ORIGINAL ARTICLE

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Study of Serum Calprotectin Levels and its Correlation with the Disease Severity in Rheumatoid Arthritis

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Abstract

Background: Calprotectin is a calcium binding protein released from activated leucocytes during inflammation. Calprotectin levels are strongly associated with disease activity in Rheumatoid arthritis (RA) patients. Clinical severity of RA is measured by various scores which include Clinical Disease Activity Index (CDAI) and Disease Activity Score (DAS) 28 (ESR).

DAS 28 has some fallacies due to the fact that, Tender Joint Count (TJC) are over weighed than Swollen Joint Count (SJC) in its formula. Also, Erythrocyte Sedimentation Rate (ESR) is variable and dependent on factors other than inflammation. The present study aimed to evaluate the serum calprotectin levels in patients of rheumatoid arthritis and correlate these levels with clinical severity of rheumatoid arthritis measured by CDAI and DAS 28 (ESR).

Material and methods: The present observational cross-sectional study was conducted in the Department of Medicine and Rheumatology Clinic, VMMC and Safdarjung Hospital, New Delhi, included 65 patients of RA satisfying the inclusion and exclusion criteria over a period of 18 months. Disease severity was calculated by CDAI and DAS 28 (ESR) and was correlated with serum calprotectin levels.

Results: The median age was 35 years, minimum age was 24 years and maximum 65 years. Females comprised of 89.23%, while males comprised of 10.77%. Female to male patient ratio was 8:1.

The present study also showed that the mean serum calprotectin level of patients with low disease activity according to CDAI and DAS 28 score was 3133.33 ng/ml and 3200 ng/ml, respectively, with moderate disease activity was 4728.93 ng/ml and 4137.5 ng/ml, respectively, with high disease activity was 7046 ng/ml and 6813.04 ng/ml, respectively and of the patients in remission, mean was 450 ng/ml and 807.14 ng/ml, respectively.

We observed that as the disease activity according to DAS 28 and CDAI increased, the serum Calprotectin levels also increased and this correlation was statistically significant as the p value was 0.0001.

Conclusion: The present study revealed a significant positive correlation between serum Calprotectin levels and disease activity according to CDAI and DAS 28. So, serum Calprotectin could be considered as a useful marker for diagnosis and monitoring of disease activity.

Introduction

Rheumatoid arthritis (RA) is a systemic auto-immune disease, with the main characteristic of persistent joint inflammation and substantial irreversible joint damage[1]. The clinical diagnosis of RA depends on the patient's clinical presentation and imaging techniques. However, imaging techniques show relative insensitivity to early bone damage with insufficiency for assessment of soft tissue changes[2].

Also, a clinical examination might lack sensitivity in patients with mild synovitis, be limited in patients with established deformities, and may overestimate severity in patients with concomitant fibromyalgia or other comorbidities. Therefore, identifying novel, sensitive serum biomarkers of RA disease activity remains challenging. Rheumatoid factor (RF) has been commonly used as a serological marker for RA; although RF had a tolerable sensitivity of 75.9% for RA, but it had a low specificity of 78.7%. Laboratory tests for prediction of disease activity include erythrocyte sedimentation rate (ESR), C reactive protein (CRP), platelet and leucocyte count[3]. However, a substantial proportion of patients with RA have normal test results[4]. In addition, the sensitivity and specificity of these tests for changes in synovial inflammation are limited[5].

Calprotectin is a calcium-binding protein, secreted predominantly by neutrophils and monocytes. Calprotectin concentration in diseased synovial fluid is 10-fold higher than concentration in serum obtained in parallel from individual patients. The resulting increase of calprotectin serum concentrations may be an important serum marker of the extent of local inflammation in the affected joints[6,7]. Clinical severity of RA is measured by various scores which

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include Clinical Disease Activity Index (CDAI) and Disease Activity Score 28 (DAS 28). DAS 28 score is calculated by online calculator, and includes ESR in its formula. DAS 28 has some fallacies due to the fact that, Tender Joint Count (TJC) are over weighed than Swollen Joint Count (SJC) in its formula. Also, ESR is variable, and dependent on factors other than inflammation. Therefore DAS 28 (ESR) may over/under represent disease activity. On the other hand, CDAI is just sum total of Swollen 28-Joint Count, Tender 28-Joint Count, Patient Global disease Activity and Evaluator's Global disease activity.

Keeping this in mind, the present study aims to measure serum Calprotectin levels in RA and correlate these levels with clinical severity of RA, measured by CDAI and DAS 28 (ESR).

Material and methods

Study setup: The present study was conducted in the Department of Medicine and Rheumatology Clinic, VMMC and Safdarjung Hospital, New Delhi.

