Original Research Article

Disease activity score 28 (DAS 28) correlation to serum prolidase as a collagen turnover marker in comparison to some pro-inflammatory markers in rheumatoid arthritis patients: clinical and sonographic study

Abstract:

objectives: Disease activity score (DAS) correlation to serum prolidase in comparison to other pro-inflammatory markers in rheumatoid arthritis (RA)cases.

Subjects ,Meterials and Methods: Forty RA cases and 40 age and sex matched controls were studied in this case-control study. Cases fulfilled the 2010 American college of rheumatology (ACR)/ European League Against rheumatism (EULAR) RA classification criteria. Disease activity was assessed by the disease activity score 28 (DAS 28). Ultrasound (US) scanning of synovitis in pre-selected joint was done in both gray scale (GS) and power doppler US (PDUS) modes.

Results: The cases'mean age was 50.05 ± 7.99 years, 52 females and 28 males participated in this study. The mean disease duration was 6.10 ± 3.89 years and the mean for the DAS-28 score was 4.85 ± 1.04. There was a significant correlation between CRPM and DAS 28 score (r=0.323, p=0.042). IL-6 correlated with GSsynovitis in the wrist (r=0.376, p= 0.017), MCP (r=0.430, p= 0.006) and PIP (r=0.653, p=<0.001**)** joints. Serum prolidase correlated with both GSsynovitis in the wrist (r= 0.486, p=0.001) and MCP (r=0.604, p=<0.001) and PDUSsynovitis in the wrist (r=0.334, p=0.035), and MCP joints (r=0.456, p=0.003). Regression analysis showed that IL-6 level had a greater impact on GS synovitis (p= <0.001), compared with serum prolidase.

Conclusions: Serum IL-6 and prolidase has significant correlations with disease activity parameters compared with other markers. Serum prolidase is an evidence on increased collagen turnover in RA cases and might be a useful tool in the assessment of joint inflammation and predicting future damage.

Keywords:Rheumatoid arthritis, serum prolidase, Disease activity score 28, ultrasound

Key points:

- High serum prolidase in RA cases associated with sonographicsynovitis
- CRPM associated with high disease activity detected with DAS 28 score
- Among all studied cytokines, serum IL-6 has the greatest impact on sonographicsynovitis

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that causes joint destruction. RA mostly affects the synovial membrane, cartilage, and bone. As a systemic inflammatory illness, RA is also linked with systemic consequences, probable disability, substantial socioeconomic expenses, and premature mortality globally. Despite advancements in understanding the biology of RA, the aetiology of the disease remains unknown. [1] RA is a complicated illness involving a large number of distinct cell types and various signalling pathways. The disease's onset and persistence are mediated by the stimulation of autoimmune processes and the generation of many cytokines. Cytokines are released by leukocytes to mediate paracrine or autocrine actions, therefore controlling a variety of cellular processes, including activation, differentiation, survival, and migration. Most extensively researched are tumour necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1 (IL-1). Prior to the beginning of RA symptoms, abnormal cytokine balance has been identified in RA casescompared with healthy persons [3,4].

TNF, IL-6, IL-1 and/or IL-1RA, GM-CSF, IL-4, IL-12, IL-17, and the eosinophil chemotactic chemokine are among these cytokines. The concentration of IL6 in serum corresponds with disease activity and radiographic joint damage. In circumstances when synovial fluid has elevated amounts of IL6, osteoclast activation corresponds with joint injury. The Interleukin-17 cytokine family includes IL-17A, IL-17B, IL-17C, and IL-17D. IL-17A was the first member of this family to be discovered and the most investigated. IL-17A contributes to the inflammatory environment in RA joints by regulating the production of pro-inflammatory cytokines, angiogenesis, and osteoclastogenesis. [8]

Inflammatory cytokines that are present in RA also induce the development of metalloproteinases. MMPs are extracellular matrix–degrading enzymes generated at high quantities by type B synoviocytes in RA, particularly at areas of erosive activity. [9] Prolidase is a metalloprotease enzyme that plays a crucial function in the metabolism of collagen. It catalysesprocollagen, collagen, hydroxyproline, and proline proteins within the cell. A rise in serum prolidase activity has been seen in several disorders, including rheumatic, hepatic, and malignant conditions. Establishing a network between lymphocytes, macrophages, fibroblasts, and hepatocytes, cytokines and acute phase proteins mutually control the expression and activity of one another. Activation of the network leads in the inflammation and gradual joint destruction typical of RA. C-reactive protein (CRP) is an indicator of systemic inflammation and is higher in RA sufferers.

