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Serum YKL-40 Levels in Patients with Rheumatoid Arthritis and Its Relationship with Disease Activity

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune synovitis. The etiopathogenesis of RA remains unidentified. Nonetheless, autoimmune mechanisms have a role in its pathomechanism. It was reported that the chronic inflammatory process in RA is associated with synovial proliferation, which is linked to cartilage and bone resorption. YKL-40 is a primary protein produced from arthritic joints by chondrocytes in vitro and in vivo. It has been demonstrated that its value is markedly increased in the context of joint disorders such as RA and osteoarthritis (OA).

Objective: This study aimed to evaluate serum YKL-40 concentrations in RA patients compared to healthy individuals and to investigate the association between serum YKL-40 levels with disease activity in RA.

Patients and Methods: This study included 35 RA patients with mean age of 42.23 ± 9.94 years, 6 (17.1%) males & 29 (82.9%) females. 35 apparently healthy individuals with mean age of 39.46 ± 8.28 years, 10 (28.6%) males & 25 (71.4%) females. Laboratory investigations were done. Serum YKL-40 was analyzed and DAS28 was evaluated.

Results: RA cases were linked to a significant increase in YKL-40 levels compared to controls. Serum YKL-40 level was significantly correlated with RA activity (P=0.001) (DAS28). There were statistically significant increases in Anti-CCP, rheumatoid factor (RF), CRP, ESR, WBCs and platelet in RA cases than in the controls.

Conclusion: Serum YKL-40 was significantly increased with RA as well as with its activity. It could be used as a valid marker in the context of RA diagnosis.

Keywords: Rheumatoid Arthritis, YKL-40, autoimmune, DAS 28.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune synovitis, which influences about one percent of population and leads to functional disability [1, 2]. The etiopathogenesis of RA remains unidentified. On the other hand, autoimmune mechanisms have a role in its pathomechanism [3].

It was suggested that the chronic inflammatory process in RA is associated with synovial proliferation which is linked to cartilage and bone resorption [4].

It has been described that biomarkers of joint metabolism and disease activities are important for proper follow-up of the disease course in RA cases [5].

It is noted that a lot of biochemical markers of joint disease metabolism in RA patients are evaluated. The most commonly utilized markers for long-term supervision of disease activity have been the erythrocyte sedimentation rate (ESR) and the serum C-reactive protein (CRP) up to now. Novel biomarkers are essential to predict the course and prognosis of disease and also to follow the response to treatment [6].

YKL-40 is a heparin-binding glycoprotein-39 having a molecular weight of 40 kDa. It is secreted in the arthritic joint by different cell types. Its name is derived from its 3 terminal amino acids i.e. tyrosine (Y), lysine (K) and leucine (L) [7].

It is formed in arthritic joints by chondrocytes in humans as well as in experimental studies. Serum and SF YKL-40 concentrations are increased in joint disorders, which include RA and OA, signifying that YKL-40 could be considered as an inflammatory marker and also a marker of tissue remodeling, while YKL-40 isn't detected in the healthy joint [8].

We aimed to evaluate serum YKL-40 concentrations in RA patients compared to healthy individuals and to assess the association between serum YKL-40 levels with disease activity in RA.

SUBJECTS AND METHODS

The present study was case-control study. Patients were enrolled from the Outpatient Clinics of the Physical Medicine, Rheumatology and Rehabilitation Department, Mansoura University Hospitals during their clinical visits from September 2021 to March 2022. The participants of this study were classified into 2 groups, 35 RA patients diagnosed according to criteria proposed by 2010 EULAR/ACR for classification of RA [9], and 35 apparently healthy volunteers of matching ages and sexes were included as control group.

Inclusion criteria: Cooperative patients and established RA patients with various disease activity.

Exclusion criteria: Patients with any other autoimmune diseases (AID) e.g., SLE, Psoriatic arthritis, Behcet's disease, Ulcerative colitis, primary osteoarthritis, pregnancy, malignant tumours, HTN, DM and Cardiac diseases.

History taking and examination including personal history, complaint of the patient**,** multisystem affection, medication, past history of medical or surgical problems.

Laboratory investigations: Complete blood count (CBC), ESR was measured in mm/hr, CRP, was measured in mg/dl, rheumatoid factor, anti-CCP, serum YKL-40 and DAS28 was evaluated.

Serum YKL-40 assessment: 5 ml blood were collected

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at room temperature by sterile venipuncture from each individual in the morning and fasting. These samples were collected in empty tubes. Centrifugation was performed at 3000 rpm for twenty minutes to separate plasma then serum YKL-40 was determined by ELISA. Using human YKL-40/CHI3L1 antibody levels were measured by ELISA based on the user manufacturer, by utilizing an ELISA plate reader.

