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The Relationship between Serum Pentraxin-3 Level and Disease Activity and Severity in Rheumatoid Arthritis Patients

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Abstract
Background: Pentraxin 3 (PTX3) is an inflammatory marker, a member of long pentraxin superfamily that has an important role in inflammation and immunity. **Objectives:** The aim of this study was to measure serum pentraxin 3 levels in RA patients and evaluate their relation with different disease parameters. **Methods:** We measured serum PTX3 levels in 40 women with RA and 40 female control subjects. PTX3concentrations were measures by ELISA. **Results:** Median serum level of pentrexin 3 was significantly higher in RA patients compared to healthy controls (36.4 ng /ml vs 12.4 ng /ml respectively) ($p<0.001$). There was a significant difference in serum PTX3 levels according to the disease activity grades. PTX3 concentration was also significantly correlated with ESR($r = 0.506$, $p=0.001$), CRP($r = 0.780$, $p<0.001$) and Larsen Score ($r = 0.784$, $p<0.001$). There was no significant difference in serum PTX3 levels and RF& anti-CCP positive and negative patients ($P >0.05$). No significant correlation ($p>0.05$) was found between serum Pentraxin-3 and age ($r = 0.095$), BMI($r = 0.059$), disease duration ($r = 0.048$) and RF titer($r = 0.240$). **Conclusions:** PTX3 can be considered as a sensitive non-invasive biomarker reflecting the disease activity and radiological damage in RA patients.

Key words: RA: Rheumatoid Arthritis, PTX3: Pentraxin 3

Introduction:

Rheumatoid arthritis (RA) is the most frequent autoimmune disease and is characterized by multiple-joint synovitis, leading to joint destruction throughout the body. The patient subpopulation is etiologically heterogeneous, with a complex interaction of genetic background with environmental causes, such as smoking, periodontal disease, and microbial flora (1).

Pentraxins are inflammatory markers of multi-functional protein groups containing long pentraxins and short pentraxins. Pentraxin 3 (PTX3) is the protype long pentraxin in pentraxins superfamily, a class of humoral pattern recognition molecules, which play a fundamental role in innate and adaptive immune response (2).

PTX3 is an acute-phase protein. Unlike CRP, PTX3 is rapidly produced locally in the inflamed tissue and released by different cell types like macrophages, vascular endothelial cells, myeloid dendritic cells, synoviocytes, chondrocytes, vascular smooth muscle cells ,fibroblasts and not hepatocytes in response to pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin-1β (IL-1β), microbial components, or biochemical substances such as oxidized low density lipoprotein (ox-LDL); Therefore, the PTX3 response is faster than the CRP response, and PTX3 is thought to more accurately present the actual inflammatory status, in addition, high serum PTX3 levels may indicate local inflammation, e.g., in the joints or in the vessels (3). Also PTX3 has a key role in innate immunity, remodeling of the extracellular matrix, cancer and development of atherosclerosis (4).

In the context of RA, PTX3 has been shown to be constitutively highly expressed by synovial fibroblasts; however, various resident or infiltrating synovial cells like macrophages or endothelial cells might also represent a source of PTX3 upon inflammatory activation. Several groups found that PTX3 is upregulated in the serum of RA patients compared to healthy donors (5). Moreover, accumulation of PTX3 seems to occur in the arthritic joint, suggesting its involvement in triggering a local inflammatory process and degeneration of bone tissue. These results indicate that PTX3 can act as an inflammatory mediator in promoting bone loss in inflammatory bone-erosive diseases (6).

In a recent meta-analysis, which evaluated a total of 21 individual studies assessing circulating PTX3 levels of RA patients, the results showed that compared to healthy controls, circulating PTX3 levels were significantly increased in RA patients, which are influenced by age, disease activity, CRP levels, ESR, and disease duration (8). Whereas recent review study summed up the roles of PTX3 in RA and recognized that PTX3 may play a crucial role in RA progression (9).