Study design: Observational cross-sectional study.

Study population: The study included patients with RA, attending the outpatient department of Medicine, Rheumatology Clinic and indoor units of department of Medicine, VMMC and SJH, New Delhi.

Study size: The study was carried out on 65 patients of RA satisfying the inclusion and exclusion criteria of the study, over a period of 18 months.

Consent and ethics clearance

Written and informed consent was taken from all subjects participating in the study. Ethics clearance was taken from the ethics committee of Safdarjung hospital, before conducting the study. The study was performed according to the principles of the declaration of Helsinki.

Inclusion criteria

1. Age above 18 years, any gender.
2. Patients fulfilling the American College of Rheumatologists (ACR)/EULAR 2010 classification criteria for Rheumatoid Arthritis[8].

Exclusion criteria

1. Any other autoimmune disorder (i.e, inflammatory bowel disease, pernicious anaemia, multiple sclerosis, psoriatic arthritis).
2. Acute or chronic kidney disease.
3. Any form of current acute or chronic infection.
4. Organ transplant recipient.
5. Current or past history of any malignancy.
6. Patients receiving any form of biological therapy.
7. Pregnant or nursing females.

All the patients included in the study were subjected to the following: History taking, thorough clinical examination laying stress on number of tender and swollen joints (28-TJC and 28-SJC) and lab investigations like CBC, ESR, RF, anti CCP, pregnancy tests for female patients and serum Calprotectin levels.

Disease severity of RA was calculated by Clinical Disease Activity Index (CDAI) and Disease Activity Score of 28 joints (DAS 28).

Then, the disease severity was correlated with serum Calprotectin levels.

For serum Calprotectin levels measurement, fasting blood samples were obtained. The serum samples were centrifuged and stored at – 80 °C until the analysis. Calprotectin was measured by a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol. The normal serum Calprotectin levels were taken to be 100 ng/ml -3000 ng/ml. ESR, anti CCP and CRP levels were measured using hospital laboratory protocols.

Results

In the present study, mean age of patients was 37.53 ± 9.6 years, the mean duration of early morning stiffness (observed in 73.85% patients) was 52.19 ± 25.16 minutes. The mean CDAI score was 15.69 ± 9.17, mean DAS 28 (ESR) score was 4.55 ± 1.33 and the mean serum Calprotectin level was 4682.31 ± 2263 ng/ml (Table I).

It was observed that, as the disease activity according to CDAI increased, the serum Calprotectin levels also increased and this correlation was statistically significant as the p value was < .0001 (Table II) (Fig. 1).

Also, as the disease activity according to DAS28 increased, the serum Calprotectin levels also increased and this correlation was statistically significant as the p value was < .0001 (Table III) (Fig. 2).

In the present study, the serum Calprotectin levels were positively correlated with CDAI score with correlation coefficient 0.7506, i.e, as the CDAI score increased the serum Calprotectin levels also increased and vice versa, and, this correlation was statistically significant as p value was < 0.0001. Also, serum Calprotectin levels were positively correlated with DAS 28 (ESR) score with correlation coefficient 0.751, i.e, as the DAS 28 (ESR) score increased the serum Calprotectin levels also increased and vice versa, and, this correlation was statistically significant as p value was < 0.0001 (Table IV).

Table I: Demographic, clinical, laboratory, CDAI, DAS 28 (ESR) and serum Calprotectin data of 65 patients of Rheumatoid arthritis.

