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Original Article

Salivary interleukin-17A and interleukin-18 as potential biomarkers of immunopathogenesis and oral health status in rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation and is often associated with poor oral health. Cytokines play a central role in RA immunopathogenesis. This case-control study investigated the involvement of salivary interleukin-17A (IL-17A) and interleukin-18 (IL-18) in RA patients in relation to oral health status. Unstimulated whole saliva samples were collected from 20 RA patients and 20 age- and sex-matched healthy controls. Oral health was assessed using plaque and gingival indices. Salivary IL-17A and IL-18 concentrations were measured by ELISA. RA patients exhibited significantly higher salivary levels of IL-17A and IL-18 compared to controls ($p < 0.05$). Both cytokines showed positive correlations with gingival index, and IL-17A was also correlated with disease activity (DAS28). Receiver operating characteristic (ROC) analysis demonstrated that both interleukins effectively discriminated RA patients from healthy controls (AUC = 0.927 for IL-17A, AUC = 0.925 for IL-18). These findings suggest that elevated salivary IL-17A and IL-18 are associated with increased oral inflammation and may serve as non-invasive biomarkers for RA immunopathogenesis and oral health deterioration.

Keywords: Rheumatoid arthritis, Oral health, Cytokines, IL-17A, IL-18.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints and various extra-articular tissues. Its development is influenced by a combination of genetic predisposition and environmental factors [1]. The initial stages of RA are marked by fatigue, signs resembling the flu, inflamed and sore joints, and stiffness in the morning, as well as raised levels of C-reactive protein and erythrocyte sedimentation rate [2]. Patients with arthritis often show oral signs, including a strong association with Sjögren's syndrome, which causes signs such as xerostomia. Other oral concerns include temporomandibular joint difficulties, methotrexate-induced ulcers, and an increased focus on gum disease [3]. There is presently a wealth of information describing and documenting the connection between dental health and overall health [4, 5, 6, 7]. Bad oral hygiene and/or low oral and periodontal health were prevalent in RA patients, and metrics like bleeds, gum inflammation, and the depth of pockets in the gums were frequently significant when compared to normal subjects. Improvements in oral hygiene and early nonsurgical periodontal therapy can also lessen the severity of systemic diseases, according to clinical trials [8, 9]. Immune and non-immune cells communicated with one another through cytokines. Therefore, the pathophysiology of a number of diseases, including RA, depends on these cytokines [10, 11].

In 2003, the Th17 subgroup of CD4 T cells was shown to produce IL-17A. It has been linked to the etiology of a number of inflammatory and autoimmune conditions, including psoriasis, gum disease, RA, and inflammatory bowel disorders [12]. One pleiotropic cytokine that is crucial to the onset and maintenance of the inflammatory response in RA is IL-18. In order to mediate bone degradation, IL-18 activates T cells in the synovium to generate inflammatory cytokines, such as RANKL [13]. Salivary diagnostics have shown enormous promise in clinical applications, and saliva has been shown to be a promising body fluid for early illness detection [14]. Thanks to combinations of biomolecules with clinical relevance and im-

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provements in detection technology, saliva may soon be the preferred first-line diagnostic sample [15]. This study was conducted to ascertain the role of salivary cytokines (IL-17A and IL-18) in the immunopathogenesis of RA with regard to oral health status because it has recently been proposed that these levels play a significant role in the pathogenesis of both systemic and oral diseases.

2. Materials and Methods

2.1. Study design

This study was designed as a case-control investigation.

2.2. Study population

A total of 40 participants were enrolled, including 20 patients diagnosed with rheumatoid arthritis (RA) and 20 age- and sex-matched healthy controls. RA diagnosis and assessment of disease activity (DAS28) were performed by a specialist rheumatologist according to the 2010 ACR/EULAR criteria. Exclusion criteria included the presence of other autoimmune or systemic inflammatory diseases, smoking, alcohol consumption, pregnancy, use of medications affecting immune function, and lack of informed consent. Ethical approval was obtained from the Scientific Committee of the College of Dentistry, University of Baghdad.