Ethical approval: The approval was obtained from The Ethical Committee of Faculty of Medicine, Mansoura University. All participants were informed about the nature of the study and they approved and signed the consents. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis: Data were analysed by utilizing IBM SPSS released 2013, V 22.0. (Armonk, NY). Qualitative data were represented as frequency and percentage. Quantitative data were represented as median (minimum and maximum) in terms of non-normally distributed data and means ± SD for normally distributed data. The normality of data was previously assessed using Kolmogorov-Smirnov test. In the context of all the previously used tests, $p \leq 0.05$ was considered significant.

RESULTS

35 rheumatoid arthritis cases with a mean age of 42.23 ± 9.94 years with 6 (17.1%) were males & 29 (82.9%) were females matched with 35 control group with a mean age of 39.46 ± 8.28 with 10 (28.6%) males & 25 (71.4%) females. There was non-statistically significant difference between the studied groups concerning age and gender for cases and control groups. (Table 1).

Table (1): Age and sex of the studied groups

	Cases group (n=35)	**Control group (n=35)**	**Test of significance**
Age (years) Mean ± SD	42.23 ± 9.94	39.46 ± 8.28	t=1.27 p=0.209
Sex (%)			
Male	6 (17.1)	10 (28.6)	χ^2=1.29
Female	29 (82.9)	25 (71.4)	p=0.255

Table (2) illustrated a significantly higher median YKL-40 among RA patients compared to controls (41.48 vs. 34.15, respectively).

Median anti-CCP was greater in RA patients than in controls (13 vs. 8) with statistically significant difference between them, median RF was also significantly higher among cases than among control group (24 vs. 10), median ESR was significantly greater in RA cases than in controls (45 vs. 20, respectively), mean WBCS count and platelet count were significantly higher among RA patients compared to controls.

Table (2): Laboratory findings and DAS score between studied groups

	Cases (n=35)	**Control group (n=35)**	**Test of significance**
YKL-40	41.48 (18.22-106.85)	34.15(22.14-40.18)	Z=3.28 P=0.001*
Anti-CCP	13(6-88)	8(5-12)	Z=5.19 P<0.001*
Rheumatoid factor (RF)	24(12-96)	10(6-16)	Z=7.07 P<0.001*
CRP (mg/L)	13(5-48)	4(2-10)	Z=6.65 P<0.001*
ESR (mm/hr)	45(20-93)	20(12-25)	Z=6.73 P<0.001*
DAS28	3.5(2-6.9)	Not applicable	
WBCs (mcL)	6.99±1.69	6.07±1.04	t=2.73 p=0.008*
RBCs (mcL)	4.67±0.58	4.46±0.54	t=1.55 p=0.125
Platelets (mcL)	299.14±73.97	260.06±44.97	t=2.52 p=0.014*

Median and Rang: non parametric test. Z: Mann Whitney U test

Table (3) and figure (1) demonstrated that AUC for YKL-40 was good in differentiating RA cases from control subjects with the best detected cutoff point was 36.57 yielding sensitivity of 68.6 % and specificity 60% and total accuracy 64.3%.

Table (3): Validity of YKL-40 in differentiating RA cases from control subjects.

	AUC	P value	cutoff value	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
YKL-40	**0.728 (0.605-0.851)**	**0.001***	**36.57**	**68.6**	**60**	**63.2**	**65.6**	**64.3**

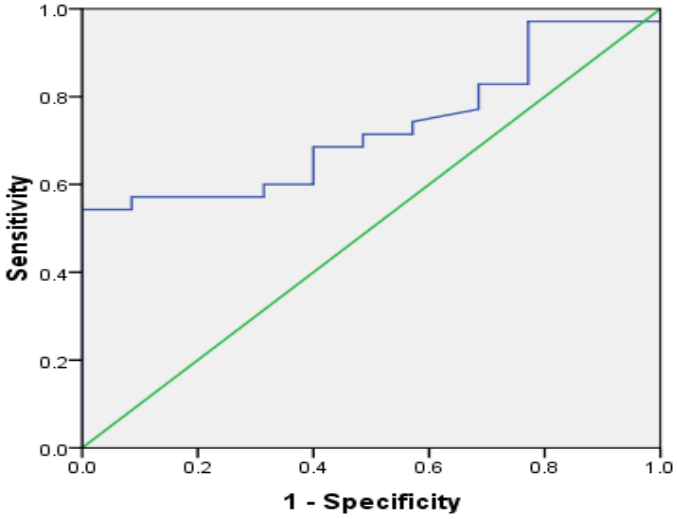

Figure (1): Validity of YKL-40 in differentiating RA cases from control subjects.

Table (5) demonstrated that there was significant positive association between YKL-40 and the following; Anti-CCP (r=0.537), RF (r=0.646), CRP (r=0.594), ESR (r=0.501) and DAS 28 (r=0.896) among cases group.

