ISSN NO: 2770-2936
Date of Publication:04-09-2022

Evaluation the role of Aggregatibacter Actinomycetemcomitans on IL-18 Production in Periodontitis Patients

Mohammed Jasim AL-Obaidi[1], Batool Hassan Al-Ghurabi[1]
[1] Master student, Department of basic science of Oral Microbiology, College of Dentistry, University of Baghdad/ Baghdad, Iraq.
ORCID : 0000-0002-7369-4272
[1] Professor, PhD in Microbiology/Immunology, Department of basic science College of Dentistry, University of Baghdad/ Baghdad,Iraq.
*Correspondence:ALobaidi.Mohammed1984@gmail.com
: batoolamms@codental.uobaghdad.edu.iq

Abstract:
Background: Aggregatibacter actinomycetemcomitans one of the most common periodontal pathogen with direct effect on periodontium, this bacterium possesses many virulence characteristics that allow it to colonize the oral cavity, invade, and evade the host's immune system. periodontitis is chronic multifactorial disease and cytokines can signal and amplify the immune responses during infection IL-18 has a role in in the body's inflammatory and immune responses in the body, it plays an essential role as a chemotactic factor for the attraction of neutrophils and lymphocytes and its expression has been reported to increase in chronically inflamed periodontal tissues.
Aim of study: This study was conducted to investigate the role of Aggregatibacter actinomyctecomtans in pathogeneses of periodontitis and their effect on serum level of IL-18 in periodontitis patient and compare with control group.
Materials and Methods: fifty-five subjects were enrolled in this case control study, their age range (23-55) years. They were 30 periodontitis and 25 healthy controls group. Sub-gingival plaque samples were collected from four deepest periodontal pockets in each subject of patients group and gingival sulcus in control group. Detection of Aggregatibacter actinomyctecomtans performed by means of RT-PCR, whereas ELISA was used to evaluate the level of IL-18.
Results: The study results revealed statically no significant differences in quantity of Aggregatibacter actinomyctecomtans and IL-18 levels between patients and controls. Moreover, there is no correlation between Aggregatibacter actinomyctecomtans and the IL-18
Conclusion: The lack of correlation between Aggregatibacter actinomyctecomtans and the IL-18 indicates that its activation is associated with inflammatory processes that are induced by a number of external factors, other than bacteria.

Key words: Aggregatibacter actinomycetemcomitans, periodontitis, IL-18.

Introduction

Periodontitis (PD) has been defined as a chronic multifactorial inflammatory disease associated with dysbiotic dental biofilms and characterized by progressive destruction of the tooth supporting apparatus. Its primary features include the loss of periodontal tissue support, manifested through clinical attachment loss and radiographically assessed alveolar bone loss, and the presence of periodontal pocketing and gingival bleeding. If untreated, severe periodontitis results in tooth loss, frequently leading to masticatory dysfunction and nutritional compromise, aesthetic impairment, altered speech, low self-esteem, and a poorer overall quality of life. Its terminal sequela is the loss of all teeth [(1)].
Furthermore, periodontitis highly prevalent with varying patterns in different populations, and it is affects about 45%-50% of adults in its mildest forms, rising to over 60% in people aged >65 years. Severe periodontitis is the sixth most common human disease [(2)].
Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) is a Gram-negative oral pathobiont that is associated with severe form of (PD). This bacterium has various virulence factors which enables the

ISSN NO: 2770-2936
Date of Publication:04-09-2022

bacterium to colonize the oral cavity, invade and evade the host defences [3]. The activation of the innate immune system is closely associated with the activation of the adaptive side [4]. Interluekine-18, a member of the IL-1 family, was primarily discovered in the *Propionibacterium acnes*-induced toxic shock model as an interferon (IFN)-γ-promoting factor, which stimulate both Th1 and Th2 cytokines, proinflammatory cytokines, chemokines, and immunoglobulin E and immunoglobulin G1 induction [5, 6, 7, 8].
Interluekine-18 can activate the secretion of other pro-inflammatory cytokines such as TNF-α, IL-1β, IL-8, and granulocyte-macrophage colony-stimulating factor, which promote the expansion, migration, and activation of PMNs during infections [9]. there is no direct evidence pointed whether IL-18 overexpression can trigger periodontitis after bacterial infection *in vivo*. Different inflammatory mediators linked with periodontitis have been determined in body fluids [7, 10]. This study was conducted to investigate the role of *Aggregatibacter actinomyctecomtans* in pathogeneses of periodontitis and their effect on serum level of IL-18 in periodontitis patient and compare with control group.