2.3. Oral examination

Clinical oral assessments were conducted, including evaluation of the plaque index (PLI) and gingival index (GI), following established protocols [16].

2.4. Saliva collection

Unstimulated whole saliva was collected from each participant by passive drooling into a sterile disposable container, avoiding stimulation or spitting. Approximately 3 mL of saliva was obtained per subject. Samples were transferred to sterile tubes, centrifuged at 2,000 × g for 3 minutes at 4°C, and the clear supernatant was aliquoted into Eppendorf tubes and stored at –20°C until analysis [17].

2.5. Measurement of interleukin levels

Salivary concentrations of IL-17A and IL-18 were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Shanghai, China). Briefly, capture antibodies specific for each biomarker were immobilized on the wells of a strip plate. Samples and standards were added and incubated, followed by the addition of biotinylated secondary antibodies. After washing, HRP-conjugate was added, and color development was achieved using a chromogenic substrate. The reaction was stopped with sulfuric acid, and absorbance was measured at 450 nm using an ELISA plate reader. Cytokine concentrations were calculated based on standard curves.

2.6. Sample size calculation

Sample size was determined using G*Power 3.1.9.7 (Franz Faul, Universität Kiel, Germany) with a power of 95% and a two-sided alpha error of 0.05.

2.7. Statistical analysis

Statistical analyses were performed using SPSS version 25. Descriptive statistics were expressed as mean ± standard deviation. The Shapiro-Wilk test was used to assess the normality of data distribution. The Chi-square test was applied for categorical variables. Comparisons between two groups were performed using the independent t-test or Mann-Whitney U test, as appropriate. Pearson and Spearman correlation coefficients were used to assess associations between clinical and cytokine parameters. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of salivary cytokines. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics of study participants

Table 1 summarizes the demographic and clinical characteristics of the 40 participants in this study. The mean age of RA patients was 47.4 ± 7.63 years, compared to 46.05 ± 6.31 years in the control group. The female-to-male ratio was 13:7 in the RA group and 12:8 in the control group. There were no significant differences between the groups in terms of age or sex distribution ($P > 0.05$). The mean DAS28 score in the RA group was 4.1 ± 0.84, indicating moderate disease activity. Additionally, both the plaque index (PLI) and the gingival index (GI) were signi-

Table 1. Demographic features and oral variables in two groups.

Demographic features	Study group	healthy group	P-value
Age			
Range	29-59	32-59	$0.272^{NS\#}$
Mean± SD	47.4±7.63	46.05±6.31	
Sex			
Female	13 (65%)	12 (60%)	$0.743^{NS\#\#}$
Male	7 (35%)	8 (40%)	
Oral health status			
PLI Mean± SD	1.61±0.49	0.43±0.05	$<0.000^{*\#}$
GI Mean± SD	1.70±0.65	0.95±0.22	$<0.000^{*\#}$
DAS28 Mean± SD	4.1±0.84	-	-

^{NS}: non-significant; *: significant; SD: standard deviation; #: t-test; ##: chi-square.

ficantly higher in RA patients than in controls ($P < 0.05$), reflecting poorer oral health status in the patient group.

3.2. Salivary cytokine levels and their correlation with clinical parameters

Regarding salivary interleukin levels, our findings demonstrated that both the mean IL-17A and the median IL-18 concentrations were significantly higher in the RA group compared to the control group ($P < 0.05$; Table 2). Furthermore, in RA patients, the mean IL-17A level showed significant positive correlations with both the gingival index (GI) and disease activity score (DAS28). Similarly, the median IL-18 level was significantly correlated with GI and with IL-17A levels (Table 3, Figure 1).

