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Original Research Article

Estimation of interleukin-17 levels in gingival crevicular fluid from healthy individuals, chronic gingivitis and chronic periodontitis patients using enzyme linked immunosorbent assay

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ABSTRACT

Background: Chronic Periodontitis (CP) is an infectious disease resulting in inflammation of supporting tissues of the teeth. A number of pro-inflammatory cytokines are formed against periodontopathogenic microorganisms. Interleukin-17 (IL-17) is a pro-inflammatory cytokine, implicated in numerous inflammatory and autoimmune conditions.
Methods: A total of 25 periodontally healthy subjects (Group 1), 25 patients with gingivitis (Group 2) and 25 patients with CP (Group 3) were included for the study based on clinical examination. Gingival index, probing pocket depth and clinical attachment loss were recorded in all subjects.
Results: The levels of IL-17 increased from healthy to gingivitis to periodontitis patients. A positive correlation was found with the IL-17 and the clinical parameters like gingival index, probing pocket depth and clinical attachment loss.
Conclusions: There is a strong association between the levels of IL-17 with periodontal disease as well as with its severity and its possible use as a biomarker for inflammatory periodontal disease.

Keywords: Periodontitis, Gingival crevicular fluid, Enzyme linked immunosorbent assay, IL-17

INTRODUCTION

CP is an infectious disease resulting in inflammation of supporting tissues of the teeth, progressive attachment loss and bone loss.[1] Periodontitis is a chronic infectious inflammatory disease that is characterized by a heavy lymphocytic infiltration into the periodontal lesions, resulting in the secretion of a variety of cytokines, that ultimately leads to the destruction of periodontal tissues including alveolar bone.[2]

A number of pro-inflammatory cytokines are formed against periodontopathogenic microorganisms. IL-17 is a pro-inflammatory cytokine which is produced by activated T helper (TH) 17 cells implicated in number of inflammatory and autoimmune conditions. CD 4+TH cells plays an essential role in regulating autoimmune disease and inflammation by secreting a novel proinflammatory cytokine, IL-17 (IL-17A); hence, these cells are termed TH17.[3] Moreover, the Th17 cells seem to antagonize T regulatory cell (Treg) development, and thereby amplify the proinflammatory responses and promotes autoimmunity.[4,5]

Levels of IL-17 are significantly higher in Gingival crevicular fluid (GCF) samples and culture supernatants

of gingival cells in periodontitis patients compared to controls.[6]

A major advance in understanding the molecular link between the immune system and bone came with the discovery of receptor activator of nuclear factor kB (RANK) and RANK ligand (RANKL), TNF superfamily receptors that are central to the regulation of osteoclastogenesis.[7] RANKL is induced by inflammatory cytokines, such as IL-17, -1b, and -6 and TNF-a, on osteoblastic stromal cells, OBs, and bone marrow stromal cells.[8-10] RANKL binds to its receptor RANK, expressed by OC progenitors, hematopoietic cells, tissue monocytes, and non-OC macrophages. Binding of RANKL to RANK drives differentiation to a mature OC phenotype with bone resorbing capability.[11]

IL-17 can modulate the RANKL/OPG ratio; IL-17 increases RANKL expression and concomitantly decreases OPG expression in osteoblastic cells in vitro and in vivo, resulting in enhanced formation of OCs and bone erosion in a mouse model of arthritis.

The role that IL-17 appears to play in regulation of immune system and its possible role in clinical disorders, identification and characterization of the molecule is of particular interest.

Objectives of the study were to estimate the levels of IL-17 in GCF of healthy individuals, gingivitis and chronic periodontitis patients and to compare the levels of IL-17 among healthy, gingivitis and periodontitis patients.

METHODS

This study was performed in the Department of Periodontology at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belagavi.

The study was conducted in the month of December 2017 and was completed in the month of November 2018.

Sample size calculations were done based on 2 methods, One for correlation between PPD and IL-17 and the other for mean difference between IL17 levels between healthy and CP (Vernel et al 2005). The higher sample size amongst them was 23 which was rounded off to 25. Hence, we finalized the sample size of 25 per group. Since we have three groups the final sample size is 75. Total of 75 subjects based on inclusion and exclusion criteria were taken, and were explained about the study in their vernacular language and after signing a written informed consent were recruited in the study. Based on clinical examination, the subjects were divided into three groups: Group 1-Healthy Individuals (n=25), Group 2-Chronic Gingivitis (n=25), Group 3- CP (n=25).

