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Association of *Porphyromonus gingivalis* with Rheumatoid arthritis

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Abstract

Background Rheumatoid arthritis (RA) is chronic systemic inflammatory disease. *Porphyromonus gingivalis (P. gingivalis)* produce peptidyle arginine enzymes, which lead to citrullination of human protein then lead to formation anticitrullinated peptide antibody (ACPA).

Objective To investigate the role of *P. gingivalis* as environmental factor for RA and association of *P. gingivalis* with development of ACPA.

Methods This study included 31 newly diagnosed RA patients with periodontitis, which included 22 females and 9 men in addition to 30 individual as healthy controls, which included 20 females and 10 men. The exclusion criteria included autoimmune disease (systemic lupus erythematosus, Bachet disease, ankylosing spondylitis, multiple sclerosis), attending Department of Rheumatology in Baghdad Teaching Hospital during period from May 2014 to January 2015. The age range was 20 to 68 years. Disease activity score 28 (DAS28) was calculated for each patient. Five ml of blood sample was taken for detection of ACPA antibody while gingival cervicular fluid was taken by paper point for detection of *P. gingivalis* by polymerase chain reaction (PCR) with specific primer for fimbrial antigen (fimA).

Results The frequency of positive cases with *P. gingivalis* were 13/31(41.90%) while in healthy controls was zero with significant P value (<0.001). The association between anti cyclic citrullinated peptide (ACCP) antibody and frequency of positive cases for *P. gingivalis* was significant (P<0.041). The association of *P. gingivalis* positivity and DAS28 was non-significant (P= 0.003).

Conclusions *P. gingivalis* showed positive association with RA in newly diagnosed patients. The frequency of positive cases for *P. gingivalis* revealed association with positivity of anti-CCP.

Key words RA, ACCP, *P. gingivalis*

List of abbreviation: *P. gingivalis* = Porphyromonus gingivalis, RA = Rheumatoid arthritis, ACPA = anticitrullinated peptide antibody, DAS28 = Disease activity score, PCR = polymerase chain reaction, ELISA = Enzyme linked immune sorbent assay, USA = United States of America, bP = base pair, UV = Ultraviolet

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting 0.2–1% of the population worldwide and is associated with progressive destruction of the joints causes' early mortality and disability. Early diagnosis is challenging because the symptoms of early RA can be non-specific (e.g., malaise, fatigue, weakness, muscle soreness, low down-grade fever, weight loss) and may actually be symptoms of other conditions. Assessment of disease activity and treatment responses is based on measurement of disease activity score28 (DAS28) [(1)].

Although the causes are indefinite, there is accumulating evidence RA is an autoimmune disease characterized by disease-specific antibodies to citrullinated protein antigens (ACPA). Citrullinated proteins are generated

by peptidyl arginine deiminases (PADs), enzymes that catalyze the modification of peptidyl-arginine to peptidyl-citrulline [2].

P. gingivalis belongs to the phylum bacteroidetes and is a non-motile, gram-negative, rod-shaped, anaerobic, pathogenic bacterium. It establishes in the oral cavity, somewhere it is implicated in confident forms of periodontal disease [3]. The Collagen degradation experiential in chronic periodontal disease consequences in part from the collagenase enzymes of this species. It has been revealed in an in vitro study that P. gingivalis can attack human gingival fibroblasts and can stay alive in them in the existence of significant concentrations of antibiotics [4].

The bacterium has concerned interest based on epidemiologic relations between RA and periodontitis and the explanation of a novel bacterial peptidyle arginine deaminase (PAD), suggesting a possible etiologic role of *P. gingivalis* in RA during the creation of citrullinated antigens. In RA, an autoimmune response develops in opposition to citrullinated peptides detected as anti-citrullinated peptide antibodies (ACPA). The existence of anti-CCP are >98% specific for the diagnosis of RA [5].

The mechanisms to citrullination that guide to RA remain unclear. A polymorphism in the PAD4 gene, which may lead to amplified citrullination has been described in populations. *P. gingivalis* is the only prokaryote recognized to have PAD, an enzyme to facilitate catalyzes the posttranslational modification of arginine residues to citrulline [6].