Multiple studies have found elevated CRP levels in blood samples of RA cases prior to the start of RA symptoms. [12] It is advised that high sensitivity CRP (hs-CRP) tests be used to detect low disease activity in RA. In RA, it is common to observe systemic low-grade inflammation that is often undetectable by regular CRP testing. [13] CRPM, a result of MMP breakdown, is an additional significant CRP metabolite. In RA, serum CRPM levels were observed to be dose- and time-dependently suppressed in response to anti-TNF and anti-IL-6 therapy. Also, associations between CRPM and RA disease activity markers such as CRP, Erythrocyte sedimentation rate (ESR), and disease Activity Score in 28 joints were shown to be statistically significant (DAS28). [14]

The key to a better result in instances of RA is disease activity assessment coupled with a treat to target strategy. [15] It is essential to use a validated disease activity measure, such as the DAS28 [16], to avoid joint degeneration at the patient group level. [17]. Ultrasound (US) and magnetic resonance imaging (MRI) are superior to clinical examination for detecting subclinical joint inflammation, and US synovitis has been demonstrated to predict radiographic progression in RA cases even during clinical remission. Multiple studies have previously established the concordance between US and clinical examination in identifying synovial inflammation in RA cases. Therefore, the purpose of this case-control study was to compare the connection between DAS and serum prolidase as a collagen turnover marker in RAcasesvs certain pro-inflammatory indicators. Both clinical and sonographic evaluations of disease activity were conducted.

Subjects,Materials and Methods:

Forty cases were recruited into this case control study fromOPC of Physical Medicine, Rheumatology and Rehabilitation Department at Tanta University hospitals. All cases fulfilled the 2010 ACR/ European League Against (EULAR) RA classification criteria. [19]Cases were excluded if they have any of the following criteria:presence of any concomitant autoimmune, inflammatory, metabolic or infectious disease.

A control group of forty healthy cases of matched age and sex were enrolled in this study. The study conforms to the provisions of the Declaration of Helsinki in 1995 and was approved by the Local Research Ethical Committee of Tanta University approved the study. All cases gave their informed consent prior to their inclusion. All participants were subjected to full medical history taking and clinical characteristics including: age, sex, arthritic symptom duration, tender joint and swollen joint count. Laboratory tests included: erythrocyte sedimentation rate (ESR), CRP, rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (anti-CCP), hsCRP, CRPM, serum prolidase, serum IL-6 and IL-17

Sampling:

Each participant's antecubital vein was aseptically accessed to collect 5ml of venous blood in a set of plain tubes. Blood samples were centrifuged at 2000 rpm for 10 minutes to separate the serum. The collected serum was aliquoted and kept at -20°C until the time of the experiment. Using a sandwich enzyme-linked

immunosorbent assay (ELISA) commercial kit, the serum concentrations of hsCRP and CRPM were measured (BioCheck, Inc. USA and Cloud-Clone Crop. USA. respectively). Interleukin 17 and interleukin 6 were measured using commercial ELISA kits supplied by RayBiotech, Inc. in the United States. All ELISA procedures were performed in accordance with the manufacturer's instructions and read using a microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, France) at 450 nm with 570 nm correction wavelength. The concentrations of unknown samples were determined using the standard curve.

Myara et al. (1984) developed a technique for determining the proline levels generated by prolidase, which was used to estimate prolidase activity. The absorbance was then measured at 515 nm using a Biosystem spectrophotometer to estimate proline (BTS 350 semiautomatic analyzer). U/L values were represented. [20]

Ultrasound examination

Ultrasonographic assessment was performed using a Samsung UGEO H60 ultrasound device and a multifrequency linear probe (7–16 MHz). The gray scale (GS) and power Doppler US (PDUS)settings were adjusted to optimize image resolution and sensitivity to detect flow.Ultrasound scanningand scoring of synovitisof the selected joints (the metacarpophalangeal (MCP), wrist, proximal interphalangeal (PIP) and metatarsophalangealjoints (MTP) was performed within 48 hours from the clinical evaluation and blood sampling.[18]

In each patient, the second to fifth MCP, second to fifth PIP, first to fifth MTP and wrist joints were scanned bilaterally using a GS and PD longitudinal scan in the midline of the joint on both the dorsal and volar aspects according to EULAR recommendations. [21]The most severe grade in every scanned region was taken as a representative of this joint. All joints were scored for GS and PD synovitisusing asemiquantitative grading from 0 to 3(normal, minimal, moderate, severe).