All subjects selected had a minimum of 20 natural teeth and with the age group of 30-60 years.

The inclusion and exclusion criteria for both the groups were as follows.

Inclusion criteria

Group 1-No clinical signs of gingival inflammation, absence of bleeding on probing, probing depth ≤3 mm, no clinical attachment loss (CAL).

Group 2-presence of clinical signs of inflammation, bleeding on probing present, gingival index (g.i) >1, probing depth ≤3 mm, no clinical attachment loss.

Group 3-Presence of clinical signs of inflammation, presence of bleeding on probing, G.I. >1, periodontal probing depth of ≥4 mm, Clinical Attachment Loss ≥3 mm.

Exclusion criteria

Included the subjects who have received periodontal therapy or antimicrobial therapy within 6 months before sampling or patients with history of any systemic diseases/conditions or patient with habit of smoke/smokeless tobacco or pregnant woman and lactating mother.

A thorough periodontal examination was carried out and parameters selected for the study were carefully recorded. Single examiner did all the recordings. The following clinical parameters were recorded.

1. Gingival index (GI) (Loe and Silness in 1963)
2. Probing depth and
3. Clinical attachment loss

Sample collection

GCF were sampled from healthy and diseased individuals. Supragingival plaque is removed via sterile cottons or curettes from the regions from which GCF was sampled. The area was isolated by cotton rolls and gently dried. The GCF samples were obtained with the help of paper points. The paper points were placed gently into the gingival crevice. Samples contaminated with blood or saliva were discarded. The collected sample were then transferred to the transport media phosphate buffer saline (PBS) and sent to central research laboratory at Maratha Mandal's N.G.H Institute of Dental Sciences and Research Centre, Belagavi. Processed GCF samples were analysed for the levels of IL-17 using ELISA.

Statistical analysis

The statistical analysis was done using SPSS 20 software.

Distribution of male and females in three study groups and distribution of patients in three study groups by age groups were done using Chi Square test. Comparison of three study groups with respect to Interleukin 17 levels In

GCF (pg/ml) scores and comparison of three study groups with respect to gingival index scores were done using Kruskal Wallis ANOVA and Mann-Whitney U test. Comparison of three study groups with respect to PPD score and comparison of three study groups with respect to CAL scores was done using One-way ANOVA and Pair wise comparisons by Tukeys multiple Posthoc procedures. Correlation between IL-17 levels with clinical parameters was done using Spearmans rank correlation coefficient.

RESULTS

There was no significant gender distribution among the three groups.

The mean age of the Group 1 (±SD) was 37.52±6.80. The mean age of the Group 2 (±SD) was 40.64±9.85. The mean age of the Group 3 was 45.92±8.56. 64% of subjects in the group 1 were in the age group of 31-40 yrs. 20% of subjects in the group 1 were in the age group of 41-50 yrs. 48% of subjects in the group 2 were in the age group of 31-40 yrs. 16% of subjects in the group 2 were in the age group of 41-50 yrs. 24% of subjects in the group 3 were in the age group of 31-40 yrs. 48% of subjects in the chronic periodontitis group were in the age group of 41-50 yrs.

Table 1 and Table 2 shows comparison of levels of IL-17 in the three groups in GCF (pg/ml) by Kruskal Wallis ANOVA and also the pair wise comparisons of three study groups with respect to Interleukin 17 levels in GCF (pg/mL) by Mann-Whitney U test. The mean value (±SD) of IL-17 in Group 1, Group 2, Group 3 are 8.19±7.61, 122.35±172.11, 178.71±199.32 respectively. The values are significant with p value of 0.0001. Also, pair wise comparisons of three study groups with respect to IL-17 levels are done which is also significant.

Table 1: Comparison of three study groups with respect to IL-17 levels In GCF (pg/mL) scores by Kruskal Wallis ANOVA.

Groups	Mean	SD	SE	Mean rank
Group 1	8.19	7.61	1.52	16.70
Group 2	122.35	172.11	34.42	41.14
Group 3	178.71	199.32	39.86	56.16
H value	41.7810			
P value	0.0001*			

*p<0.05.

Table 3, Table 4 shows comparison of 3 groups with respect to gingival index scores and also the pair wise comparison of groups with respect to gingival index scores. Statistically significant scores were seen when Group 1 and Group 2, Group 1 and Group 3, Group 2 and Group 3 were compared with p value less than 0.001.