Material And Method

Subjects

Fifty-five (55) subjects were enrolled in this case control study, (27 males and 28 female) their age range (23-55) years. They were from the attendants to Taji primary health care centers from November 2021 to January 2022. A questionnaire was used to record special notes regarding these subjects.

Including and excluding Criteria: The participants enrolled in this study and considered eligible must have met the following criteria: presence of at least 20 or more natural teeth, and patients with periodontitis had periodontal pockets equal or more than 3 millimeters in at least two non-adjacent teeth, with loss of attachment more than 3 mm. exclusion criteria include any previous extensive periodontal therapy or being currently under active periodontal treatment. Patients were receiving antibiotic treatment and include individuals without systemic conditions as well as pregnant and menopausal women.

Oral Hygiene Index

By analyzing clinical periodontal data, the periodontal health state was determined by using a periodontal probe of William's graduation.

Dental Plaque Sampling: Samples of sub-gingival plaque were taken from the four periodontal pockets that were the deepest in each patient's mouth of periodontitis subjects and gingival sulcus in control subjects. A single vertical stroke was used to collect samples from pocket, and these samples were then transferred to an eppendorf tube containing 0.5 ml of TE buffer. (10 mM Tris- HCl, 1 mM EDTA, pH 7.6)**,** [11] by vigorously agitating the tip. Afterward, the sample was kept at a cold temperature (-40 degrees Celsius) until DNA could be extracted.

Real-Time PCR

According to the Geneaid extraction methodology, genomic DNA was extracted from plaque samples. Lyophilized primers were provided by Macrogen Company. We prepared a stock solution of lyophilized primers, dissolved in nuclease-free water at a concentration of 100pmol/µl. These primers were diluted to a concentration of 10 pmol/µl by mixing 10 l of primer stock solution (stored at -20 C) with 90 µl of nuclease-free water. *A. actinomycetemcomitans*16SrRNAA-F1: 5’-CTTACCTACTCTTGACATCCGAA-3’, *A.actinomycetemcomitans*16SrRNAA-R1: 3’-ATGCAGCACCTGTCTCAAAGC-5’
In the qPCR experiment, the standard curve method makes use of a dilution series of a known template copy number. The standard curve is obtained by doing a linear regression between log concentration (copy µl -1) and CT.
Real time PCR amplifications were carried out using a magnetic induction cycler (Mic RT-qPCR), with 10µl volumes containing 0.5 µl for each primer; 2.5 µl nuclease free water; 1.5 µl template of DNA and finally 5 µl Master Mix.

Collection of Blood Samples: Under sterile conditions, three milliliters of the subject's venous blood will be collected from each individual. After transferring the blood into a sterile plain tube, the serum was separated from the blood by centrifugation at 3000 rpm for 10 minutes. The serum was then divided into small aliquots and stored at -20 degrees Celsius until it was used for analysis.

Measurement of IL-18: Using ELISA to quantified IL-18 concentration.

ISSN NO: 2770-2936
Date of Publication:04-09-2022

Statistical analysis

The data was non-parametric and calculated by Mann-Whitney test, p-value of p>0.05 was considered non-significant**.**

Results

The demographic pictures of patients and controls groups were presented in table (1),

Table 1: Case-control differences in age and sex.

