Evaluation of serum anti-cyclic citrullinated peptide antibodies level in rheumatoid patients with and without periodontitis

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ABSTRACT

Background: Rheumatoid arthritis (RA) and periodontitis (PD) are widely prevalent diseases and are characterized by tissue destruction due to chronic inflammation. Recently, there is growing evidence that the two diseases share many pathological features. An association between periodontitis and rheumatoid arthritis has been considered many years ago. The current study was established to evaluate the serum ACCP levels in rheumatoid arthritis patients with and without periodontitis, in addition to study the correlation between serum ACCP levels and periodontal parameters.

Subjects and methods: A total of 95 subjects were enrolled in this study, age ranged (35 – 55) years divided into 3 groups: Group I consist of 45 rheumatoid arthritis patients with periodontitis, while group II consist of 35 rheumatoid arthritis patients without periodontitis and group III which consist of 15 apparently- healthy controls. . Periodontal parameters used in this study were plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). Blood samples were collected from each subject to determine serum concentrations of ACCP by means of enzyme-linked immune-sorbent assay (ELISA).

Results: present study revealed that serum ACCP levels were significantly higher in rheumatoid patients with periodontitis ($P<0.001$) than that in other two groups. Regarding the correlation between serum ACCP levels and periodontal parameters, the current study showed positive correlation between serum ACCP and each of plaque index, gingival index and probing pocket depth.

Conclusion: The current results provide strong evidence of association between periodontitis and rheumatoid arthritis, and suggested that periodontitis may play an important role in activation and triggering immune response.

Key words: Periodontitis, rheumatoid arthritis, anti-ccp antibodies, p. ginivalis. (J Bagh Coll Dentistry 2012; 24(4):83-87).

INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common autoimmune disease and the most frequent chronic inflammatory arthropathy. It affects around 1% of the world population, 75% of patients are female .It is characterized by inflammation of the synovial membrane which spreads symmetrically from the small to large joints leading to the destruction of the joints [1]. On the other hand, periodontitis (PD) is a multi factorial infection characterized by a destructive inflammatory process affecting tooth supporting tissues and resulting in periodontal pocket formation and alveolar bone resorption, which might eventually lead to tooth loss [2].Host tissue damage in PD is mainly due to the action of oral microbes and associated host immune-inflammatory responses [3-5] whereas, combination of environmental and genetic factors with antibodies directed against cyclic citrullinated peptide (ACCP) has been associated with the onset of RA[6].

Citrullination or deamination is the term used for a genetic modification of the amino acid arginine in a protein into the amino acid citrulline and caused by enzymatic activity through Peptidyl-Arginine Deaminases (PAD) enzyme. It has been found that P. gingivalis is currently the only known bacterium with the expression of PAD which is involved in citrullination [7]. ACCP are highly specific for RA and have been implicated in disease etiology, it may be detected in roughly 50-60% of patients with early RA [8]

Intriguingly, both PD and RA share comparable clinical and pathological characteristics. For instance, in either condition, both connective tissue and bone are involved, and immune-inflammatory mediators are responsible for the host tissue damage, thus elevation of bone resorptive cytokines in PD and RA suggests common pathological pathway shared by the two diseases [9]. Significant associations between the clinical parameters of PD and RA have been observed and it was suggested that both diseases possess a common underlying dysregulation of the inflammatory responses within the host [10]. So the current study was established to evaluate the serum ACCP levels in rheumatoid arthritis patients with and without periodontitis, in addition to study the correlation between serum ACCP levels and periodontal parameters.

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SUBJECTS, MATERIALS AND METHODS

In a cross sectional study, ninety-five (95) subjects, aged ranged (35-55) years were recruited in this study. They were from attendants seeking treatment in the rheumatology clinic in Baghdad Teaching Hospital, Baghdad.

Patients were subjected to questionnaire about name, age and gender with full registration of periodontal parameters.

From each subjects, 2ml of venous blood was harvested, centrifuged and then sera were separated and stored at (-20c) for later analysis for quantitative determination of ACCP (IgG) in serum by ELISA according to manufacturers protocol (EUROIMMUN (Leubeck, Germany), LOT.NO. E111027AM). The laboratory test was done in the Teaching Laboratories of Baghdad Medical City, Baghdad.

