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Correlation between chronic periodontitis and rheumatoid arthritis: A periodontist perspective

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ABSTRACT

In this study, we evaluate the relationship between rheumatoid arthritis (RA) and chronic periodontitis on the basis of clinical attachment present and severity of attachment loss in both the cases. First of all Diagnosis of rheumatoid arthritis and chronic periodontitis was performed, thereafter bacterial DNA extraction from blood serum sample and subgingival dental plaque of each group through PCR and later DNA purification through Spin protocol was performed, oligonucleotide primer was used to detect t.forcythia and PCR amplification was done to detect T. Denticola for both the groups .PBDNA was detected in both SGP and serum samples of both the groups. In SGP samples, Tannerella forsythia was more frequently detected as compared to serum samples of both the groups. In result theclinical attachment Level (CAL) was observed to be higher in RA group as compared to CP group.

Comparison of CAL according to severity was also observed in both the groups which suggested that RA group has mild periodontitis as compared to CP group in which moderate to severe periodontitis was seen, Detection of periodontal bacterial DNA by PCR assay PBDNA was detected in both SGP and serum samples. In SGP samples, Tannerella forsythia was more frequently detected as compared to serum samples of both the groups. So these are two common chronic inflammatory diseases with a similar host-mediated pathogenesis. Current evidence suggests that an association exists between periodontitis and RA. Well-designed multicenter longitudinal clinical trials and studies with sufficient sample sizes are needed to ascertain the relationship between these two diseases and whether periodontal treatment can reduce the severity of RA or prevent its onset.

1. Introduction

Chronic Periodontitis (CP) is an infective disease characterized by an irreversible loss of connective tissue and bone of the periodontal apparatus. (Napimoga MH et al. 2009).[1] Although the etiology of CP is multifactorial, accumulation of dental biofilm on the tooth and gingival surfaces is considered as the primary etiological factor in CP with specific increase in pathogenic and virulent strains of microorganisms such as Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola which constitute the "red complex" (Lang NP et al.2009).[2]

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease primarily affecting the joints that, if left untreated, results in functional disability concordant with radiographic progression. It has a higher predilection for females than males (2–3:1) (Gabriel et al. 1999, Symmons et al. 2002),[3] starts in the fourth or fifth decade

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of life.

It is well recognized that both these diseases share pathogenic similarities. Bone metabolism is integral in both the diseases, and the role of the receptor activator of nuclear 'factor-kappa B ligand (RANKL) and its inhibitor osteoprotegerin(OPG) in bone/jaw resorption and joint erosions, is well established .(Napimoga MH.2009 Schett G. 2005. Le XK.2009).[4,5] In addition, these two conditions share common genetic and environmental epidemiologic risk factors (Marotte H 2006, Bonfil JJ.1999).[6] Indeed, several observational studies demonstrated a high prevalence of periodontitis in patients with RA compared to the general population. Moreover, the presence of periodontitis in subjects with RA was associated with an increased level of acute-phase reactants (c-reactive protein and ESR) and tender and/or swollen joints (Abou-Raya A .2005, Bingham CO III.2009).[7–9] So, based on these studies, it was postulated that periodontitis may be a risk factor for both the onset and progression of RA. The red-complex bacteria which constitutes Porphyromonasgingivalis, Treponema denticolaand Tannerella forsythia have been strongly implicated in the etiology of chronic periodontitis. Among these, Porphyromonas gingivalis is the only known bacterium expressing the peptidyl arginine deiminase (PAD) which is responsible for the post translation and conversion of arginine to citrulline, leading to the production of antibodies to cyclic citrullinated peptides (ACCPA). ACCPA are considered as specific markers for RA, since they are found in 80% of the sera of patients with RA, with a 99% specificity (van Venrooij WJ et al.2002).[10] This supported the hypotheses that periodontopathogens involved in periodontitis may also contribute to the etiology of RA (Lundberg K et al.2010).[11] The aim of this study was to detect the presence of Tannerella forsythia from both the subgingival plaque (SGP) and serum samples from patients affected by RA and periodontitis. Moreover, the correlation of this bacterium in these two diseases and how these two diseases are interrelated was also explored.

2. Materials and Methods

This study involved 120 subjects, divided into two groups i.e., Group I&II. Recruitment of the subjects was undertaken at the Department of Periodontology, Faculty of Dental Sciences, in collaboration with Department of Rheumatology. Informed and voluntary written consent from patients was obtained before start of the clinical examination according to the ethical principles. Subjects involved in this study were both males and females in the age group between 25-65 yrs, divided in two groups, one having RA and other having CP. Subjects suffering from connective tissue disorder, smokers, pregnant and lactating women were excluded from the study.

