

Effect of MMP-13 levels on Disease Modifying Antirheumatic Drugs(DMARDs) and Corticosteroids on Rheumatoid Arthritis Patients with Chronic Periodontitis - A Biochemical Analysis

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To investigate the effect of anti-rheumatic DMARD and anti-inflammatory steroids in rheumatoid arthritis patients with chronic Periodontitis and also to estimate the levels of inflammatory biomarker MMP-13 in rheumatoid arthritis patients with chronic Periodontitis. A total of 90 subjects participated in the study. They were divided into three groups, Group I- 30 RA patients with CP who are consuming DMARD medications, Group II- 30 RA patients with CP who are consuming steroids and Group III- 30 population controls. The medications used by the rheumatoid arthritis patients were confirmed by a rheumatologist from the patients clinical records, based on the duration of the diseases, use of DMARDs, use of steroids, serological markers of RA, ACPA (anti-citrullinated peptide antibody), RF(rheumatoid factor) and no of swollen tender joints were determined. The Disease activity score(DAS 28) was calculated from the no of tender and swollen joints (28 joint count). Subsequent analysis for mmp-13 was done by enzyme linked immunosorbent assay (ELISA). The serum MMP -13 levels in the serum of the healthy control group had significantly lower mean and standard deviation when compared to group I and II. The MMP-13 levels were higher in patients taking DMARDs when compared with the patients on steroid medications, which were statistically significant ($P < 0.001$). In our study, MMP-13 levels are raised in DMARD group and decreased in the corticosteroid group with an increase in the periodontal parameters such as pocket depth and CAL. The possibility of periodontal destruction would have happened much before and the treatment on steroids would have led to remission, thereby reduction in the MMP13 levels was noted.

Keywords: Chronic periodontitis, DAS 28, MMP-13, Rheumatoid arthritis, Pocket probing depth, Disease modifying anti-rheumatic drugs, Corticosteroids.

Periodontitis is a chronic, multifactorial, slowly progressing inflammatory disease within the supporting structure of the teeth, causing clinical attachment loss and ultimately leads to destruction of the alveolar bone.¹ It has been evidenced from previous studies that the proinflammatory cytokines, MMPs, NO and other inflammatory

mediators have been increased and plays a major role in the pathogenesis of periodontal disease. The main etiological agent in Periodontitis is the subgingival biofilm.² The dysregulation in the host inflammatory response plays a significant role in the development of Periodontitis, therefore, host modification is only an adjunctive treatment for Periodontitis. The causative agent is still not identified in Rheumatoid arthritis (RA), host modification is the primary treatment of choice.

The balance between bone formation and bone resorption is the normal physiological

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remodeling process, when there is imbalance exists it leads to various systemic diseases such as Rheumatoid arthritis (RA). Rheumatoid arthritis is defined as chronic autoimmune inflammatory disease which is characterized by acquisition of inflammatory infiltrate in the synovial membrane leading to destruction of joints architecture and synovitis.³ RA occurs in 1% of the world wide population with the predominance of females, three times more than males.^{4,5}

In the management of rheumatoid arthritis, the use of conventional synthetic Disease modifying anti-rheumatic drugs (DMARDs), biological DMARDs, low dose steroids and NSAIDs (Non-steroidal anti-inflammatory drugs) are known to suppress active inflammation.^{6,7} Also patients who are suffering from rheumatoid arthritis are said to have an increased risk for infection. Periodontitis have been associated with rheumatoid arthritis and it has even been suspected to be a triggering factor for rheumatoid arthritis eruption. The most commonly used drug in rheumatoid arthritis is methotrexate, because of the disadvantage of toxicity, their use in Periodontitis is minimal.

The gram negative micro-organisms which is responsible in causing Periodontitis, leading to tissue destruction, induce the host cells to release Matrix Metalloproteinase (MMPs). MMPs are enzymes which act both in the physiologic development and also in the pathologic destruction.⁸ The cells that release MMPs in Periodontitis and Rheumatoid arthritis are polymorphonuclear leucocytes, monocytic phagocytes, macrophage and fibroblast. Five major group of MMPs have been identified of which MMP-13 is an interstitial collagenase which is involved in periodontal and rheumatoid tissue destruction.^{9,10,11} Earlier studies by Fuller and chambers have shown that the MMP-13 was expressed in the osteoblastic cell lines located adjacent to osteoclasts at sites of active bone resorption.¹²

It was hypothesized that association with rheumatoid arthritis and chronic Periodontitis exists that the severity of periodontal inflammation is masked by the drugs which have been used in the management of rheumatoid arthritis, also the modification in the serum levels of MMP-13 may partially explain the plausible mechanism acting

in the influence of these two chronic inflammatory diseases.

