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Role of Serum Calprotectin as a Predictor for Disease Activity in Inflammatory Arthritis

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ABSTRACT

Background: Serum calprotectin (CLP) has an important role in regulating innate and adaptive immune response. Its evaluation in rheumatic diseases can offer better outcomes for controlling and predicting disease activity

Aim of the work: This work aimed at assessing serum CLP in patients with rheumatic diseases and estimating its relation with clinical, laboratory and musculoskeletal ultrasound.

Patients and methods: Our randomized study included 60 patients of both sexes, with rheumatoid arthritis (RA), psoriatic arthritis (PSA) and ankylosing spondylitis (AS) and a control group of 20 apparently healthy volunteers . All patients were subjected to serum CLP evaluation besides clinical and musculoskeletal ultrasound assessment.

Results: Serum CLP was significantly higher in all patients with significant difference between patients and control group. No significant correlation was detected between calprotectin and disease activity parameters other than significant negative correlations with inflammatory markers in RA & AS and positive correlation with DAPSA score.

Conclusions: CLP serum levels were particularly high in RA, PSA and AS and can be used to differentiate these autoimmune diseases. CLP offers a good alternative when CRP is negative.

Keywords: Rheumatoid arthritis, Ankylosing spondylitis, Psoriatic arthritis, Serum calprotectin.

INTRODUCTION

Calprotectin (S100A8/S100A9 protein) or damage-associated molecular pattern (DAMP) protein mainly denotes neutrophil activation. It represents ~45% of the cytoplasmic proteins in neutrophils, are released under inflammatory conditions and form a stable heterodimer. CLP can be detected in the plasma of healthy subjects with an estimated range between 0.1 and 1.6 μg/ml **(1, 2)**.

It's released immediately in response to local inflammation. In contrast, other inflammatory markers are generated by downstream pathways and need to be synthesized de novo leading to delays or other factors influencing response (3).

Activation of the innate immune system and tissue damage are the most powerful stimuli for CLP production. CLP is elevated in inflammatory bowel disease, rheumatic diseases, sepsis, acute coronary syndromes, cystic fibrosis, infection, inflammation and cancer (3).

In peripheral arthritis like RA and PSA, CLP is a promising serum marker of inflammatory activity as local production of CLP from activated synovial cells and activated macrophages reflect the extent of inflammation. Moreover, the small size of calprotectin molecule (36.5 kDa) allows easy diffusion from inflamed joints into the circulation, where it can be easily measured accurately (4).

In axial spondyloarthritis (SPA) including AS, PSA and inflammatory bowel disease (IBD)-related spondylitis. CLP was reported to be expressed by macrophages and neutrophils in synovial tissue of AS patients, and the colon of patients with IBD.

Both serum and fecal calprotectin was reported to be associated with SPA although the pathogenesis is not clear activation of the innate immunity pathways is evident (2). Moreover, higher calprotectin serum levels were associated with more severe forms of the disease but this result still under observation (3).

We conducted this study to evaluate the levels of serum calprotectin in RA, PSA and AS patients and its relation with disease activity parameters.

PATIENTS AND METHODS

This study was carried out on 60 patients with inflammatory arthritis (RA, AS & PSA), in addition to 20 apparently healthy volunteers matched in age and sex. Patients were selected from the Outpatient Clinics of Physical Medicine, Rheumatology, and Rehabilitation Department of Tanta University Hospitals.

Inclusion criteria: Patients diagnosed as inflammatory arthritis according to diagnostic and classification criteria of each group **(5-8)**.

Exclusion criteria: Patients with thyroid, diabetes mellitus and hepatic or renal diseases. Patients with history of infections as tuberculosis, malignancy and demyelinating disease. Patients with symptoms of IBD.

Patients groups: Group I: Involved 20 patients with RA diagnosed by 2010 ACR/EULAR classification criteria for RA score for patients (5).

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Group II: Involved 20 patients with AS diagnosed by Assessment of Spondylo-arthritis International Society (ASAS) classification criteria for axial SPA [6].
Group III: Involved 20 patients with PSA diagnosed by Classification criteria for Psoriatic Arthritis (CASPAR) criteria [7, 8].
Control group: Involving 20 apparently healthy volunteers matched with patients in age and sex.