Diagnosis of RA was performed by a rheumatologist in accordance with the criteria of the American rheumatism association (ARA) 1987 revised criteria, 1987. All patients were under two or more DMARDs, most of them with nonsteroidal anti-inflammatory drugs and low doses of steroids.

Diagnosis of chronic periodontitis was made in acc to AAP workshop, 1999 and it was determined by measuring pocket depth and clinical attachment loss indexes (Rodrı´guez-Martı´nez et al. 2006). These indexes were obtained by using a UNC- 15 periodontal probe graduated in millimetres (0–15 mm). Clinical attachment loss index was measured from the epithelial attachment to the cement – enamel junction.

2.1. Serum sample

Ten milliliters of peripheral blood from the cubital vein was obtained within the citrated vacuum tubes. 5ml of the blood was collected for bacterial DNA extraction and remaining 5ml was collected in clot accelerator vaccum tube for ACCPA evaluation. It was centrifuged at 275g for 8 min. to obtain the serum.

2.2. Subgingival plaque sample (SGP)

SDP was collected with a Gracey curette from the buccal, mesial, palatal and distal sulcus and placed into an eppendorf tube with 1ml of phosphate-buffered saline (PBS). Subgingival dental plaque was obtained after collection of blood samples, to avoid transitory bacteraemia which might influence the presence of the different bacterial species in serum. SDP and peripheral blood samples were transported on ice and were stored at -40 C until PCR were performed.

2.3. Bacterial DNA extarction

All samples were processed with aseptic requirements to prevent contamination from both environment and during the DNA extraction method for PCR assays. The DNA was extracted with the help of QiAgen DNA extraction kit (QiAgen, Inc., vAlenciA, CA).

2.4. DNA Purification from Blood and subgingival dental plaque (SDP) using Spin Protocol)

20 μl QIAGEN Protease (or proteinase K) were pipetted into the bottom of a 1.5 ml micro centrifuge tube each for serum and SGP sample. 200μl PBS were added to serum sample and 400 μl PBS to SGP. 200 μl Buffer AL were added to the serum sample and 400μl Buffer AL to SGP sample. Mixed by pulse-vortexing for 15 s. And was Incubated at 56°C for 10 min. Than 1.5 ml micro centrifuge tube was Briefly centrifuged to remove drops from the inside of the lid. 200μl ethanol (96–100%) were added to the serum sample and 400 μl ethanol (96–100%) were added to the SGP sample, and mixing was done by pulse-vortexing for 15 s. After mixing,

the 1.5 ml micro centrifuge tube was briefly centrifuged to remove drops from the inside of the lid. Complete mixture of serum sample and 700μl of the mixture of SDP sample was carefully applied to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim and was centrifuge at 8000 rpm for 1 min. the QIAamp Mini spin column was place in a clean 2 ml collection tube, and the tube containing the filtrate was discarded. The QIAamp Mini spin column were carefully opened and 500μl Buffer AW1 were added to both sample without wetting the rim. and was centrifuge at 8000 rpm for 1 min. QIAamp Mini spin column were place in a clean 2 ml collection tube, and the collection tube containing the filtrate was discarded. Than QIAamp Mini spin column were open and 500μl Buffer AW2 was added to both sample without wetting the rim and was centrifuge at full speed 14,000 rpm for 3 min. And was recommended to Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. And was Centrifuge at full speed for 1 min. QIAamp Mini spin column were place in a clean 1.5 ml microcentrifuge tube, and the tube containing the filtrate was discarded. the QIAamp Mini spin column were open carefully and 200μl Buffer AE or distilled water was added and Incubated at room temperature (15–25°C) for 1 min, and was centrifuge at 8000 rpm for 1 min.

2.5. Oligonucleotide primers

Species-specific oligonucleotide primer 16S rDNA was used to detect the T. forsythia. Targeting signature sequence was forward-5'-GCG TAT GTA ACC TGC CCG CA-3' and reverse-5'-TGC TTC AGT GTC AGT TAT ACC T-3' (21). The expected size of the T. forsythia-specific PCR product is 641 bp.

2.6. Polymerase chain reaction (PCR) Amplification

PCR assay was carried out in 25 ml of a reaction mixture containing 1.0U Taq DNA polymerase (Roche, Indianapolis, IN, USA) 0.5 mM of oligonucleotides, 0.2mM of dNTPs, 1.5mM of MgCl2 and 10 ng of DNA template.