Many previous studies have given contradicting results regarding the association between chronic Periodontitis and rheumatoid arthritis. However, a significant association between the two common chronic diseases has been reported recently.^{13,14,15,16} Not many studies have been done on the treatment for rheumatoid arthritis in patients suffering from chronic Periodontitis. This study was to investigate the effect of anti-rheumatic DMARDs, and anti-inflammatory steroids in rheumatoid arthritis patients with chronic Periodontitis, also to estimate the levels of inflammatory biomarker MMP-13 in rheumatoid arthritis patients with chronic Periodontitis.

MATERIAL AND METHODS

A total of 90 subjects participated in the study, and they were divided into three groups

Group I- 30 RA patients with CP who are consuming DMARDs medications. Group II- 30 RA patients with CP who are consuming steroids. Group III- 30 population controls.

All the participants underwent a general rheumatological and full mouth periodontal examination. Rheumatoid arthritis patients were selected by a rheumatologist from the department of rheumatology, Saveetha medical college, Chennai. They were selected based on the American college of Rheumatology (ACR) criteria 2010.¹⁷ The medications used by the rheumatoid arthritis patients were confirmed by a rheumatologist from the patients clinical records, based on the duration of the diseases, use of DMARDs, use of steroids and, serological markers of RA, Anti-citrullinated peptide antibody (ACPA), Rheumatoid factor (RF), no of swollen tender joints were determined. The Disease activity score (DAS 28) was calculated from the no. of tender and swollen joints (28 joint count). Steroid was very commonly used medication in the treatment of RA with long duration, so our study aimed in comparing steroid with DMARDs. The criteria for chronic Periodontitis was selected based on the American academy of periodontology in 1999¹⁸, an intraoral examination of periodontal examination was conducted which included periodontal

pocket depth, clinical attachment level, bleeding on probing and tooth mobility. Oral hygiene maintenance was assessed by questionnaire; all the patients were on mechanical plaque control with the use of toothpaste and toothbrush, brushing once a day. Clinical periodontal parameters were recorded at six sites/tooth and the site with the deepest probing depth and CAL were taken into consideration. The healthy controls were age/gender matched without any systemic disease and no sign of periodontal disease, were selected from Thai Moogambigai dental college, Chennai and were enrolled in the study.

Informed consent was obtained from all the participants and the study protocol was approved by the Saveetha medical college, ethical committee, Chennai.

2ml of blood was collected from the antecubital fossa of each patients by venipuncture, using a 20 gauge needle with heparin tubes and immediately transferred to laboratory. The blood was allowed to clot at room temperature and after 1hour, serum was separated from blood by centrifuging for 20 minutes at 3000 revolutions/minute (RPM) and subsequent analysis for mmp-13 was done by enzyme linked immunosorbent assay (ELISA).

MMP-13 Analysis

Serum MMP-13 levels were measured using commercially available kit, according to the manufacturer's instructions using Human Matrix Metalloproteinase-13 (MMP-13) ELISA kit obtained from Bioassay Technology Laboratory (218, Ningguo Road, Yangpu District, Shanghai, China) used to detect the enzyme levels in the sample in duplicates. The kit made use of biotinylated anti-human MMP-13 antibody and Avidin-Biotin-Peroxidase Complex. Absorbance of the substrate color reaction was read on Micro well Plate ELISA Reader (ThermoFisher Scientific Instrument Co., Ltd, Shanghai Shi, China) using 450 nm wavelengths.

The sensitivity of the assays was 0.04 ng/ml. Intra-assay coefficient of variation was <8% and inter-assay coefficient of variation was <10.0%. Results are expressed as ng/ml.

