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ORIGINAL ARTICLE

Study of Serum and Synovial Fluid Levels of Calprotectin of Rheumatoid Arthritis Patients in Correlation with Disease Activity and Severity

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ABSTRACT

Background: *Rheumatoid arthritis [RA] is one of the most common chronic, inflammatory autoimmune disease with a frequency of 0.5–1.0% between the adult population of developed countries. It is marked by chronic inflammation of synovial tissue and accompianed by damage of the articular cartilage and adjecent bone, leading to substantial disability.* ***Objectives****: The aim of this study is to determine serum and synovial fluid levels of calprotectin in rheumatoid arthritis patients and to determine its relation with disease activity and severity.* ***Methodology:*** *This study was carried out on 40 rheumatoid arthritis patients who were admitted to Rheumatology, Rehabilitation and Physical Medicine Outpatient' clinic and Inpatient Department of Benha University Hospital .Also Thirty age and sex matched(28 females and 2 males) apparently healthy volunteers were included in the study as a control group . All patients were assessed by full medical history, clinical examination, functional assessment, laboratory investigations including CBC, ESR ,CRP, liver functions, RF, Anticcp antibody, and X-rays were done to both hands. Serum and synovial levels of calprotectien were measured using the ELISA technique.* ***Results:*** *Serum levels of calprotectien were significantly higher in RA patients than healthy subjects [p<0.001], also there was a highly statistically significant increase in the mean synovial fluid calprotectin levels than mean serum calprotectien levels [p<0.001]. Local and systemic levels of calprotectin correlate with clinical, immunological and instrumental assessments of disease activity and the inflammatory degree of the joint.* ***Conclusion:*** *Calprotectin could be used as a new biomarker for monitoring the disease activity and severity of RA. Larger sets are needed to confirm the diagnostic and prognostic accuracy of calprotectin in RA*

INTRODUCTION

Rheumatoid arcthritis [RA] is a chronic autoimmune disease characterized by inflammation of the synovium, which outlines the inner cavity of synovial joints except for cartilage surfaces. It is a heterogeneous disease spanning several disease subsets with potential distinct pathogenic pathways. [1]

There is a general consensus that RA is a multi factorial disease, resulting from the interaction of both genetic and environmental factors, which contribute to its occurrence and expression.[2]

Calprotectin is a calcium-binding proteins display in the cytoplasm about neutrophils and expressed on the membrane of monocytes. Upon neutrophil activation or endothelial adhesion of monocytes, calprotectin is released and may be discharged in serum or body fluids as a potentially incendiary clinical inflammatory marker. [3]

During the past two decades there has been increasing interest in calprotectin, a major leucocyte protein, which constitutes about 40–60% of the soluble cytosolic protein content in neutrophilic granulocytes, as well as being a major monocyte/macrophage protein. Calprotectin is released during the interaction of monocytes with inflammatory activated endothelium, and it binds to endothelial cells and modulates transendothelial migration of leucocytes.[4]

The aim of this study is to estimate serum calprotectin levels and level of calprotectin in the synovial fluid of rheumatoid arthritis patients and to determine its correlation with disease activity and severity.

METHODOLOGY

This study was carried out on 70 individuals attending Inpatients and Outpatients Clinics of Rheumatology and Rehabilitation Department, Benha University Hospital.

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They were divided into two groups:

- *Group 1:* Included fourty (40) adult RA patients 37 females and 3 males clinically diagnosed and classified according to the American College of Rheumatology/European League against Rheumatology EULAR 2010 classification criteria for RA at base line [5].
- *Group 2:* Thirty (30) age and sex matched group 28 females and 2 males apparently healthy volunteers were included in the study as a control group

Exclusion criteria

- Patients with other connective tissue diseases
- Patients with degenerative arthritis
- Pervious joint infection

Ethical considerations:

Institutional review board approval of protocol as well as an informed written consents of patients and controls for this study were obtained.

Statistical Analysis:

The clinical data were recorded on a report form. These data were tabulated and analysed using the computer program SPSS [Statistical package for social science] version 20 to obtain: In the statistical comparison between the different groups, we use the following tests to determine the significant difference .Student's *t*-test used to compare mean of two groups of quantitative data of parametric. Mann-Whitney test used to compare mean of two groups of quantitative data of non-parametric ANOVA test [F value] and kruskal-wallis test:-Used to compare mean of more than two groups of quantitative data of parametric and non-parametric respectively.

Inter-group comparison of categorical data was performed by using fisher exact test [FET]. A P value <0.05 was considered statistically significant [*] while >0.05 statistically insignificant P value <0.01 was considered highly significant [**] in all analyses.

