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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: *An autoimmune, persistent, inflammatory synovitis is rheumatoid arthritis (RA). RA's etiopathogenesis is still unknown. However, part of its pathomechanism involves autoimmune mechanisms. According to a report, RA's chronic inflammatory process is connected to synovial proliferation, which is connected to the resorption of bone and cartilage. One major protein that chondrocytes make from arthritic j[1]oints both in vitro and in vivo is called YKL-40. It has been shown that when joint problems such osteoarthritis (OA) and RA are present, their value increases significantly.****Objective:*** *This study aimed to evaluate serum YKL-40 concentrations in RA patients compared to healthy individualsand to investigate the association between serum YKL-40 levels with disease activity in RA at Makkah hospital, Saudi Arabia.* ***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 validmarker in the context of RA diagnosis.*

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

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INTRODUCTION

About 1% of the population suffers from rheumatoid arthritis (RA), a chronic inflammatory autoimmune synovitis that impairs function (1, 2). RA's etiopathogenesis is still unknown. Conversely, its pathomechanism involves autoimmune mechanisms (3). It was proposed that synovial proliferation, which is connected to cartilage and bone resorption, is linked to the chronic inflammatory process in RA (4).

It has been noted that in order to properly monitor the progression of the disease in RA cases, biomarkers of joint metabolism and disease activities are crucial (5). It is observed that many biochemical indicators of joint disease metabolism are assessed in patients with RA. Up until now, serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have been the most often used markers for long-term monitoring of disease activity. In order to track a patient's reaction to treatment and forecast the trajectory and prognosis of their illness, novel biomarkers are crucial (6).

YKL-40 is a heparin-binding glycoprotein-39 having a molecular weight of 40 kDa. It is secreted in the arthriticjoint by different cell types. Its name is derived from its3 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 markerand also a marker of tissue remodeling, while YKL-40 isn't detected in the healthy joint [(8)].

We aimed to evaluate serum YKL-40 concentrationsin 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, Makkah Hospitals during their clinical visits from March 2022 to september 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 autoimmunediseases (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 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 weremeasured by ELISA based on the user manufacturer, byutilizing an ELISA

plate reader.

Ethical approval: The approval was obtained from The Ethical Committee. 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 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 normallydistributed 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 consideredsignificant.

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 significantdifference between the studied groups concerning age and gender for cases and control groups. (Table 1).

	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 Female	6 (17.1) 29 (82.9)	10 (28.6) 25 (71.4)	χ^2=1.29 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 greaterin RA cases than in controls (45 vs. 20, respectively), mean WBCS count and platelet count were significantlyhigher among RA patients compared to controls.

	Cases(n=35)	**Controlgroup (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*

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%.

	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

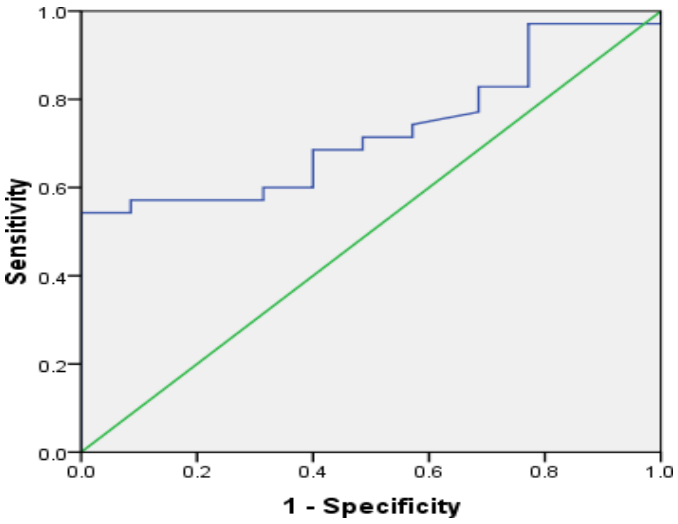

Table (5) demonstrated that there was significant positive association between YKL-40 and the following; Anti-CCP (r=0.537), RF (r=0.646), CRP (r=0.594), ESR (r=0.501) and DAS 28 (r=0.896) among cases group.

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

Table (6) illustrated that YKL-40 and RF are statistically significant predictors of DAS-28 score among studied caseswith 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).

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

Our research thus sought to assess serum YKL-40 levels in RA cases relative to normal individuals and to examine the relationship between serum YKL-40 levels and RA disease activity. In terms of demographic data, our analysis showed that there were negligible variations between the two groups for every attribute, suggesting that the groups were similar in every way. Jafari-Nakhjavani and associates also (12).

With regard YKL-40 level, the current study demonstrated that RA cases were linked to a significantincrease 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 RAcases and 1,041 control subjects) and have demonstratedthat 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 revealedthat there was a significant positive correlation betweenserum 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)].

In terms of laboratory results, we found that RA cases had significantly higher levels of Anti-CCP, RF, CRP, ESR, WBCS, and platelets than controls. In terms of YKL-40's validity, we showed that its AUC was effective in distinguishing patients from controls; the best identified cut off level was 36.57, resulting in a sensitivity of 686.6%, specificity of 60%, and overall accuracy of 64.3%. Likewise, **Jafari-Nakhjavani et al. [(12)]** have displayed that the ROC curvefor 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. Jafari-Nakhjavani et al.'s (12) study, however, disagreed with ours in that their investigation found no significant correlation between YKL-40 and ESR, CRP, or anti-CCP. Furthermore, in opposition to the current investigation, Narayan et al. (15) have shown that, in RA cases, serum YKL-40 concentrations did not correlate with indices of RA activity such as DAS-28, VAS, CRP, or ESR. Serum YKL-40 concentrations in RA subjects were found to be negatively connected with serum IGF-I concentrations but positively correlated with serum IL-6 and CRP concentrations (Matsumoto & Tsurumoto, 16). There was also a link between YKL-40 concentrations and the radiologic score

. As the functional disability of patients became severe, circulatory YKL concentrations are increased. In addition, the present study demonstrated thatYKL-40 and RF are statistically significant predictors ofDAS-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 itsactivity. Additionally, it could be used as a valid markerin the context of RA diagnosis.

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