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Emerging roles of interleukin and receptor activator nuclear factor ligand and osteoprotegerin levels in periodontal disease: An original research

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Abstract--Introduction: Very few studies are done to evaluate IL-34 levels together with GCF RANKL and OPG levels in periodontitis patients before and after non-surgical periodontal treatment (NSPT). Materials and Methods: We included 150 patients to be equally divided to case and the controls. GCF and clinical periodontal recordings were investigated at the baseline and 6 weeks after non-surgical periodontal treatment NSPT. ELISA was used for quantifying of GCF IL-34, RANKL and OPG levels and their relative ratios were calculated. Results: Higher levels of GCF IL-34 and RANKL levels were

found in the both the groups than in control group at baseline, whereas GCF OPG levels were statistically lower at baseline. GCF IL-34 and RANKL levels lowered in the 6th week after therapy in the both periodontitis groups, while the concentration OPG levels statistically amplified ($P < 0.05$). Significantly positive correlations among the IL-34 with RANKL, sampled-site clinical attachment level (CAL), and gingival index (GI), whereas negative correlation with OPG were reported ($P < 0.05$). Conclusions: Evaluation of the IL-34 levels together with RANKL/OPG ratio in GCF maybe valuable in identifying high risk individuals with periodontitis patients.

Keywords---Periodontitis, Interleukin-34, RANKL, OPG.

Introduction

Receptor activator of nuclear factor- κ B ligand (RANKL) is the essential cytokines in differentiation of osteoclasts that cause bone resorption. Activation of osteoclast that triggers bone destruction is regulated by RANKL, RANK and osteoprotegerin (OPG). The effects of RANKL is modified by OPG by blocking RANKL/RANK interaction. RANKL and OPG can be discovered in gingival tissue, gingival crevicular fluid (GCF), saliva, serum. It has also been attributed to periodontal disease; the blockage of the CSF-1 receptor (CSF-1R) leads to reduced alveolar bone loss. [1, 2] The lack of CSF-1R results in osteopetrosis, diminished mononuclear phagocyte and reproductive defect indicating the function of CSF-1 is through CSF-1R.[4-6] The cytokine interleukin 34 (IL-34) may play a role in the pathogenesis of chronic periodontitis. The concept of targeting CSF-1/CSF-1R and RANK signaling pathway may need to be considered in the regulation of IL-34. The interaction between IL-34 levels and RANKL/OPG ratio in GCF might disclose a new bone devastation pathway in periodontitis. There are very few studies that revealed these mediators before and after the periodontal therapy. Hence in the present study we aim to study the roles of interleukin and receptor activator nuclear factor ligand and osteoprotegerin levels in periodontal disease.

Material and Methods

We conducted a prospective observational study at the department of periodontics in our institution. The ethics approval was taken and the patient concern was taken. The study was done for a period of 2 years. The subjects selected for the study were 150, who were divided to equal groups of 50 each as cases (B,C) and controls. In the case they were further divided as those with Stage 3-grade B, C periodontitis. Those patients who had periodontitis according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions were included in the study. We excluded the patients who had other unrelated systematic condition, smoking habits on antibiotics etc.

Periodontal status of participants was assessed by evaluating plaque indices, gingival indices, clinical attachment level and BOP. The periodontal bone loss was determined by taking full-mouth periapical radiographs. All the patients with periodontitis included a rigorous hygiene phase and full-mouth scaling and root

planning and ultrasonic instruments. NSPT was performed twice a week for two weeks. Clinical data were obtained from the both of periodontitis groups again after six weeks SRP. GCF samples were collected from two sites and mesiobuccal or distobuccal sites of single-rooted teeth from each participant in the all groups. The collection of samples were performed at baseline from whole groups and 6 weeks after NSPT from patients with periodontitis. The sites, PPD < 3 mm and absence of CAL and BOP were chosen for CTRL group samples. For periodontitis groups, the samples were collected from sites with PPD 6 mm, CAL 5 mm and 30% bone loss. ELISA assay to examine the total quantities of IL-34, OPG and RANKL was done. The values that were obtained were compared keeping $p < 0.05$ as significant.