Statistical analysis

It was calculated using version 20.0 of the statistical package for the social sciences software. For continuous variables, case data were given as mean standard deviation or median (minimum–maximum) and as number (percent) for categorical variables. The Chi-square test was utilised to analyse group comparisons for categorical variables (Fisher or Monte Carlo). To compare two groups with regard to quantitative variables with normal and atypical distributions, the Student t and Mann Whitney tests were utilised, respectively. Consideration was given to the Spearman correlation coefficient and logistic regression analysis. P value less than 0.05 was deemed statistically significant.

The characteristics of the 80 study participants (52 women and 28 men) are shown in (Table 1). The cases' mean age was 50.05 ± 7.99 years, the mean disease duration was 6.10 ± 3.89 years and the mean for the DAS-28 score was 4.85 ± 1.04. Anti-CCP antibodies were positive in all cases, while 80% had positive RF. There were no significant differences between cases and control cases with regard to mean age and sex.

Ultrasound detected GS and PD synovitis scores are described in (Table 2)Sonographicsynovitisis shown in (figure 1). Correlations between the variable studied inflammatory markers, cytokines, serum prolidase and both DAS 28 score and sonographic parameters are shown in(Table 3 and 4).Linear regression analysis represented in (Table 5) revealed that serum IL-6 had the higher

influence on ultrasound detected synovitis, where the PIP joints were the most influenced followed by MCPs and lastly the wrist joint.

Discussion:

Rheumatoid arthritis (RA) is a chronic inflammatory illness that primarily affects the synovial tissue and is characterised by pannus hyperplasia and gradual bone deterioration, which ultimately results in the loss of joint function. [22]

The diagnosis of RA is mostly based on clinical symptoms, radiographic abnormalities, and laboratory tests, such as inflammatory markers and autoantibodies. [23] Early identification of RA is essential because it aids in the effective control of disease activity, decreases the risk of disability, halts disease progression, and improves long-term outcomes. [24] Prior to the clinical start of RA, a growing body of evidence identifies cytokine disturbances. Cytokines stimulate synovial stromal cells to maintain an inflammatory environment. [5] The purpose of this study was to compare the influence of several pro-inflammatory mediators (hs CRP, CRPM, IL-6, and IL-17) vs serum prolidase as a metalloproteinase on clinical and sonographic disease activity in instances of RA.

52 females and 28 men, with a mean age of 50,00.5 7,99 years, participated in this study. The mean duration of the illness was 6.10 3.89 years, and the mean DAS-28 score was 4.85 1.04. The median grey scale wrist synovitis score in the U.S. was 2. The median wrist joint PDUS synovitis score was 1.

In the current investigation, all inflammatory markers were elevated in RA casescompared withcontrol. Regarding blood levels of hs-CRP and CRPM, Sennels H et al. [25] and Maijer KI [26] discovered comparable outcomes. The acute phase protein of the liver is a significant characteristic of several inflammatory disorders. In RA, it is believed that CRP and its products direct a portion of complement activation, beginning new joint involvement and contributing to joint destruction. [27] Regarding cytokines, the levels of IL-6 and IL-17 were greater in RA casescompared withcontrol. It is widely recognised that IL-6 and IL-17

contribute to joint inflammation and structural damage in RA. The correlation between elevated levels of both cytokines in blood, synovial fluid, and different tissues and higher RA disease activity has been shown. [28] [29]

Prolidase is an MMP that catalyses the rate-limiting step of collagen metabolism, and oxidative stress influences its activity. [30] Previous research has demonstrated that oxidative stress levels are greater in RA cases than in control. There are at least 16 forms of collagen in the human body; type I collagen is most typically found in bone. It has also been demonstrated that RA cases have a quicker bone turnover rate than persons without RA. Increased bone turnover and oxidative stress may result in elevated prolidase levels in RA cases. [31] Consistent with our findings, Ugan et al. discovered an increase in serum prolidase activity in RA casescompared withcontrol. Ucar et al. found no significant difference between the patient and the control group. [32]

