Salivary level of RANKL and OPG in chronic periodontitis

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ABSTRACT

Background: Periodontal diseases are initiated by microbial plaque, which accumulates in the sulcular region and induces an inflammatory response. The Receptor activator of nuclear factor-kappa B ligand / osteoprotegerin (RANKL/OPG) axis is involved in the regulation of bone metabolism in periodontitis, in which an increase in receptor activator of nuclear factor-kappa B ligand or a decrease in osteoprotegerin can tip the balance in favor of osteoclastogenesis and the resorption of alveolar bone that is the hallmark of periodontitis. This study was performed to investigate the role of salivary levels of RANKL and OPG in pathogenesis of chronic periodontitis.

Subjects and Methods: Fifty five subjects with chronic periodontitis with ages range from 24-64 years and 25 apparently healthy volunteers their ages and sexes were matched with the patients were participated in this study. Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. Saliva samples were collected from all patients and controls. Enzyme-linked immunosorbent assay was carried out for estimation the salivary level of RANKL and OPG in studied groups.

Results: The present data revealed that the median salivary levels of RANKL was elevated in patient as compared with control group ($P<0.001$), whereas the salivary levels of OPG doesn't show any significant differences between the study groups ($p>0.05$). In contrast the ratio of RANKL/OPG ratio was significantly higher in patients when compared with the ratio in control group. Furthermore, negative significant correlation was noticed between RANKL and OPG. Regarding correlation between salivary (RANKL and OPG) and clinical periodontal parameters, RANKL levels was showed significant positive correlation with each of probing pocket depth and clinical attachment level. Otherwise no association between OPG levels and clinical parameters of periodontitis was found. Moreover; the ratio RANKL/OPG was showed significant positive correlation with each of gingival index, probing pocket depth and clinical attachment level.

Conclusion: This study demonstrates that salivary levels of RANKL and OPG play a crucial role in pathogenesis of periodontitis, and the relative RANKL/ OPG ratio appears to be indicative of disease occurrence.

Keywords: chronic periodontitis, RANKL, OPG. (J Bagh Coll Dentistry 2015; 27(1):189-194).

INTRODUCTION

Periodontitis has a multifactorial etiology, and the characteristic tissue destruction is mediated mainly by the aberrant immune response of the host to periodontopathic bacteria. The role of oral microflora in the etiology of various inflammatory periodontal diseases has been well established, and specificity may vary between bacterial etiologies and different forms of periodontal disease [(1,2)]. The chronic form of periodontitis, termed chronic periodontitis, is the most prevalent disease type [(3)].

While headways have been made in various areas concerning the molecular pathogenesis of periodontitis, one area which of increasing importance is the involvement of the (RANKL/OPG) axis in bone remodeling and bone loss in periodontitis. RANKL and its receptor "Receptor Activator of Nuclear Factor-kappa B" (RANK) have been recognized as key factors regulating osteoclast formation [(4)].

RANKL a membrane-bound or soluble protein belonging to the tumor necrosis factor (TNF) superfamily that is primarily produced in osteoblastic lineages and activated T cells. RANKL stimulates osteoclast differentiation and activation, and inhibits osteoclast apoptosis. Binding of RANKL to RANK expressed on the surfaces of osteoclasts and their precursors, promotes osteoclast differentiation and activation [(5)].

OPG is a soluble TNF receptor–like molecule, is the inhibitor of osteoclast differentiation. It binds to RANKL and blocks RANKL from interacting with RANK and neutralizes its activity by inhibiting the cell-to-cell signaling between osteoblast/bone stromal cells and osteoclast precursor cells, resulting in the inhibition of osteoclast formation. RANKL and OPG are crucial molecules that act as positive and negative regulators, respectively, in osteoclastogenesis and bone resorption. Under normal physiologic conditions, there is a balance between bone resorption and bone formation. Upregulation of RANKL has been seen in inflamed periodontal tissues, indicating that RANKL strongly participates in the processes of periodontal tissue destruction [(6)]. Therefore this study was performed to investigate the role of salivary levels of RANKL and OPG in pathogenesis of chronic periodontitis.

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SUBJECTS AND METHODS

Fifty five patients with chronic periodontitis (38 male and 17 female) were enrolled in this study, their age range from 24 to 64 years. They were from attendants seeking treatment in the department of periodontics, College of Dentistry, Baghdad University from Novmber 2012 to Janraury 2013. Diagnosis was made by specialized dentists in the College.

Oral examination was performed at four surfaces of each tooth except 3rd molar according to the following criteria: Assessment of dental plaque by (PI) of Silness and Loe [7]. Assessment of gingival condition by (GI) [8]. Probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). All the cases had received no treatment with no complain of chronic or systemic diseases.