Results

We observed that genders were equally distributed among the groups. The various clinical parameters were statistically higher in the cases than the control groups. Table 1 We observed that there was significant difference of the IL-34 level, RANKL, CAL and GI among the groups. Correlation coefficients are presented in Table 2. The correlation analyses were performed by using the total amounts for the elimination of GCF volume. A significantly positive correlation was demonstrated among the total of IL-34 level, RANKL, CAL and GI ($P < 0.05$), whereas IL-34 levels was negative correlated with OPG. RANKL levels with also negative correlated with OPG and OPG levels with negative correlated with sampled site CAL and GI ($p < 0.05$) when every group were examined together. IL-34 levels also positive correlated with RANKL/ OPG ratio when all groups were examined together ($P < 0.05$).

Table 1 Comparison of the clinical Parameters among the groups

Groups	PPD	CAL	PI	GI	BOP	GCF Volume
CTRL						
Full-mouth	2.40 (1.65–2.98)	2.43 (1.65–3.64)	0.09 (0.02–0.21)	0.17 (0.01–0.38)	7.15 (4.15–9.42)	0.28 (0.23–0.35)
Sampled sites	2 (1–2)	2 (1–2)	0 (0–1)	0 (0)	0 (0)	
Periodontitis						
Stage 3-grade B						
Full-Mouth						
Before Treatment	4.92 [§] (3.18–5.88)	5.45 [§] (3.98–6.76)	2.15 [‡] (1.65–2.89)	2.30 [§] (1.59–2.72)	85.20 [§] (56.40–100)	
After Treatment	2.78 [§] (2.08–3.32)	3.32 [‡] (2.18–4.46)	0.46 [§] (0.12–1.21)	0.79 [†] (0.13–1.34)	20.49 [§] (11.46–32.81)	
Sampled Sites						
Before Treatment	6 [§] (5–8)	6 [§] (5–8)	2 [‡] (2–3)	2 [‡] (2–3)	100 [§] (100)	0.84 [‡] (0.75–0.95)
After Treatment	2 [§] (2–3)	3 [§] (3–4)	0 [†] (0–1)	0 [†] (0–1) 0 [†] (0–1)	0 [†] (0)	0.54 [§] (0.48–0.63)
Periodontitis						
Stage 3-grade C						
Full-Mouth						
Before Treatment	4.13 [§] (3.35–6.97)	4.53 [§] (3.42–7.83)	2.21 [‡] (1.19–3.00)	2.20 [§] (1.23–3.00)	71.57 [§] (40.97–100)	
After Treatment	3.58 [§] (2.85–5.64)	4.21 [‡] (3.33–7.92)	1.26 [§] (0.35–2.71)	1.55 [§] (0.64–2.54)	14.47 [§] (6.80–31.76)	
Sampled Sites						
Before Treatment	7.5 [§] (6–9)	7.5 [§] (6–9)	2 [‡] (2–3)	2 [‡] (2–3)	100 [§] (100)	0.89 [§] (0.77–0.96)
After Treatment	4 [‡] (3–6)	5 [§] (4–7)	0 [†] (0–1)	0 [†] (0–1)	20 [§] (0–100)	0.57 [§] (0.48–0.69)

Table 2 The Correlation (r) Among Groups of the various biochemical parameters.

	IL-34 to RANKL	IL-34 to OPG	RANKL to OPG	IL-34 to CAL	RANKL to CAL	OPG to CAL	IL-34 to GI	RANKL to GI	OPG to GI	IL-34 to RANKL/OPG
CTRL	-0.113	-0.329	0.083	-0.145	-0.184	-0.044	0.228	-0.236	0.033	-0.036
Periodontitis Stage 3-grade B	-0.200	-0.274	0.041	0.246	-0.116	-0.089	0.021	0.143	0.099	0.200
Periodontitis Stage 3-grade C	0.066	-0.356	0.003	0.383	-0.152	-0.080	0.063	-0.022	0.196	0.212
All groups	0.666**	-0.627**	-0.440**	0.694**	0.582**	-0.402**	0.683**	0.590**	-0.355**	0.779**

