Biochemical Analysis of C-X-C Motif Chemokine Ligand 10 (CXCL10) as a Biomarker in Patients with Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is characterized by chronic inflammation and synovial hyperplasia that eventually leads to the destruction of the joints. CXCL10 has been originally identified as a pro-inflammatory chemokine that mediate leukocyte trafficking and modulate innate and adaptive immune responses. It plays a critical role in the inflammatory response and is involved in several biological processes. The aim of the study was to assess the diagnostic efficacy of serum CXCL10 levels in early RA patients. Patients and methods: The study included 60 RA patients; 30 of them were early diagnosed, and 30 longstanding RA and 30 healthy controls. Clinical examination was done for all patients. Measurement of serum CXCL10 level was done by ELISA, while assessment of disease activity in patients was done using disease activity score (DAS-28). Serum levels of CXCL10 were significantly higher in RA patients than controls (*P*< 0.001), and was more elevated in early diagnosed than longstanding RA patients, with a a significant positive correlation with DAS-28 ESR (r=0.361, *P*=0.005), number of tender joint (r=0.319, *P*=0.013), and number of swollen joint (r=0.280, *P*=0.030). A cutoff at 470.0 pg/ml was able to recognize longstanding RA with a sensitivity of 88.3% and a specificity of 90% , while a cutoff of 793 pg/ml was able to diagnose early RA with 65% sensitivity and 77% specificity (p=0.009). in conclusion, serum CXCL10 may be a useful biomarker for diagnosis of early RA and determination of disease activity.

Rheumatoid arthritis (RA) is the most common inflammatory arthritis with an approximate incidence of 1% worldwide [1, 2]. It has a female: male ratio of about three: one and although age of onset is commonly over fifty years, it may appear at any age. It is a systemic disease with predominant joint involvement and typically involves the small joints of the hands and feet [2, 3].

C-X-C motif chemokine 10 (CXCL10), named interferon (IFN)-ɣ-inducible protein 10, also called IP-10, belongs to the non-ELR CXC family due to the fact that it lacks the ELR motif before the forward half-cystine residue within the N-terminus. It is therefore a chemoattractant for activated T lymphocytes but not neutrophils [4-6]. CXCL10 is secreted by a number of cell types including immune cells (leukocytes, neutrophils, eosinophils, monocytes and dendritic cells) and non-immune cells (epithelial cells, endothelial cells, keratinocytes, fibroblasts, astrocytes and stromal cells) in response to IFN-ɣ [7, 8]. CXCL10 has been detected within sera, synovial fluid, and synovial tissue in patients with RA [9, 10] Furthermore, its concentrations in RA synovial fluid have been reported to be greater than in osteoarthritis (OA) synovial fluid and greater than serum concentrations among

patients including RA [9]. CXCL10 is expressed mainly by using infiltrating macrophage-like cells and fibroblast-like synoviocytes of RA synovium [9, 11]. CXCL10 slightly increases receptor activator of nuclear factor-κB ligand (RANKL) expression in RA synoviocytes and markedly increases RANKL expression in CD4+ T cell and RANKL promotes CXCL10 expression in osteoclast precursors [12].

RA is considered a major economic burden. It is therefore essential to diagnose patients as early as possible and target expensive therapeutics to those who are most likely to respond. There is evidence to support a 'window of opportunity' hypothesis in early RA whereby patients diagnosed within 3 months of disease onset Early RA are more likely to benefit from treatment with disease modifying anti-rheumatic drugs (DMARDs) and various biological agents than patients diagnosed later in disease course [13]. Early treatment appears to result in reduced disease activity and the achievement of clinical remission [14]. The psychosocial consequences of disease such as work place disability leading to loss of employment as well as patient fatigue, pain and emotional stress may also be prevented by earlier treatment.

Therefore, the purpose of this study was to assess serum CXCL10 levels in RA patients and to investigate the role of CXCL10 as a marker of disease activity.

