Role of Serum Calprotectin in Rheumatoid Arthritis as a Biomarker of Inflammation and its Correlation with Disease Severity

Original Article

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ABSTRACT

Aim of the Work: The present study attempted to assess the level of serum calprotectin CLP in patients with rheumatoid arthritis as well as its correlation with clinical, laboratory radiological and ultrasonographic parameters of disease activity and severity.

Patients and Methods: 40 RA patients treated with conventional DMARDS were enrolled as a study group and 40 age and sex matched healthy participants, as a control group. ELISA was used to measure serum calprotectin (CLP) levels, while (Anti-CCP), (CRP), (ESR), and (RF) were also measured. The disease activity was evaluated using the DAS28, the disease severity by Rheumatoid arthritis Severity Scale (RASS). Affected joints were sonographically assessed. Plain x-ray of wrists as well as hands were evaluated using the modified Larsen score (MLS).

Results: The level of serum CLP was remarkably higher in RA patients treated with conventional DMARDS compared to controls (*P<0.001*). There was a significant positive association between serum CLP level in patients with RA and DAS28, CRP, ESR, RF, RASS, MLS Anti-CCP as well as ultrasonographic findings.

Relation between patient's results and types of graft used showed no statistically significant differences between them.

Conclusion: The CPL is incremented in the cases with RA, it correlates with activity and severity and may be a candidate for biomarker.

Key Words: Calprotectin, disease activity, disease severity, rheumatoid arthritis, ultrasonography.

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INTRODUCTION

Rheumatoid arthritis (RA) is regarded as a chronic systemic inflammatory autoimmune disease, which is highlighted by a group of articular as well as extra-articular aspects. Moreover, RA is the most familiar inflammatory arthritis, which affects about 1% of the general populations. It affects two to three folds more females than males. The main feature of this disease is constant symmetric polyarthritis, which affects the feet, wrists, and hands, nonetheless nearly all diarthrodial joints are involved. The severity as well as activity of RA often increases by the time; however chronic RA usually leads to a gradual development of multiple degrees of deformity and joint destruction, a remarkable decline in functional status as well as early death [1].

Ultrasonography (US) in RA plays a significant role in assessing the peripheral joints for active inflammation and disease severity in the form of; synovial thickening, effusion, increased vascularity, and bone erosions [2].

Calprotectin (CLP) is a hyperdynamic of two calcium-binding proteins existing in the cytoplasm of neutrophils and expressing the monocyte of membrane. It is also known as myeloid-related protein {MRP}-8/14, S100A8/A9 [3].

Fecal calprotectin FC has been confirmed as one of the most sensitive noninvasive, and reliable diagnostic tools for inflammatory bowel disease IBD in clinical practice both in adults and children [4], however; serum calprotectin is excreted from activated granulocytes and monocytes/macrophages in the synovium and synovial fluid during inflammation, so multiple studies were performed to assess the clinical benefit of serum calprotectin as an inflammatory marker for diagnosing and assessing the rheumatoid arthritis activity as well as its severity [3-5].

Moreover, Serum Calprotectin is an indication for diagnosing as well as monitoring autoimmune inflammatory arthritis disease activity, for instance; systemic lupus erythematosus (SLE), juvenile rheumatoid arthritis, psoriatic arthritis and spondyloarthropathies [5].

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Aim of The Work:

The current work attempted to assess the level of serum calprotectin in patients with rheumatoid arthritis as well as its correlation with clinical, laboratory radiological and ultrasonographic parameters of disease activity and severity.

PATIENTS AND METHODS:

Study population

The present study enrolled 40 RA patients treated with conventional DMARDS in (Group I), in addition to 40 healthy participants matched in age and gender as a control group (Group II). The research team obtained an informed consent from all volunteers, moreover, the ethical committee of the Faculty of Medicine at Tanta University also approved the current study Approval code:32150/02/18. All the patients were selected from the Physical Medicine, Rheumatology as well as Rehabilitation clinics at Tanta University Hospitals.

Inclusion criteria: RA patients were diagnosed according to the American College of Rheumatology (ACR) as well as European League Against Rheumatism (EULAR) 2010 Criteria for RA [6]. All patients was treated with conventional DMARDS Methotrexate., Hydroxychloroquine, Leflunomide. Exclusion criteria: Inflammatory bowel disease patients [7], patients with cancer and any other autoimmune diseases [8,9].

