Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 11:661-665

Detection of *Porphyromonas gingivalis* DNA in the synovial fluid of rheumatoid arthritis patients by real-time PCR

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ABSTRACT

Microbial infections are believed to play an important role in the initiation and perpetuation of rheumatoid arthritis. This study aimed to investigate the relationship between the presence of Porphyromonas gingivalis DNA in the synovial fluid and rheumatoid arthritis. The synovial fluid samples were collected from 22 patients with rheumatoid arthritis and 20 patients with not suffering from rheumatism, overall 42 patients were investigated. The presence of P. gingivalis DNA was evaluated by the real-time PCR method. There was a significant relationship between rheumatoid arthritis and non-rheumatoid arthritis with the DNA number (Pv< 0.05). P. gingivalis DNA were detected in 3 of 20 (13.6%) rheumatoid arthritis patients, but there was no significant relationship between P. gingivalis DNA and rheumatoid arthritis (Pv> 0.05). DNA of periodontal pathogens can be found in the synovial fluid of rheumatoid arthritis patients. It shows oral bacteria may play a role in the pathogenesis of rheumatoid arthritis.

Keywords: *Porphyromonas gingivalis*, Synovial fluid, Rheumatoid arthritis, Real-time PCR

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease that affecting 1–2% of the general population. The disorder is most typically prevalent in the female and elderly (1). It is believed that approximately half of the risk factors for RA are attributed to genetic factors such as the human leukocyte antigen (HLA) alleles while the other half of the risks are environmental factors such as infections and smoking (2). Environmental factors together with genetic factors play roles in the development of RA, possibly through the loss of tolerance to citrullinated peptides (3). Citrullination is a process involved in the pathogenesis of RA, and the conversion of arginine to citrulline allows for a high-affinity antigen interaction with the HLA-DRB1*04 allele (4). Microbial infections are believed to play an important role in the initiation and perpetuation of RA. Clinical studies have shown the association of microbial infections and RA. Several studies using animal models have also found that microbial infections can induce and/or exaggerate the symptoms of experimental RA. *Porphyromonas gingivalis* (*P.gingivalis*), a periodontal anaerobic intracellular pathogen, has been associated with RA and the pathogenesis of the disease (5, 6), mainly due to the ability of the bacterium to citrullinate host and bacterial peptides (7), unique in the prokaryotic world. Moreover, in the previous studies, DNA of *P. gingivalis* has been detected in the SF (synovial fluid) samples of patients with RA (8, 9).This study aimed to investigate the presence of *Porphyromonas gingivalis* DNA in the synovial fluid of rheumatoid arthritis patients.

MATERIALS AND METHODS

2.1. Patients: The investigation was performed on 22 patients with RA and 20 patients not suffering from RA (6 artificial joint prostheses, 12 suspected joint infections, and 2 joint surgeries). Overall demographic information of 42 patients (20 controls and 22 cases) was been investigated from May 2015 till Jun 2016, at the Rheumatology ward of the Emam Reza Hospital, Tabriz, Iran. The patients with RA were diagnosed by an experienced rheumatologist. Written informed consent was obtained from all the patients prior to entering the study, and the study was approved by the Regional Ethics Committee, Tabriz University of Medical Sciences (Tabriz, Iran; no REC. 1394.202, May 4, 2015).

2.2. Assessment of *P.gingivalis* DNA in SF: In the present study,3ml SF was taken from joints of RF patients and controls, and analyzed for protein, glucose, color, and turbidity by the standard methods. As well as, RF factor, RBC, and WBC were evaluated.

The Qiagen kit (Qiagen GmbH, Hilden, Germany) was used for DNA extraction according to the manufacturer's instructions. To determine the average concentrations of the DNA and their purity, we used the spectrophotometer **(**Nano drop, 2000c Thermo Scientific, U.S.A) at a wavelength of 260nm and 280nm**.**Real-time polymerase chain reaction (PCR) was carried out in a total volume of 25µl by the Real-Time PCR-AB (Applied Bio system) Step One Plus kit, California, USA. The distilled water was used as a negative control. *P. gingivalis* ATCC 33277was used as a positive control strain.

2.3. Statistical analysis: Data were analyzed by SPSS 18.0 software (SPSS Inc., Chicago, IL), and variables were described in terms of means. The data were analyzed for normal distribution using the Kolmogorov–Smirnov and Shapiro–Wilk test. The Chi-square and Fisher's exact tests were performed to compare cases and controls. In general, P-values ≤0.05 were accepted as statistically significant.

RESULTS

A total of 42 SF specimens from case and control groups were collected. Table 1 illustrates the demographic and clinically profiles of the both groups. The mean age of patients with RA was 47.86 years and in non-arthritis group was 50.00 years, including 14 males and 28 females. In this study, statistical analysis did not show a significant difference between the gender and age in patients with RA and non-arthritis (Pv>0.05). The glucose levels in patients with RA were significantly higher than non-arthritis group (Pv 0.011). However, the averages of protein levels were 4.70 and 5.30 in patients with RA and non-RA, respectively .There was no significant difference between protein levels and RA and non-arthritis (Pv 0.07). There was a significant relationship between RA and non-RA with the DNA quantity, RBC, and WBC counts Pv< 0.05 (Table 1).