Materials and Methods

Patients

Patients with RA, who were diagnosed according to ACR/EULAR (2010) Classification Criteria for RA [15] , were recruited from the rheumatology clinics at the (Suez Canal university hospital, Ismailia, Egypt). The study was performed on sixty RA patients. We enrolled; 30 early RA patients with disease duration < 2 years and another 30 longstanding RA patients with disease duration ≥ 2 years at study entry. Thirty healthy subjects were included as a control group. Informed consents were taken from each patient and control. The study was approved by Suez Canal university medical ethical committee.

Blood specimen collection and clinical data

Clinical data collection included disease duration, medication history, duration of morning stiffness and body mass index (BMI). Clinical evaluation of disease activity was done by assessing the following parameters: swollen joint counts in 28 joints (SJC 28), tender joint counts in 28 joints status (TJC 28), CRP, erythrocyte sedimentation rate (ESR), Disease Activity Score in 28 joints (DAS-28) Mild ≤ 3.2, moderate ≥ 3.2 but > 4.4, severe. All the RA patients were under medical treatment as Methotrexate (MTX), hydroxychloroquine (HCQ), Leflunomide and steroid.

Laboratory investigations included complete blood count (CBC), rheumatoid factor (RF) and anti-CCP. Quantitative detection of CXCL10 level in serum of the patients and control was done. Serum was separated from whole blood via density gradient centrifugation at 4°C within 4 hours of blood collection and promptly saved into 200 μL aliquots at −80°C till experimentation.

Measurement of CXCL10 level

Serum CXCL10 concentrations were measured by sandwich enzyme linked immunosorbent assay (ELISA) kit, from Bio kit (Quantikine human CXCL10, Shanghai Sunred Biological Technology Co., Ltd). The results are automatically calculated using the straight-line regression equation of the standard curve with the standard density and the OD values and are expressed in pg/mL. This assay has high sensitivity and excellent specificity for detection of CXCL10.No significant cross-reactivity or interference between CXCL10 and analogues was observed. The sensitivity of CXCL10 level measurement range extends from 75pg/mL to 6000pg/mL. The lowest protein concentration value of the measurement range is 35.11pg/mL.

Statistical Analysis

Analysis of data was performed by IBM computer using statistical package for social science (SPSS) version 19. Data had been expressed as mean, standard deviation, frequencies and percentages were used to describe qualitative variables. The comparison between two groups with parametric

variables was done using independent sample t-test (t). ANOVA test was used to assess the statistical significance of the difference between more than two study group means. The correlation coefficient between two parametric parameters was calculated by using Pearson and spearman correlation coefficient. Chi-Square test was used to examine the relationship between two qualitative variables. The ROC Curve (receiver operating characteristic) provides a useful way to evaluate the Sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. In all tests if ($P > 0.05$) it is non significant, if ($P< 0.05$) it is significant and if ($P <0.001$) it is highly significant.

Results

The studied groups were age and gender matched. Group 1 included 30 RA patients with disease duration < 2 years, they were 27 females (90.0%) and 3 males (10.0%), they had a mean age of 40.60±11.99 years. group 2 included 30 RA patients with disease duration ≥ 2 years, they were 28 females (93.3%) and 2 males (6.7%), they had a mean age of 45.43±9.17 years. The control group included 30 normal subjects, 27 females (90.0%) and 3 males (10.0%), they had a mean age of 42.87±9.31 years.

In this study, there was no statistically significant difference between early and longstanding RA patients regarding BMI, No. of tender joint, No. of swelling joint, and treatment (Table 1). Moreover, there was no statistically significant difference between early and longstanding RA patients regarding RF, Anti-CCP, CRP, ESR, and DAS28.ESR (Table 2).

Table 1. Clinical data of early diagnosed and longstanding RA patients

Characteristics	Early RA (n=30)	Longstanding RA (n=30)	*P*-value
Duration of disease (yrs)[#] (Mean ± SD)	1.30±4.31	10.06±7.25	<0.001*
BMI kg/m^{2} [#] (Mean ±SD)	29.92±5.79	29.03±	NS
No. of joint tender[#] (Mean ± SD)	10.30±8.82	10.63±9.6	NS
No. of joint swelling [#] (Mean ± SD)	1.83±1.78	2.20±2.1	NS
Treatment No. (%)[@]			
MTX	11(36.7%)	10(33.3%)	
MTX and HCQ	7(23.3%)	12(40%)	
MTX, HCQ and steroid	5(16.7%)	4(13.3%)	
Leflunomide	7(23.3%)	4(13.3%)	