Demographic Characteristics	**Study groups**		***P*-value**
	Periodontitis n=30	**Healthy control n=25**	
Age (years)			
Range	(23-52)	(24-50)	0.210^{NS}
Mean ± SD	38.50±7.50	36.88±6.50	
Sex			
Female	14 (47%)	14 (56%)	0.723^{NS}
Male	16 (53%)	11 (44%)	

Table (2) shows that there are significant differences in plaque index as between PD patients group and control group (P<0.05)

Table 2: intergroup comparisons of mean values of plaque index between study groups.

Grouping	**Mean difference**	**Tukey's HSD (*P*-value)**
Plaque index		
Periodontitis vs. Control	1.58	0.0000^{**}

In addition, significant differences were found between PD patients and the control group (P<0.05) in gingival index, as shown in table (3).

Table 3: intergroup comparisons of mean values of gingival index between study groups.

Grouping	**Mean difference**	**Tukey's HSD (*P*-value)**
Gingival index		
Periodontitis vs. Control	1.76	0.0000^{**}

Intergroup comparisons of mean values of BOP between groups revealed that there were significance differences between PD patients and control group (P<0.05), table (4).

Table 4: intergroup comparisons of mean values of bleeding on probing index between study groups.

Grouping	**Mean difference**	**Tukey's HSD (*P*-value)**
Bleeding on Probing		
Periodontitis vs. Control	53.67	0.0000^{**}

The descriptive statistics of the mean values for both PPD and CAL parameters of both groups are illustrated in table (5). The mean of the PPD was (3.45±0.56) and the mean of CAL was (3.01±0.79), whereas the control group was free from PPD and CAL

ISSN NO: 2770-2936
Date of Publication:04-09-2022

Table 5: intergroup comparisons of mean values of pocket depth and clinical attachment lose index between study groups.

Periodontal Parameters	**Study groups**	
	Periodontitis (30)	**Control (25)**
Pocket Depth		
Mean± SD	3.45±0.56	000
CAL		
Mean± SD	3.01±0.79	000

This finding showed that there was no statistically significant difference between both study groups (PD and control) when comparing the mean rank values of *A. actinomycetemcomitans*, table (6).

Table 6: Inter groups comparisons of the mean rank values of A.A bacterial count between study groups.

Grouping	Mean Rank difference	Mann Whitney Test (*P*-value)
A.A bacterial load		
Periodontitis vs. Control	4.13	0.514^{NS}

In regard the PD group, the findings of the present study revealed that there is no significant correlation between bacterial count and each PI, GI and PPD (r=0.111; p=0.056; r=0.0.57; p=0.761; r=0.094; p=0.619, respectively). However, there was positive correlation with CAL and BOP (r=0.466; p=0.0006; r=0.547; p=0.001).as shown in table (7).

Table 7: Correlation between A.A bacterial load and clinical periodontal parameters in periodontitis patients

A.A bacterial load	R-value	*P*-value
PI	0.111	0.556^{NS}
GI	0.057	0.761^{NS}
PPD	0.094	0.619^{NS}
CAL	0.466	0.006^{**}
BOP	0.547	0.001^{**}

Figure (1) displays the mean rank values of IL-18 for the patient and control groups. There were significant differences (P<0.05) between patient and control group. Meanwhile, PD patients group with a mean value of (43.8 pg/ml), and control group with a mean value of (23.16 pg/ml).
According to table (8), intergroup comparisons of IL-18 levels across all pairs of groups, there was a significant difference (P<0.05) between PD group and control group.