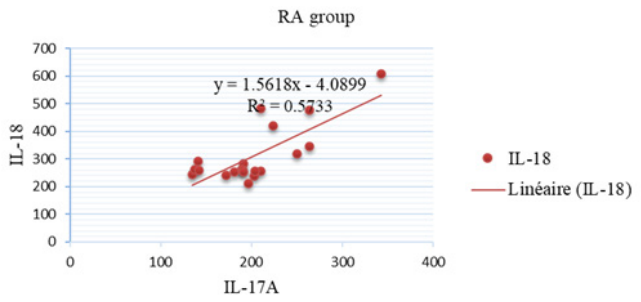

Fig. 1. Scatter plot depicting the correlation between the cytokine levels among the patient group. Each red dot represents a data point for an individual subject in this group. The line represents the linear regression line fitted to the data. The coefficient of determination (R^2), indicating the proportion of the variance in IL-18 that is predictable from IL-17A, is 0.573 for this linear model.

3.3. Diagnostic performance of salivary cytokines

To evaluate the sensitivity and specificity of salivary interleukins, a receiver operating characteristic (ROC) analysis was performed to distinguish RA patients from healthy controls. Both IL-17A and IL-18 demonstrated excellent discriminatory power, as shown in Figure 2 and Table 4. The area under the curve (AUC) values indicated

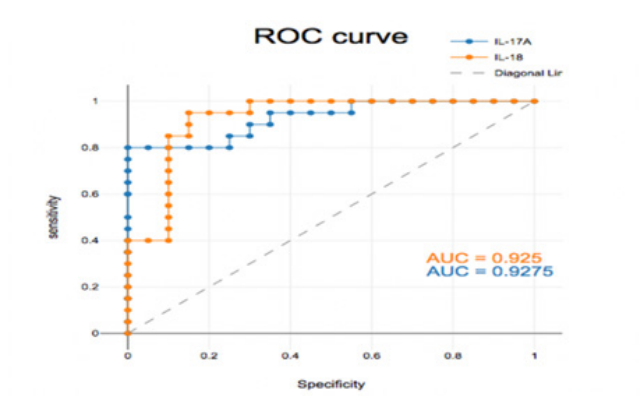

Fig. 2. Receiver operating curves (ROC) for salivary cytokines in patients vs. controls.

Table 2. Salivary concentrations of cytokines in the two groups.

Salivary cytokines	study group	healthy group	P-value
IL-17			
Min-Max	135-343	85.17-149.84	
Mean± SD	201.95±50.42	129±19.06	0.000*#
Median	194	135	
IL-18			
Min-Max	212.84-607.99	143.50-282.77	
Mean± SD	311.31±104.01	199.41±37.54	
Median	261.66	201.06	0.000*+

*: significant; SD: standard deviation; #: t-test; +: Mann-Whitney Test.

Table 3. Correlation between cytokines and clinical parameters in the patient group.

Pearson's correlation	RA group			
	IL-17A	PLI	GI	DAS28
IL-17A		r=0.228 p=0.332	r=0.485 p=0. 030*	r=0.458 p=0.041*
PLI	r=0.228 p=0.332		r=0.283 p=0.225	r=0.088 p=0.711
GI	r=0.485 p=0. 030*	r=0.283 p=0.225		r=0.227 p=0.335
DAS28	r=0.458 p=0.041*	r=0.088 p=0.711	r=0.227 p=0.335	
Spemann's correlation	RA group			
	PLI	GI	DAS28	IL-17A
IL-18	r=0.332 p=0.151	r=0.790 p=0. 000*	r=0.368 p=0.110	r=0.547 p=0. 012*

Table 4. Comparison of the diagnostic properties of interleukins between the two groups.

Comparison	Test result variables (s)	AUC	P-value	Optimal cut-off point	sensitivity	specificity
RA Vs. Controls	IL-17A	0.927	0.000*	238	100%	%95
	IL-18	0.925	0.000*	172	100%	%95

that both interleukins are highly effective in differentiating between the two groups.