#Quantative data are represented by Mean/SD and tested by t test,

@Qualitative data represented by chi-Square Test. $P > 0.05$ is not statistically-significant (NS). BMI = Body mass index. MTX=Methotrexate; HCQ= Hydroxychloroquine

Table 2. Laboratory data of early diagnosed and longstanding RA patients

Characteristics	Early RA (n=30)	Longstanding RA (n=30)	*P*-value
Rheumatoid factor (IU/mL) (Mean ± SD_	49.98 ± 5.55	46.73± 3.54	NS
Rheumatoid factor			
Positive, n(%)	20(66.7%)	24(80%)	NS
Negative, n(%)	10(33.3%)	6(20%)	
Anti-CCP (U/mL)			
Positive, n (%)	12(40%)	19(63.3%)	NS
Negative, n (%)	18(60%)	11(36.7%)	
CRP (mg/L) (Mean ± SD)	15.19±17.17	14.83±15.95	NS
ESR (ml/hr) (Mean ± SD)	54.73±27.23	59.07±31.84	NS
DAS28.ESR (Mean ± SD)	5.12±1.60	4.93±1.61	NS

Anti-CCP: Antibody to cyclic citrullinated peptide; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; DAS28: Disease Activity Score 28 joints; *P* > 0.05 is not statistically-significant (NS).

Serum CXCL10 was significantly higher in RA patients than control with highly statistically significant difference (*P*<0.001). The serum levels of CXCL10 were more elevated in early RA more than established RA (Table 3).

Table 3. serum levels of CXCL10 in early diagnosed, longstanding RA patients and healthy control

Variable	Early RA (n=30)	Longstanding RA (n=30)	Healthy control (n=30)	*P*-value
CXCL10(pg/mL)	1448.86±1253.38	726.84±316.27	376.50±296.00	<0.001*

P < 0.05 is statistically-significant.

CXCL10 serum levels had a highly statistically significant positive correlation with DAS-28 ESR (r=0.361, *P*=0.005), Number of tender joints (r=0.319, *P*=0.013), and Number of swollen joints (r=0.280, *P*=0.030). There was no statistically significant correlation between serum CXCL10 and disease duration (r= -0.217, *P*=0.096), ESR (r=0.023, *P*= 0.864) and CRP (r =0.234, *P*=0.062) (Table 4).

Table 4.Correlation of RA patients' parameters with serum CXCL10 level

Characteristics	CXCL10	
	r	*P*-value
Age	-0.121	NS
BMI	0.074	NS
Duration of disease (years)	-0.217	NS
Treatment	0.105	NS
CRP	0.234	NS
ESR	0.023	NS
DAS-28 ESR	0.361	0.005*
Anti-CCP	-0.084	NS
No. of tender joints	0.319	0.013*
No. of swollen joints	0.280	0.030*
Rheumatoid factor	0.136	NS

P > 0.05 is not statistically-significant (NS).

Receiver-operating characteristics (ROC) curve was done to investigate the ability of CXCL10 as a biomarker for diagnosis of RA. The best cut-off value was 470.0 pg/ml for serum CXCL10 that indicated the presence of RA with 88.3% sensitivity and 90% specificity (*P*=<0.001). Another cut-off value was determined at 793 pg/ml for diagnose of early RA with 65% sensitivity and 77% specificity (*P*=0.009) figure 1

