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Frequency of fimA Genotypes of *P. gingivalis* in Rheumatoid Arthritis Patients and Control group

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ABSTRACT

Background: Rheumatoid arthritis (RA): Chronic inflammatory polyarthritis, an autoimmune response targeting citrullinated antigens, due to destructive synovial joint inflammation. Porphyromonas gingivalis, a gram-negative bacterium residing in the subgingival tissues of the oral cavity, has been implicated in the association with RA, mainly due to the identification of a bacterial enzyme known as peptidyl arginine deaminase (PAD). The presence of this enzyme in P. gingivalis suggests its potential role in RA development by generating citrullinated antigens. **Aims**: The association between P. gingivalis and the development of anticitrullinated peptide antibody (ACPA) and if its role as an environmental risk factor of RA. **Methods:** This case-control study involved a total 140 participants, collection of blood and gingival crevicular fluid (GCF) samples from the participants, various tests were conducted, Specific PCR primers targeting fimA genotypes of P. gingivalis were utilized to amplify the relevant genetic regions,statistical analysis used: Statistical Package for the Social Sciences (SPSS) version 26, a T-test and Chi-square (χ^2) test were utilized. **Results:** The p-values for ESR, ACCP, and IL-17 were 0.0001, 0.02, and 0.0001, respectively, indicating strong statistical significance for RA patients. Among the RA patients with P. gingivalis infection and the RA patients without P. gingivalis infection the analysis did not reveal a statistically significant difference in mean of ACCP and all biomarkers between the two groups (p-value=1.000). **Conclusions**: No association between P. gingivalis and the development of ACPA, the rise in ESR levels as disease severity escalates underscores the potential practicality of using ESR as an indicator to evaluate disease activity in RA, a trend towards a higher occurrence of gum problems among RA patients especially in category of patients developed gum problems before the onset of RA**.**

Keywords: Anti-cyclic Citrullinated Peptide, Disease Activity Score-28 (DAS28), Interleukin-17, Porphyromonas gingivalis, Rheumatoid arthritis.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that affects between 0.5_1% of people worldwide, RA characterized by persistent inflammation of the synovial membrane which results in the destruction of cartilage and bone [1,2]. *Porphyromonas gingivalis (P. gingivalis*) is a Gram-negative anaerobic bacterium that predominantly inhabits the subgingival tissues in the oral cavity [3].

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The association between *P. gingivalis* and RA:

stems from the discovery of a bacterial enzyme called peptidyl arginine deaminase (PAD). This enzyme suggests that *P. gingivalis* may contribute to the development of RA by producing citrullinated antigens. Epidemiological links between periodontitis and RA have also highlighted the significance of this bacterium. In RA, an autoimmune response is triggered by citrullinated peptides that are recognized by ACPA are particularly specific (98%) for diagnosing RA [4]. While human PADs are responsible for citrullination in normal physiological processes and inflammation, *P. gingivalis* is the only known prokaryote with PAD. Therefore, it plays a crucial role as a pathogen in periodontitis [5]. In susceptible individuals, anti-citrullinated peptide antibodies may be produced in response to *P. gingivalis* infection, and human PADs in the joints may continue to citrullinate host proteins, sustaining antibody production and contributing to the onset of chronic arthritis. Blocking bacterial and human PADs has the potential to be an effective treatment for RA by preventing the production of antigens that initiate and perpetuate autoimmunity [6].

The objectives of this study: (1)To determine the frequency of *fimA* genotypes of *P. gingivalis* in RA patients and health control groups by using the PCR method was the primary goal. (2) Evaluate the ESR, RF, and ACCP,IL-17 levels in the serum of RA patients and control groups.

METHODS

This study was a case-control investigation conducted on Iraqi participants with rheumatoid arthritis (RA) who visited the rheumatology division of Al-Sadr Medical City in Najaf. Additionally, Najaf residents in good health were also included in the study. A total of 140 participants were involved, with 70 of them diagnosed with RA, confirmed by rheumatologists using the ACR/EULAR 2010 Criteria and serological testing. The remaining 70 participants served as healthy controls. Data was gathered between October and December 2022, involving 70 rheumatoid arthritis (RA) patients - 64 female and 6 male, aged 20 to 70. A corresponding healthy control group of 70 individuals was included, with 6 men and 64 women, aged 20 to 70. Information was collected through a comprehensive questionnaire.

Inclusion criteria (patients and healthy group) :

Patients group: Patients who will be enrolled in the research are thoroughly examined to ensure that they satisfy the precise diagnostic standards necessary for RA all patients between the ages of 20 and 70 who have a rheumatologist-diagnosed inflammatory arthritic condition and get a score of >or =6 on the 2010 ACR/EULAR Criteria and without Alzheimer`s disease or any other auto immmune disease.

