NON SURGICAL PERIODONTAL TREATMENT EFFECT ON IL-17 AND IL-18 LEVELS IN GINGIVAL CREVICULAR FLUID OF STAGE III/IV, MOLAR/INCISOR PATTERN PERIODONTITIS

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ABSTRACT

Main purpose: to determine the levels of Interleukin-17 and Interleukin-18 in the gingival crevicular fluid (GCF) of stage III/IV, molar/incisor pattern periodontitis patients matched to their levels in periodontally healthy individuals.

Subjects and methods: Fifty subjects were included; 25 of them are diagnosed with stage III/IV, molar/incisor pattern periodontitis patients and 25 are periodontally healthy subjects. Probing depth (PD), clinical attachment level (CAL), plaque index (PI) and gingival index (GI) were recorded for all the enrolled subjects at baseline and after three months following non surgical treatment in the tested periodontitis group. GCF levels of IL-17 and IL-18 were analyzed by enzyme-linked immunosorbent assay at baseline and after three months following non surgical treatment in the stage III/IV, molar/incisor pattern periodontitis group.

Results: Clinical parameters and GCF levels of IL-17 and IL-18 were higher in stage III/IV, molar/incisor pattern periodontitis than periodontally healthy subjects . Both IL-17 and IL-18 were decreased after non surgical treatment of the stage III/IV, molar/incisor pattern periodontitis group. So, this proved that both IL-17 and IL-18 are positively correlated to the clinical parameters and both interleukins are positively correlated with each other and have a role in periodontal disease pathogenesis.

Conclusion: IL-17 and IL-18 are important proinflammatory cytokines of periodontal disease and either one of them can be used as a potential biomarker of stage III/IV, molar/incisor pattern periodontitis.

KEYWORDS: Stage III/IV , Molar/Incisor pattern , GCF

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INTRODUCTION

Periodontal diseases are the diseases that can affect gingiva, periodontal ligament, cementum and bone. According to rate of progression, they are classified into either slowly progressing diseases (chronic) or rapidly progressing diseases (Aggressive) *(Armitage 1999)*. Aggressive periodontitis is not only characterized by rapid progression but also by the characteristic pattern of rapid attachment loss and bone resorption around incisors and first molar, absence of systemic diseases and young age of onset *(Albandar 2014)*. Many factors as specific genetic phenotypes, host immune deficiencies and certain bacterial infections have been proposed as causative factors of aggressive periodontitis *(Kulkarni and Kinane 2014)*.

One of the relatively new   presented pro-inflammatory cytokines is IL-17 which has an extensive range of defensive and destructive effects **(McKenzie et al., 2006; Gaffen and Hajishengallis 2008; Ohyama et al., 2009).** IL-17 has a critical role in polymorphonuclear cells' homeostasis and can prompt epithelial cells, fibroblasts and osteoblasts to discharge a variety of inflammatory chemokines that can kill extracellular bacterial invaders *(Khader et al., 2009; Hajishengallis 2014; Zenobia and Hajishengallis 2015)*.

IL-18 is another proinflammatory cytokine re-leased by T cells, B cells, macrophages, keratino-cytes, oral epithelial cells, dendritic cells, and os-teoblasts *(Sugawara et al., 2001, Niyonsaba et al., 2005)*. It may have a role in the development of the inflammatory responses due to its proinflammatory, angiogenic, and chemotactic characteristics.

Caspase-1 is responsible for intracellular activation of immature IL-18 to mature IL-18 *(Johnson and Serio 2005)*.Some studies confirmed an association between the periodontal disease severity and levels of IL-18 *(Orozco et al., 2006, Figueredo et al.,2008)*.

AIM OF THE STUDY

Objectives were to compare and correlate the clinical parameters and biochemical parameters (IL-17 and IL-18 levels in GCF) in stage III/IV, molar/incisor pattern periodontitis patients (At baseline and after 3 months of nonsurgical treatment) versus periodontally healthy subjects so that, we can clarify the value of these makers in diagnosis of periodontal disease.