In terms of correlations, serum IL-6 concentrations shown substantial positive associations with grey scale synovitis in the wrist, MCP, and PIP joints. While serum prolidase was favourably connected with grey scale synovitis in the wrist, MCP, PIP, and PDUS synovitis in the wrist and MCP joints, it was negatively correlated with PIP synovitis in the wrist and MCP joints. Both inflammatory cytokines and metalloproteinases interact to create a sonographically detectable local inflammatory state. In cultured synoviocytes, Trabandt et al. found that the inflammatory cytokines present in RA increase the synthesis of metalloproteinases. Analysis of synovial tissue from cases with RA demonstrated especially high metalloproteinase staining in the intimal lining layer and interface areas of erosive activity. [15] In both treated and untreated cases of RA, US observations of synovial hypertrophy and PD vascularity were favourably linked with various synovial fluid cytokines and growth factors, including IL-6 and IL-17A. [33]

CRPM is the sole biomarker that shown a significant connection with the DAS-28 score; two additional investigations [14] reached comparable conclusions. [26] This may be explained by the fact that pro-inflammatory cytokines drive and control RA to a large extent. Although the variance in cytokine levels and subsequent signalling pathways might be substantial, end products such as CRPM are well-established indicators of tissue damage. These indicators quantify the disease burden by reflecting the amount of cartilage that is being lost, and they are strongly related with disease activity. [34]

Linear regression analysis demonstrated that IL-6 has the strongest impact on ultrasound detected synovitis followed by the serum prolidase. Multiple studies support this result.[35,36] Baillet et al stated that IL-6 impact

on synovitis in RA is found in both treated and untreated cases. Thereby, IL-6 release from inflamed synovium in RA cases could be a surrogate marker of synovitis. [35] IL-6 is a pleiotropic cytokine capable of mediating cartilage and bone damage through induction of acute phase proteins, synoviocytes and osteoclast proliferation. [36]

The limitations of this study include its small sample size, cross sectional design, and the absence of another high-sensitivity imaging such as MRI. Also, the current study didn't measure cytokines concentration in the synovial fluid and only serum levels were done. Future studies investigating the large panel of MMPs is recommended. Also, adding an erosion score to sonographic evaluation is important to predict the impact on bone damage and joint destruction.

Conclusions:

The current study highlighted the role of serum prolidase in the pathogenesis of RA. Prolidase levels can be easily assessedin both serum and synovial fluid. Serum prolidase is an evidence on increased collagen turnover in RA cases and can be a useful tool in the assessment of disease activity as well as predicting joint damage and erosions. Also, blocking serum prolidase activity might be a promising tool in the treatment of RA cases.

Other study findings include: significantly higher levels of various inflammatory markers such as hs-CRP, CRPM, IL-6 and IL-17 in RA casescompared withcontrol. Serum IL-6 and prolidase has significant correlations with clinical and sonographic disease activity compared with other markers. US is a reliable tool in RA, able to give additional informations on disease activity beyond that given by clinical score.

List of abbreviations:

CRP	C-reactive protein
Hs CRP	High sensitivity c-reactive protein
CRPM	CRP matrix metalloproteinase degradation product
IL	Interleukin
RA	Rheumatoid arthritis
ACR	American college of rheumatology
EULAR	European League Against rheumatism
DAS 28	Disease activity score 28
US	Ultrasound
PDUS	Power Doppler ultrasound
GS	Grey scale

TNF	Tumor necrosis factor
MMP	Matrix metalloproteinase
MCP	Metacarpophalangeal
PIP	Proximal interphalangeal
MTP	Metatarsophalangeal

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Table 1: Comparison of the demographic features and the different inflammatory markers in the two studied groups

parameter	Cases (n = 40)	Control (n = 40)	**P**
Age (years)			
Mean ± SD.	50.05 ± 7.99	49.32 ± 5.58	0.639
Median (Range)	52 (32 – 63)	48 (43 - 60)	
Sex			
Female	30 (75%)	22 (55%)	0.061
Male	10 (25%)	18 (45%)	
Occupation			
Housewife	19 (47.5%)	12 (30%)	0.108
Manual worker	21 (52.5%)	28 (70%)	
ESR			
Mean ± SD.	45.90 ± 26.91	11 ± 5.91	**<0.001**
Median (Range)	35 (5 – 105)	10 (5 – 20)	
CRP			
Positive	30 (75%)	4 (10%)	**<0.001**
Negative	10 (25%)	36 (90%)	
hscRP (mg/L)			
Mean ± SD.	6.51 ± 4.22	1.27 ± 0.31	**<0.001**
Median (Range)	4.99 (1.80 – 15.80)	1.15 (0.90 – 2)	
CRPM (ng/ml)			
Mean ± SD.	5.03 ± 4.31	0.52 ± 0.33	**<0.001**
Median (Range)	2.92 (0.98 – 16.61)	0.54 (0.05 – 1)	
IL-6 (ng/ml)			
Mean ± SD.	30.58 ± 17.09	11.67 ± 5.63	**<0.001**