The specific primer used for T denticola in the study is indicated in the Table 1.

PCR reaction was performed in a thermal cycler (Applied Biosystem, ABI Fostercity, USA) with the cycling parameters reported (Ashimoto et al. 1996, Tran &Rudney 1996, Stubbs et al. 1999, Suzuki et al. 2001).[12,13] Positive controls were included in each PCR set by using DNA of the bacterial strains: T. forsythia (ATCC 43037). A negative control was also included in each PCR set, it was a blank sample with only deionized water (instead of patient sample) to know if unspecific products were amplified. The PCR products were analysed by electrophoresis in a 2% agarose gel using Tris– boric acid–EDTA buffer, using a 100 bp DNA ladder marker (New England Biolab, Beverly, MA, USA) to estimate the molecular size. Each gel was stained with ethidium bromide (0.5 mg/ml) and photographed under UV light (Bio-Rad Gel documentation system).

3. Results

Table 1: Clinical attachment level (CAL)

	RA Group (n=60)	**CP Group (n=60)**	**P value**
	Mean ± SD	Mean ± SD	
CAL in mm	2.74 ± 0.90	3.73 ± 0.92	<0.001

Table 2: Comparison of CAL in severity of chronic periodontitis

CAL in mm	**RA Group (n=60)**	**CP Group (n=60)**
	Mean ± SD	Mean ± SD
Mild Periodontitis	2.24 ± 0.30	2.56 ± 0.53
Moder/ate Periodontitis	3.10 ± 0.54	3.63 ± 0.70
Severe Periodontitis	6.34 ± 0.21	5.42 ± 1.12

3.1. Type of periodontitis according to CAL

Mild type of chronic periodontitis was more frequently diagnosed (55%) in the RA group than moderate and severe stages (41.7% & 3.3% respectively) while in the CP group, moderate stage (86.7%) was more frequently seen than mild and severe stages (5% & 8.3% respectively).

3.2. Detection of periodontal bacterial DNA by PCR assay

PBDNA was detected in both SGP and serum samples. In SGP samples, Tannerella forsythia was more frequently detected as compared to serum samples of both the groups

Table 3: Periodontal bacterial DNA detected in DP and SE

T.forsythia presence in plaque	**RA Group**		**CP Group**		**P value**
	N	%	N	%	
Present	43	71.7	44	73.3	0.83

T.forsythia presence in blood	**RA Group**		**CP Group**		**P value**
	N	%	N	%	
Present	19	31.7	10	16.7	0.055

4. Discussion

The aetiology of Chronic Periodontitis is multifactorial in nature of which Gram-negative bacteria is the most

important. Periodontitis has been associated with RA whose aetiology is still unknown, although some reports have indicated that an infectious agent in a susceptible host could be one possible trigger factor. (Carty et al. 2004).[14] Unlike RA, the possible common aetiopathogenesis of these two entities and the immunological profile of periodontitis (Pd) have not been studied extensively in the literature. In this study, the presence of Tannerella forsythia in serum and subgingival dental plaque of CP and RA patients was evaluated and whether any possible correlation exists between these two diseases. The bacterial DNA (PBDNA) by PCR in subgingival dental plaque (SDP), and serum of rheumatoid arthritis (RA) with periodontal disease (PD) patients were investigated by Martinez-Martinez RE 2009.[15] The prevalence of chronic periodontitis in rheumatoid arthritis in the study is based on numerous data which suggests a correlation between the two diseases. Many well-conducted studies have shown that patients with RA have an increased suspectibilty of expressing mild to severe Periodontitis. The mean of CAL describes the prevalence and severity of periodontal disease which is based on the concept that all individuals and all sites within an individual are equally susceptible to periodontal breakdown (Baelum V 1988).[16] In the present study, the mean clinical attachment level in both the groups was 2.74 mm and 3.73 mm respectively which was clinically significant. RA Group patients were mostly affected by mild periodontitis (55%) followed by moderate (41.7%) and (severe periodontitis (3.3%), respectively. In the CP group, patients were mostly affected by moderate periodontitis (86.7%) followed by severe periodontitis (8.3%). These data are in accordance with the findings of Eduardo de Paula Ishi (2008).[17] In the present study, CAL was compared to assess the severity of periodontitis. RA group patients with moderate and severe periodontitis were found to be highly significant $p<0.001$ and patients with mild periodontitis were found to be statistically insignificant with $p>0.05$. (Martinez-Martinez RE 2009, Mercado et al. 2001).[15] Mean CAL in CP patients group II was found higher than group I. This was due to fact that all the RA patients in our study used DMARDs while some used non steroidalanti-inflammatory drugs. These drugs might protect the periodontal tissues from destruction. (Holzhausen M 2002)[18] Periodontal bacterial DNA was detected in SGP samples and in the serum samples. By using PCR, the presence of T.forsythia in plaque was found to be 71.7% and 73.3% respectively in RA and CP groups which was statistically insignificant. The presence of T.forsythia in serum was 31.7% and 16.7% respectively in both the groups, which was statistically insignificant. Similar findings were observed by Martinez-Martinez RE 2009[15] where patients showed PBDNA in SGP and 83.5% in serum.

