ORIGINAL ARTICLE

ASSOCIATION OF PERIODONTITIS AND RHEUMATOID ARTHRITIS BIOMARKER ANTI-CYCLIC CITRULLINATED PEPTIDES/PROTEINS

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Background: Rheumatoid arthritis (RA) is a chronic multi-system disease that causes damage to bone and joint connective tissues. Periodontitis (PD) is an infectious disease and a leading cause of chronic periodontal inflammation in which microbial plaque causes damage to alveolar bone and surrounding connective tissue which leads to loss of tooth. Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies are potentially important substitute markers for diagnosis and prognosis in rheumatoid arthritis (RA). The objective of this study was to determine the possible relationship of Rheumatoid Arthritis and Periodontitis with the help of Biomarker Anti-CCP. **Methods:** Total 120 individuals were recruited for this study and were equally divided into 4 groups: Rheumatoid Arthritis (RA), Periodontitis (P), Rheumatoid Arthritis with Periodontitis (RAP) and Healthy controls (C). Oral examinations were done for all patients prior to the sampling. Patients' serum sample was analysed for the selected biomarker (serum anti-CCP) by using anti-CCP ELISA IgG kit. Patients were recruited from both in-patients and out-patients departments of Ghurki Trust Hospital, Lahore. For healthy controls, individuals with no history of RA and Periodontitis or any other systemic illness were included in this study. **Results:** In RA and RAP group mean anti-CCP level was quite high as compared to that of C and P group respectively. But no statistically significant differences were observed between anti-CCP level in RA and RAP groups. **Conclusion:** Mean anti-CCP level in RA and RAP group was quite high compared to that of C and P group respectively. Use of anti-CCP antibodies may allow the clinical rheumatologist to better predict the diagnosis and prognosis of individual patients with RA.

Keywords: Anti-CCP, Periodontal Disease, Rheumatoid Arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterised by the accretion of an inflammatory infiltrate in the synovial membrane that leads to synovial membrane inflammation and destruction of bony structure. RA may also present with some extra-articular manifestations such as the pulmonary, ocular, vascular and some other systemic diseases.[1] Periodontitis (PD) shows diffused inflammatory destruction of the periodontal attachment with alveolar bone involvement, which may be due to both genetic as well as acquired predispositions. Periodontitis is the most common oral disease.[2] It eventually results in soft and hard periodontal tissue destruction which results in tooth loss.[3] Researches have shown that patients with chronic RA have a noticeable increased incidence of PD in comparison to healthy individuals.[4–6] A positive and strong association has been found between the presence of PD and development of RA.[7] Rheumatoid factor (RF) is currently used to diagnose rheumatoid arthritis (RA) but RF is not accurate as anti-Cyclic Citrullinated Peptide (anti-CCP). RF and anti-CCP antibodies have shown to be present prior to the development of clinical symptoms of arthritis suggesting an initial immune dysregulation in RA that occurs years before the disease becomes symptomatic. Moreover, anti-CCP has shown to be a specific marker for monitoring disease prognosis and predict the erosive or non-erosive progression of the disease. Thus, it's a useful tool for optimal therapeutic management of RA patients.[8–10] Anti-CCP antibodies have higher diagnostic specificity and positive predictive value than RF and anti-MCV antibodies. Anti-MCV antibodies have the higher sensitivity in comparison to anti-CCP antibodies and RF.[11]

The objective of this study was to determine the possible relationship of Rheumatoid Arthritis and Periodontitis with the help of Biomarker Anti-CCP.

PATIENTS AND METHODS

This was a prospective cross-sectional study. It was conducted in Ghurki Trust Hospital, Lahore and in the Centre for Research in Molecular Medicine, The University of Lahore. A group of 30 Rheumatoid Arthritis (RA) patients, 30 periodontitis (P) patients, 30 Rheumatoid Arthritis with periodontitis (RAP) patients and 30 age and gender matched healthy controls (C) were targeted for participation. The duration of study was 9 months which included 5 months for sample collection and 4 months for experimental procedures, and data analysis. Patients of each group were recruited from both in-patients and out-patients department of Ghurki Trust Hospital, Lahore. For healthy controls, individuals with no history of RA and Periodontitis or

any other systemic illness were included. Written informed consent was taken from all the participants of the study. Informed consent was obtained from all participants. Basic demographics were recorded using a questionnaire. The confounding factors were ruled out by detailed history and examination. Blood samples for the analysis of serum biomarkers including Anti-CCP were drawn from an ante-cubital vein from the patients of each group. The blood samples were immediately placed in EDTA tubes between ice packs and transported to Biochemistry and Molecular Biology Laboratory of CRIMM, The University of Lahore. Data entry and analysis was done on SPSS-21.