Subjects and methods

Ethical Approval: Research ethical committee in Faculty of Oral and Dental medicine, Future University in Egypt has accepted this clinical study with number (FUE.REC(4)/2-2023) and was also listed at https://clinicaltrials.gov/ under the number (**NCT05297084**).Oral consent was taken from patients as many of them can't read or write

Recruitment and eligibility criteria:

A total of 50 individuals were enrolled and categorized as; **group A** that included 25 stage III/IV, molar/incisor pattern periodontitis patients and **group B** which included 25 periodontally healthy control subjects.

Inclusion criteria: ASA type I subjects *(Doyle et al., 2022)* from both genders with age range between 18 to 30 years old were included.

Exclusion criteria: Subjects with periodontal surgeries in the last 6 months, Subjects with prior use of antibiotics in the last 6 months ,Smokers, Pregnant and lactating females were excluded.

Clinical parameters: Plaque Index (PI) *(Silness and loe 1964)***,** gingival Index *((loe and Silness 1963)***,** Probing depth (PD) and Clinical Attachment level (CAL) *(Glavind and Löe 1967)* were recorded. PD and CAL were measured in 6 points (3 buccal and 3 lingual/palatal) using William's graduated periodontal probe.

GCF sampling: In **group A**, the site with deepest probing depth was selected for GCF sampling while

in **group B**, any site was sampled using PerioPaper strips[**] according to guidelines provided by *Bıyıkoğlu et al., 2013* and *Kinney et al., 2014.*

Phase I periodontal therapy: After baseline GCF sample collection and clinical measurements, group A patients received phase I periodontal therapy.

Analysis of IL-17 and IL-18 levels in GCF: Enzyme linked immunosorbent assay (ELISA) was used to examine GCF according to kit directions.

Statistical Analysis

Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check data normality. IL-17 and IL-18 levels data showed parametric distribution so; Student's t-test was used. Non-parametric distribution was observed in PI, GI, PD, CAL so; Mann-Whitney U test was used. Numerical data were presented as mean and standard deviation (SD) values. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Base line characteristics: There was no statistically significant difference between mean age values in the two groups. There was also no statistically significant difference between gender distributions in the two groups, table (1).

Plaque Index (PI): At baseline; stage III/IV, molar/incisor pattern periodontitis group showed statistically significant higher mean PI than control group (*P*-value = 0.001, Effect size = 0.633). After three months; there was no statistically significant difference between the two groups (*P*-value = 0.209, Effect size = 0.001). There was a statistically significant decrease in mean PI after three months in stage III/IV, molar/incisor pattern periodontitis group (*P*-value <0.001, Effect size = 0.768), table (2).

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* *PerioPaper, ProFlow, Amityville, NY, USA

TABLE (1) Descriptive statistics and results of Student's t-test and Chi-square test for comparison between base line characteristics in the two groups

	StageIII/IV, M/I periodontitis (n = 25)	Control (n = 25)	*P*-value
Age			
Mean (SD)	36.4 (3.4)	36.2 (3.7)	0.843
Gender [n (%)]			
Male	7 (28%)	8 (32%)	0.467
Female	18 (72%)	17 (68%)	

*: Significant at P ≤ 0.05

Gingival Index (GI): At baseline; stage III/IV, molar/incisor pattern periodontitis group showed statistically significant higher mean GI than control group (*P*-value <0.001, Effect size = 1.375). After three months; there was no statistically significant difference between the two groups (*P*-value = 0.558, Effect size = 0.146). There was a statistically significant decrease in mean GI after three months in stage III/IV, molar/incisor pattern periodontitis group (*P*-value = 0.001, Effect size = 0.643), table (2).

Probing Pocket Depth (PPD): At baseline as well as after three months; stage III/IV, molar/incisor pattern periodontitis group showed statistically significant higher mean PPD than control group (*P*-value <0.001, Effect size = 3.333) and (*P*-value <0.001, Effect size = 3.255), respectively. There was a statistically significant decrease in mean PD after three months in stage III/IV, molar/incisor pattern periodontitis group (*P*-value <0.001, Effect size = 0.852), table (2).