Controls group: The number-age-gender was matched with patients apparently healthy individuals without joint discomfort or issues or Alzheimer`s disease or any autoimmune disease and those without a family history of RA.

Exclusion criteria (patients and healthy group)

- Patients with other autoimmune diseases (systemic lupus erythematosus, Bachet disease, ankylosing spondylitis and multiple sclerosis)
- Exclusion of Alzheimer's disease.
- patients over the age of 70 and those under the age of 20.
- Patients who taking an antibiotic (because the bacteria may be die).

Samples Collection

-Blood samples

In this study venipuncture was used to obtain (5 ml) of blood from each patient and healthy control, (3 ml) of this blood were then separated by centrifugation and a serum sample was divided into two aliquots in an Eppendorf tube after that isolated and stored at (-20 °C) until it was needed by ELISA, while1.6 ml of blood was added to 0.4 sodium citrate to measure the ESR.

- Gingival cervicular fluid samples

By inserting sterile paper points (F1 and F2) into each site for 20 seconds a sample was taken from the supragingival region each sample was put into a microtube with (1 mL) of Ringer's solution (8.6 g NaCl, 0.3 g KCl, and 0.33 g CaCl2HO in 1000 mL HO) and kept at -80°C until it was needed DNA extraction was done according to the protocol kit of Wizard® Genomic DNA Purification Kit from promega. The method used for detecting fimA of *P. gingivalis* was PCR, as described by Alnasrawy, [7] the primers set was utilized for the detection of *P. gingivalis* in Table No1.

Ethical approval

The College of Medicine's ethics committee gave its approval to this work. Additionally, approval from the Rheumatology Unit of Al-Sadr Medical City was received prior to the research project's start, as well as informed consent from each participant. All patients were informed about the study and gave their consent for researchers to collect a blood sample ,Gingival crevicular fluid sample and complete a questionnaire for them.

Statistical analysis

Data was processed using Statistical Package for the Social Sciences (SPSS) version 26 for Windows (GraphPad Software, San Diego, California, USA) for statistical analysis. Used to compare the means between two groups, a T-test , while ANOVA was used for comparisons among multiple means. Statistical significance was defined as a p-value of <0.05, and high significance was considered if the p-value was <0.001. Chi-square (χ^2) test was utilized to compare two categorical variables.

Table No.1:The primers set was utilized for the detection of *P. gingivalis.*

Gene name	Primer name	Sequences (5 to 3)	Product size(bp)	Referenes
Type Ib fimA	Type Ib fimA –F	CAG CAG AGC CAA AAA CAA TCG	271	*Alnasrawy et al.* 2014 [7]
	Type Ib fimA-R	TGT CAG ATA ATT AGC GTC TGC		
Type II fimA	Type II fimA –F	ACA ACT ATA CTT ATG ACA ATG G	257	*Alnasrawy et al.* 2014 [7]
	Type II fimA –R	AAC CCC GCT CCC TGT ATT CCG A		

RESULTS

The distribution of highly virulent genes, namely II fimA and Ib fimA, in both patients and control groups. Table No.2 presents the findings, indicating that 2.9% of RA patients had Ib fimA genes and 1.4% had II fimA genes. Interestingly none of these genes were detected in the control group and as a result no significant differences between the two groups.

The study included a total of 43 RA patients with gum problems and 27 RA patients

without gum problems. Among the RA patients with gum problems, before the onset of RA and patients developed gum problems after the onset of RA. The statistical analysis was p-value of 0.09, indicating a trend towards a higher occurrence of gum problems among RA patients. However, the difference between RA patients with and without gum problems did not reach statistical significance.

Furthermore, we compared RA patients with *P. gingivalis* infection to RA patients without *P. gingivalis*, focusing on various parameters such as DAS 28, RF, ACCP, and IL-17 levels. Out of the total sample, three patients (4.3%) had *P. gingivalis* infection, while 67 patients (95.7%) were without *P. gingivalis*. The analysis did not reveal any statistically significant differences in the mean values of all biomarkers between the two groups (p-value = 1.000). Additionally, the statistical analysis showed no significant association between the presence of *P. gingivalis* gene types (Ib fimA and II fimA) and disease activity in RA patients (p-value = 1.000). Among the RA patients with *P. gingivalis* infection and the RA patients without *P. gingivalis* infection the analysis did not reveal a statistically significant difference in mean of all biomarkers between the two groups (p-value = 1.000).

Table No.2: The distribution of *P. gingival* genes Ib fimA, II fimA in patients and controls.