The objectives of this study was to investigate the role of *P. gingivalis* as environmental factor for RA and association of *P. gingivalis* with development of ACPA

Methods

This study included 31 newly diagnosed RA patients with 30 persons as healthy controls. The RA patients attended Rheumatology Department in Baghdad Teaching Hospital were examined by rheumatologist during the period from May 2014 to January 2015 and DAS28 was calculated for each patient according to ACR.

Blood samples

In this study 5 ml of blood were taken from each patients and healthy control by venipuncture, then 3 ml of blood were separated by centrifuge and serum sample was isolated and stored at (-20 °C) until used while 1.6 ml of blood was add to 0.4 of sodium citrate for erythrocyte sedimentation rate (ESR).

ELISA test for anti-CCP:

The anti-CCP was done by ELISA technique (indirect method) according to instructions manual by Human company/Germany .

DNA-extraction from gingival cervicular fluid samples

The wizard genomic purification by promega/USA.

Monoplex PCR for detections fimA of *P. gingivalis* was done according to Nakagawa [7].

The primer set was used in detection of *P. gingivalis* as followed:

Gene	Primer	Product size (bp)	Reference
Type 1b fimA	CAGCAGAGCCAAAAACAAT GCTGTCAGATAATTAGCGTC TGC	250	Nakagawa ,et al [7]
Type 11 fimA	ACAACTATACTTATGACAAT GGAACCCCGCTCCCTGTATT CCGA	200	Amano,et al [8]

Statistical analysis

Prevalence of infection was compared between different variable by Chi-squared test. Significance was attributed to probability $P \leq 0.05$. Computer SPSS and Microsoft were used for determination of probability values.

Results

The mean age in newly diagnosed RA patients was 46.3 years versus 43.6 in controls and the p value non-significant as in table (1).

Table 1. Mean age in newly diagnosed rheumatoid arthritis patients

Age (yr)	Control	Newly diagnosis RA patients
Mean	43.6	46.03
Standard Deviation	11.28	12.35
Median	43	50
Minimum	18	20
Maximum	65	67
P value	>0.05	

Table (2) showed distribution of severity state (high, moderate, mild, remission) in newly diagnosed RA patients.

Table 2. The DAS28 score in treated and newly diagnosed RA patients

		Newly diagnosis RA patients
Remission	Count	3
	%	9.7%
Mild	Count	6
	%	19.4%
Moderate	Count	9
	%	29.0%
High	Count	13
	%	34.5%
Total	Count	29
	%	100%
P value	0.665	

The frequency of positive cases for anti-CCP were 23 out of 31 RA patients while in controls was zero with significant association (P<0.001) as in table (3).

Positivity of *P. gingivalis* in newly diagnosed RA patients was 13 (41.9%) out of 31, while negative in all cases of control with significant difference (P value <0.001) as in table (4) and (Figure 1, 2).

Table 3. Serum anti-CCP positivity in newly diagnosed RA patients versus controls

		Control	Newly diagnosis RA patients
Positive	Count	0	23
	%	0.0%	74.2%
Negative	Count	30	8
	%	100%	25.8%
Total	Count	30	31
	%	100%	100%
P value	<0.001		

Table 4. The frequency of positive cases with P. gingival in newly diagnosed patients and healthy controls

		Control	Newly diagnosis RA patients
Positive	Count	0	13
	%	0.0%	41.9%
Negative	Count	30	18
	%	100%	58.1%
Total	Count	30	31
	%	100%	100%
P value	<0.001		

Table (5) shows significant association between positive cases of *P. gingivalis* and anti-CCP positivity with P value <0.05.

Current study revealed no association between *P. gingivalis* infection and DAS score with P value =0.003 as in table (6).