4. Discussion

The mean PLI and GI values in RA patients were increased as compared to healthy controls, according to this study. This indicates that the gingival tissue among patients was more inflamed, and it may be connected to the rise in plaque, which is the cause of gingival inflammation [18], which concurs with this outcome. GI and PLI are likely caused by increased likelihood of temporomandibular joint involvement in RA patients, significant arthritis-related hand malfunction that restricts the patient's ability to move and, concurrently, decreased saliva from secondary Sjogren's syndrome, both of which lead to plaque buildup. As well as RA patients' emotional depression about their condition, which deteriorates their attention to personal hygiene [18,19]. Saliva is now a widely accessible, non-invasive biofluid for identifying biomarkers in a number of conditions, including RA. A few advantages of using saliva for diagnostic purposes include ease of collection, reduced patient discomfort, and frequent monitoring. In the context of RA, salivary cytokines may provide valuable insights into the progression of illness and the efficacy of treatment [20].

The current results were consistent with a prior study [21, 22], which revealed that the RA patients had higher mean levels of IL-17A than the control group, and also observed that cytokine expression levels were out of balance, with RA patients having higher levels of IL-17 than controls. In addition, Atwa et al. [23] reported that when RA patients were compared to healthy controls, their levels of IL-17 were noticeably higher in RA cases. Moreover, Moran et al. [24] found that RA disease activity is driven by high production of IL-17A level in the inflamed joint. This supports our finding regarding the positive correlation between IL-17A and DAS28. Thus, evidence points to the significance of IL-17A during RA, emphasizing its potential for prognosis and disease surveillance. It is worth mentioning that studies conducted by Rosu et al. [25] and Jain and associates [26] showed that IL-17A is essential to the pathophysiology of RA. This could be the result of the inflammatory response. The immune system is overactive in those patients, which results in higher levels of IL-17A as part of the body's reaction to inflammation. Its elevation suggests the presence of an ongoing inflammatory process, which is particularly prominent in patients whose disease activity is high.

Similar to the results from numerous studies, the present results found that RA patients' group had statistically significant increase in median IL-18 level when compared to control group [27, 28], revealing that RA patients' IL-18 levels were higher than controls. Moreover, Shao et al. [29] showed that IL-18 biological activity in their blood, synovial fluid, and tissue than control groups. Broz and Dixit [30] reported that the activation of inflammasomes may be the cause of elevated salivary IL-18. To present various data, recent findings discovered that the median salivary IL-18 level showed no discernible difference between RA patients and controls [31]. This disparity could result from the distinct population under investigation or the various sample kinds.

The positive correlation between IL-17A and IL-18 in this study may indicate the involvement of these interleukins in the inflammatory process in the periodontal tissues in RA patients. On the other hand, the positive correlation between interleukins (IL-17A and IL-18) and GI in RA patients, this could be due to the fact that increased production of inflammatory interleukins involved in the inflammation periodontal tissues in oral cavity and indicates that a large number of RA patients struggle with dental self-care and dental hygiene. Additionally, Salivary IL-17A and IL-18 demonstrated excellent clinical accuracy in discriminating RA patients from healthy individuals.

This study revealed significantly higher levels of salivary IL-17A and IL-18 in rheumatoid arthritis (RA) patients compared to healthy controls, with these elevations correlating positively with increased Plaque Index (PLI) and Gingival Index (GI) scores. Such associations underscore the interplay between systemic inflammation in RA and oral health status. These findings reinforce the established link between periodontal disease and RA, suggesting that poor oral health may exacerbate RA progression. Consequently, incorporating diligent oral hygiene practices and periodontal treatments into the standard care for RA patients is imperative to potentially mitigate both oral and systemic inflammatory burdens.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Authors' contributions

BHAG and AAA were involved in the conception and design of the study, in the literature search, clinical analysis, data analysis, statistical analysis, and in manuscript preparation and manuscript reviewing. All authors were involved in the conception and design of the study, in data analysis, and in manuscript preparation and manuscript reviewing. All authors have read and approved the final manuscript.

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