	Sample size	Mean ± SD	Median	Minimum - Maximum	Inter quartile range
Age (Yrs)	65	37.54 ± 9.6	35	24 - 65	30 - 43
Duration of morning stiffness (Minutes)	48	52.19 ± 25.16	45	15 - 120	30 - 60
No. of joints involved	65	12.89 ± 5.68	12	2 - 28	8 - 18
Duration of joint pain (Yrs)	65	4.63 ± 3.61	4	1 - 20	2 - 5.25
Pulse (Per minute)	65	79.85 ± 5.97	79	70-92	75 - 85
SBP (mmHg)	65	133 ± 32	133	165 - 101	116 - 148
DBP (mmHg)	65	93 ± 32	93	125 - 60	86 - 109
Swollen joint count (out of 28)	65	2.69 ± 3.04	2	0 - 16	0 - 4
Tender joint count (out of 28)	65	4.25 ± 3.34	4	0 - 16	2 - 6
Patient global assessment of disease activity (out of 10)	65	4.82 ± 2.07	5	1 - 8	3 - 7
Provider global assessment of disease activity(out of 10)	65	4.11 ± 2.01	4	1 - 7	2 - 6
CDAI score	65	15.69 ± 9.17	14	2 - 39	8 - 21.25
Haemoglobin (gm/dl)	65	12.1 ± 1.42	12	10 - 15	11 - 13
TLC (10^3/ml)	65	7.37 ± 1.47	7.5	4 - 11	6.3 - 8.2
Platelet count (10^5/ml)	65	2.84 ± 0.63	2.7	1.6 - 4	2.4 - 3.4
ESR (mm/st hour)	65	39.05 ± 21.26	34	10 - 98	23 - 48.75
Serum bil. (mg/dl)	65	0.6 ± 0.1	0.6	0.2 - 0.9	0.6 - 0.6
SGOT (units/l)	65	47.95 ± 10.54	45	20 - 84	45 - 47.25
SGPT (units/l)	65	46.17 ± 8.49	45	27 - 71	45 - 49
ALP (IU/l)	65	75.49 ± 21.61	76	25 - 129	58.75 - 87.75
Serum creatinine (mg/dl)	65	0.54 ± 0.21	0.5	0.1 - 0.9	0.4 - 0.7
Blood urea (mg/dl)	65	25.83 ± 3.58	26	15 - 42	24 - 28
Serum sodium (meq/l)	65	137.97 ± 3.62	138	130 - 145	135 - 141
Serum potassium (meq/l)	65	4.59 ± 0.62	4.7	3.5 - 5.5	4 - 5.1
Rheumatoid factor (U/l)	60	53.68 ± 16.27	54	28 - 90	41 - 66
Anti CCP (units/ml)	65	54.77 ± 17.5	56	24 - 92	41 - 69.5
DAS 28	65	4.55 ± 1.33	4.72	2.02 - 7.5	3.673 - 5.492
Serum calprotectin levels (ng/ml)	65	4682.31 ± 2263.56	4500	200 - 8900	3400 - 6100

Table II: Correlation between serum Calprotectin levels and disease activity, according to CDAI.

Disease activity, according to CDAI					
	Low	Moderate	High	Remission	P value
	Serum Calprotectin levels (ng/ml)				
Sample size	21 (32.3%)	28 (43.07%)	15 (23.07%)	1 (1.5%)	
Mean ± SD	3133.33 ± 1969.09	4728.93 ± 1662.96	7046 ± 1296.71	450 ± 0	< .0001
Median	3100	4400	7300	450	
Min-Max	200-6800	900-8000	4500-8900	450-450	
Inter quartile range	1425-4725	4000-6000	6000-8000	450-450	

Table III: Correlation between serum Calprotectin levels and disease activity, according to DAS 28 (ESR).

Disease activity, according to DAS 28 (ESR)					
	Low	Moderate	High	Remission	P value
	Serum calprotectin levels (ng/ml)				
Sample size	3 (4.6%)	32 (49.2%)	23 (35.3%)	7 (10.7%)	
Mean ± SD	3200 ± 264.58	4137.5 ± 1440.6	6813.04 ± 1281.2	807.14 ± 532.63	<.0001
Median	3100	4250	6700	600	
Min-Max	3000-3500	900-6800	4000-8900	200-1500	
Inter quartile range	3025 - 3400	3450 - 5000	6000 - 7972.500	412.500 - 1375	

Table IV: Spearman's rank correlation between serum Calprotectin levels vs. CDAI score and DAS 28 (ESR).

	Sample size	Correlation co-efficient r	Significance level	95% confidence interval for r
Serum calprotectin levels vs CDAI score	65	0.7506	< 0.0001	0.6202 to 0.8406
Serum calprotectin levels vs DAS 28 (ESR)	65	0.751	< 0.0001	0.6208 to 0.8409

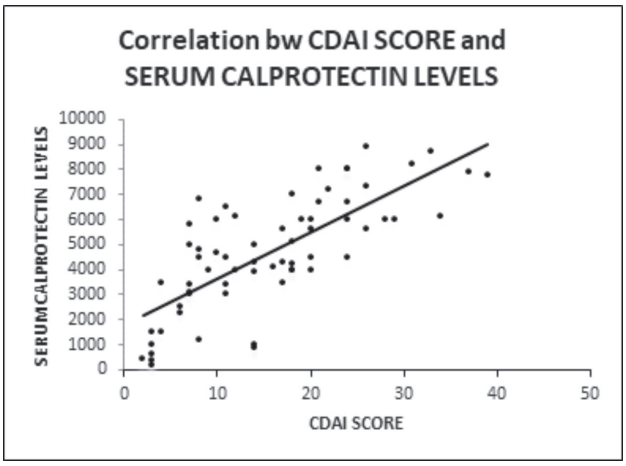

Fig. 1: *Correlation between serum Calprotectin levels and disease activity, according to CDAI.*