DNA of *P. gingivalis* in SF was detected more frequently in specimens obtained from patients with RA in comparison to non-RA (Pv> 0.05). In total, *P.gingivalis* DNA were detected in 3 RA patients (13.6%).

DISCUSSION

In the present study, most patients with RA were females (66.6%), and it was in accordance with the knowledge that RA affects more females than males (1, 2).

In the current study ,*P. gingivalis* DNA in SF was more in specimens obtained from patients with RA in comparison to non-RA but did not observe an association between *P. gingivalis* DNA and RA. Gram-negative anaerobic bacilli may cause infections in the body; the most common types included oral, dental, pleuropulmonary, intra-abdominal, female genital tract, skin, and soft and bone tissue infections (10). Clinical and animal model studies have suggested that infections due to *P. gingivalis*, *Mycoplasma*, Epstein–Barr virus (EBV), and *Proteus mirabilis* may becontribute to the pathogenesis of RA (11-13). A significant increase in the incidence of the periodontal disease has been observed in patients with chronic-active RA compared to healthy subjects, and the prevalence of RA is higher in periodontal disease patients compared to individuals without periodontal disease (7, 14, 15).

The oral bacteria or their genetic material can reach the joints. Moen et al, found more genetic material in the synovial fluid than in plasma, but they observed no relationship between DNA of microorganisms with the

parameters of the inflammatory status such as CRP, white blood cells, and platelets counts(16).Previously significant associations between infections due to *P. gingivalis*, and periodontal disease and RA have reported. *P. gingivalis* is the only known prokaryotic organism that contains enzyme peptidyl arginine deiminase which is essential for the generation of citrullinated autoantigens (6, 7, 17). Martinez-Martinez et al found *P. gingivalis* more frequently in the SF than in serum among various oral pathogens and presented the possibility of a free DNA transport to the joint. They confirmed an oral genetic material transport to the joints (16).

Table1. Demographic and clinical profiles of the studied groups

The variables	Arthritis (n=22)	Non-arthritis (n=20)
Mean age ±SD, years	47.86±18.61	50.00±15.20
Mean RBC count ±SD, mm^3	2505.45±2925.80	1007.50±1674.72
Mean WBC count ±SD,mm^3	4089.27±3158.46	123.20±129.08
Mean glucose ±SD, mg/mL	118.27±56.15	82.45±23.91
Mean protein ±SD, mg/mL	4.70±1.02)	5.30±1.08
Mean DNA nanodrop ±SD, ng/µL	91.86±59.57	28.79±27.96
Male, %	27.3	40.0
Female, %	72.7	60.0
Synovial fluid color, %		
Yellow	77.3	75.0
Red	22.7	5.0
Turbid	59.1	30
Semi turbid	22.7	5
Semi clear	18.2	35
Clear	0	30
Rheumatoid factor, %		
(-)	0	100
(+)	45.5	0
(++)	31.8	0
(+++)	22.7	0

SD, standard deviation; RBC, red blood cell; WBC, white blood cell

Our findings are supported by a previously published case-control study which DNA of *P. gingivalis* was found in SF in patients with RA (16, 19). In this study, there was no significant difference between the DNA of *P. gingivalis* with RA (Pv>0.05); this finding is dissimilar to Reichert et al study (18). On the other hand, Fagundes et al reported that *P. gingivalis* was the most frequent species detected in SF (42.1%), and this rate was higher than the *P. gingivalis* DNA detected in our finding, but like our study they did not found any statistically significant differences between presence of *P. gingivalis* DNA and RA (19).The differences between studies may be due to the number of patients, the studied groups, detection method and the variety of strains.

The method of detection in other studies was PCR. To our knowledge, the present study is the first report of *P. gingivalis* DNA detection by the real-time PCR method in SF of RA patients. The sample size of our study was low; we suggest more studies are necessary to be performed. The occurrence of periodontal disease in many patients with RA is well established, and *P. gingivalis* bacteria can induce inflammatory cytokines and proteases by mononuclear cells or fibroblasts. As well as *P. gingivalis* DNA was most frequently detected in both gingival plaques in periodontitis patients(19-21).To obtain a whole evaluation on the relation between *P. gingivalis* and RA, it is better, DNA detection of *P. gingivalis* not only in SF but also done in gingival plaque diseases by the real-time PCR method.

Based on the results of this study, the real-time PCR method can detect the *P. gingivalis* DNA in the SF of patients with rheumatoid arthritis both quantitative and qualitative. This finding may help the specific treatment of rheumatoid arthritis in the future.

In conclusion, our data indicate that *P. gingivalis* DNA can be detected in SF. It shows oral bacteria may play a role in the pathogenesis of rheumatoid arthritis. Therefore, *P. gingivalis* may be contributed to the systemic inflammation.

Acknowledgments
This article was written based on a dataset of M. Sc thesis registered at Tabriz University of Medical Sciences, Tabriz, Iran. This project was financially supported by Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences.

Conflict of Interest
There is no conflict of interest.

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