P.gingivalis	**Category**	**Patients**		**Controls**		**p. value**
		No.	**Percent %**	**No.**	**Percent %**	
Gene type I b fimA	**Positive**	**2**	**2.9**	**0**	**0.0**	**1.00**
Gene type II fimA	**Positive**	**1**	**1.4**	**0**	**0.0**	**1.00**

Table No.3: Distribution of *Porphyromonas gingivalis* genes in RA patients according to DAS28-ESR.

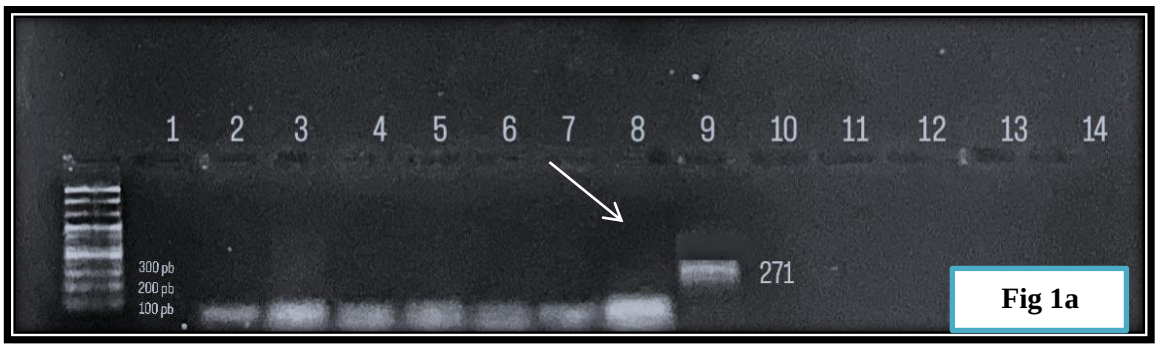
p.gingivalis			**DAS-ESR**				**Total**	**P.value**
			Remission N=25	**Mild N=20**	**Moderate N=20**	**Severe N=5**		
Gene Type Ib fimA	**Positive**	**No.**	**1**	**1**	**0**	**0**	**2**	**1.000**
		%	**1.47**	**1.47**	**0.0**	**0.0**	**2.9%**	
Gene Type II fimA	**Positive**	**No.**	**1**	**0**	**0**	**0**	**1**	
		%	**1.44**	**0.0**	**0.0**	**0.0**	**1.4%**	

Table No.4: Distribution of periodontal problems in patients with Rheumatoid arthritis.

Variables	Category	RA patients with gum problems N=43 patients			RA patients without gum problems N=27	P.value
		5Patients ≤ 4 year N= 11	5Patients >4 year N= 32	Total		
RA Patients with Gum Problems	Before RA	4	20	24 (34.3%)	27 (38.6)	0.09
	After RA	7	12	19(27.1%)		
RA patients with tooth loss	No.	4	1	5	0	
	%	80.0	20.0	100%	0.0	

Table No.5: Impact of P. gingivalis Infection on Disease Activity and Biomarkers in RA Patients.

Variables	RA patients with *P. gingival* N= 3 (4.3)	RA patients without *P. gingival* N= 67 (95.7)	p. Value
Mean DAS 28	2.4	2.93	1.000
Mean RF	30.5	38.01	1.000
Mean ACCP	7.28	7.45	
Mean IL-17	13.1	12.48	



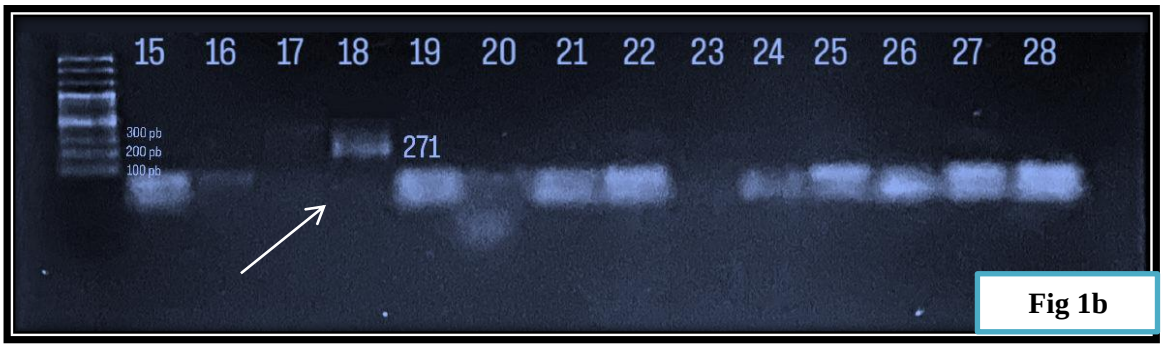


Figure 1a, b: *P. gingivalis* positive periodontal samples for type Ib fim A.M:1000bp marker lane (9, 18) & the size of product 271 bp. In Gel electrophoresis for PCR visualized under UV light (time 90 mint., 50 volt).