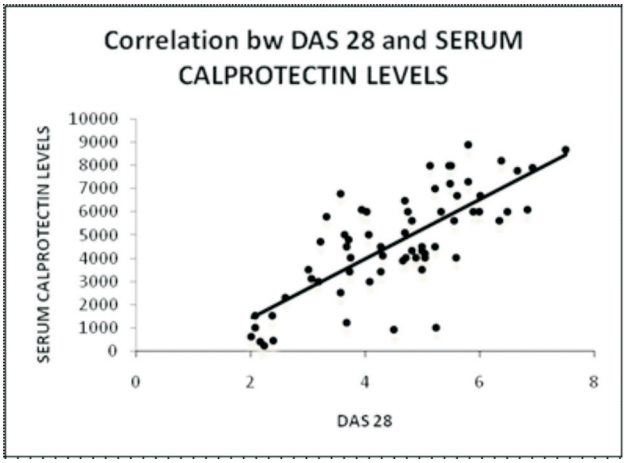

Fig. 2: *Correlation between serum Calprotectin levels and disease activity, according to DAS 28 (ESR).*

Discussion

In the present study, serum Calprotectin levels correlated significantly with laboratory parameters, as well as with clinical examination, including CDAI score and DAS 28 (ESR).

The mean duration of morning stiffness was 52.19 ± 25.16 minutes, the median range of ESR was 34 mm (39.05 ± 21.26), CRP was 12 mg/l (13.57 ± 7.79) and of serum Calprotectin levels was 4500 ng/ml (4682.31 ± 2263.56) (Table I).

In a study conducted by Hammer SB *et al*, the median (range) levels of the laboratory markers were, ESR 15 (2 - 63) mm in the first hour[9], CRP 4 (1 - 49) mg/l, and Calprotectin 1.8 (0.3 - 8.7) mg/l.

The serum Calprotectin levels were positively correlated with CDAI score with a correlation co-efficient of 0.7506, i.e, as the CDAI score increased the serum calprotectin levels also increased and vice versa and this correlation was statistically significant as p value was < 0.0001 (Table IV).

In 2015, Jose´ Inciarte-Mundo *et al*, also observed that serum Calprotectin levels were significantly lower in patients with low disease activity or remission, compared with moderate or high disease activity.

The serum Calprotectin levels were positively correlated with DAS 28 score with correlation co-efficient of 0.751, i.e, as the DAS 28 score increased the serum Calprotectin levels also increased and vice versa and this correlation was statistically significant as p value was < 0.0001 (Table IV).

In the study by Hammer SB *et al* disease activity according to DAS 28 (ESR) correlated positively with serum Calprotectin levels as the correlation co-efficient was 0.55 ($p < 0.001$).

In 2014, a study conducted by Kwi Young Kang *et al* found that disease activity according to DAS 28 (ESR) correlated positively with serum Calprotectin levels as the correlation co-efficient was 0.55 ($p < 0.05$)[10].

Limitations

Our study had some limitations. First, the design was cross-sectional. Second, disease duration was short. Third, a relatively small number of patients were included in this study.

Also, Calprotectin level was not measured in the synovial fluid. However, the protein is small and can pass easily into the circulation thus serum Calprotectin level can reflect the degree of inflammatory activity in the joints. Also, the correlation between serum Calprotectin level and the degree of radiographic joint damage was not evaluated.

Conclusion

A total of 65 patients who fulfilled the EULAR/ACR criteria

of RA were studied.The maximum number of patients were in the age group of 18 - 50 years with the mean age of 37.54 ± 9.8 years. Female patients made up the bulk of patients with female to male ratio of 8:1. Symmetrical joint pain was the most common presenting symptom, accompanied by EMS. 11 out of 65 patients had some deformities. 60% patients had both small and large joint involvement. 75.38% patients were on triple drug combinations, while 4 patients were taking Ayurvedic treatment also.

The present study revealed a significant positive correlation between serum Calprotectin levels and other markers of disease activity, i.e., ESR and CRP. Serum Calprotectin levels did not differ with age and disease duration but correlated with disease activity .32.3% patients were found to have low disease activity, 43.07%, moderate, 23.07%, high and 1.5% were in remission, based on CDAI. There was significant positive correlation between serum Calprotectin levels and disease activity according to CDAI. 4.6% patients were found to have low disease activity, 49.2% moderate, 35.3%, high and 10.7%, were in remission, based on DAS 28 (ESR).There was significant positive correlation between serum Calprotectin levels and disease activity according to DAS 28 (ESR).

To the best of our knowledge, this is the first study in India to measure serum Calprotectin levels in RA and correlate it with disease activity, accoording to CDAI and DAS 28 (ESR). Since this is a small study, we need larger studies to explore serum Calprotectin as a biomarker of disease activity in RA patients.

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