

# Assessment of disease activity in Rheumatoid Arthritis using urinary CTx-I levels as a marker of bone destruction and serum IL-6 as a marker of inflammation.

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## Citation

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## Abstract

**Background and Objectives:** Urinary C-terminal cross linking telopeptide of type 1 collagen (CTX-I) is a specific marker of bone resorption. Also, IL-6 in the synovial cavity may contribute to that in the serum and may raise its levels. The aim of this study was to measure urinary CTx-I and serum interleukin-6 (IL-6) levels in rheumatoid arthritis (RA) patients and to use these markers to assess the disease activity by comparing them with the existing markers of disease activity. **Methods:** 35 RA patients, 18 patients suffering from osteoarthritis (OA) with / without inflammatory synovitis and 18 age - matched healthy controls were included in the study. Urinary CTx-I and serum IL-6 levels were measured in all of them. **Results:** A total of 71 subjects were included in the study. A positive correlation was found between CTx-I and age ( $p = 0.044$ ,  $r = 0.362$ ), DAS28 ( $p = 0.007$ ,  $r = 0.451$ ), swollen joint count ( $p = 0.006$ ,  $r = 0.452$ ) and tender joint count ( $p = 0.006$ ,  $r = 0.453$ ). Levels did not correlate with erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Rheumatoid factor and disease duration. IL-6 levels were found to have significant associations with age ( $p = 0.012$ ,  $r = 0.42$ ), CRP ( $p = 0.048$ ,  $r = 0.337$ ), DAS28 ( $p = 0.024$ ,  $r = 0.382$ ), swollen joint count ( $p = 0.001$ ,  $r = 0.555$ ) and tender joint count ( $p = 0.04$ ,  $r = 0.349$ ). No correlations were found between IL-6 and ESR, Rheumatoid factor and disease duration. A positive correlation was also seen between CTx-I and IL-6 ( $p = 0.03$ ,  $r = 0.352$ ). **Conclusions:** Serum IL-6 and urinary CTx-I levels are markedly elevated in RA with active disease and their increased levels correlate with the existing markers of increased disease activity.

## INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic multisystem disease of unknown etiology. It is characterized by persistent inflammatory synovitis, usually involving multiple joints in a symmetrical distribution. The potential of the synovial inflammation to cause cartilage and bone erosions and subsequent changes in joint integrity is the hallmark of the disease.<sup>1</sup> The overall prevalence of RA is around 1 % in the general population.<sup>2</sup> India harbors around 5.4 % of the global burden of RA.<sup>3</sup>

Osteoclastic activation has been suggested to be the dominant process leading to bone resorption in Rheumatoid arthritis (RA). Most biochemical indices of bone resorption are related to collagen breakdown products such as hydroxyproline or the various collagen cross links and telopeptides.<sup>4</sup> Of these, urinary C-terminal cross linking telopeptide of type 1 collagen (CTX-I) is a more specific marker of bone derived type 1 collagen fragments in urine in

RA.<sup>5</sup> Cross linking compounds pyridinoline (PYD) and deoxypyridinoline (DPD) levels in urine have previously been studied to assess collagen degradation<sup>6</sup> and disease activity in RA. But CTX- I levels in urine have not been adequately studied.

The appearance of pro-inflammatory cytokines in joint tissue/synovial fluid and serum/plasma of patients with arthritic conditions, suggest that they play a pivotal role in the local and systemic inflammatory response. However in many cases it has not been possible to relate known in vitro effects of pro-inflammatory cytokines to clinical and laboratory observations. IL-6 mediates the synthesis of acute phase proteins.<sup>7</sup> Many attempts at establishing a correlation between this cytokine and the disease activity in RA have been made but the results obtained were inconsistent. However a recent animal trial on mice with antigen-induced arthritis confirms the important role of IL-6 in the development of disease.<sup>8</sup> Also the synovial fluid in

rheumatoid arthritis contains substantially increased amounts of IL-6.<sup>9</sup> Using cytokine probes, it has been claimed that IL-6 in rheumatic synovial fluid originates from type B synovial lining cells and fibroblasts.<sup>1011</sup> This IL-6 in the synovial cavity may contribute to that in the serum and may raise its levels. Thus the present study was conducted to prove that levels of CTx-I in urine and IL-6 in serum of RA patients with active disease are elevated.