Table (5): Association between YKL-40 and age & laboratory findings among studied cases group.

Among cases		YKL-40
Age (years)	r	0.000
	p	0.997
Anti-CCP	r	**0.537**
	p	**0.001****
RF	r	**0.646**
	p	**0.001****
CRP (mg/L)	r	**0.594**
	p	**0.001****
ESR (mm/hr)	r	**0.501**
	p	**0.002****
DAS28	r	**0.896**
	p	**0.001****
WBCs (mcL)	r	0.193
	p	0.267
RBCs (mcL)	r	0.050
	p	0.776
PLT (mcL)	r	0.040
	p	0.817

r: Spearman correlation co-efficient, **statistically significant

Table (6) illustrated that YKL-40 and RF are statistically significant predictors of DAS-28 score among studied cases with 81.8% of DAS -28 score can be predicted by both factors using the following relation (DAS 28 =0.850+ 0.08*YKL-40-0.032* RF).

Table (6): Linear regression of prediction of DAS 28 among studied cases.

	Unstandardized Coefficients		Standardized Coefficients	t	P
	β	Std. Error	Beta		
(Constant)	.850	.318		2.674	.012*
YKL-40	.080	.013	1.318	6.192	.001*
Anti-CCP	-.002	.009	-.017	-.187	.853
RF	-.032	.014	-.568	-2.371	.025*
CRP	.022	.028	.143	.798	.431
ESR	-.002	.009	-.026	-.191	.850
F=26.03 , P<0.001* **R^2=0.818** **Prediction equation** **(DAS 28 =0.850+ 0.08*YKL-40-0.032* RF)**					

DISCUSSION

RA is an inflammatory and AID featured by chronic inflammation principally affecting the joints. The exact pathomechanisms, which provoke the autoimmune response, ultimately causing joint damage, aren't completely understood. On the other hand, at the initial disease phases, some antigens expressed to T-lymphocytes by APCs, and amazingly, one such candidate autoantigen, which can elicit an immune response, is YKL-40 [1, 2].

YKL-40 is produced by various cells in the arthritic joints. It is a primarily protein produced by chondrocytes. On the other hand, it could be detected in the chondrocytes from arthritic joints in humans. YKL-40 adjusts the inflammatory and immune responses and can be linked to cellular reorganization. YKL-40 binds to an unidentified receptor, and various inflammatory cytokines regulate its expression [10, 11].

Thus, our work aimed at evaluating serum YKL-40 values in RA cases compared to normal individuals and at investigating the correlation between serum YKL-40 levels with RA disease activity. Concerning demographic characteristics, our study revealed that both groups revealed insignificant differences regarding all demographic features, which indicated that both groups were comparable as regards all such parameters. Likewise, **Jafari-Nakhjavani *et al.*** [12].

With regard YKL-40 level, the current study demonstrated that RA cases were linked to a significant increase in YKL-40 values compared to the controls. This came in accordance with **Jafari-Nakhjavani *et al.*** [12] who examined 156 RA patients during a one year. They demonstrated that serum YKL-40 concentrations were significantly greater among RA cases than in controls (951.63 ± 639.98 versus 444.92 ± 150.37 pg/mL). Similarly, **Lee and Song** [13] conducted a meta-analysis study, which comprised 9 studies (707 RA cases and 1,041 control subjects) and have demonstrated that YKL-40 concentrations were significantly greater among RA cases compared to controls (95% CI=0.726~1.417, p<0.001).

With regard to disease activity, our study revealed that there was a significant positive correlation between serum YKL-40 values and disease activity (P=0.001) (DAS28). Likewise, **Jafari-Nakhjavani *et al.*** [12] have displayed that serum YKL-40 values were positively correlated with RA activity (p=0.007). Also, **Aleksandrova *et al.*** [14] have revealed that serum YKL-40 concentrations showed positive correlation with DAS 28. Additionally, **Lee and Song** [13] have demonstrated in their Meta-analysis study that YKL-40 values were positively correlated with DAS28 (p<0.05). Moreover, YKL-40 could have a main function in the context of cartilage damage in arthritic joint. In RA cases, circulatory YKL-40 might reflect an association of cartilage metabolism and local inflammation compared to serum CRP and ESR. Different cell types such as synovial cells, chondrocyte, osteoblast, macrophage, and neutrophils form YKL-40 in RA cases, however it is challenging to identify which cell type is responsible for the increased YKL-40 values in the serum [12].

Regarding laboratory findings, we revealed significant increases in Anti-CCP, RF, CRP, ESR, WBCS and platelet in RA cases in comparison with controls.

Regarding validity of YKL-40, we displayed that AUC for YKL-40 was good in differentiating patients from controls with the best detected cut off level was 36.57 yielding sensitivity of 68.6 % and specificity of 60% and total accuracy of 64.3%. Likewise, **Jafari-Nakhjavani *et al.*** [12] have displayed that the ROC curve for RA diagnosis had an AUC of 0.797 (p<0.05) indicating a high probability of properly predicting RA.