The patients were divided into three main groups:

Group I consist of forty-five (45) RA patients who fulfill the 1987 ACR criteria for RA with chronic periodontitis (CP). Whereas , group Π consist of thirty- five(35) RA patient without CP .Finally group ΠI which consist of fifteen (15) age, sex matched apparently- healthy volunteers.

The periodontal Examination

Oral examination was performed at four surfaces of each tooth except 3rd molar according to the following criteria:

1. Assessment of dental plaque by (PL.I) of Silness &Loe (1964) [11].
2. Assessment of gingival condition by (G.I) of Loe &Silness (1963) [12].
3. Probing Pocket Depth (P.P.D): It is the distance from the gingival margin to the point at which periodontal probe stop in the gingival crevice. Williams's periodontal probe was allowed to fall by its own weight without any pressure.
4. Clinical Attachment Level (CAL): It is the distance from the cemento-enamel junction (CEJ) to the location of the inserted probe tip (bottom of periodontal pocket).The distance was measured indirectly by subtracting the distance from the gingival margin to the CEJ from PPD. In some cases when there was gingival recession ,distance measured by adding the distance from the gingival margin to the CEJ to the PPD. Estimation of CAL was done by using William periodontal probe
5. Bleeding on probing (B.O.P): A blunt periodontal probe inserted to the bottom of the gingival pocket and is moved gently along the root surface. If bleeding occur within 30 seconds after probing, the site was given positive score (1), and a negative score for the non bleeding site.

RESULTS

The current study revealed highly significant differences in the means of periodontal parameters between the studied groups at P< 0.001 as shown in table -1

Furthermore, table-2 tested the comparisons by using Least significant difference (LSD) method which showed highly significant differences among the studied groups regarding all periodontal parameters at p<0.001 with the exception of non significant differences reported between group I and group Π regarding G.I and B.O.P at p> 0.05.Also, the present study revealed that mean serum ACCP levels were statistically higher in group I than other two groups (96.32± 92.24 ; 29.14± 36.31; 1.03± 0.35) , respectively, Table -3.

Table -4 observed highly significant differences in ACCP values between group I and the other two groups at p<0.001, and showed significant differences between group Π and group III at p < 0.05.

Regarding correlation between serum ACCP values and clinical periodontal parameters ,table -5 showed weak positive correlation in group I with each of PL.I ,G.I at p<0.001, p<0.05,respectively.

DISCUSSION

Pathophysiologically, the same immune mechanisms, cytokines, inflammatory mediators, and degradative enzymes operate in both periodontal disease and RA[13] The similarity between RA and PD has prompted several studies as early as 1930s, regarding the periodontal status in patients with RA , these studies have been noted significantly high level of plaque in RA patients in comparison with the healthy controls.

Supporting the above findings ,the present study revealed highly significant differences between the studied groups regarding PL.I; G.I; P.P.D; CAL and B.O.P at p <0.001.That is probably because patients with RA might be more likely to obtain tempromandibular joint involvement, severe hand dysfunction (caused by arthritis) which hinder the patient's oral hygiene practices due to restriction of movements, at the same time, decreased saliva from secondary Sjögren's syndrome all enhances plaque accumulation, as well as RA patients may be emotionally depressed about their illness causing the deterioration of the attention to the personal hygiene. Moreover, chronic use of corticosteroids all impact oral health of patients with RA[14]. This

result was consistent with result reported by kasser et al. [15] who showed that RA patients had higher rate of BOP, greater PPD, and greater CAL compared with the healthy controls, with the exception of non significant differences between group I and group Π regarding G.I and B.O.P, this is probably because RA patients are usually on multiple drug treatment including NSAIDs and corticosteroids which are anti-inflammatory in action. These drugs may mask the effects of inflammation such as gingival bleeding and swelling interfering with the criteria of BOP and G.I giving erroneous inferences.