Apparently healthy periodontium volunteers their ages and sexes were matched to patients consisted of 25 individuals who were considered as control (19 males and 6 females). The saliva obtained from patients and healthy controls were analyzed for RANKL and OPG by using commercially available ELISA and performed as recommended in leaflet with kits, (Human RANKL and OPG ELISA Kit/ Cusabio/China).

Statistical analysis

It was assessed using P (Mann-Whitney-test), P (Bonferroni-test). Correlation between the different parameters was calculated by the spearman test and p values of P<0.01and P<0.05 were considered significant.

RESULTS

In the present study the mean age of patients was 40.15 ± 10.53 years, and there was male's predominance among patients, about (69.1%) of CP patients were males, while only (30.9%) were females. Furthermore, (34.5%) of patients had positive family history of chronic periodontitis, while (65.5%) showed negative family history as clearly shown in table (1). The differences in clinical periodontal parameters in patients and healthy controls are summarized in table (2).

Table (3) revealed a highly significant elevation in level of RANKL among patients (56.8 pg/ml) in comparison to that of healthy control (2.21 pg/ml), (p<0.001). While there is no significant differences (p>0.05) in median level of OPG between patients and healthy control groups (15 ng/ml; 17.99 ng/ml) respectively, as shown in table (4).

Determination the ratio of RANKL / OPG in current study revealed, that there was high difference between two study groups. The median RANKL / OPG ratio in chronic periodontitis patients (3.61) was significantly higher (P<0.001) in comparison to the ratio in healthy control (0.12), according to tables (5).

The results of correlation between RANKL and OPG are clearly shown in table (6). An anticipated, salivary RANKL level was showed significant negative correlation with OPG (r=-0.331, p=0.024). On the other hand the current study found that RANKL/OPG ratio was positively correlated with RANKL(r=0.724, p=0.001), whereas negatively correlated OPG (r=-0.300, p=0.006).

Regarding the correlation of RANKL and OPG with clinical periodontal parameters, RANKL level was showed significant positive correlation with each of PPD (r=0.387, P=0.004) and CAL (r=0.267, P=0.049), as observed in table (7). In contrast, there is no association between OPG levels and other clinical parameters of periodontitis was found (p>0.05), table (8).

Moreover; the current study found that the RANKL/OPG ratio was positively correlated with each of GI, PPD and CAL (r= 0.250, P=0.024; r=0.409, p<0.001; r=0.334, P=0.002) respectively, as shown in table (9).

Table 1: Demographic Characteristic in CP Patients and Healthy Control

		Study groups		P-value
		CP Patients n=55	Healthy control n=25	
Age and Sex				
Age (years)	Range	(24-64)	(20-51)	
	Mean ± SD	40.15±10.53	37.38±9.10	0.254^{NS}
Gender type	Female	17 (30.9%)	6 (24%)	0.128^{NS}
	Male	38 (69.1%)	19 (76%)	0.672^{NS}

NS=Not significant (p>0.05).
**** = Highly significant difference (p≤0.001).**

Table 2: Clinical Periodontal Parameters in CP Patients and Healthy Control.

	Study groups		P-value
	CP Patients n=55	Healthy control n=25	
Clinical periodontal Parameters (Mean ± SD)			
Plaque index	1.50±0.62	0.85±0.37	<0.001**
Gingival Index	1.26±0.49	0.74±0.29	<0.001**
Probing Pocket Depth (mm)	2.10±0.66	1.17±0.62	<0.001**
Clinical Attachment Loss	3.08±3.74	0.00±0.00	<0.001**
Bleeding on Probing (BOP)	28.85±30.57	6.12±8.08	<0.001**

**** = Highly significant difference (p≤0.001).**

Table 3: Differences in saliva concentration of RANKL between patients and healthy controls

	CP patients (n=55)	Healthy control (n=25)	P (Mann-Whitney)
Saliva RANKL			
Range	(6.21-284.34)	(0-10.18)	
Median	56.8	2.21	<0.001**
Inter-quartile range	(15.99-85.4)	(1.2-6.27)	
Mean Rank	53.15	15.29	

Table 4: Differences in saliva concentration of OPG between patients and healthy controls

	CP patients (n=55)	Healthy control (n=25)	P (Mann-Whitney)
Saliva OPG			
Range	(2.3-57)	(4.57-28.3)	
Median	15	17.99	0.070NS
Inter-quartile range	(10.25-19.3)	(12.68-20.91)	
Mean Rank	37.84	47.69	