Clinical and laboratory assessment: A full medical history was obtained from participants, general examination, locomotor system examination, assessment of activity according to the Disease Activity Score 28 (DAS28): DAS28 = $0.56\sqrt{}$ (TEN28) + $0.28\sqrt{}$ (SW28) + 0.70 Ln (ESR) + 0.014 (GH) [10]. Assessment of severity according to the rheumatoid arthritis severity scale (RASS): It consists of three visual analogue scales (Disease activity, Functional impairment, and physical damage). All three domains are assessed using a range from 1-100, with a score of 1 meaning no evidence of condition and 100 meaning maximum progression [11].

Laboratory assessment: RF by latex agglutination methods (bioscience catalogue no 1300501) [12], anti-CCP by ELIZA methods (orgentec diagnostica 601) [13], ESR by Westergren methods [14], CRP by latex agglutination methods (BioMed, catalogue no 301040) [15], Laboratory assessment of serum Calprotectin level.

Venous blood samples were gathered from all volunteers using sterilized disposable syringes in a sterilized tube, then samples were centrifuged for 15min at 1000g to separate serum using a clean and dry Pasteur pipette. Serum samples were kept at −70°C until used to test the level of serum CLP using ELISA technique [16].

Radiographic assessment

Plain X-ray were obtained for both hands and wrists, and a radiological assessment was carried out using the modified Larsen score MLS 1995, sixteen joints were evaluated in each hand as well. The final score of both hands ranges from 0 to 160 [17].

Ultrasonographic assessment

Musculoskeletal US was used to assess the most clinically affected wrist joint, whereas systematic multi-planar gray-scale US (GSUS) as well as power Doppler (PDUS) examinations were performed based on an integrated manner on 40 wrist, according to the European League against Rheumatism (EULAR) guidelines.

All regions of the joint were evaluated based on parameters of inflammation and joint damage (synovial thickening, joint effusion, PD activity and bone erosion) [18,19].

1. Synovial thickening: Defined by US as an unnatural tissue within a hypo-echoic intra-articular which is incapable of displacement as well as poorly compressible visualized in longitudinal and transversal planes, and it is measured in millimeters.

2. Joint effusion: It is defined as a compressible region within the anechoic intra-capsule and examined semi-quantitatively like the following: Grade 0: no effusion; Grade 1: minimum amount; Grade 2: mild amount of fluid (without distension of the joint capsule); Grade 3: large amount of fluid (with distension of the joint capsule).

3. Bone erosion score: Defined as an obstruction to the surface of bone on two orthogonal planes, and it is evaluated as follows: Grade 0: normal surface of bone; Grade 1: irregularity of the bone surface without seeing the defect in two planes; Grade 2: surface defect in two planes; Grade 3: defect of the bone resulting in significant bone damage.

4. Vascularity by Power Doppler (PD): The semi-quantitative scores for the evaluation of PD, were assessed like the following: Grade 0: no flush into the synovium; Grade 1: single vessel signals; Grade 2: less than half of the area of the synovium is filled with vessel signal; Grade 3: more than half of the area of the synovium is filled with vessels.

Statistical analysis:

performed using SPSS software, version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA), the statistical package for social sciences. Demographic data were compared between patients and controls were compared, using the χ^2, Mann-Whitney U test, Kruskal

Wallis test and unpaired Student's t-tests when appropriate, as well as Pearson correlation coefficient (r) to determine the correlation between plasma CLP concentrations, in addition to clinical and radiographic parameters. Data are expressed as mean ± SD. *P values* less than 0.05 were considered statistically significant for differences and correlation [20].

RESULTS:

The age of RA patients studied ranged from 28-50 years with a mean age of 39.30 ± 6.81. Thirty-seven patients with RA were females and three patients were males, while the age of the control group ranged from 35.0 – 50.0 years, with a mean age of 41.70 ± 4.0. All controls were females. Disease activity (DAS28) in RA patients ranged from 2.53 – 5.96 with a mean of 4.61 ± 1.23; most of the patients were with moderate and severe disease activity. Disease severity ranged from 40.0 – 100.0 with a mean of 77.50 ± 18.03. Radiological assessment by MLS showed a range of 20.0 – 64.0 with a mean of 34.30 ± 14.06, while ultra-sonographic finding revealed grade 3 joint synovitis in 16 patients (40%), grade 3 bone erosion in 14 patients (35%), and Doppler activity grade 3 in 16 patients (40%) (Table 1, Fig. 1). The serum CLP levels of the patients with RA ranged from 560.0 – 980.0 ng/ml with a mean of 825.50 ± 126.93, which was significantly higher than that in controls $p < 0.001^*$ (Table 2). There was a remarkable association between the level of serum Calprotectin and number of tender joints $p<0.001^*$, swollen joints $p<0.001^*$, pain by VAS $p<0.001^*$, acute phase reactant ESR $p<0.001^*$,CRP $p=0.001^*$, RF $p<0.001^*$, Anti-CCP $p<0.001^*$, DAS 28 $p<0.001^*$, RASS $p<0.001^*$, MLS $p<0.001^*$ and ultrasound findings for effusion $p=0.004^*$, erosion $p=0.016^*$ and Doppler activity $p=0.049^*$ (Table 3).