The aim of this study was to measure serum pentraxin 3 levels in RA patients and evaluates their relation with different disease parameters.

Patients and methods:

This study included 40 adult RA patients attending inpatients and outpatients clinics of Rheumatology and Rehabilitation Department, Benha University Hospital and fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis (ACR/EULAR) (10). All patients were females, their ages ranged from 22 to 63 years. Forty healthy adult females of matched ages ranged from 21 to 61 years served as control. The exclusion criteria of the study included patients who were under the treatment of daily steroid (more than 5mg prednisone) or pulse steroid, having major medical co-morbidities like cancer, diabetes melitus, heart disease, hepatitis or chronic liver disease, having other rheumatic or autoimmune diseases, being pregnant or lactating or under the age of 18 years. The study protocol was approved by Benha University, Faculty of Medicine Ethics Committee and a prior written consent was taken from each patient and the control group included in the study.

All patients were subjected to full history taking, full clinical examination and locomotor system examination. The disease activity score (DAS28) was calculated and graded as a score of DAS28 below 2.6 indicate remission, between 2.6 and 3.2 indicates low, 3.2-5.1 indicates moderate, and >5.1 indicates high disease activity. Laboratory investigations were performed including ESR, CRP, rheumatoid factor (RF) and anti-cyclic citrullinated peptide (Anti-CCP).Hand radiograph were obtained and evaluated according to Larsen index score. The scores were summed up giving a maximum score of 150 when all joints of both hands are fully destroyed (11).

Measurement of serum Pentraxin 3 levels by ELISA

Blood samples were collected from the RA patients and control subjects after an overnight fast. Serum was separated by centrifugation and was stored at – 20 °C until PTX3 estimation by ELISA using (human PTX3 ELISA kit E-01807hu, Cloud clone corp., USA). Serum PTX3 levels were measured according to the manufacturer's recommendations using an ELISA plate reader (Quantikine, R&D Systems, Minneapolis, MN).

Statistical analysis

Data were analyzed using SPSS software, version 22.0 (IBM, Armonk, NY, USA) for Windows. Categorical data were presented as number and percentages. Normally distributed variables were expressed as mean ±standard deviation and analyzed by Student "t" test for 2 independent groups, or ANOVA for 3 independent ones. While non-parametric variables were presented as median and inter-quartile range (IQR), and analyzed by Mann Whitney U test or Kruskal Wallis (KW) test for 2 independent groups or more respectively. Linear association between variables was assessed by Person's or Spearman's correlation coefficients for parametric and non-parametric ones respectively. The accepted level of significance in this work was stated at 0.05 (P ≤0.05 was considered significant) (12).

Results

All patients and control were females. There was no significant difference between RA and control groups in terms of age, and body mass index (BMI) (p>0.05) **(Table 1)**. Median disease duration was 4.5 years with IQR 3 - 10 years.

Laboratory and radiological data of RA patients were shown in (Table 2).

Table (1) Distribution of demographic characteristics of RA and control groups

Variable		RA patients (n=40)	Controls (n=40)	St."t"	P
Age (ys)	Mean±SD	43.0±12.1	38.5±11.8	1.65	0.101 (NS)
	Range	22-63	21-61		
BMI (kg/m^2)	Mean±SD	29.5±3.8	30.1±4.5	0.71	0.48 (NS)
	Range	22.4-36	21.5-37.1		

Table (2) Laboratory and radiological data of RA patients

Variable	RA patients (n = 40)
	Median (IQR)
ESR (mm/h)	67.5 (40 - 80)

CRP (mg/dl)	12	(6 - 24)
RF titer (n=32)	64	(16 -128)
Larsen score	15	(10 - 30)

Median serum level of pentrexin 3 was significantly higher in RA patients compared to healthy controls (36.4 ng /ml vs 12.4 ng /ml respectively) (p<0.001) **(Fig 1).**