Statistical analysis

The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results reveal that some variables (Age, MMP 13 levels, Disease

duration, ESR, DAS 28, and CRp) follow Normal distribution and some variables (PD, CAL, PI and Missing teeth) do NOT follow Normal distribution. Therefore to analyse the data both Parametric and Non parametric methods are applied. To compare the mean values between all three groups one way ANOVA is applied followed by Tukey's HSD post hoc tests for multiple pairwise comparisons. To compare the mean values between two groups independent sample t-test is applied. To compare proportions between groups Chi-Square test is applied. For variables which do NOT follow Normal distribution, to compare between Groups Kruskal Wallis test is used followed by Bonferroni adjusted Mann Whitney test for multiple pair wise comparison. To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp. Released 2013) is used. Significance level is fixed as 5% ($\alpha = 0.05$).

RESULTS

Demographic data gender was given in table 1. Statistically significant ($P=0.001$) difference was observed in relation to proportions of genders between groups. There was increase in the number of females in the RA group when compared with the controls.

On comparing the clinical parameters involved in the study group on table 2, result showed that statistically significant ($P<0.001$) difference was observed among all groups in relation to Probing depth, clinical attachment levels, plaque index and missing teeth.

The mean values and standard deviation for the laboratory markers of RA were given in Table 3. The RA patients with chronic Periodontitis were grouped according to the medication taken for the remission of RA. The duration of disease activity in RA, was found to show a statistically significant ($P<0.001$) difference between groups I and II. T-Test comparison analysis exhibited no significant correlation among the laboratory markers of RA

The serum MMP-13 levels were depicted in Table 4. RA. The serum MMP-13 levels in the serum of the healthy control group had significantly lower mean and standard deviation when compared to group I and II. The MMP-13 levels were higher in patients taking DMARDs when compared with

Table 1. Demographic Data on the Basics of Gender Difference

Gender	Group						Total		P value
	Group-I		Group-II		Group-III		N	%	
	N	%	N	%	N	%	N	%	
Male	8	26.7	4	13.3	17	56.7	29	32.2	0.001
Female	22	73.3	26	86.7	13	43.3	61	67.8	
Total	30	100.0	30	100.0	30	100.0	90	100.0	

Table 2. Clinical Periodontal Parameters of the Study Groups

Variables	Group	N	Mean \pm Std dev	Mean Rank	P-Value
Probing depth	Group-I	30	6.87 \pm 0.90	59.23	<0.001
	Group-II	30	7.07 \pm 1.081	61.77	
	Group-III	30	1.53 \pm 0.507	15.50	
CAL	Group-I	30	8.80 \pm 1.031	60.83	<0.001
	Group-II	30	8.87 \pm 1.332	60.17	
	Group-III	30	0.33 \pm 0.479	15.50	
Plaque index	Group-I	30	2.67 \pm 0.390	63.15	<0.001
	Group-II	30	2.52 \pm 0.447	57.78	
	Group-III	30	0.72 \pm 0.449	15.57	
Missing teeth	Group-I	30	1.07 \pm 1.596	46.83	0.005
	Group-II	30	1.13 \pm 0.993	54.80	
	Group-III	30	0.37 \pm 0.669	34.87	

Table 3. Laboratory Markers In Patients With RA

Variables	Group	N	Mean	Std. Dev	t-Value	P-Value
Disease duration (yrs; mean \pm SD)	Group-I	30	1.67	.758	4.334	<0.001
	Group-II	30	2.77	1.165		
DAS 28 score	Group-I	30	3.3647	1.96772	1.024	0.310
	Group-II	30	3.8230	1.46193		
ESR (mm/hour; mean \pm SD)	Group-I	30	36.57	23.354	0.896	0.374
	Group-II	30	42.13	24.734		
CRP ((mg/l; mean \pm SD)	Group-I	30	36.633	7.0183	0.256	0.799
	Group-II	30	36.183	6.5684		

Table 4. Biochemical Data (MMP13) Obtained From Serum Samples

Biochemical Variable	Medication Group	N	Mean	Std. Dev	F-value	P-value
MMP13 Levels	DMARDs medication(Group-I)	30	2.9075	.63542	68.569	<0.001
	STEROID medication (Group-II)	30	1.9553	.50443		
	HEALTHY CONTROLS(Group-III)	30	1.2780	.47042		
	Total	90	2.0469	.85926		

the patients on steroid medications, which were statistically significant ($P < 0.001$).