All the patients were assessed by the following:
A. Clinical assessment: I- Full medical history taking. II- Locomotor system examination. III-Assessment of diseases activity using (DAS, ASDAS, DAPSA) [9-11]. IV- laboratory findings (ESR, CRP, CBC RF, ACPA & serum calprotectin).

Intended use: The kit used a double-antibody sandwich **enzyme-linked immunosorbent assay** (ELISA) supplied by Sun Red technology to assay the level of calprotectin in serum samples of human calprotectin in blood samples. Catalog No. 201-12-546 D (CALPRO) [12]. Assay range:1.56 – 100 ng/ml
VI - Musculoskeletal ultrasound: Patients were examined at ultrasonography unit of Rheumatology, Rehabilitation and Physical Medicine Department using SAMSUNG MEDISON (UGEO H60), with linear array transducers (7.5-16 MHz). EULAR certified MSK US operator blinded to the study performed the following US scores.

- **Activity of diseases by US7 for arthritis** [13] .
- **Madrid score for enthesites in relation to serum calprotectin** [14].

Ethical Approval: Written informed consents to participate were acquired from all patients. The study was in accordance to the principles of Helsinki declaration and was approved by Local Research Ethics Committee of Faculty of Medicine Tanta University.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The used tests were:

- **Chi-square test:** For categorical variables, to compare between different groups.
- **Monte Carlo correction:** Correction for chi-square when more than 20% of the cells have expected count less than 5.
- **Mann Whitney test:** For abnormally distributed quantitative variables, to compare between two studied groups.
- **Kruskall Wallis test:** For abnormally distributed quantitative variables, to compare between more than two studied groups and followed by Post Hoc test (Dunn's for multiple comparisons test) for pairwise comparison
- **Spearman correlation test (r_s):** was utilized to study the relationship (direction and power) of nonparametric variables. Correlation considered weak when it was from 0.0 to less than 0.25, moderate from 0.25 to less than 0.75 and strong from 0.75 to 1.0.
- **Receiver operating characteristic (ROC) curve:** was used for measuring the accuracy, sensitivity & specificity. Areas under the curve represents the accuracy, it ranges from zero up to one (100%).

RESULTS

The demographic data of our participants are delineated in **tables (1,2)** and showed that : RA patients were predominantly female 80% in contrast to AS that affect 100% males and in PSA 65% were females . According to laboratory investigations, rheumatoid factor (RF) was positive in 80% in RA and 25% in PSA. AntiCCP was elevated in 50% in RA and 35% in PSA. The mean delay in diagnosis in our patients are 7.0 ± 5.26 years in RA , 9.44 ± 11.08 years in AS and 18.65 ± 8.95 years in PSA.

High serum CLP levels were detected in patients group with mean values of 5.89 ± 2.73 in RA, 7.50 ± 4.40 in AS, and 4.85 ± 2.46 in PSA and there was significant difference with the control group. Also there was significant difference between As ,RA and PSA with higher levels detected in As group **(table3).** In evaluation of serum CLP as a bio marker for these autoimmune rheumatic diseases our result showed that serum CLP has specificity of 70% at level of 1.6 microgram/ml (**table 4)**. Serum CLP had a negative correlation with CRP in RA, ESR and CRP in AS and in PSA significant positive correlation with peripheral arthritis DAPSA **(table 5).**