RESULTS

The healthy control 'C' group includes 63% healthy males and 37% healthy females with mean age of 31.33±6.52, and BMI 22.53±4.22. The group 'P' included 47% healthy males and 53% healthy females with mean age of 40.03±8.86, and BMI 26.27±1.81. The 'RA' group included 40% healthy males and 60% healthy females with mean age of 45.83±16.06, and BMI 24.86±3.61. The 'RAP' group included 40% healthy males 40% and 60% healthy females with mean age 41.43±11.96, and BMI 24.69±2.32. (Table-1)

Table-1: Descriptive statistics

Parameter	C (30)	P (30)	RA (30)	RAP (30)
Males (%)	63	47	40	40
Females (%)	37	53	60	60
Age (Years)	31.33 ± 6.52	40.03±8.86	45.83±16.06	41.43±11.96
BMI	22.53±4.22	26.27±1.81	24.86±3.61	24.69±2.32

Serum levels of Anti-CCP in group C, P, RA, and RAP was 1.4380±0.22675, 2.7437±0.61332, 34.4167±11.80788, and 42.2070±12.91378 (pg/mL) respectively. (Table-2)

Table-2: Anti-CCP in groups (pg/mL)

	Mean±SE	Range
C	1.44±0.23	0.40–5.98
P	2.74±0.61	0.34–15.68
RA	34.42±11.81	0.00–265.34
RAP	42.21±12.91	0.78–252.83

Serum levels of Anti-CCP in all four groups were also assessed and inter group analysis was done. The results showed that there was a significantly difference in concentrations of Anti-CCP in groups C, P, RA and RAP at the 0.001 level.

Anti-CCP concentrations in individuals of group P when compared with those of group C, any statistically significant difference has not been observed. Group RA and RAP showed statistically significant difference in anti-CCP when compared with those of group C (p=0.001 and 0.009 respectively). Likewise concentrations of anti-CCP in group P showed significant differences when compared with group RA (p=0.012). (Table-3)

Table-3: Analysis of variance of Anti-CCP concentrations in C, P, RA AND RAP Groups

Groups	Vs	*p*
C	P	0.916
	RA	0.009*
	RAP	0.001*
P	C	0.916
	RA	0.012*
	RAP	0.002*
RA	C	0.009*
	P	0.012*
	RAP	0.530
RAP	C	0.001*
	P	0.002*
	RA	0.530

*Significant

DISCUSSION

Periodontal disease (PD) is a risk factor for RA.[12] The anti-CCP are produced locally in the area of inflamed synovium of RA patients suggesting that citrullinated proteins are present in the inflamed synovium. Rheumatoid arthritis and adult PD have nearly similar pathogenic mechanisms and immunologic and pathological findings. Porphyromonas gingivalis (P. gingivalis) is a pathogen of oral cavity which is strongly related in the pathogenesis of PD and has a unique microbial enzyme, peptidyl arginine deiminase (PAD). PAD enzyme has been identified as a susceptibility factor for RA.[13]

Rheumatoid arthritis is a common, chronic, systemic autoimmune disease, which ultimately leads to destruction of the joint architecture and consequent movement disability. Although the aetiology of RA remains unknown, but accumulating studies have recognised a strong association between RA and Periodontitis (PD). Anti-cyclic citrullinated peptide autoantibody and citrullinated peptide have been involved in breaking of self-tolerance and development of autoimmune in RA.The anti-CCP antibodies, form immune complexes with citrullinated proteins, which can later be bound by inflammatory cells via their Fc receptors. A complex cascade involving complement activation mediates the role of these immune complexes and inflammatory cells. These mechanisms finally result in release of mediators of inflammation and joint destruction ultimately leading to the onset of RA. This hypothesis reveals that oral bacterial infection may play a role in peptide citrullination, which might be involved in loss of self-tolerance and development of autoimmune in RA.[14]

In our study it was observed that in RA and RAP group mean anti-CCP level was high compared to that of C and P group respectively. But no statistically significant differences were observed between anti-CCP level in RA and RAP group. The combined detection of IgM and IgA RFs in serum is a strong indicator of RA.[15]

Studies comparing the sensitivity and specificity of RFs and anti-CCP antibodies for the diagnosis of RA have used the CCP1 assay.[16] Generally, the sensitivity of anti-CCP has been comparable to RF (50–75%) with a higher specificity (90–95%). Studies that have used the CCP2 assay show higher sensitivity for RA than CCP1, with equally high specificity.[17]

CONCLUSION

Mean anti-CCP level in RA and RAP group was quite high as compared to that of C and P group respectively. No statistically significant difference was observed between anti-CCP level in RA and RAP group. Use of anti-CCP antibodies may allow the clinical rheumatologist to better predict the diagnosis and prognosis of individual patients with RA. Either this or other serologic tests will allow a more rational therapeutic decision-making and hence influence the long-term outcome of the disease.

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