## MATERIAL AND METHODS

Before the commencement of the study an approval from the K.E.M. Hospital Ethics Committee for Research on Human Subjects was sought. (No. EC/114/2007)

35 consecutive patients of active RA as defined by the ACR criteria attending the Rheumatology Services OPD of K.E.M. Hospital, Mumbai, over a period of 100 days (May 2007- August 2007) were included in the study. 18 patients suffering from Osteoarthritis (OA) with / without inflammatory synovitis and 18 age- matched healthy controls were also included in the study.

To evaluate disease activity score, haemoglobin (Hb), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), number of swollen joints, tender joints were measured and the 28 joint disease activity score (DAS28) values were calculated. Levels of CTx-I determined by ELISA in spot urine samples were measured (Table 1). Serum IL-6 levels were also measured using commercial ELISA type kits (Table 1).

### Figure 1

Table 1: CTx-I and IL-6 values in the 3 groups

	CTx-I			IL-6		
	Mean	SD	Median	Mean	SD	Median
RA patients	1948.587	736.917	2059.45	19.814	11.379	7.86
OA patients	1715.947	863.88	1943.52	6.330	1.743	6.243
Controls	799.06	528.466	666.78	4.956	4.025	4.939

All data were analyzed for normality using the Kolmogorov Smirnov Test. Mann Whitney U Test was used to compare CTx-I and IL-6 levels in RA, OA patients and controls. Kruskal Wallis H Test was used to compare the 3 groups together. The Karl Pearson test was used to correlate the levels of CTx-I and IL-6 with DAS28, ESR, age, CRP, rheumatoid factor (RF), disease duration, swollen and tender joint count. No formal sample size calculations were done for this study. All analysis was done using the SPSS software version 15.0. We considered  $p < 0.05$  as significant.

## RESULTS

A total of 71 subjects were included in the study. Their mean age was  $42.82 \pm 8.97$ . There were 60 females and 11 males. (M: F = 1: 5.45). Among the RA patients 30 were females and 5 were males (M: F = 6:1), amongst OA 15 were females and 3 were males (M: F = 5:1), controls: 15 females, 3 males (M: F = 5:3). Mean age of RA patients was  $41.29 \pm 10.21$  yrs, of OA patients, mean age was  $47.7 \pm 4.24$  yrs and of controls, mean age was  $40.83 \pm 8.38$  yrs. Mean disease duration at the time of the study was  $3.47 \pm 3.13$  yrs.

CTx – I levels in RA patients were compared with that in OA ( $p = 0.362$ ), RA patients compared to controls ( $p < 0.0001$ ) and OA patients with controls ( $p = 0.001$ ). On comparing all the 3 groups together (RA, OA and controls), a significant difference was found between the 3 groups. A post hoc Bonferoni's test showed significant difference between RA and controls and OA and controls. The difference between RA and OA was not significant.

### Figure 2

Table 2: Markers of disease activity and their relation with CTx-I and IL-6

VARIABLE	MEAN $\pm$ SD	MEDIAN	Correlation with CTx-I	Correlation with IL-6
Age (yrs)	40.914 $\pm$ 9.319	40.00	$p=0.044$ , $r=0.362$	$p=0.012$ , $r=0.42$
ESR (mm at the end of 1 hour)	47.914 $\pm$ 18.687	46.00	$p=0.767$ , $r=0.052$	$p=0.888$ , $r=0.025$
CRP	28.901 $\pm$ 47.533	12.03	$p=0.369$ , $r=0.162$	$p=0.048$ , $r=0.337$
RA Factor (IU / I)	71.06 $\pm$ 97.854	16.53	$p=0.434$ , $r=0.137$	$p=0.928$ , $r=0.016$
DAS28	7.113 $\pm$ 0.886	7.11	$p=0.007$ , $r=0.451$	$p=0.024$ , $r=0.382$
Swollen joint Count	16 $\pm$ 7.227	16	$p=0.006$ , $r=0.452$	$p=0.001$ , $r=0.555$
Tender joint Count	18.257 $\pm$ 7.273	18	$p=0.006$ , $r=0.453$	$p=0.04$ , $r=0.349$
Disease duration (Yrs)	3.471 $\pm$ 3.126	3	$p=0.740$ , $r=0.058$	$p=0.927$ , $r=0.016$

Same tests were performed for IL-6 levels. IL-6 values were significantly different in RA patients compared to controls ( $p < 0.0001$ ), in OA patients as compared to controls ( $p = 0.008$ ), and in RA patients as compared with OA patients ( $p = 0.015$ ). On comparing all the 3 groups together (RA, OA and controls), a significant difference was found between the 3 groups. A post hoc Bonferoni's Test showed that the difference was significant between RA and controls. OA and

controls and between RA and OA was not significant.