RESULTS

This study include 40 adult RA patients they were 37 females and 3 males clinically diagnosed and classified according to the American College of Rheumatology / European league against Rheumatology EULAR 2010 classification criteria for RA at base line [5]. Their ages ranged between 23and 70 years with a mean of 45.33±11.79 years. Thirty Patients of them had unilateral knee effusion and 10 patients had bilateral knee effusion . Also 30 age and sex matched group were included in the study they were 28 females and 2 males apparently healthy volunteers were included in the study as a control group, their ages ranged between 31 and 65 years with a mean of 50.2±7.67years.

The present study revealed that the mean serum level of calprotectin were significantly higher in RA patients in comparison to control groups [p<0.001] as shown in table (1), also there was a highly statistically significant increase in the mean synovial fluid calprotectin levels than in mean serum calprotectin levels (p<0.001).

Also there was a statistically significant correlation between disease activity and serum and synovial fluid levels of calprotecin**.** Table (2)

Table 1: Calprotectin level in serum and synovial fluid of RA and control groups

	Case group (40)	Control group (30)	Statistical test (MW)	P value
Serum calprotectin median (IQR)	3.26(1.29-4.78)	0.42(0.38-0.54)	6.99	<0.001**

Table 2: Correlation between activity , serum and synovial levels of calprotectin

	Mild (5)	Moderate (15)	Severe (20)	Statistical test (FET)	P value
Serum calprotectin median (IQR)	1.09 (1.02-1.09)	2.34 (0.92-2.78)a	4.78 (3.74-5.94)ab	KW= 21.34	<0.001**
Synovial calprotectin mean ±SD (range)	0.92 (0.51-5.83)	3.45 (1.02-6.78)	4.89 (0.92-5.77)	KW= 1.38	0.501

a=sig & mild b= sig & moderate KW: kruskal-wallis

Correlative Analysis:

Table 3: Correlation between serum, synovial calprotectin levels and other variables

	Serum calprotectin		Synovial calprotectin	
	Rho	**P value**	**rho**	**P value**
DAS-28	0.67	<0.001**	0.31	0.049*
X ray grading	0.26	0.103	0.39	0.012*
HAQ	0.504	0.001**	0.049	0.77
BMI	0.156	0.337	0.055	0.734
Disease duration	0.471	0.003**	0.435	0.006**
RF	0.38	0.015*	0.32	0.045*
Anti-CCP	0.332	0.037*	0.06	0.72
CRP	0.483	0.002**	0.25	0.13
ESR	0.649	<0.001**	0.14	0.41
HGG/dl	-0.357	0.024*	-0.008	0.96
HCT	-0.15	0.37	-0.11	0.50
RBCs	-0.30	0.058	-0.08	0.61
RDW cv	0.37	0.018*	0.22	0.17
WBCs/103mm3	0.09	0.58	-0.15	0.37
Plat /103mm3	0.08	0.60	-0.17	0.28

There was astatistically high significant positive correlation between serum levels of calprotectin and DAS 28, HAQ disease duration, ESR, CRP, and significant positive correlation between serum calprotectin levels and Anti ccp, RF, HGG and RDW as shown in table 3 and fig 1.

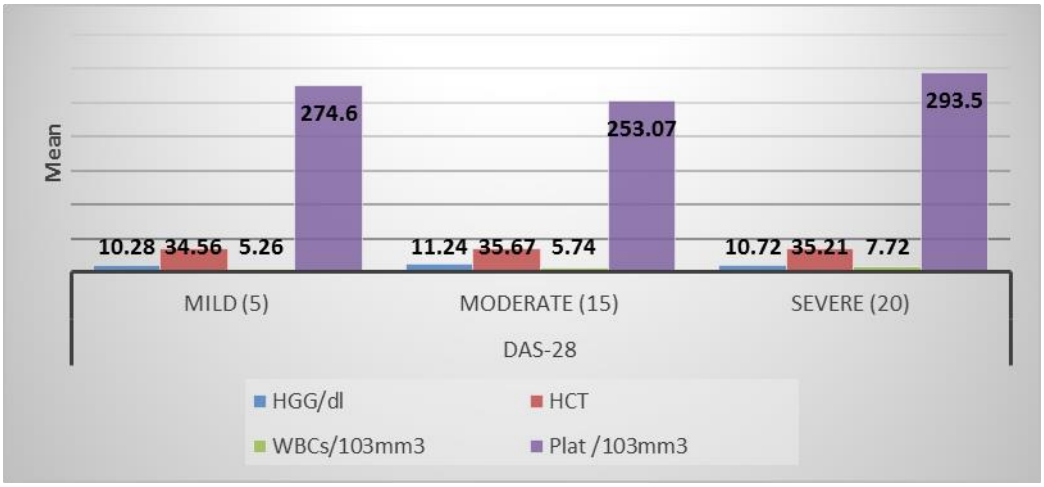

Fig. 1: Correlation between disease activity and hemoglbobin, hematocrite,white blood cell and platelets

DISCUSSION

The present study revealed that the mean serum level of calprotectin was a highly significant increase of the serum calprotectin in RA patients compared with control groups (p<0.001).