Table 5: Differences in ratio of RANKL\OPG between patients and healthy controls

	CP patients (n=55)	Healthy control (n=25)	P (Mann-Whitney)
Salivary ratio RANKL/OPG			
Range	(0.3-34.24)	(0-1.54)	
Median	3.61	0.12	<0.001**
Inter-quartile range	(1.24-9.04)	(0.06-0.43)	
Mean Rank	52.9	15.83	

Table 6: Correlation between RANKL and OPG among patients

Variables	RANKL	OPG	RANKL/OPG
RANKL	1	r=-0.331 p=0.024*	r=0.724 p=0.001**
OPG	r=-0.331 p=0.024*	1	r=-0.300 p=0.006*
RANKL/OPG	r=0.724 p=0.001**	r=-0.300 p=0.006*	1

Table 7: Correlation between saliva level RANKL and clinical periodontal parameters in patients

	RANKL	
	Correlation	P- value
Plaque index	0.119	0.388^{NS}
Gingival Index	0.047	0.733^{NS}
Probing Pocket Depth (mm)	0.387	0.004*
Clinical Attachment Loss	0.267	0.049*
Bleeding on Probing (BOP)	-0.108	0.432^{NS}

Table 8: Correlation between saliva level OPG and clinical periodontal parameters in patients

	OPG	
	Correlation	**P- value**
Plaque index	0.077	0.574 NS
Gingival Index	-0.072	0.600 NS
Probing Pocket Depth (mm)	0.019	0.892 NS
Clinical Attachment Loss	0.015	0.913 NS
Bleeding on Probing (BOP)	0.072	0.600 NS

Table 9: Correlation between serum level RANKL\OPG and clinical periodontal parameters in patients

	RANKL/OPG ratio	
	Correlation	**P value**
Plaque index	0.209	0.062NS
Gingival Index	0.250	0.024*
Probing Pocket Depth (mm)	0.409	<0.001**
Clinical Attachment Loss	0.334	0.002*
Bleeding on Probing (BOP)	0.214	0.055 NS

DISCUSSION

Inflammation and bone loss are hallmarks of periodontal disease. Accumulated evidence demonstrates that periodontitis involves bacterially derived factors and antigens that stimulate a local inflammatory reaction and activation of the innate immune system. Proinflammatory molecules and cytokine networks play essential roles in this process. IL-1 and TNF-α seem to be primary molecules that, in turn, influence cells in the lesion. Antigen-stimulated lymphocytes (B and T cells) also seem to be important. Eventually, a cascade of events leads to osteoclastogenesis and subsequent bone loss via the RANK–RANKL–OPG axis. This axis and its regulation are not unique to periodontitis but rather are critical for pathologic lesions involving chronic inflammation [5].

The current results denoted a predominance of CP among males than a female which is comparable with other Iraqi study conducted by Fadil and Al- Ghurabi in 2012 [9], whereas, disagree with the result reported by Ali and colleagues [10] who observed that periodontitis in males less than as in females. The large prevalence of periodontitis among males may be due to that male less interest in oral hygiene than males. Positive family history was observed in 34.4% of patients with CP. This high rate of a positive family history in the present study raises the possibility of a genetic basis for CP transmission. However; this percentage which is in agreement with a local study reported by Ali *et al.*, [10] who found 32% of patient had positive family history, and disagree with broad studies [11, 12].

The findings of the present study indicate that the RANKL levels in saliva of chronic periodontitis patients were increased compared to controls, whereas the OPG levels were unchanged. These results are in agreement with other studies [13, 14, 15], who found an increase in soluble RANKL concentrations without a corresponding change in OPG levels in individuals with chronic periodontitis compared to healthy controls. Conversely, a reciprocal relationship was also found, in which RANKL protein expression was higher and OPG levels were lower in diseased gingival tissues compared to healthy controls [16]. In consistent with our results Wara and colleagues, pointed out to that although the exact levels of RANKL and OPG varied from study to study, the trend was generally the same; the RANKL/OPG ratio was higher in periodontitis patients than in healthy controls [15]. These findings correspond well with the critical role of RANKL in driving osteoclastogenesis and bone loss in periodontitis [17].

Similarly, Corrti and colleagues showed that study on RANKL versus OPG concentrations in gingival tissue extracts clearly demonstrated a trend toward a higher RANKL/OPG ratio in individuals with periodontitis than in healthy controls.

In a cross-sectional study, Bostanci *et al.* [18] quantified the RANKL and OPG levels in GCF from 21 healthy subjects, 22 gingivitis, 28 CP, 25 generalized aggressive periodontitis and 11 chronic periodontitis immunosuppressed patients, detecting that RANKL levels increased and OPG decreased in periodontitis patients compared with either gingivitis or healthy individuals, and concluded that RANKL/OPG ratio may predict disease occurrence.