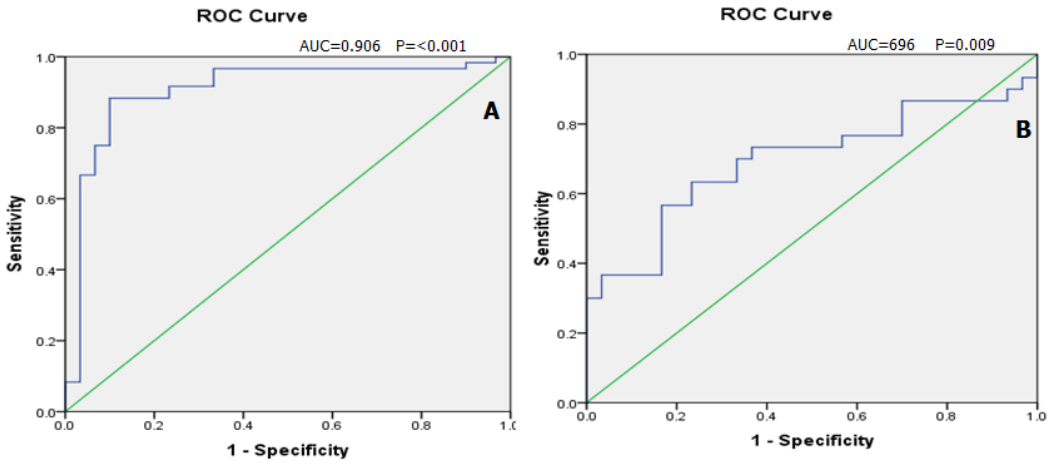

Figure 1. ROC curves for serum CXCL10. A: for diagnosis of RA disease B: for prediction of early RA.

Discussion

In our study, the measured serum level of CXCL10 was significantly higher in RA patients compared to healthy control. Our results were in agreement with [16] who demonstrated that CXCL10 was significantly increased in the serum of RA patients. Similarly, [17] reported that CXCL10 levels were significantly higher in patients with autoimmune diseases when compared to control group. Also this was in agreement with [18] who examined the expression of selected pro- and anti-angiogenic chemokines and their receptors in systemic sclerosis (SSc), The expression of CXCL10 was significantly elevated in SSc serum compared to normal control.

In this work, there was no correlation between serum levels of CXCL10 and CRP as well as ESR. This was in accordance with the study done by [19] who found that CXCL10 was not correlated with the inflammatory markers CRP or ESR. In contrast [20] found positive significant correlation between CXCL10 levels and ESR as well as CRP. Similarly, [21] found that levels of CXCL10 was significantly correlated with ESR.

Regarding the correlation between serum CXCL10 and DAS-28 ESR, our results revealed a highly positive significant correlation. In agreement with our finding, [20] showed a positive significant correlation between DAS-28 ESR and serum CXCL10 levels. On the other hand, the study by [22], who studied blood chemokines profile in 43 untreated early RA patients. They reported that CXCL10 level correlated with multiple disease activity measures including as DAS-28 CRP.

Interestingly, Our study reported that there was a significantly positive correlation between serum levels of CXCL10 and the number of tender and swollen joints in RA patients where the tender and swollen joints count is the most specific clinical measure to assess disease activity in RA patients. Thus CXCL10 may play a central role in early RA inflammation and may serve as a disease activity marker in early RA. In contrast, in established RA it was reported no correlation with clinical disease activity measures such as swollen joint counts, CRP, and ESR [23]. Another study in established RA did not observe any correlation between CXCL10 and clinical disease activity measures [24].

In the current study, we observed no significant correlation between serum CXCL10 level in RA patients with RF or anti-CCP. In contrast to our findings study by [25] who measured CXCL10 in RA patients who were initiating TNF inhibitor therapy and correlated chemokine level with therapeutic response. They reported that CXCL10 level was higher in anti-CCP-positive patients than in anti-CCP-negative patients. In agreement with our result, they found that no significant difference in CXCL10 levels between RF-positive and RF-negative patients.

When ROC curve was done for serum CXCL10 for prediction of the diagnosis of RA, The area under the curve (AUC) was 90.6 %. The best cut-off value for the diagnosis of RA was > 470.0 pg/ml with sensitivity and specificity were 88.3 % and 90 % respectively. These results indicated good validity of CXCL10 as a diagnostic marker for RA. Also, [25] reported that CXCL10 showed significant predictive ability based on AUC of 83.0 %.

In conclusion, serum CXCL10 level is significantly elevated in RA patients especially in early RA and correlated with

disease activity. It can act as a biomarker for diagnosis of rheumatoid arthritis disease at cut-off value of > 470.0 pg/ml with sensitivity 88.3 % and specificity 90 %.

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