Also there was highly statistically significant increase in the mean synovial fluid calprotectin levels than that mean serum calprotectin levels (p<0.001)

This is in agreement with the result of Shumnalieva et al .[6] results who reported that the mean serum levels were higher in RA patients compared to healthy controls (p<0.001).

Also Cerezo LA et al.[7] results were matched with our findings where they reported that the mean serum levels of calprotectin in RA patients were highly significantly increased compared to control patients (p<0.0001).

This also goes in agreement with Chen et al. [8] who found higher levels of calprotectin in patients with RA and this was in keeping with the high calprotectin gene expression reported in patients with such a disease.

As regard the mean serum and synovial fluid levels of calprotectin in RA patients, there was a highly statistically significant increase in the mean calprotectin synovial fluid levels than calprotectin serum levels ($p<0.001$)

This coincides with the study done by Shumnalieva et al.[9] who also compared the serum calprotectin levels with its synovial fluid level in RA patients and found that the synovial fluid levels of calprotectin showed a highly significant increase than their serum level in the study group.

Also Berntzen et al[10] demonstrated significantly elevated synovial fluid, and circulating calprotectin levels, in patients with RA compared with control individuals . Synovial fluid calprotectin levels were significantly higher than that of serum counterparts, suggesting local release of the protein. Also they reported a positive association between calprotectin, inflammatory and serological markers, as well as with the RA joint disease activity score.

In our study there was asignificant correlation between the mean serum calprotectin level and disease duration ($p>0.003$), and between the synovial levels of calprotectien and disease duration ($p>0.006$) The present study also revealed statistically significant correlation between the mean serum and synovial fluid levels of calprotectien and CRP, ESR, RF, anticcp DAS and x ray grading by Larsen score ($p<0.031$).

These results were in consistent with the results of Lucie et al.[11] who found that the mean serum calprotectien levels was significantly correlated with ESR, CRP, RF and Anti-CCP patients ($p = 0.001$, $p=0.0001$, $p= 0.009$, $p = 0.042$, respectively) and insignificantly correlated with the VAS ($p= 0.15$)

The correlation of calprotectin with the acute phase reactants suggested that calprotectin is a better biomarker to predict disease progression as it correlates with acute phase reactants; thus, combining measurement of CRP with that of calprotectin may be a useful strategy whice predicts disease progression Young-Min.[12]

The present study revealed significant positive correlations between serum calprotectin and both ESR and CRP in RA patients.

Also this finding is in accordance with De Seny et al.[13] and Hammer et al.[14] who reported that calprotectin may behave like an acute phase protein. However an improved face validity of calprotectin as a marker is that the protein is released from activated leucocytes which are derived mainly from the inflamed synovium in patients with RA. In contrast, the acute phase proteins CRP and ESR are primarily produced in hepatocytes after induction by interleukins released during inflammation. Thus calprotectin is a more advantageous marker compared to acute phase proteins by directly reflecting the amount of activated leucocytes in the inflamed joints. Hammer et al. [15]

A remarkable finding of the current study is the significant correlation between serum calprotectin and RF levels which agree with the finding of Hammer et al.[16] who reported a significant positive correlation between the two markers.

In our study there was asignificant correlation between synovial calprotectin level and x ray grading which was determine by Larsen score

The association between calprotectin and joint damage is biologically plausible. Calprotectin may be released from activated granulocytes and macrophages in the inflamed synovium as well as from the high number of granulocytes in the synovial fluid during inflammation. . This supports the finding of high levels of calprotectin in synovial fluid in several inflammatory joint diseases, where significant correlations were found between the levels in plasma and synovial fluid.

Conclusion and recommendations

The level of systemic and local calprotectin are correlated with clinical, immunological and instrumental assessments of disease activity, joint inflammation and disease progression. Calprotectin could be used as anew biomarker to monitor the disease activity and severity of RA. Larger sets are needed to confirm the prognostic and diagnostic efficacy of calprotectin in RA.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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