Table (1): Demographic and laboratory data of the three studied groups

	RA (n = 20)		AS (n = 20)		PSA (n = 20)	
Sex						
Male	4	20.0%	20	100.0%	7	35.0%
Female	16	80.0%	0	0.0%	13	65.0%
Age (years)						
Min. – Max.	40.0 – 70.0		18.0 – 58.0		30.0 – 59.0	
Mean ± SD.	52.60 ± 9.85		32.0 ± 14.55		43.85 ± 10.09	
Smoking						
No	13	65.0%	12	60.0%	17	85.0%
Yes	7	35.0%	8	40.0%	3	15.0%
Medication						
NSAID	0	0.0%	12	60.0%	4	20.0%
Conventional	20	100%	0	0.0%	14	70.0%
Biological	0	0.0%	8	40.0%	2	10.0%
ESR 1st Min. – Max.	7.0 – 70.0		8.0 – 25.0		10.0 – 80.0	
Mean ± SD.	37.25 ± 22.19		19.0 ± 6.77		23.35 ± 19.96	
CRP -ve	6	30.0%	12	60.0%	8	40.0%
+ve	14	70.0%	8	40.0%	12	60.0%
Min. – Max.	6.0 – 49.0		6.0 – 12.0		12.0 – 24.0	
Mean ± SD.	13.30 ± 10.87		9.0 ± 3.21		14.64 ± 4.94	
RF -ve	4	20.0%	20	100.0%	15	75.0%
+ve	16	80.0%	0	0.0%	5	25.05
Min. – Max.	5.0 – 128.0		–		4.0 – 40.8	
Mean ± SD.	53.22± 40.81		–		26.0 ± 15.2	
Anti-CCP -ve	10	50.0%	20	100.0%	13	65.0.0%
+ve	10	50.0%	0	0.0%	7	35.0%
Min. – Max.	10.0 – 200.0		–		–	
Mean ± SD.	88.93 ± 73.43		–		–	
Duration (years) Min. – Max.	1.0 – 15.0		0.20 – 30.0		7.0 – 30.0	
Mean ± SD.	7.0 ± 5.26		9.44 ± 11.08		8.95	

Table (2): Clinical assessment of 3 studied groups in relation to activity according to DAS28, ASDAS, and DAPSA score.

	DAS28		**ASDAS**		**DAPSA**	
Patients	No.	%	No.	%	No.	%
Remission (< 2.6)	4	20.0	0	0	0	0
Low disease activity (≤3.2 & >2.6)	0	0.0	0	0	0	0
Moderate disease activity (>3.2 - ≤ 5.1)	16	80.0	12	60	3	15
High disease activity (>5.1)	0	0.0	8	40	17	85
Min. – Max.	1.40 – 5.10		1.70_4.10		26.0-58.0	
Mean ± SD.	3.77 ± 1.28		2.56±1		47.10±11.19	

Table (3): Comparison between the four studied groups according to serum calprotectin

Calprotectin	RA (n = 20)	AS (n = 20)	PSA (n = 20)	Control (n = 20)	H	p
Min. – Max.	3.88 – 15.90	3.60 – 15.77	3.16 – 10.48	0.11 – 2.40	50.038*	<0.05*
Mean ± SD.	5.89 ± 2.73	7.50 ± 3.00	4.85 ± 2.46	1.10 ± 0.75		
Significance between groups	P1 0.066	P2 0.728	P3. 0.029*			

P1 between RA and AS. P2 between RA and PSA. P3 between AS and PSA.

There is a significant difference between the studied groups and the control group regarding serum calprotectin with significant difference between AS and PSA groups.

Table (4): ROC curve for calprotectin as a marker for autoimmune diseases.

AUC	p	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
1.00	<0.001*	**>2.78**	**100.0%**	**100.0%**	100.0%	100.0%	100.0%
1.00	<0.001*	**>1.6**	**100.0%**	**70.0%**	90.9%	100.0%	92.5%

AUC: Area Under a Curve NPV: Negative predictive value PPV: Positive predictive value *p≤0.05 (Statistically significant).

Table (5): Correlation between serum Calprotectin and different parameters in three studied groups

	Calprotectin RA		Calprotectin AS		Calprotectin PSA	
	rs	p	rs	p	rs	p
DAS28	0.184	0.438				
Synovitis						
Gray	-0.078	0.743	-0.975	<0.001*	0.073	0.760
Doppler	0.215	0.364	-0.894	<0.001*	0.058	0.808
Erosion	-0.082	0.730	-0.707	<0.001*	0.248	0.291
RF	-0.431	0.096				
Anti-CCP	0.288	0.419			0.520	0.019*
ESR	0.031	0.898	-0.564	0.010*	0.580	0.079
CRP	-0.672	0.008*	-0.598	0.005*	0.010	0.966
MASI score			0.040	0.867	0.309	0.184
ASDAS			-0.051	0.830		
DAPSA					0.474	0.035*

Correlation 0.1-0.2 weak 0.2- 0.75 moderate 0.75-more strong.

There is a significant positive correlation between serum calprotectin and DAPSA score and significant negative correlation with CRP in RA group , ESR and CRP in AS group.