ISSN NO: 2770-2936
Date of Publication:04-09-2022

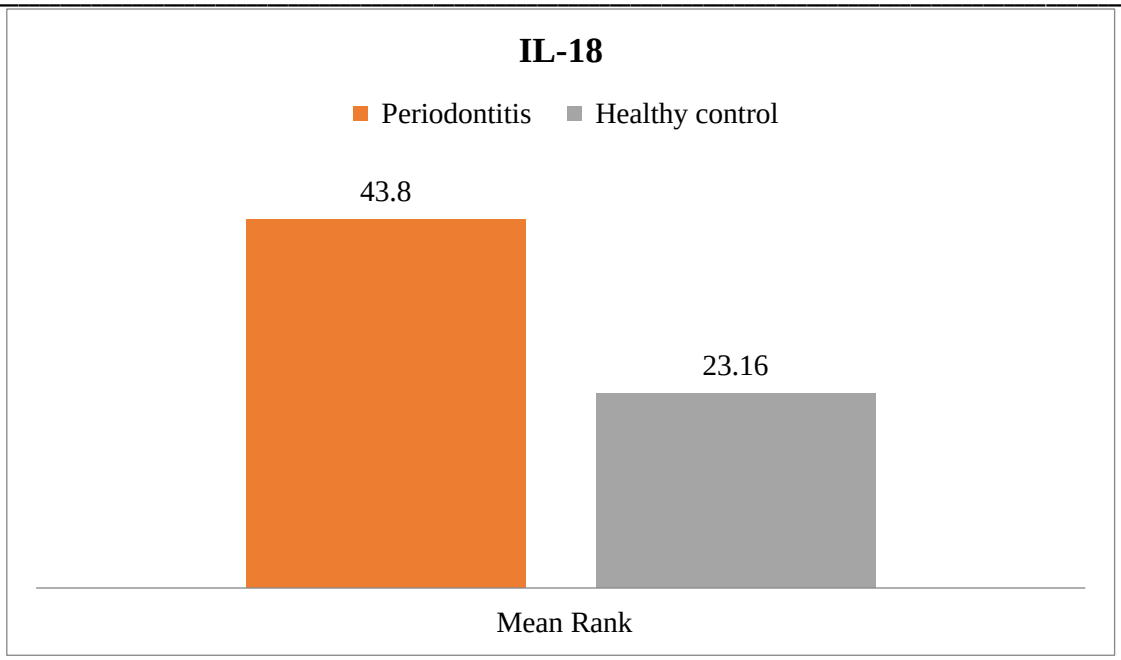

Figure- 1: The difference in mean rank values of serum IL-18 among study groups

Table 8: Inter groups comparisons of the mean rank values of IL-18 between study groups.

IL-18	Mean Rank difference	Mann Whitney Test (*P*-value)
Periodontitis vs. Control	20.64	0.002**

Table (9) show that there is no correlation between IL-18 and each of PI, GI, CAL and BOP, while there is significant correlation with PPD (r= 0.478; p = 0.007) among PD patients. Furthermore, the present results revealed no significant correlation between bacterial loads and IL-18 in periodontitis patients.

Table 9: Correlation between IL-18 level and clinical periodontal parameters in Periodontitis patients

IL-18	**R-value**	***P*-value**
PI	0.161	0.393^{NS}
GI	0.019	0.919^{NS}
PPD	0.478	0.007**
CAL	0.014	0.937^{NS}
BOP	0.235	0.209^{NS}

Discussion

In this study, RT-PCR method was successfully used to detect and count *A. actinomycetemcomitans* in subgingival microbial samples. The result revealed that *A. actinomycetemcomitans* was detected in both periodontal healthy subjects and. These results are in agreement with other results reported by Socransky et al., who found that microbial complexes are repeatedly found together in subgingival biofilm with and without periodontal disease [(12)]. Another important finding in this study was the significant correlation between the bacterial count and clinical attachment loss (CAL) in patients group. These results coincide with Sanchez and colleague who found that a higher CAL value was associated with *A. actinomycetamcomitans* (Sanchez et al., 2015). While disagree with Tomita et al. (2013)[(14)] who prove that *A. actinomycetamcomitans* counts showed no significant correlation with PPD or CAL. Generally, the interaction between different virulence factors of *A. actinomycetemcomitans* and host immune system's response can progress the resorption of bone in periodontal disease [(15)].

The present study revealed a significant increase in the serum levels of IL-18 in patient group compared to the control group. IL-18 is considered to be a pro-inflammatory cytokine which plays a role in the regulation

ISSN NO: 2770-2936
Date of Publication:04-09-2022

of inflammation and metabolism [16, 17]. This may explain the increase levels of IL-18 in the serum of PD patients as observed in present study. This result was in agreement with other studies [18, 19].