Figure 1 shows comparison of three study groups with respect to PPD scores by One Way ANOVA-Statistically significant scores were seen when healthy and chronic periodontitis group were compared with p value 0.0001. Statistically significant scores were seen when gingivitis and chronic periodontitis groups were compared with p value 0.0001.

Table 2: Pair wise comparisons of three study groups with respect to IL-17 levels In GCF (pg/ml) scores by Mann-Whitney U test.

Groups	Mean	SD	Mean rank	U value	Z value	P value
Group 1	8.19	7.61	16.66			
Group 2	122.35	172.11	34.34	91.50	-4.2880	<0.001
Group 1	8.19	7.61	13.04			
Group 3	178.71	199.32	37.96	1.00	-6.0440	<0.001
Group 2	122.35	172.11	19.80			
Group 3	178.71	199.32	31.20	170.00	-2.7649	0.0057*

*p<0.01.

Figure 2 shows comparison of three study groups with respect to CAL scores by one-way ANOVA- Statistically significant scores were seen when healthy and chronic periodontitis group were compared with p value 0.0001. Statistically significant scores were seen when gingivitis and chronic periodontitis groups were compared with p value 0.0001.

Table 3: Comparison of three study groups with respect to gingival index scores by Kruskal Wallis ANOVA.

Groups	Mean	SD	SE	Mean rank
Group 1	0.37	0.27	0.05	13.02
Group 2	1.94	0.73	0.15	43.22
Group 3	2.74	0.55	0.11	57.76
H-value	54.9670			
P-value	0.0001*			

*p<0.05.

Table 4: Pair wise comparisons of three study groups with respect to gingival index scores by Mann-Whitney U test.

Groups	Mean	SD	Mean rank	U value	Z value	P value
Group 1	0.37	0.27	13.00			
Group 2	1.94	0.73	38.00	0.00	-6.0634	<0.001
Group 1	0.37	0.27	13.00			
Group 3	2.74	0.55	38.00	0.00	-6.0634	<0.001
Group 2	1.94	0.73	18.18			
Group 3	2.74	0.55	32.82	129.50	-3.5507	<0.001

Table 5: Correlation between Il-17 levels In GCF (pg/ml) with gingival index, PPD and CAL scores with in three study groups by Spearman's rank correlation coefficient.

Groups	Parameters	Correlation between Interleukin 17 levels in GCF (pg/ml) with			
		N	Spearman R	t value	P level
Group 1	Gingival index	25	0.8490	7.7043	<0.001
	PPD	25	0.7826	6.0286	<0.001
	CAL	25	--	--	--
Group 2	Gingival index	25	0.9823	25.1506	<0.001
	PPD	25	0.6633	4.2511	<0.001
	CAL	25	--	--	--
Group 3	Gingival index	25	0.9170	11.0278	<0.001
	PPD	25	0.9992	122.1738	<0.001
	CAL	25	0.9954	49.7260	<0.001

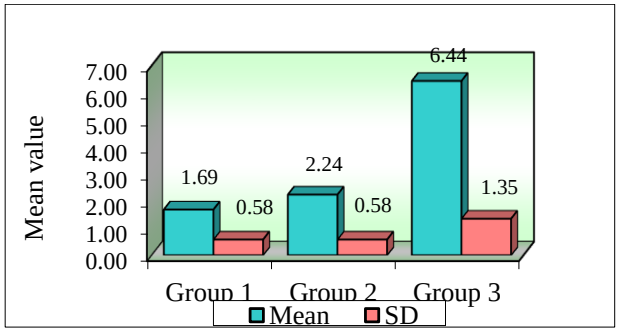

Figure 1: Comparison of three study groups with respect to PPD scores.

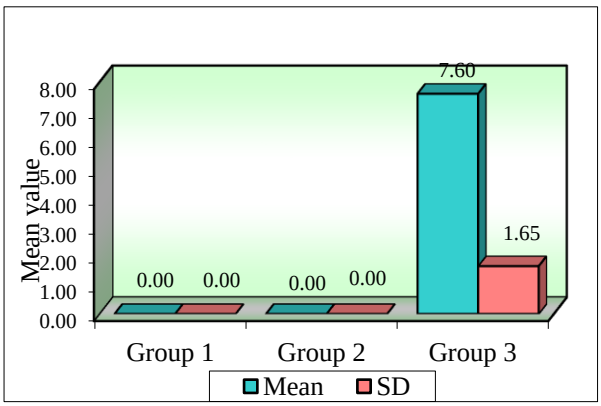

Figure 2: Comparison of three study groups with respect to CAL scores.