Another interesting finding in this study was the significant elevation of serum ACCP levels in antibodies in sera of RA patients associated with PD than those in other two groups (RA patients without PD and healthy control). Correspondingly several studies observed high levels of ACCP among RA patients with PD [17, 18]. Molitor and colleagues reported that ACCP titters were considerably higher in RA patients with moderate to severe PD than in RA patients without PD [17]. The possible explanation for the higher levels of serum ACCP may be due to the fact that P. gingivalis is the common oral pathogen strongly implicated in the pathogenesis of PD. P. gingivalis, a gram-negative anaerobic bacterium that is recognized to be the only bacteria known to express PAD enzyme which has been identified as a susceptibility factor for RA. P. gingivalis may, therefore, play a role in peptide citrullination and involved in loss of self-tolerance and development of RA (Lundberg et al., 2008). In contrast the results conducted by Hamevose et al., [19] were at variance with our results. In conclusion the current results provide strong evidence of association between PD and RA, and suggested that PD may play an important role in activation and triggering immune response.

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Table 1: Summary statistics of periodontal parameters at the three studied groups

Groups		No.	Mean	Std. Dev.	Std. Error	p-value
PL.I	RA + PD	45	2.16	0.64	0.10	P<0.001**
	RA	35	1.74	0.44	0.07	
	Control	15	0.67	0.49	0.13	
G.I	RA + PD	45	1.98	0.50	0.07	P<0.001**
	RA	35	1.80	0.41	0.07	
	Control	15	0.57	0.31	0.08	
P.P.D.(mm)	RA + PD	45	4.59	0.44	0.07	P<0.001**
	RA	35	2.91	0.22	0.04	
	Control	15	1.78	0.25	0.06	
CAL(mm)	RA + PD	45	6.67	0.92	0.14	P<0.001**
	RA	35	2.82	0.31	0.05	
	Control	15	0.00	0.00	0.00	
B.O.P. (Mean of %)	RA + PD	45	0.73	0.45	0.07	P<0.001**
	RA	35	0.71	0.46	0.08	
	Control	15	0.07	0.26	0.07	

** Highly significant at p <0.001

Table 2: Inter group Comparison of the mean of clinical periodontal parameters among the three studied groups

Multiple Comparisons				
Parameters	Group (I)	Group (J)	P-value	C.S.(*)
PL.I	RA + PD	RA	0.001	HS
		Control	0.000	HS
	RA	Control	0.000	HS
G.I	RA + PD	RA	0.077	NS
		Control	0.000	HS
	RA	Control	0.000	HS
P.P.D.	RA + PD	RA	0.000	HS
		Control	0.000	HS
	RA	Control	0.000	HS
CAL	RA + PD	RA	0.000	HS
		Control	0.000	HS
	RA	Control	0.000	HS
B.O.P.	RA + PD	RA	0.840	NS
		Control	0.001	HS
	RA	Control	0.002	HS

(*) HS: Highly Significant at P< 0.001 ; NS: Non Significant at P>0.05

Table 3: Mean serum level of ACCP differences among different groups

Groups	No.	Mean	Std. Dev.	Std. Error	p-value
RA + PD	45	96.32	92.24	13.75	0.001 HS
RA	35	29.14	36.31	6.14	
Control	15	1.03	0.35	0.09	

Hs: Highly significant at p<0.001

Table 4: Inter group comparison regarding ACCP values among the three studied groups

Multiple Comparisons(*)			
Group	Group	P-value	C.S.(*)
RA + PD	RA	0.000	HS
	Control	0.000	HS
RA	Control	0.018	S

(*) HS: Highly Significant at P< 0.001; S: Significant at P<0.05

Table 5: Spearman's correlation coefficient with probability levels for Periodontal Parameters and ACCP values in the three studied groups

Groups	ACCP value	PL.I	G.I	P.P.D.	CAL	B.O.P.
RA+ PD	Corr. Coeff.	0.472	0.335	-0.019	0.041	-0.170
	Sig.(2-tailed)	0.001	0.024	0.902	0.787	0.263
	No.	45	45	45	45	45
RA	Corr. Coeff.	0.023	0.219	0.316	0.012	-0.003
	Sig(2-tailed)	0.897	0.205	0.065	0.944	0.986
	No.	35	35	35	35	35