Clinical Attachment Level (CAL): There was a statistically significant decrease in mean CAL after three months in stage III/IV, molar/incisor pattern periodontitis group (*P*-value <0.001, Effect size = 0.85), table (2).

TABLE (2). The mean, standard deviation (SD) values, results of Mann-Whitney test and Wilcoxon signed-rank test for comparison between clinical parameters in the two groups and the changes within each group

Clinical parameter	Time	StageIII/IV , M/I periodontitis (n = 25)		Control (n = 25)		P-value	Effect size (d)
		Mean	SD	Mean	SD		
PI	Base line	1.68	0.8	1	0.59	0.001*	0.633
	3 months	0.8	0.58	1	0.59	0.209	0.001
	Change	0.88	0.73		-	-	-
P-value (Effect size)		<0.001* (d =0.768)					
GI	Base line	1.64	0.91	0.56	0.51	<0.001*	1.375
	3 months	0.68	0.63	0.56	0.51	0.558	0.146
	Change	0.96	1.1		-	-	-
P-value (Effect size)		0.001* (d = 0.643)					
PD (mm)	Base line	7.2	1.32	1.36	0.57	<0.001*	3.333
	3 months	5	1.61	1.36	0.57	<0.001*	3.255
	Change	2.2	1.19		-	-	-
P-value (Effect size)		<0.001* (d =0.852)					
CAL (mm)	Base line	8.72	1.28		-	-	-
	3 months	6.64	1.63		-	-	-
	Change	2.08	1.15		-	-	-
P-value (Effect size)		<0.001* (d = 0.85)					

: Significant at P ≤ 0.05

Interleukin-17 (IL-17) level: At baseline as well as after three months; stage III/IV, molar/incisor pattern periodontitis group showed statistically significant higher mean IL-17 level than control group (*P*-value <0.001, Effect size = 0.79) and (*P*-value = 0.006, Effect size = 0.145), respectively. There was a statistically significant decrease in mean IL-17 after non-surgical treatment for three months in stage III/IV, molar/incisor pattern periodontitis group (*P*-value <0.001, Effect size = 0.842), table (3).

Interleukin-18 (IL-18) level: At baseline as well as after three months; stage III/IV, molar/incisor pattern periodontitis group showed statistically significant higher mean IL-18 level than control group (*P*-value <0.001, Effect size = 0.822) and (*P*-value <0.001, Effect size = 0.756), respectively. There was a statistically significant decrease in mean IL-18 after non-surgical treatment for three months in stage III/IV, molar/incisor pattern periodontitis group (*P*-value = 0.001, Effect size = 0.57), figure (1), table (3).

Correlation between IL-17 and IL-18 levels in group A: There was a statistically significant direct positive correlation between IL-17 and IL-18 levels at baseline as well as after three months ($r = 0.414$, *P*-value = 0.040) and ($r = 0.483$, *P*-value = 0.015), respectively, figure (2), table (4).

TABLE (3) The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between Interleukin 17 and 18 levels in the two groups and the changes within each group

| Interleukins (pg/ml)	Time	StageIII/IV, M/I periodontitis (n = 25)		Control (n = 25)		P-value	Effect size (Partial η^2)
		Mean	SD	Mean	SD		
IL-17	Base line	51.6	10.07	21.52	4.91	<0.001*	0.79
	3 months	26.8	7.85	21.52	4.91	0.006*	0.145
	Change	24.8	9.31	-		-	-
P-value (Effect size)		<0.001* (Partial η^2 = 0.842)					
IL-18	Base line	268	85.37	13.43	4.66	<0.001*	0.822
	3 months	132.16	48.5	13.43	4.66	<0.001*	0.756
	Change	135.84	62.76	-		-	-
P-value (Effect size)		0.001* (Partial η^2 = 0.57)					