Table 5 shows correlation between Interleukin 17 levels In GCF (pg/ml) with gingival index, PPD and CAL scores with in three study groups by Spearman's rank correlation coefficient. Statistically significant values were found between the IL-17 levels and G.I., PPD and CAL with p value less than 0.001.

DISCUSSION

The periodontal activity is mainly mediated by immune responses and a complex network of inflammatory mediators. GCF is an exudate from periodontal tissue and assessment of biomarkers in GCF has been introduced as a non-invasive method to monitor the progression of periodontal diseases.[12,13]

IL-17 is emerging as potentially important players in pathogenesis of periodontitis. IL-17 is of particular interest in the pathogenesis of periodontitis because of its involvement in both inflammation and protective antimicrobial immunity.[14] IL-17 has potent osteoclastogenic properties in part due to its capacity to stimulate RANKL expression by osteoblasts and other stromal cells and therefore, a focal point of interest in bone related disease such as rheumatoid arthritis, osteoporosis and periodontal disease.

The present study was conducted to estimate the levels of IL-17 in the GCF of healthy, gingivitis and chronic periodontitis patients using ELISA.

There was no statistically significant gender predilection obtained from our study. The mean age for the chronic periodontitis individuals was 45.92±8.56. A significant relation has been seen in age distribution between the group. The mean age in healthy group is 37.52±6.80. Azman, 2014 in his study on clinical association between IL-17 family cytokines and periodontitis confirmed the elevation of serum, saliva and GCF levels of IL-17A in periodontitis patients and positive correlation with clinical parameters and age.[15]

The levels of IL-17 are increased in chronic periodontitis compared to gingivitis and healthy group. Pair wise comparison between the groups with respect to levels of IL-17 levels in GCF was done which came out to be statistically significant. Significant difference between the levels of IL-17 between healthy and periodontitis individuals is obtained with p value less than 0.001 and also significant difference between levels of IL-17 in gingivitis and chronic periodontitis group is seen in the present study. This was in accordance to study conducted by Vernal 2005, Ravindra Reddy Nagireddy 2013.[6,16] Vernal et al 2005 in his study of levels of IL-17 in GCF and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis concluded that the total amount of cytokine IL-17 in GCF samples were significantly increased in periodontal disease.[6]

Akio Mitani 2015 conducted a study on IL-17 and IL-35 in chronic periodontitis patients. GCF samples were analysed for the levels of these cytokines in healthy and chronic periodontitis subjects.[17] The levels of IL-35 and IL-17 were significantly higher in GCF from the patients with periodontitis than healthy participants.

Husniah Batool et al 2018 in a study of salivary levels of IL-6 and IL-17 as an indicator of disease severity in patients with calculus associated Chronic Periodontitis concluded that the levels of IL-6 and Il-17 were significantly increased in calculus associated periodontitis as compared to healthy controls and their levels increased with progression of Chronic periodontitis.[18]

Arief 2017 conducted a study on serum Interleukin 17 in chronic periodontitis. Blood serum were obtained from healthy as well as chronic periodontitis individuals and the concentration of cytokine IL-17 was analysed by ELISA.[19] The results showed that subjects with periodontitis presented with significantly higher concentration of IL-17 3.6pg/ml when compared to control 3.1pg/ml.

In, summary our data showed higher cytokine IL-17 levels in GCF from periodontitis subjects than in healthy subjects, and also its co relation with clinical parameters like gingival index, probing pocket depth and clinical attachment loss, suggesting the role of IL-17 in pathogenesis of chronic periodontitis. The effect of periodontal treatment and levels of IL-17 was not evaluated in the study.

CONCLUSION

The objective of the present study was to estimate the levels of Interleukin 17 in healthy, gingivitis and CP patients using ELISA. The study concluded that the levels of IL-17 increased from healthy to gingivitis. The levels of IL-17 increased from gingivitis to chronic periodontitis patients. Highly statistically significant difference was found with the levels of IL-17 in chronic periodontitis patients as compared to the healthy group. A positive correlation was found between the levels of IL-17 and the clinical parameters like gingival index, probing pocket depth and clinical attachment loss.

Thus, the result showed that there is a strong association of Interleukin 17 with periodontal disease as well as with its severity and that it could be used as a novel marker for periodontal disease.

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