In accordance, our work revealed that YKL-40 concentrations had a positive correlation with anti-CCP, RF, CRP, ESR and DAS 28 in RA cases. However, **Jafari-Nakhjavani *et al.*** [12] study was in disagreement with our study regarding the association between YKL-40 and ESR, CRP and anti-CCP being non-significant in their study [12]. Also, in contrast to the present study **Narayan *et al.*** [15] have demonstrated that serum YKL-40 concentrations had no correlation with measures of RA activity including DAS-28, VAS, CRP, and ESR in

RA cases. **Matsumoto and Tsurumoto [(16)]** have demonstrated that serum YKL-40 concentrations in RA cases had a positive correlation with serum IL-6 and CRP concentrations, but were negatively correlated with serum IGF-I concentrations. The radiologic score had also a correlation with YKL-40 concentrations. As the functional disability of patients became severe, circulatory YKL concentrations are increased.

In addition, the present study demonstrated that YKL-40 and RF are statistically significant predictors of DAS-28 score among studied cases with 81.8% of DAS -28 score can be predicted by both factors using the following formula (DAS 28 =0.850+ 0.08*YKL-40-0.032* RF).

CONCLUSION

In the context of RA, serum YKL-40 was significantly increased. In addition, it seemed to be significantly correlated with RA as well as with its activity. Additionally, it could be used as a valid marker in the context of RA diagnosis.

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REFERENCES

1. **Guo Q, Wang Y, Xu D *et al.* (2018):** Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Research, 6 (1): 15. doi: 10.1038/s41413-018-0016-9.
2. **van Bilsen J, van Dongen H, Lard L *et al.* (2004):** Functional regulatory immune responses against human cartilage glycoprotein-39 in health vs. proinflammatory responses in rheumatoid arthritis. Proceedings of the National Academy of Sciences, 101 (49): 17180-85.
3. **Choy E (2012):** Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. Rheumatology, 51 (5): 3-11.
4. **Jimenez-Boj E, Redlich K, Türk B *et al.* (2005):** Interaction between synovial inflammatory tissue and bone marrow in rheumatoid arthritis. The Journal of Immunology, 175 (4): 2579-88.
5. **Karsdal M, Woodworth T, Henriksen K *et al.* (2011):** Biochemical markers of ongoing joint damage in rheumatoid arthritis-current and future applications, limitations and opportunities. Arthritis Research & Therapy, 13: 1-20.
6. **Kazakova M, Sarafian V (2013):** YKL-40 in health and disease: a challenge for joint inflammation. Biomedical Reviews, 24: 49-56.
7. **Johansen J, Høyer P, Larsen L *et al.* (2007):** YKL-40 protein expression in the early developing human musculoskeletal system. Journal of Histochemistry & Cytochemistry, 55 (12): 1213-28.
8. **Güngen G, Ardic F, Fındıkoğlu G *et al.* (2012):** The effect of mud pack therapy on serum YKL-40 and hsCRP levels in patients with knee osteoarthritis. Rheumatology International, 32 (5): 1235-44.
9. **Aletaha D, Neogi T, Silman A *et al.* (2010):** rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & Rheumatism, 62 (9): 2569-81.
10. **Johansen J (2006):** Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. Dan Med Bull., 53 (2): 172-209.
11. **Recklies A, White C, Ling H (2002):** The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. Biochemical Journal, 365 (1): 119-26.
12. **Jafari-Nakhjavani M, Ghorbanihaghjo A, Bagherzadeh-Nobari B *et al.* (2019):** Serum YKL-40 levels and disease characteristics in patients with rheumatoid arthritis. Caspian Journal of Internal Medicine, 10 (1): 92-97.
13. **Lee Y, Song G (2019):** YKL-40 levels in rheumatoid arthritis and their correlation with disease activity: a meta-analysis. Journal of Rheumatic Diseases, 26 (4): 257-63.
14. **Aleksandrova E, Novikov A, Luchikhina E *et al.* (2021):** AB0837 serum YKL-40 levels in patients with early rheumatoid arthritis: Relation to disease activity and joint destruction. BMJ Journal, 21: 1442. https://doi.org/10.1136/annrheumdis-2021-eular.2069
15. **Narayan V, Pallinti V, Ganesan N (2019):** A study of serum YKL-40 and its correlation with traditional biomarkers in rheumatoid arthritis patients. Indian Journal of Rheumatology, 14 (3): 200-205.
16. **Matsumoto T, Tsurumoto T (2001):** Serum YKL-40 levels in rheumatoid arthritis: correlations between clinical and laboratory parameters. Clinical and Experimental Rheumatology, 19 (6): 655-60.