Finally, results of our study suggested that there were no major differences in the compared groups. The severity of CAL was found to be statistically significant in patients of moderate and severe periodontitis. The presence of periodontopathic bacteria in both the groups suggests an association between the two diseases i.e. rheumatoid arthritis and chronic periodontitis. This information can be of value for future studies with larger sample size and elucidated if PBDNA could be one possible trigger for RA development.

5. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

6. Source of Funding

None.

References

1. Napimoga MH, Benatti BB, Lima FO, Alves PM, Campos AC, Pena-Dos-Santos DR, et al. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. *Int Immunopharmacology*. 2009;9(2):216–22.
2. Lang NP, Schätzle MA, Löe H. Gingivitis as a risk factor in periodontal disease. *J Clin Periodontol*. 2009;36(s10):3–8.
3. Gabriel SE. The epidemiology of rheumatoid arthritis. *Rheumatic Dis Clin North Am*. 2001;27(2):269–81.
4. Schett G, Hayer S, Zwerina J, Redlich K, Smolen JS. Mechanisms of disease: the link between RANKL and arthritic bone disease. *Nat Clin Pract Rheumatol*. 2005;1(1):47–54.
5. Le XK, Laflamme C, Rouabhia M. Porphyromonasgingivalis decreases osteoblast proliferation through IL-6-RANKL/OPG and MMP-9/TIMPs pathways. *Indian J Dent Res*. 2009;20(2):141–9. doi:10.4103/0970-9290.52884.
6. Marotte H, Farge P, Gaudin P, Alexandre C, Mougin B, Miossec P, et al. The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. *Ann Rheumatic Dis*. 2006;65(7):905–9.
7. Abou-Raya A, Abou-Raya S, Abu-Elkheir H. Periodontal disease and rheumatoid arthritis: is there a link? *Scand J Rheumatol*. 2005;34(5):408–10.
8. III COB, Giles JT, Jones PE, Bathon JM, and SJB. High prevalence of moderate to severe periodontal disease in rheumatoid arthritis patients. *Arthritis Rheum*. 2007;56:420.
9. Bonfil JJ, Dillier FL, Mercier P, Reviron D, Foti B, Sambuc R, et al. A "case control" study on the role of HLA DR4 in severe periodontitis and rapidly progressive periodontitis. *J Clin Periodontol*. 1999;26(2):77–84.
10. Van Venrooij WJ, Hazes JM, Visser H. Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. *Neth J Med*. 2002;60(10):383–8.
11. Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA-the citrullinated enolase connection. *Nat Rev Rheumatol*. 2010;6(12):727–30.
12. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol*. 1996;11(4):266–73.
13. Tran SD, Rudney JD. Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of Actinobacillusactinomycetemcomitans and Porphyromonasgingivalis. *J Clin Microbiol*. 1996;34(11):2674–8.

14. Carty SM, Snowden NEIL, Silman AJ. Should infection still be considered as the most likely triggering factor for rheumatoid arthritis. *Ann Rheumatic Dis*. 2004;63(2):46–9.
15. Martinez-Martinez RE, Abud-Mendoza C, Patiño-Marin N, Rizo-Rodríguez JC, Little JW, Loyola-Rodríguez JP, et al. Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. *J Clin Periodontol*. 2009;36(12):1004–4.
16. Baelum V, Fejerskov O, Manji F. Periodontal diseases in adult Kenyans. *J Clin Periodontol*. 1988;15(7):445–52.
17. Ishi EDP, Bertolo MB, Rossa C, Kirkwood KL, Onofre MA. Periodontal condition in patients with rheumatoid arthritis. *Braz Oral Res*. 2008;22(1):72–7.
18. Holzhausen M, Rossa C, Marcantonio E, Nassar PO, Spolidório DM, Spolidório LC, et al. Effect of selective cyclooxygenase-2 inhibition on the development of ligature-induced periodontitis in rats. *J Periodontol*. 2002;73(9):1030–6.

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