DISCUSSION

Chronic Periodontitis and RA are two chronic inflammatory diseases, with a common pathologic deregulatory pathway which occurs mainly in synovial joint and in gingival crevice, finally leading to destruction of bone. Both of these are chronic inflammatory diseases. They share multifactorial etiology, and the RA patients are treated with anti-inflammatory therapy and DMARDs which will suppress the prostaglandin production, thereby reducing periodontal Inflammation.¹⁹ The literature review showed that the basic difference between both diseases is that RA is an inflammatory autoimmune disease, while PD is an immune-inflammatory disease of bacterial origin.²⁰

Various experimental and observational studies in animals and in humans have been conducted to confirm the association between periodontal inflammation and RA. Previous studies have proposed that, there was not much effect with the use of non-steroidal inflammatory agents in both these chronic inflammatory diseases. The evolution of new anti-rheumatic agents, including synthetic DMARDs and biological DMARDs, which act by targeting the specific cells in the inflammatory pathway, most likely to slow down or inhibit the progression of these inflammatory conditions.

Corticosteroids are powerful anti-inflammatory and immunosuppressive agents that have been used in the treatment of RA, which will reduce the inflammation, pain and will slow down the destruction of the joints. Disease modifying antirheumatic drugs, including methotrexate, leflunomide, hydroxychloroquine, sulphasalazine, and minocycline, and immunosuppressants are frequently given to slow the progression of erosive articular damage over a time period.

Earlier reports by a Dutch compared prednisolone 10 mg daily with placebo and reported a clinical improvement over 12 weeks period.²¹ In spite of this, they showed a rebound deterioration when the dose of prednisolone was reduced. Finally, the Arthritis and Rheumatism Council did a study on 128 subjects, who were

randomly selected to consume prednisolone 7.5 mg daily or placebo in addition to nonsteroidal and disease modifying agents.²²

A metaanalysis was conducted to compare the prednisolone at a dose of 2.515 mg daily with placebo or nonsteroidal antiinflammatory drugs.²³ They showed a great improvement with the low dose of prednisolone when compared with NSAIDs. There was a tremendous improvement in joint tenderness, pain, and strength. Regardless, the results was in concurrence with many rheumatologists that prednisolone at these doses is an effective antiinflammatory agent.

The most traditional and commonly used biomarker for RA is ESR and CRP, both found to be associated with the severity of the diseases. Several other markers have also been implicated for their prognostic evaluation in RA. One such is the matrix metalloproteinases (MMPs), zinc dependent proteases that regulate extracellular matrix proteolysis and are involved in the cleavage of cytokines, chemokines, thus they play a vital role in inflammation. Various types of MMPs have been found in both periodontal and rheumatoid arthritis inflammation. The three classical collagenases, interstitial collagenase (MMP1), neutrophil collagenase (MMP8), and collagenase 3 (MMP13), all cleave a specific scissile bond in the triple-helical collagens at one specific site. This redundancy, also observed for the stromelysins, ensures that the biological processes of ECM remodeling can take place under various conditions by different cell types.

In this study, we examined on the effect of the use of antirheumatic medication and glucocorticoids on the clinical periodontal parameters, also the effect of these drugs on MMP-13 were also observed. Therefore, we included 30 patients consuming synthetic DMARDs and another group of 30 patients consuming steroids, and 30 healthy controls. It has been suggested that both these drugs works on the principal to suppress the body's hyperactive immune and/or inflammatory response, there by leads to decrease pain and inflammation, eventually will reduce or prevent joint damage, and to preserve the structure and function of the joints, to make a better standard of living in RA patients. To the best of our understanding, this is the first study to evaluate MMP-13 levels in drugs such as DMARDs and

steroid medications in serum samples of patients with RA with chronic Periodontitis.

In this study, 22 females and 8 males participated (73.3%) in the DMARDs group and 26 females and 4 males participated (86.7%) in the corticosteroid group, where as in the controls 17 males and 13 females (43.3%). There was a female prediction in the RA group compared with controls, which was in accordance with other studies which reported that females were three times more likely to develop RA than males .