Table 1: Disease activity (DAS28), severity (RASS), and radiological data (MLS, US) in RA patients studied

Disease activity score (DAS 28)			
Min. – Max.			2.53 – 5.96
Mean ± SD.			4.61 ± 1.23
Median			4.99
Grading of DAS 28		No	%
Clinical remission		4	10.0
Low disease activity		4	10.0
Moderate disease activity		16	40.0
High disease activity		16	40.0
Disease severity	Min. – Max.	Mean ± SD.	Median
RASS	40.0 – 100.0	77.50 ± 18.03	70.0
Radiological assessment	Min. – Max.	Mean ± SD.	Median
Modified Larsen score MLS	20.0 – 64.0	34.30 ± 14.06	34.0
Ultrasonographic assessment		No.	%
Synovial thickness			
Min. – Max.		2.30 mm– 6.40mm	
Mean ± SD.		3.42 ± 1.0mm	
Median		3.20mm	
Joint effusion			
Grade 0		4	10.0
Grade 1		8	20.0
Grade 2		12	30.0
Grade 3		16	40.0
Bone erosions			
Grade 0		6	15.0
Grade 1		8	20.0
Grade 2		12	30.0
Grade 3		14	35.0
Doppler activity			
Grade 0		4	10.0

Grade 1	6	15.0
Grade 2	14	35.0
Grade 3	16	40.0

Table 2: Serum calprotectin levels in RA patients and controls

Serum calprotectin (ng/ml)	Group I (RA) (n = 40)	Group II (Control) (n= 40)	U	*P*
Min. – Max.	560.0 – 980.0	11.0 – 60.0	0.0*	*<0.001**
Mean ± SD.	825.50 ± 126.93	30.0 ± 14.70		
Median	880.0	31.50		

*Significant at *p<0.05*. U: **Mann-Whitney U test**

Table 3: Correlation between serum calprotectin levels and demographic, clinical, laboratory, and radiological data in RA patients

		Serum calprotectin		
	r_s	U	H	*P*
Sex		19.0		0.546
Age	0.397			0.083
No. of tender joints	0.777			*<0.001**
No. of swollen joints	0.839			*<0.001**
VAS (0-100)	0.747			*<0.001**
ESR	0.819			*<0.001**
DAS 28 score	0.811			*<0.001**
RASS	0.709			*<0.001**
CRP (mg/dl)	0.682			*0.001**
RF (IU)	0.767			*<0.001**
ANTI-CCP (IU)	0.667			*0.001**
Modified Larsen score	0.735			*<0.001**
Synovial thickness	0.772*			*<0.001**
Effusion			H=3.341*	0.004*
Erosions			H=10.319*	0.016*
Doppler signal			H=7.878*	0.049*

H: H for **Kruskal Wallis test** U: **Mann-Whitney U test** * significant at *p<0.05*.

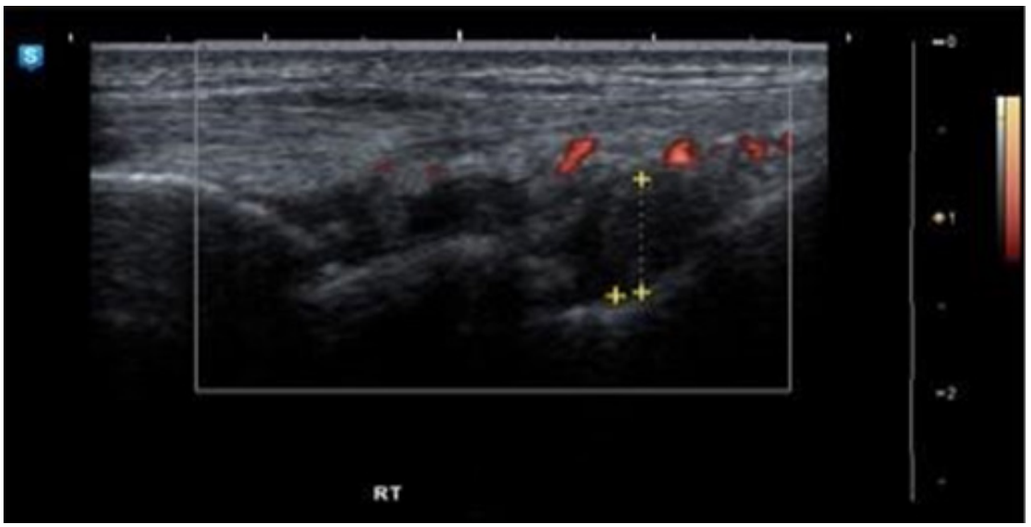

Fig. 1: Ultrasound examination of the wrist joint of RA patient showed synovial thickness 396 6.40mm, joint effusion grade 3, Doppler activity grade 2 and bone erosion grade 1.