Median (Range)	24.80 (10.40 – 64.20)	10.30 (5.12 – 23.80)	
IL-17 (ng/ml)			
Mean ± SD.	43.71 ± 21.61	22.68 ± 5.77	**<0.001**
Median (Range)	35.80 (18.90 – 79.90)	21.95 (15.60 – 34)	
Serum prolidase (U/L)			
Mean ± SD.	607.22 ± 166.92	417.18 ± 144.93	**<0.001**
Median (Range)	560.65 (340.40 – 890.80)	469.65 (30.70 – 543.10)	

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, hsCRP: high sensitivity CRP, CRPM: metalloproteinase degradation product of CRP, IL-6: Interleukin-6, IL-17: Interleukin-17. Bold values are significant at ≤ 0.05

Table 2: Ultrasound parameters characteristics (median and range) in RA patients (n = 40)

	No. (%)
Ultrasound (B-mode synovitis)	
Wrist	
Median (Range)	2 (0 – 3)
MCP	
Median (Range)	2 (0 – 3)
PIP	
Median (Range)	1 (0 – 3)
MTP	
Median (Range)	2 (0 – 3)
PDUS synovitis	
Wrist	
Median (Range)	1 (0 – 2)
MCP	
Median (Range)	0 (0 – 2)
PIP	
Median (Range)	0 (0 - 0)
MTP	
Median (Range)	0 (0 - 0)

MCP: metacarpo-phalangeal, PIP: proximal inter-phalangeal, MTP: metatarso-phalangeal joints, PDUS: power doppler ultrasonography.

Table 3: Correlations of different inflammatory markers with sonographic parameters in RA patients

Parameter	Ultrasound (B-mode synovitis)								PDUS synovitis			
	Wrist		MCP		PIP		MTP		Wrist		MCP	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
hs-CRP (mg/L)	0.034	0.833	-0.150	0.357	-0.164	0.312	-0.137	0.399	0.069	0.673	0.024	0.884
CRPM	0.079	0.630	-0.022	0.892	-0.157	0.334	-0.033	0.839	0.148	0.364	0.306	0.054
IL-6	**0.376**	**0.017**	**0.430**	**0.006**	**0.653**	**<0.001**	0.203	0.208	0.273	0.088	0.227	0.158
IL-17	0.046	0.779	-0.044	0.790	-0.125	0.442	0.239	0.137	0.133	0.414	0.095	0.558
Serum prolidase (U/L)	**0.486**	**0.001**	**0.604**	**<0.001**	**0.489**	**0.001**	0.241	0.134	**0.334**	**0.035**	**0.456**	**0.003**

hs-CRP: high sensitivity CRP, CRPM: metalloproteinase degradation product of CRP, IL-6: Interleukin-6, IL-17: Interleukin-17. Bold values are significant at ≤ 0.05

Table 4: Correlation between DAS28 and different serum markers in patient group (n = 40)

	DAS28	
	r	**p**
Hs-CRP (mg/L)	0.292	0.068
CRPM (ng/ml)	0.323	**0.042**
IL-6 (ng/ml)	-0.047	0.773
IL-17 (ng/ml)	0.194	0.230
Serum Prolidase (U/L)	0.066	0.688

r: Pearson coefficient. Bold values are significant at ≤ 0.05

Table 5: Linear regression analysis for inflammatory markers affecting ultrasound detected synovitis

Ultrasound	p	95% C.I	
		L.L	U.P
Wrist synovitis			
IL-6	0.282	-0.007	0.024
Serum prolidase (U/L)	**0.031**	0.00	0.003
MCP			
IL-6	0.330	-0.010	0.028
Serum prolidase (U/L)	**0.002**	0.001	0.005
PIP			
IL-6	**<0.001**	0.019	0.046
Serum prolidase (U/L)	0.185	0.000	0.002

CI: Confidence interval, LL: Lower limit,UL: Upper Limit, MCP: metacarpo-phalangeal, PIP: proximal inter-phalangeal. Bold values are significant at ≤ 0.05

Figure1: grade II PD signal in the radiocarpal (wrist) joint with grade II GS synovitis and extensor tenosynovitis