Similarly, Banu and colleagues found that IL-18 levels were 46% higher in patients with periodontitis as compared to control [20]. On the other hand, Cheng et al in (2014) [21] reported that the IL-18 has been proposed to be biomarkers for periodontitis. Contrary to this study, Chitrapriya et al in (2015) [22] and Abbas in (2019)[23] found elevated serum levels of IL-18 in healthy subjects compared with periodontitis samples.

Another study done by Correa *et al*. they reported no significant difference of GCF IL-18 levels between pre- and post—periodontal treatment in healthy group with periodontitis. These contradictory results among these studies may be due to subject selection and also different methods used for the detection of IL-18 in samples.

Considering the correlation of serum IL-18 with clinical periodontal parameters, there was significant correlation with PPD in chronic periodontitis group and this was in agreement with Johnson and Serio, 2005 and Nair et al., 2016 [19, 24] who observed concomitant rise in the level of IL-18 as the PPD increased.

On other hand the present study showed non-significant correlations with other clinical periodontal parameters and this was in agreement with Johnson and Serio, 2005 and Panezai et al.,2017 [24, 25].

Other Iraqi study found significant correlation with PPD in chronic periodontitis group and indicated that IL-18 expression is closely associated with periodontal destruction [23].

The current study found a non-significant correlation between bacterial loads and (IL-18), These results could be attributed to that the periodontitis is not an infectious condition, but rather a "dysbiotic disease", that is associated with an alteration in the abundance or influence of individual species within the polymicrobial community, relative to their abundance or influence in health [26, 27].

Conflict of interest: None.

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of periodontology. 2018 Jun;89:S173-82.
2. Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H, Ellwood R, Giacaman RA, Herrera D, Herzberg MC, Könönen E. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. Journal of clinical periodontology. 2017 Mar;44:S5-11.
3. Ashwath B, Anitha V, Shanmugam M. Aggregatibacter actinomycetemcomitans-A periodontopathogen. IP International Journal of Periodontology and Implantology. 2021 Jul 15;6(2):61-7.
4. Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: Toward a better understanding of complex mechanisms. Cell discovery. 2020 Jun 9;6(1):1-22.
5. Hoshino T, Wiltrout RH, Young HA. IL-18 is a potent coinducer of IL-13 in NK and T cells: a new potential role for IL-18 in modulating the immune response. The Journal of Immunology. 1999 May 1;162(9):5070-7.
6. Hoshino T, Yagita H, Ortaldo JR, Wiltrout RH, Young HA. In vivo administration of IL-18 can induce IgE production through Th2 cytokine induction and up-regulation of CD40 ligand (CD154) expression on CD4+ T cells. European journal of immunology. 2000 Jul;30(7):1998-2006.
7. Yoshinaka K, Shoji N, Nishioka T, Sugawara Y, Hoshino T, Sugawara S, Sasano T. Increased interleukin-18 in the gingival tissues evokes chronic periodontitis after bacterial infection. The Tohoku journal of experimental medicine. 2014;232(3):215-22.
8. Lv K, Wang G, Shen C, Zhang X, Yao H. Role and mechanism of the nod-like receptor family pyrin domain-containing 3 inflammasome in oral disease. Archives of Oral Biology. 2019 Jan 1;97:1-1.
9. Sahoo M, Ceballos-Olvera I, del Barrio L, Re F. Role of the Inflammasome, IL-1 β, and IL-18 in Bacterial Infections. TheScientificWorldJournal. 2011 Oct 1;11:2037-50.