The higher RANKL/ OPG ratio could explain in part the amount of bone loss in periodontitis patients, and this either be related to lower levels of OPG or higher levels of RANKL in periodontitis patients compared with healthy control. However, an increased RANKL/OPG ratio also may be associated with the clinical severity of periodontitis. The current study found that the RANKL/OPG ratio was positively correlated with each of GI, PPD and CAL, this result was consistent with that reported by Fatemeh *et al*., [19] in which there was statistically significant correlation between the concentration of RANKL and CAL in chronic periodontitis patients, there was also negative correlation between OPG concentration and CAL in those patients.

Meanwhile, Bostanci *et al*., stated that although periodontitis is associated with an increased RANKL/OPG ratio compared to healthy controls, the ratio may not necessarily distinguish between mild, moderate, and severe forms. Nevertheless, results show that RANKL contributes to alveolar bone loss in periodontitis and tooth loss, other findings have suggested that the increased RANKL/OPG ratio may serve as a biomarker that denotes the occurrence of periodontitis, but may not necessarily predict ongoing disease activity [14].

Microbial stimulation with *Aggrigatibacter* in chronic periodontitis induced RANKL expression on the surface of CD4 + cells and this may explain the elevation of RANKL/OPG ratio in periodontitis [20]. In conclusion this study demonstrates that salivary levels of RANKL and OPG play a crucial role in pathogenesis of periodontitis, and the relative RANKL/ OPG ratio appears to be indicative of disease occurrence.

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الخلاصة

الخلفية العلمية: تنشا امراض ما حول اللثة عن طريق الصحيفة الجرثومية التي تتراكم في منطقة التلمية والتي تحث الاستجابة الالتهابية. ان محور RANKL وOPG يساهم في عملية تنظيم ايض العظم في مرض النساغ والتي فيها الزيادة في الرانكل والنقصان في الاوبجي يمكن ان يرجح التوازن باتجاه عملية نقض العظم وارتشاف العظم النسغي و التي هي السمة المميزة لمرض النساغ. تم إجراء هذه الدراسة لبحث دور مستويات اللعابية من RANKL وOPG في التسبب في اللثة المزمنة.

المرضى وطرق العمل: خمسة وخمسون مريضا مصابا بمرض النساغ المزمن تتراوح أعمارهم بين 24-64 عاما و 25 من المتطوعين الاصحاء كانت اعمارهم و اجناسهم متطابقة مع المرضى شاركوا في هذه الدراسة. وكانت معلمات ما حول الاسنان المستخدمة في هذه الدراسة هي مؤشر الصحيفة الجرثومية و مؤشر التهابات اللثة و عمق جيوب اللثة و وفقدان الانسجة الرابطة والنزيف اثناء الفحص. تم جمع عينات من اللعاب من مجموعتي المرضى والسيطرة. وأجري فحص مقايسة الانظيم المرتبط الممتز المناعية لتقدير المستوى اللعابي من RANKL وOPG في مجموعتي الدراسة.

النتائج: كشفت البيانات الحالية أن متوسط المستويات اللعابية من RANKL قد ارتفع في مجموعة المرضى مقارنة مع مجموعة التحكم (P <0.001)، في حين أن المستويات اللعابية من OPG لم تظهر أي فروق ذات دلالة إحصائية بين مجموعات الدراسة (P> 0.05) . في المقابل كانت نسبة RANKL / OPG أعلى بكثير لدى المرضى بالمقارنة مع النسبة في المجموعة الضابطة. وعلاوة على ذلك، لوحظ ارتباط كبير سلبي بين RANKL وOPG. وفيما يتعلق بالارتباط بين المستويات اللعابية من (RANKL وOPG) ومعلمات اللثة السريرية، وقد أظهر مستويات RANKL ارتباط إيجابي كبير مع كل من عمق الجيوب وفقدان الانسجة الرابطة. ولم يلاحظ وجود علاقة بين مستويات OPG والمعلمات السريرية لالتهاب اللثة. وعلاوة على ذلك، قد أظهرت نسبة RANKL / OPG ارتباط إيجابي كبير مع كل من مؤشرالتهاب اللثة، عمق الجيوب وفقدان الانسجة الرابطة. الاستنتاجات: توضح هذه الدراسة أن مستويات اللعابية من RANKL وOPG تلعب دورا حاسما في التسبب في امراض اللثة، وان نسبة RANKL / OPG قد تكون مؤشرا على حدوث المرض.

كلمات المفتاحية: التهاب اللثة المزمن، RANKL، OPG