Table 5. Relationship between *P. gingivalis* positivity and anti-CCP of newly diagnosed RA patients

P. gingivalis	No.	Mean±SD of CCP
Positive	13	168.46 ± 34.33
Negative	18	78.94 ± 25.53
LSD value	----	85.603
P value	----	0.0410

* Significant (P<0.05)

Table 6. The frequency of positive cases with *P. gingivalis* in newly diagnosed patients

DAS28	*P. gingivalis*			
	Negative		Positive	
	No.	%	No.	%
Remission	1	2.8	2	7.7
Mild	1	2.8	5	19.2
Moderate	6	16.7	3	11.5
High	10	27.8	3	11.5
Total	18	51.1	13	49.9
P value	0.003			

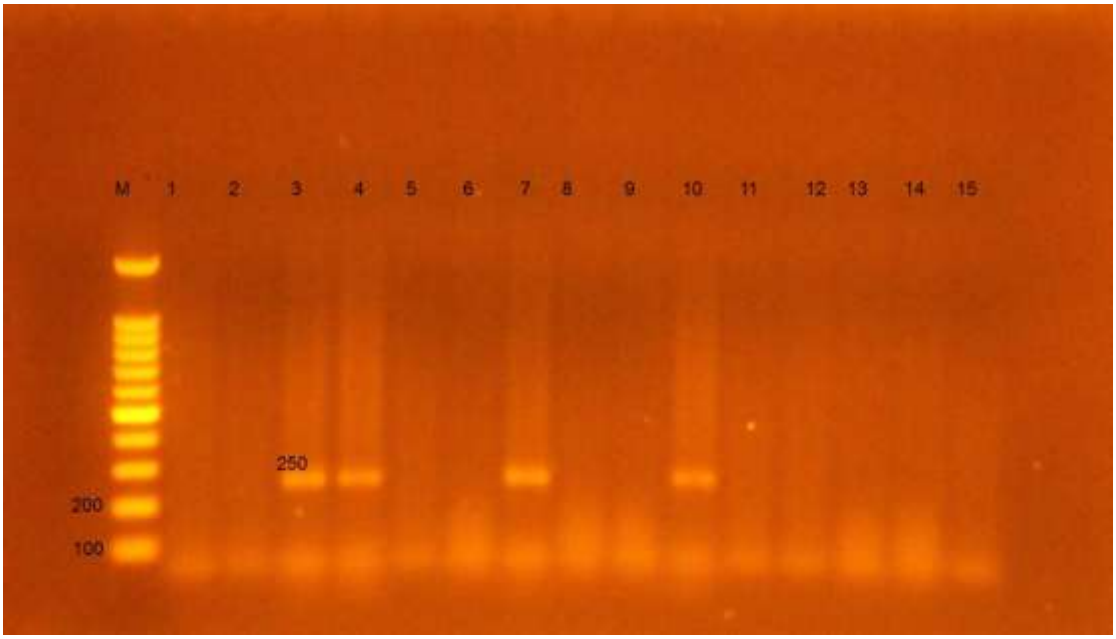

Fig. 1. Gel electrophoresis for PCR product of *P. gingivalis* type Ib fim visualized under UV light.M:1000 bp marker lane (3,4,7,10) were positive for type Ib . The size of product was 250 bp (time 90 mint., 50 volt)

Discussion

Porphyromonus gingivalis genome detection was done for newly diagnosed RA cases by PCR to detect FimA (fimbrillin antigen), which consider highly specific for *P. gingivalis;* two type of FimA were used (FimA type 1b, FimA type II) to increase specificity and to diagnosis bacteria from gingival cervcular fluid [9]. The positive cases were (41.90%) of thirty one newly diagnosed RA patients. Relationship of *P. gingivalis* infection with RA could be illustrated by mechanisms of secreted enzyme peptidyle arginine deaminase, which lead to citrullination and permanent post-translation of arginine to citrulline as a consequence for this event will lead to accumulation of citrullinated peptide with development of anti citrullinated peptide antibody, which concerned in RA and useful diagnostic marker for RA. This agree with Hitchon, *et al* who proposed that citrullination

of human protein by bacterial enzymes will lead to production of ACPA which used as good diagnostic markers for RA [10].