Levels of CTx-I and IL-6 were tested for correlation with the existing disease activity markers (Table 2). A positive correlation was found between CTx-I and age ( $p = 0.044$ ,  $r = 0.362$ ), DAS28 ( $p = 0.007$ ,  $r = 0.451$ ), swollen joint count ( $p = 0.006$ ,  $r = 0.452$ ) and tender joint count ( $p = 0.006$ ,  $r = 0.453$ ). Levels did not correlate with ESR, CRP, RF and disease duration. IL-6 levels were found to have significant associations with age ( $p = 0.012$ ,  $r = 0.42$ ), CRP ( $p = 0.048$ ,  $r = 0.337$ ), DAS28 ( $p = 0.024$ ,  $r = 0.382$ ), swollen joint count ( $p = 0.001$ ,  $r = 0.555$ ) and tender joint count ( $p = 0.04$ ,  $r = 0.349$ ). No correlations were found between IL-6 and ESR, RF and disease duration. A positive correlation was also seen between CTx-I and IL-6 ( $p = 0.03$ ,  $r = 0.352$ ).

## DISCUSSION

The erosive nature of RA is well documented. In this study, it was found that urinary CTx-I levels in RA and OA patients were markedly elevated compared to that in controls, indicating increased collagen degradation due to active bone destruction. There was a positive correlation between CTx-I and DAS28, swollen joint count, tender joint count and the age of the patient. This association implies that an increase in the extent of arthritis is followed by an increase in collagen type -1 degradation. Past studies on serum CTx-I have shown similar results.<sup>12</sup> Numerous studies conducted using other collagen degradation products (PYD, DPD, ICTP, NTX-1) have shown similar results.<sup>5613141516</sup>

It was also found that serum IL-6 levels in RA patients were significantly higher than those in OA and controls. This finding is in confirmation with those of Madhok et al, Cohick et al, Senturk et al and Sharma et al.<sup>17181920</sup> IL-6 levels were found to be significantly associated with age, CRP, DAS28, swollen and tender joint counts.

A positive correlation was also present between CTx-I and IL-6 levels indicating a possible role of IL-6 in increased collagen degradation due to bone resorption. A possible mechanism may be increased stimulation of production of matrix metalloproteinase's (MMP-9, MMP-2) and inhibition of tissue inhibitors of metalloproteinase's (TIMP-1)<sub>21</sub> by IL-6 which may result in increased collagenase activity and bone resorption. Future studies involving larger numbers of subjects need to be performed to confirm this finding.

DAS28, a disease activity marker in RA is a clinical index

calculated using swollen, tender joint counts, ESR and VAS score. The values of these variables may be low in cases of early RA or in cases where symptomatic treatment with NSAIDs has been given. In these cases though the value DAS28 will be low, active destruction of bone occurs in the body which may go unnoticed. In these cases the disease activity can be better assessed using biochemical markers. Identifying RA patients at high risk of progression of joint and bone damage at a very early stage is of particular importance for the management of the disease. Indeed these patients may be candidates for treatment with appropriate aggressive DMARD's<sup>222324</sup> which have been shown to reduce the progression of joint damage but may not be optimal for all patients. IL-6 and CTx-I can be effectively used to determine the level of inflammatory response and degree of collagen degradation, respectively and may thus be useful in identifying individual RA patients at high risk of progression very early in the disease.

This study has its limitations due to a small cohort. More longitudinal studies, with larger number of subjects are necessary to better determine the clinical value of these biomarkers. In these studies the predictive ability of biomarkers associated with cartilage breakdown measured at diagnosis, and before treatment, should also be evaluated. A documentation of decrease in these biomarkers when the patient goes in remission will be more helpful to correlate them with disease activity. In general, the identification of biomarkers that can be used as prognosis tools in daily practice to predict the onset and progression of joint damage remains the goal of these studies in RA, and also in OA where the ability to predict progression of cartilage destruction and outcome is perhaps of even greater importance.

## CONCLUSION

Serum IL-6 and urinary CTx-I levels in RA patients are markedly elevated. Their increased levels correlate with the existing markers of increased disease activity. Further studies are required to evaluate their value as prognostic markers of the disease.

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