DISCUSSION

Calprotectin (CLP); is a heterodimer made up of two proteins, S100A8 and S100A9, that are produced by neutrophils and active monocytes in the blood and inflammatory tissues. Although CLP's involvement in the inflammatory process has already been established, its significance in the pathophysiology, diagnosis, and management of rheumatic illnesses has recently drawn considerable attention [21].

The CLP, is a candidate biological indicator for monitoring disease activity in many autoimmune disorders, as it can portend response to therapy or disease deterioration [21].

The ages of the RA patients studied ranged from 28-50 years. Thirty-seven patients with RA were females and three patients were males, while the age of the control group ranged from 35.0 – 50.0 years. All the controls were females.

In current study, there was no remarkable association between demographic data (sex and age) and serum CLP levels in the RA patients, denoting that age and sex have no significant influence on the serum CLP levels.

The present study demonstrated that the serum CLP levels in RA patients were remarkably higher compared to the control group. These findings align with those of Brun, *et al*. [22], Chen, *et al*. [23], Baillet, *et al*. [24], Cerezo, *et al*. [25], Soliman, *et al*. [26] and Mansour, *et al*. [27]. In RA, stimulated phagocytes in the synovial membrane express CLP with intense expression at the cartilage–pannus junction [28]. CLP levels are approximately ten times greater in synovial fluid compared to serum in participants with active inflammatory arthritis, therefore this may indicate the existence of an intraarticular origin of inflammation [29].

Association between serum CLP and disease activity of RA has been confirmed in recent years. Circulating CLP levels are high in active RA and are significantly related to Disease Activity Score based on a 28-joint count (DAS28), simplified Disease Activity Index (SDAI) and Clinical Disease Activity Index (CDAI). In some RA patients, the levels of CRP or ESR were normal, under this circumstance, CLP, prior to CRP, is a satisfactory predictor of CDAI activity in linear regression analyses [30].

According to Brun, *et al*. [22], Cerezo, *et al*. [25], Berntzen, *et al*. [31], Madland, *et al*. [32], and Garcia, *et al*. [33] it was found that serum CLP is positively related to RA disease activity, supporting the idea that CLP is involved in the pathogenesis of RA. The findings of the current study support the claim that there is a strong association between serum CLP level and disease activity in patients with RA, and thus supporting that CLP is a marker of inflammation.

The positive association between disease activity parameters and serum CLP level, is attributed to the fact that CLP is an inflammatory-related protein released from leukocytes, macrophages, and monocytes, contributing to the process of several inflammatory diseases, including rheumatoid arthritis (RA) [34]. In the case of inflammation, CLP is released from activated inflammatory cells, both granulocytes as well as macrophages are recruited into the synovial and synovium fluid. Such cells include massive amounts of CLP, which is released during both activation as well as cell death. This protein is tiny, and can easily push through the circulatory system, consequently the levels of CLP serum are indicator of inflammatory activity in the joints [31]. Moreover, CLP is also confirmed to be an alarm, which amplifies the inflammation cascade [35].

The results of the current study are compatible with the findings of Cerezo, *et al*. [21], Garcia, *et al*. [33], Kane, *et al*. [36], Adel, *et al*. [37], and Hammer, *et al*. [38], who found a remarkable positive association between CLP and acute phase reactants (ESR and CRP) in patients with RA. They demonstrated that this correlation is important as CLP interacts as an acute phase protein, as it was released from activated leucocytes from the inflamed synovium in RA. Nonetheless, the acute phase proteins ESR and CRP are essentially generated in hepatocytes during inflammation. Therefore, serum CLP is a more beneficial indicator rather than acute phase proteins through reversing the number of activated leucocytes in the inflamed joints [37,38].