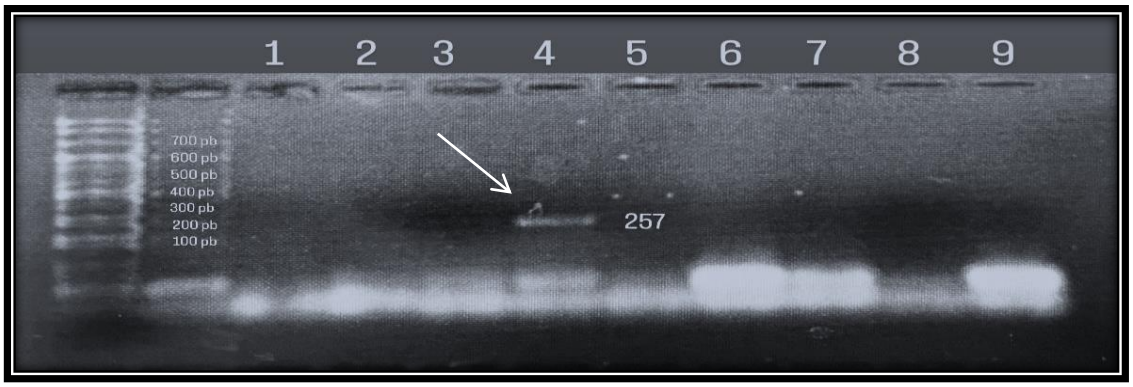


Figure 2: *P. gingivalis* positive periodontal samples for type II fim .M:1000bp marker lane (4) & the size of product 257 bp. In Gel electrophoresis for PCR visualized under UV light (time 90 mint., 50 volt).

DISCUSSION

These findings suggest that there is no substantial association between the presence of *P. gingivalis* gene types (fimA) examined in both patients (61.4% had gum problems) and controls (12.5%gum problems), comparision with study of Castellar-Mendoza et al,[2] that found suggesting no significant difference of *P.gingivalis* in 53.3% from patients of RA and 44.4% in healthy control.

The distribution of *P. gingivalis* based on gene types Ib and II (fimA) in patients categorized by the severity of their rheumatoid arthritis (RA) as measured by DAS-ESR , the calculated p-value for this analysis is 1.000 indicating no statistically significant difference in the presence of *P. gingivalis* based on gene (type I & type II) fimA among the different severity categories comparison with Hachim et al, [8] found (41.9%) of patients in the gene

type Ib (fimA) and gene type II (fimA) in remission (two patients), mild (five patients), moderate (three patients) and severe (three patients) categories, however the patients with RA exhibited a lower proportion of healthy pocket epithelium compared to the controls, indicating poorer oral health status furthermore, there was a tendency towards a higher inflammatory state in patients with RA who had severe periodontitis it is important to note that all patients with RA were receiving anti-inflammatory medication while none of the controls were using such drugs.

The study observed that RA patients with *P. gingivalis* had a mean ACCP level of 7.28 while those without *P. gingivalis* had a mean ACCP level of 7.45, the calculated p-value for comparing the two groups did not show a statistically significant difference in the mean values of all biomarkers (p-value = 1.000) these findings align with previous research by Hachim et al, (2016) [8] which suggested that *P. gingivalis* may be a potential risk factor for RA and correlated with the presence of anti-CCP antibodies, in addition to the results by Cheng et al, [9] found In contrast to controls, anti-CCP positive at-risk patients had dysbiotic subgingival microbiomes and higher concentrations of P. gingivalis, which is consistent with the hypothesis that the oral microbiome, and more especially P. gingivalis, play a significant role in the development of RA.

In our study potential association between the presence of *P. gingivalis* and increased disease activity as indicated by higher DAS 28 scores, higher levels of RF and ACCP, and higher IL-17 levels however, it should be noted that these results do not establish a causal relationship between *P. gingivalis* and disease activity in RA. Additionally, considering other potential confounding factors, such as medication usage and lifestyle factors and assessing the impact of periodontal treatment on RA disease activity would provide a more comprehensive understanding of these associations. The immune response in RA is modulated by antirheumatic drugs to prevent the development or progression of the disease therefore, it is likely that the ACCP parameter which is associated with *P. gingivalis* may also be influenced by the use of rheumatic medication , however, the potential impact of these medications was not taken into account in the current study [10] .

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