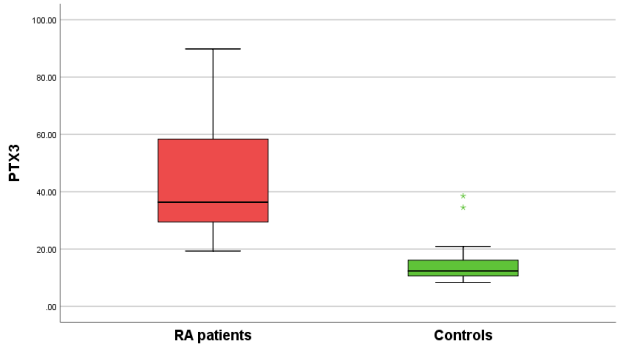

Fig (1) Comparison of serum pentraxin 3 (PTX3) levels between RA patients and control.

The PTX3 level was significantly different according to the DAS28 grade (p <0.001) **Fig. 2**.
There was no significant difference in serum PTX3 level and RF& anti-CCP positive and negative patients (P >0.05) **Table 3**.

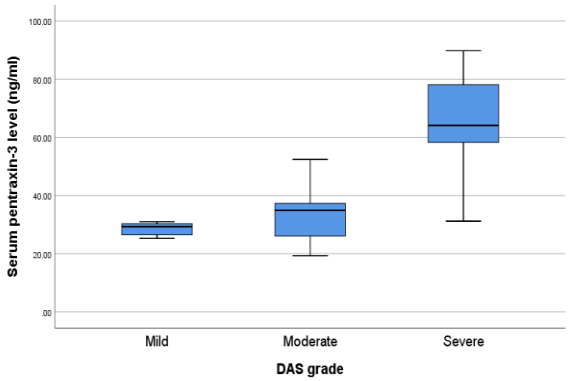

Fig (2) Comparison of serum pentraxin 3 (PTX3) levels based on disease activity grades (DAS28) in RA patients.

Table (3) PTX3 values in RF and anti-CCP-positive and -negative patients

Variable		n.	Serum Pentraxin-3 level		Z_{MWU} test	P value
			Median	IQR		
RF	Negative	8	36.3	29.8-55.9	0.203	0.84
	Positive	32	40.8	25.5-77.8		(NS)
Anti -CCP	Negative	9	35.0	26.7-52.4	1.63	0.102
	Positive	31	48.8	34.3-77.5		(NS)

PTX3 concentration was significantly correlated with ESR (r = 0.506, p=0.001), CRP (r = 0.780, p<0.001), p=0.002) and Larsen score (r = 0.784, p<0.001) **(Table 4)**.
No significant correlation **(p>0.05)** was found between serum Pentraxin-3 and age (r = 0.095,p=0.56), BMI (r = 0.059, p=0.71), disease duration (r = 0.048, p=0.76) and RF titer (r = 0.240, p=0.18). **(Table 4)**

Table (4) Correlation between serum Pentraxin-3 level and the studied variables

PTX3 with	Patient group (n=40)	
	rho	P
Age (years)	0.095	0.56
BMI (kg/m^2)	0.059	0.71
Disease duration (years)	0.048	0.76
ESR(mm/hr)	0.506	**0.001** (HS)

CRP(mg/dl)	0.780	**<0.001 (HS)**
RF titer (u/ml)	0.240	0.18
Larsen score	0.784	**<0.001 (HS)**

Discussion

In our study, all RA patients were females and this supported by predominance of RA among females due to hormonal effect as female to male ratio 3:1 (13).

In our study, There were high statistically significant increase in serum levels of pentraxin 3 (PTX3) in RA patients compared to healthy controls ($p<0.001$). This finding was in agreement with Ekin et al. who found that the serum PTX3 levels in RA patients were highly significant compared to the healthy individuals (14). Also this was consistent with, Asanuma et al. who found that Plasma PTX3 levels were significantly higher in Japanese female RA patients than in female controls (15). In addition, the results of the studies done by Balbaloglu et al., Sharma et al., Sağ et al. and Gittaboyina et al were in agreement with our finding as they showed that serum Pentraxin 3 concentration was significantly increased in RA patients compared to healthy controls (5, 16-18).