** Statistically significant (P < 0.05).

Discussion

In our study the total amount of a components in the GCF and interactions between the GCF components and periodontal diseases were observed. [3,7,8]. We observed that the total RANKL levels in GCF were higher in the both of periodontitis group than CTRL group and this levels were decreased after NSPT and the total amount of OPG were statistically lower in the both of periodontitis groups than CTRL group at baseline. But there were no difference in the total GCF OPG levels after NSPT in periodontitis. A higher RANKL/OPG ratio is addressed symptomatic of the presence of untreated periodontitis. It is proposed that this ratio might potentially serve as a molecular diagnostic mediator for the disease [5]. In a previous study unchanged RANKL/OPG ratio after NSPT and their result did not associate with clinical parameters, despite the improved clinical outcome in the patients with chronic periodontitis and aggressive periodontitis. While in some studies a positive correlation between RANK/OPG ratio and sampled sites PPD and CAL parameters in localized aggressive periodontitis. Balli et al. [9] showed higher RANKL/OPG ratio in periodontitis before therapy and the RANKL/OPG ratio lowered after NSPT, but this decrease was not significant in their study. Similar to their study in our study higher RANKL/OPG ratio levels in GCF in the both of periodontitis groups than control group at baseline and this ratio significantly lowered together with a significant enhancement in clinical parameters in periodontitis groups after NSPT.

In the study of Clark et al. [10] showed that in periodontitis increased CSF-1 levels in gingival tissue from periodontitis patients compared to controls, whereas IL-34 expression was similar. Batra et al. [11] reported that levels of IL-34 in GCF showed a rising level from healthy followed by chronic periodontitis group and aggressive periodontitis group and this levels positive correlated with PI, GI, PPD and CAL. In unison to this study in our study significantly greater IL-34 levels in GCF in the those of periodontitis groups than CTRL group. Additionally, total and concentration GCF IL- 34 levels were greater in patients with stage 3-grade C than stage 3- grade B patients. This might recommend an eventual cellular hyperactivity that may support periodontal damage in grade C periodontitis and this may cause more destruction of periodontal tissues in stage 3-grade C than in stage 3-grade B.

Ertugrul et al. [12] reported that aggressive periodontitis patients showed greater GCF cytokine levels than patients with chronic periodontitis and they explained these result as follows that this increase was owing to gene polymorphisms cod in

for the produce of inflammatory markers. Contrary to this study in our study we evaluated effects of NSPT on GCF IL-34 levels in patients with stage 3-grade B and stage 3-grade C and compared to these results with GCF RANKL and OPG levels. Total amounts and concentration GCF IL-34 levels significantly decreased after NSPT and GCF IL-34 levels positive correlated with GI, CAL and RANKL/OPG ratio in GCF in our study. Total IL-34 GCF levels also showed statistically a positive correlation with RANKL GCF levels, while a negative correlation with OPG GCF levels when all groups were examined together. The results demonstrate that periodontal treatment can notably lower IL-34 levels and RANKL/ OPG ratio in GCF, indicating a significant difference in all clinical periodontal parameters among both periodontitis groups after treatment. We can propose that IL-34 exhibits a pro-inflammatory property and plays pivotal role in the development of periodontitis. Considering the GCF IL-34 results of the present, IL-34 may be a valuable detection marker in individuals at high risk of developing periodontal disease and may be a potential indicator for better efficient treatment. The limitation of our study is that the CSF-1 and CSF-1R levels in GCF were not assessed. The potential association of among IL-34, CSF-1, CSF-1R, TNF- α and RANKL deserves further investigation.

Conclusion

We can conclude from our study that RANKL/OPG ratio may be lowered after the therapy. Evaluation of the IL-34 levels together with RANKL/OPG ratio in GCF maybe valuable in identifying high risk individuals with periodontitis patients.

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