ISSN NO: 2770-2936
Date of Publication:04-09-2022

10. Al-Ghurabei BH, Shaker ZF, Fadhel R, Al-Khayli NG, Mustafa LK. Serum levels of interlukine-1beta and interlukine-2 in chronic periodontitis. Al-Mustansiriya J. Sci. 2012;23(3):55-62..
11. Borges MA, Figueiredo LC, Brito Jr RB, Faveri M, Feres M. Microbiological composition associated with vitamin D receptor gene polymorphism in chronic periodontitis. Brazilian Oral Research. 2009;23:203-8.
12. Socransky SS, Haffajee AD, Cugini MA, Smith CK, Kent Jr RL. Microbial complexes in subgingival plaque. Journal of clinical periodontology. 1998 Feb;25(2):134-44.
13. Sánchez GA, Acquier AB, De Couto A, Busch L, Mendez CF. Association between Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis in subgingival plaque and clinical parameters, in Argentine patients with aggressive periodontitis. Microbial pathogenesis. 2015 May 1;82:31-6.
14. Tomita S, Komiya-Ito A, Imamura K, Kita D, Ota K, Takayama S, Makino-Oi A, Kinumatsu T, Ota M, Saito A. Prevalence of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia in Japanese patients with generalized chronic and aggressive periodontitis. Microbial pathogenesis. 2013 Aug 1;61:11-5.
15. Gholizadeh P, Pormohammad A, Eslami H, Shokouhi B, Fakhrzadeh V, Kafil HS. Oral pathogenesis of Aggregatibacter actinomycetemcomitans. Microbial pathogenesis. 2017 Dec 1;113:303-11.
16. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature reviews immunology. 2011 Feb;11(2):85-97.
17. Stensvold D, Slørdahl SA, Wisløff U. Effect of exercise training on inflammation status among people with metabolic syndrome. Metabolic syndrome and related disorders. 2012 Aug 1;10(4):267-72.
18. Banu S, Jabir NR, Mohan R, Manjunath NC, Kamal MA, Kumar KR, Zaidi SK, Khan MS, Tabrez S. Correlation of Toll-like receptor 4, interleukin-18, transaminases, and uric acid in patients with chronic periodontitis and healthy adults. J Periodontol. 2015 Mar;86(3):431-9. doi: 10.1902/jop.2014.140414. Epub 2014 Oct 27. PMID: 25345339.
19. Nair V, Bandyopadhyay P, Kundu D, Das S. Estimation of interleukin-18 in the gingival crevicular fluid and serum of Bengali population with periodontal health and disease. Journal of Indian Society of Periodontology. 2016 May;20(3):260.
20. Banu S, Jabir NR, Mohan R, Manjunath NC, Kamal MA, Vinod Kumar KR, Zaidi SK, Khan MS, Tabrez S. Correlation of Toll-like receptor 4, interleukin-18, transaminases, and uric acid in patients with chronic periodontitis and healthy adults. Journal of Periodontology. 2015 Mar;86(3):431-9.
21. Cheng WC, Hughes FJ, Taams LS. The presence, function and regulation of IL-17 and Th17 cells in periodontitis. Journal of clinical periodontology. 2014 Jun;41(6):541-9.
22. Chitrapriya MN, Rao SR, Lavu V. Interleukin-17 and interleukin-18 levels in different stages of inflammatory periodontal disease. Journal of Indian society of periodontology. 2015 Jan;19(1):14.
23. Abbas R. F. Analysis of Nuclear Factor Kappa and Toll-Like Receptor-4 Genes Polymorphisms and there Associations with Selected Immunological Biomarkers in Iraqi Population with severe Chronic Periodontitis 2019 (RAGHAD)
24. Johnson RB, Serio FG. Interleukin-18 concentrations and the pathogenesis of periodontal disease. Journal of periodontology. 2005 May;76(5):785-90.
25. Panezai J, Ghaffar A, Altamash M, Sundqvist KG, Engström PE, Larsson A. Correlation of serum cytokines, chemokines, growth factors and enzymes with periodontal disease parameters. PloS one. 2017 Nov 30;12(11):e0188945.
26. Lamont RJ, Hajishengallis G. Polymicrobial synergy and dysbiosis in inflammatory disease. Trends in molecular medicine. 2015 Mar 1;21(3):172-83.
27. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nature reviews microbiology. 2018 Dec;16(12):745-59.