In the present study, There were no statistical significant correlation ($p>0.05$) between the pentraxin 3 level and the age. This result was consistent with studies done by Ekin et al. and Asanuma et al. who found that no correlations between the pentraxin 3 level and age (14-15).

In our study, the median disease duration of RA patients was 4.5 years; there were no statistically significant correlation between serum levels of pentraxin 3 and disease duration. Our finding was in agreement with Asanuma et al. who found that no correlations between the pentraxin 3 level and disease duration (15). In contrast to our results, the study done by Atzeni et al. showed that pentraxin 3 levels were significantly higher in patients with longer disease duration; supporting the idea that pentraxin 3 may take part in the chronic inflammatory process in established RA (19).This discrepancy may be due to different sample size and longer mean disease duration than described in our study.

In the present study ,we found that when the RA patients were divided into groups according to DAS28 activity (low, moderate and high), there were high statistically significant differences between these groups as regard the median serum level of pentraxin 3 ($p<0.001$). Our findings were similar to the study done by Jafari et al. who showed that there was a significant difference in serum PTX3 level according to the disease activity (remission, low, moderate and high) reflecting the probable association of PTX3 with disease activity (20).

Also, we found that there were statistically significant positive correlation with the serum level of pentraxin 3 in RA patients and acute phase reactants denoting disease activity, namely ESR and CRP. These results were in concordance with Boutet et al. who found that serum levels of PTX3 were significantly correlated with ESR in naïve RA patients at baseline being a strong marker of disease activity (21). Also our findings consistent with Targonska et al. who found that PTX3 concentration was correlated with inflammatory markers including C-reactive protein (CRP) and ESR suggesting the role of PTX3 as an inflammatory marker of the joint disease activity(22) . Also Jafari et al**.** found a significant correlation between serum PTX3 level with disease activity and CRP suggesting the probable association of PTX3 with disease activity and the conventional marker, CRP (20).

In the present study, there was no significant difference ($P>0.05$) in median serum levels of PTX3 between RF positive and negative patients and also no significant difference in anti CCP positive and negative patients was found. These results were in agreement with Balbaloglu et al. who showed that there was no statistically significant difference in PTX3 level between seropositive and seronegative RA patients(5).

In contrast to our results, Targonska et al. showed that PTX3 concentration was significantly higher in patients with anti-CCP positive compared with anti-CCP negative and this discrepancy might be due to different sample size and longer disease duration than described in our study (22).

In our study, there were no significant correlation ($p>0.05$) between serum Pentraxin-3 and rheumatoid factor titre and this consistent with Jafari et al. who found no significant correlation between serum PTX3 level and the RF (20).

In our study, we found high significant positive correlation ($p<0.001$) between serum Pentraxin-3 and Larsen Score. Our results were in agreement with Asanuma et al. who found that radiological scoring was positively correlated with plasma PTX3 levels in RA patients, concluded that pentraxin 3 may be a useful biomarker for predicting progressive joint destruction in patients with RA (15). Also our finding was in concordance with Boutet et al**.** who reported that PTX3 serum levels

measured at the onset of RA and prior to treatment modification correlates with radiographic damage observed at 12 months follow-up (21). Moreover, accumulation of PTX3 seems to occur in the arthritic joint, suggesting its involvement in triggering a local inflammatory process and degeneration of bone tissue. These results indicate that PTX3 can act as an inflammatory mediator in promoting bone loss in inflammatory bone-erosive diseases (6).

In conclusion, our data suggests a potential role of Pentraxin 3 (PTX3) as a sensitive non-invasive biomarker reflecting the disease activity and radiological damage in RA patients.

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