-inflammatory cytokines like TNF-α, IL-1b, IL-6, IL-11, and IL-17 during an inflammatory response can trigger osteoclastogenesis by down-regulating OPG synthesis and up-regulating RANKL expression in stromal cells and osteoblasts [(31)], this may be regarded as the principal mechanisms controlling the degeneration of local bone in periodontal disease [(32)].

Additional research shows that the level of OPG was unchanged in periodontitis patients [(21) (33)]. The results of our study also revealed that the OPG level decreased significantly in hypothyroidism groups both with and without periodontitis in comparison to the control group. This may be related to the fact that OPG level decrease in disease due to pro-inflammatory cytokines like IL-1b, IL-6, IL -11, IL-17, and TNF-α that up-regulating RANKL expression and down-regulating OPG synthesis in the inflammatory process [(31)], and also fewer cells supplying OPG, which will decrease the amount of these modulators [(28)].

In hypothyroidism, the proinflammatory cytokines increased [(34)]; this may explain the OPG low level in hypothyroidism patients. This difference in results may be explained as these studies use serum rather than saliva. Other study showed that there is a non-significant difference of OPG level in hypothyroid patient [(35)]. When correlate the level of salivary OPG with clinical periodontal parameters, results indicate that there was a positive non-significant correlation between OPG and BOP. While in periodontitis group there were a non-significant negative correlation between OPG and PI, PPD and CAL, because the OPG in the periodontal patients GCF decreased as the severity of the condition increased. Patients with chronic periodontitis have less bone-surrounding tissue and less bone-associated cells because of bone resorption. As a result, there would be fewer cells generating OPG, which would decrease the amount of this modulator released into the GCF [(28)].

CONCLUSION

OPG has a protective role in disease processes, in which the level of OPG decreased in all diseases group and increase in health. Hypothyroidism affects the level of salivary OPG which decreased in this disease and this may lead to decrease bone density. More studies are needed to determine the OPG role in hypothyroidism.

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