*: Significant at P ≤ 0.05
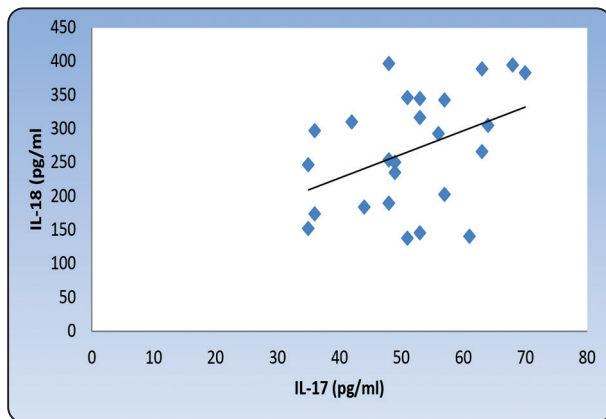
Fig. (1). Scatter diagram representing direct correlation between IL-17 and IL-18 levels at base line
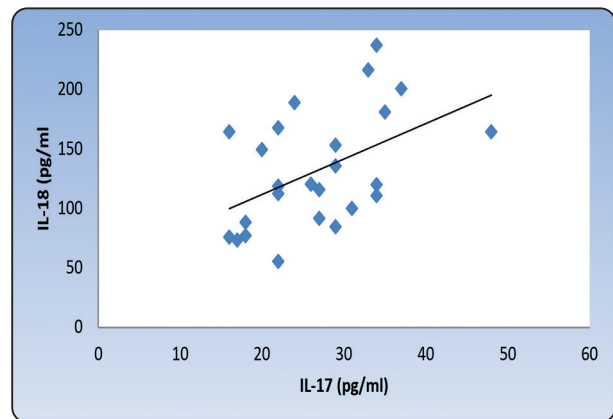

Fig. (2). Scatter diagram representing direct correlation between IL-17 and IL-18 levels after three months

TABLE (4) Results of Pearson's correlation coefficient for the correlation between IL-17 and IL-18 levels

Time	Correlation coefficient (r)	P-value
Base line	0.414	0.040*
3 months	0.483	0.015*

*: Significant at P ≤ 0.05

DISCUSSION

Damage of periodontal tissue can be a result of the interaction between host immune system and periodontal pathogens (*Johnson et al., 2004*). Although infection by pathogens is needed for beginning of periodontal disease, following progression in large part appears to be due to cytokine profile and host immune response. It has been proposed that T-cell have an important role in the individual's vulnerability to advanced and possible aggressive periodontal destruction (*Salvi and Lang 2005; Borch et al., 2009*).

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The current study was performed on stage III/IV molar/incisor pattern periodontitis patients (previously known as Aggressive periodontitis according to AAP *(Armitage 1999)*. Staging in the new periodontal disease classification refers to degree of periodontal disease severity or amount of periodontal tissue destruction. Stage III/IV periodontitis patients suffer from interdental clinical attachment loss ≥ 5mm with teeth loss ranging from 4 teeth (Stage III) to 5 teeth or more (Stage IV). Distribution of affected teeth as molars and incisors has been used instead of the term aggressive *(Armitage 1999; Caton et al., 2018)*.

The current study showed that, IL-17 and IL-18 levels were higher in the stage III/IV molar/incisor pattern periodontitis patients more than their levels in the healthy group. Similar results reported higher levels of IL-17 in the GCF of periodontitis patients *(Vernal et al., 2005; Azman 2014)*. Another study reported more increased amounts of IL-17 in the GCF of generalized aggressive periodontitis patients more than periodontally healthy controls and accredited the variance to the potential part of these proinflammatory cytokines in the periodontal pathogenesis *(Cifcibasi et al., 2015)*. On the other side and regarding IL-17, some studies reported conflicting results to the current study *(Yetkin et al., 2009; Szkaradkiewicz et al., 2011; Ay et al., 2012)*.

The present study also showed higher levels of IL-18 in GCF of periodontitis patients compared to their levels in periodontally healthy subjects and their decrease after treatment and this was consistent with results of *Horwood et al., (2001), De Campos et al., (2012) and Nair et al., (2016)*.

CONCLUSION

Levels of IL-17 and IL-18 in GCF could be suspected to play an important role as inflammatory biomarkers in stage III/IV molar/incisor pattern periodontal disease pathogenesis.

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