In the same vein, Chen, *et al*. [23], and Hammer, *et al*. [38], found a marked positive association between RF, anti – CCP titers as well as serum CLP level, supporting the idea that CLP is involved in the pathogenesis of RA. On contrary, Garcia, *et al*. [33] studied 60 patients with different levels of disease activity evaluated before and after treatment, they did not detect any relationship between serum CLP level and anti-CCP titers. This can be illustrated by the fact that the RF levels are influenced by RA activity than anti-CCP titers. Liao, *et al*. [39], and Hammer, *et al*. [40], found a marked positive association between RASS and serum CLP level in patients with RA. In addition, they reported that CLP was elevated in serum of RA patients with erosive and disabling disease and it was considered a marker of inflammation. Liao, *et al*. [39], in a study assessing disease biomarkers in RA patients, synovial fluid and serum were collected from erosive and non-erosive RA patients, clinical and laboratory assessment of RA patients was performed, which included DAS 28, RF, CRP and radiographic imaging to assess bone erosions, using the modified Larsen score in RA patients. They found that CLP was significantly correlated with MLS, and they reported that CLP molecules were elevated

in the serum of patients with radiographic bone erosions, which is a strong predictor of disability, compared to patients with non-erosive RA or healthy individuals. They also found a significant correlation between CLP, DAS-28, CRP, and RF, supporting that CLP is a biomarker for diagnosing the activity and severity of RA.

Hammer, *et al*. [40], included in their study 45 patients with RA, who were clinically, and laboratory assessed for CLP, CRP, DAS 28, and radiographic imaging (plain hand radiographs using the modified Sharp score), they found that CLP was positively associated with all disease activity parameters and (modified Sharp score). They reported that radiographic damage is indicative in numerous clinical studies about RA. Moreover, it has been associated with the long-term development of physical disability.

About radiological correlation, Liao, *et al*. [39], Hammer, *et al*. [40] and Hammer, *et al*. [41] found a marked positive association between radiographic joint damage score as well as serum CLP level in RA patients, evaluated by modified Larsen Score. These findings support the hypothesis that CLP may be implicated in pathogenesis of RA and may contribute to the bone erosion in RA patients indicating that the mechanism by which CLP plays a destructive role in the joints of RA patients, responsible for cartilage destruction and joint damage. Furthermore, they illustrated that CLP has been reported to predict radiographic joint damage in RA patients and may explain that the decrease in CLP serum level over time, correlated with a decrease in disease activity, would lead to further suppression of structural damage to the joints. In contrast, Madland, *et al*. [32] no correlation was found between serum CLP and modified Larsen score.

The findings of the current study are also consistent with the those of Hammer, *et al*. [42], Hurnakova, *et al*. [43], Inciarte-Mundo, *et al*. [44], Hurnakova, *et al*. [45], and Nordal, *et al*. [46], it was found that the level of serum CLP was positively correlated with ultrasonographic assessment (effusion, synovial thickness, erosions, and Doppler activity), which is a useful tool for reflecting activity in RA patients more than with clinical examination.

Hammer, *et al*. [42], studied 20 patients with RA and started treatment with adalimumab, patients were clinically examined by US at baseline after 1, 3, 6 and 12 months. They found a significant correlation between serum CLP, DAS 28, CRP as well as ultrasound synovitis (joint effusion and synovial thickness), besides, they reported that CLP is a better predictor of ultrasound synovitis. Hurnakova, *et al*. [43], 37 patients with RA were clinically examined by US to assess synovitis (joint effusion and synovial thickness) as well as synovial vascularity using power Doppler (PD) ultrasound via a semi quantitative grading from 0-3. The levels of serum CLP, CRP and ESR were assessed during the ultrasound assessment. They found a positive correlation between serum CLP level, laboratory, and clinical markers of disease activity (DAS 28, CRP) and US synovitis score in RA patients. They found that CLP may be a better predictor of ultrasound-determined synovial inflammation than CRP. Inciarte-Mundo, *et al*. [44] also found a significant correlation between serum CLP and ultrasonographic parameters and showed that CLP may contribute to diagnose disease activity detected by a power Doppler in RA patients. They demonstrated that serum CLP discriminates disease activity from remission in patients with RA.

The limitation in our study as a preliminary study is limited by small number of RA cases, and non of our patients treated with biological drug. further studies with large sample size are needed in the future.

CONCLUSION

The serum level of calprotectin is significantly higher in RA patients than in normal population. This level was positively associated with disease activity parameters (DAS 28, CRP and synovitis assessed by US) and with parameters of disease severity (RASS, and bone erosions assessed by MLS and US). These findings indicate that CLP may be an advantageous marker for reflecting disease activity and severity in RA. More studies are needed to explore the role of CLP in the pathogenesis of autoimmune diseases aiming to search for a novel therapeutic target.

CONFLICT OF INTEREST

There are no conflicts of interest.

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