In our study, the periodontal finding in RA patients were different in both the groups, there was an increase in periodontal pocket depth, clinical attachment levels and missing teeth was found to be higher in patients consuming steroids, not much difference was observed in the plaque score between the group I and group II, but it was found to be reduced in the control group. Our findings were in contradiction with Sjostrom et al²⁴ suggested that the periodontal findings in RA and control group were similar. The study results are in agreement with various previous reports and this may be due to raised production of pro-inflammatory mediators in both RA medication group.^{25,26,27}

In an another study, Miranda et al²⁸ recorded clinical periodontal parameters, ESR, and CRP in juvenile idiopathic arthritis (JIA) and compared with controls, significantly higher values of ESR and CRP in the arthritis group. The same has been observed in our study that there is an increase in the ESR values in the steroid medication group compared with the DMARDs group, but not much difference was noted in the CRP levels. CRP level was initially thought to be correlated with the severity of periodontal disease expressed in terms of clinical periodontal measurements^{29,30} and it is considered a marker of systemic inflammatory activity in RA.³¹ In our present findings, there was no significance in the serum CRP levels between both the medication groups, hence our study also do not support for the hypothesis that serum levels of CRP are associated with periodontal attachment loss. For that reason, considerable difference in the levels of CRP depress its application as a reliable marker for periodontal tissue destruction.

In this study all patients in the steroid group were consuming wysolone (5mg) daily, and

the DMARDs group were using Methotrexate and sulfasalazine regularly, and the duration of the disease was long with steroid medication (2.77 ± 1.165 years), compared with the DMARDs. In our study, there is an increase in the serum MMP-13 levels in the DMARDs group compared with the steroid group, might correlate the presence of the active form of this enzyme with periodontal bone loss and teeth loss occurring during this disease duration . Early treatment for RA starts with the synthetic DMARDs and in non-responsive patients or in long term RA, the patients are started on corticosteroids, possibly leading to a reduction in the periodontal destruction because of its anti-inflammatory property, which probably be the reason for the reduction in MMP-13 levels.

One possibility that the RA patients are at increased risk for periodontal inflammation, could be due to the long duration of RA. Since the periodontal pathogens which is also present in the systemic circulation through vascular transportation, respond to the increased levels of inflammatory mediators and can cause destruction in the periodontium. Likelihood, inadequate biofilm removal because of improper brushing technique due to the restricted hand-finger movement, can also increase the risk for causing RAPID (Rheumatoid arthritis periodontal inflammatory disease).

Contrarily, the long-term usage of antirheumatic drugs might have hindered the clinical symptoms of Periodontitis in RA, this could be one of the reason for the absence of difference in the severity of periodontal inflammation between the patients with RA and the systemically healthy controls. This is in agreement with the findings of Ezel et al³² who found that medication such as corticosteroids may decrease gingival inflammation, but it is in disagreement with Gleissner et al³³, no correlation between the medication used and periodontal parameters.

DMARDs and corticosteroids can regulate plaque-associated gingivitis. Data related to plaque index in our study showed a higher value compared to controls, but no statistical difference was observed between group I and group II, this may have been due to the prolonged use of anti-inflammatory and anti-rheumatic drugs. Another possibility is that patients in the control group,

can be selected on biased by their oral hygiene status, which would not be effective in the reduction of marginal inflammation.

Thus, the finding of increased clinical attachment level among patients using these anti-inflammatory medications can be interpreted as indication of the strength of the effect of RA on the pathogenesis of periodontal diseases, this finding is supported by a Biyikoglu B *et al*³⁴ MMP-13 is considered as a marker of disease activity progression, primarily proceed towards the function and relevance of this metalloproteinase during the progression of Periodontitis in RA patients.

CONCLUSION

In our study, MMP-13 levels are raised in DMARDs group and decreased in the corticosteroid group with an increase in the periodontal parameters such as pocket depth and CAL, possibility of periodontal destruction would have happened much before and the treatment on steroids would have lead to remission, thereby reduction in the MMP13 levels. In RA patients, in spite of the fact that anti-inflammatory drugs may reduce the gingival inflammation, no matter what amount of local factors present, resulting in an unfavourable co-relation between plaque index and serum MMP-13 levels, suggests that synthesis and degradation of MMP-13 in RA patients may be dissimilar. Further studies needed to investigate MMP-13 levels in RA with or without drug medication on healthy subjects receiving anti-rheumatic and anti-inflammatory medication, may be helpful in understanding the mechanism of the synthesis and degradation of MMP13 in the pathogenesis of periodontal diseases.

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