There were 41.90% of newly diagnosed RA patients have infection with P. gingivalis infection as well as there is high titer of ACPA in newly diagnosed RA patients, this finding reflect the role of *P. gingivalis* as one of environmental factor for RA and stimulation for ACPA formations. *P. gingivalis* infection showed a significant association with DAS, so the bacteria may responsible on initiating autoimmune process, which end with development of RA as well as infection with bacteria may exacerbate RA. This finding agree with Al-katma, *et al* (2007), who hypothesized that control of periodontal infections and gingival inflammation by scaling roots planning and plaque control in subjects with periodontal disease reduce the severity of RA [11]. However, this result disagree with Lugli *et al* (2014), who proposed that *P. gingivalis* infection, which causes periodontitis was associated with development of RA not with severity because severity of disease depend on autoimmune process not on infection with *P. gingivalis* [12].

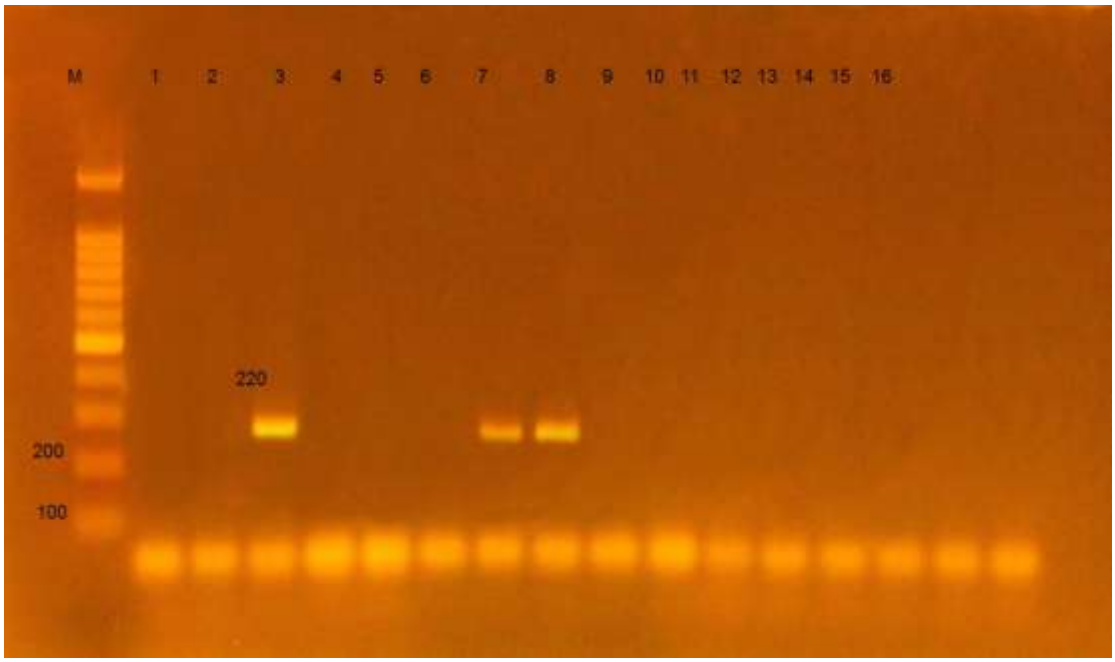

Fig. 2. Gel electrophoresis for PCR product of *P. gingivalis* type II fim visualized under UV light.M:1000bp marker lane (3,7,8) were positive for type II fim . the size of product 220 bp (time 90 mint., 50 volt)

Molecular mimicry between citrullination of protein by bacterial enzyme and human protein may be responsible for production of ACPA [13]. ACPA in newly diagnosed RA patients was high in association with positivity of *P. gingivalis* infections this proved role of bacteria as environmental factor for RA when individual infected with these bacteria will have periodontal disease then there is high tendency to affect with RA [14].

This study concluded that *P. gingivalis* may be consider as risk factor for RA and showed correlation with positivity of anti-CCP.

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Author contribution

Sadeq: Data collection and drafting of the article. Ahmed: Design of the work, data interpretation, drafting and critical revision of the article. Mohammed: samples collection.

Conflict of interest

The authors declare no conflict of interest.

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