DISCUSSION

CLP plays multiple roles in innate immunity due to its intrinsic cytotoxic and pro inflammatory properties, as it controls cell differentiation, proliferation and netosis [15]. It also regulates adaptive immune responses through inducing CD8-T cells, acting on the over expression of dendritic cells, function as an endogenous ligand of CD69, and is a costimulatory raiser of CD40/CD40L, activating T-cells [15, 16]. It has been studied as an acute-phase protein in autoimmune diseases such as RA, autoimmune vasculitis, systemic lupus erythematous and Sjogren's syndrome. [15,17,18].

All of our patients had higher serum CLP levels than the control group, with mean values of 5.89 ± 2.73 in RA, 7.50 ± 4.40 in AS, and 4.85 ± 2.46 in PSA and there was significant difference between level of serum CLP in rheumatic diseases and control group. **Hanson *et al.* [19]** stated that calprotectin is a pro-inflammatory factor of innate immunity that functions as an endogenous damage-associated molecular pattern molecule through the activation of Toll-like receptor 4. It has been suggested that almost every autoimmune diseases have higher levels of serum CLP.

Besides we found significant difference between AS, RA and PSA with higher levels detected in A group. **Cayper *et al.* [20]** noted that, CLP is secreted not only in the joints, but also in other inflamed tissues such as the gastrointestinal mucosa. Up to 50% of patients with SPA have subclinical bowel inflammation, and this might explain the elevation of serum CLP in AS [21].

Regarding CLP correlation with diseases activity, no significant correlation was detected with ultrasonography and clinical parameters except for DAPSA score in PSA patients showed positive correlation with serum CLP and laboratory markers except negative correlation with ESR and CRP in RA and AS. These results contradict with other researchers ([22-24]. This could be partially explained as most of our patients were in active state in RA patients, 80% had moderate activity according to DAS28, in AS group, 60% of our patients had moderate activity and 40% were in severe activity according to ASDA, in PSA group 15% were in moderate and 85% were in severe activity according to DAPSA. Given the fact that CLP was high in all of our patients even those in remission, this lack of association might be for the severity of the activity. In our opinion, CLP might serve more in assessment of disease severity and prognosis rather than disease activity but still more studies is needed to support this hypothesis.

In addition nature of elevation and decent of inflammatory markers as ESR and CRP is different than that of serum CLP, which is released immediately after synovial inflammation in contrast to the relative delay of acute phase reactants due to de novo synthesis in response to disease activity.

Regarding CRP, although we had only 4 patients in remission and 56 patients between moderate to severe activity according to clinical scoring, 26 patients had negative CRP and 34 patients had positive CRP distributed in our three groups. Also, we detected negative correlation between serum CLP & ESR and CRP. This agrees with the study of **Torgutalp *et al.*** [25] who found that serum calprotectin in RA is a strong acute phase reactant that increases in synovitis and activity, making serum calprotectin more specific than CRP in RA, particularly in patients with liver issues. **Gialouri *et al.* [26]** concluded the same opinion that CRP shouldn't be used as a marker of PSA activity as 52% of his patients with moderate or high DAPSA score displayed negative CRP values. **Other researchers** stated that CRP or ESR is elevated in about 40 to 50 % of patients with AS and normal ESR or CRP does not rule out AS disease activity [27, 28]. **Eltwaab *et al.* [29]** is in accordance with our results as they found that serum levels of CLP in patients with RA with negative CRP were significantly higher than that of the healthy controls.

It has been revealed that more than 40% of RA patients have a normal CRP level. Therefore, for patients with a normal CRP level, serum CLP may be used to accurately reflect diseases activity and prognosis [30].

According to the results of ROC curve analysis in our patients (RA, AS & AS) and control group, CLP had 70% specificity in relation to control group. This specificity reached 100% at a value of 2.7 micrograms that suggest serum CLP can be used as good biomarker to differentiate autoimmune diseases with cut off values greater than 2.7 micrograms. However, concomitant non-autoimmune inflammatory conditions must be considered and ruled out first.

LIMITATIONS

Our study has several limitations. First, no longitudinal samples were collected, and base line evaluations were not available to compare calprotectin levels. Another potential limitation is the long disease duration of our participants. Lastly, the small size sample in each group.

CONCLUSION

CLP serum levels were particularly high in RA, PSA and AS and can be used to differentiate these autoimmune diseases. It offers a good alternative when CRP is negative to monitor disease activity.

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Conflict of Interest: Nil.

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