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Assessment of Glycoprotein YKL-40 in Rheumatoid Arthritis Patientsin Iraq Population

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory condition characterized by synovial joint inflammation. It leads to bone loss and painful cartilage breakdown.YKL-40 is a heparin-human cartilage glycoprotein-39 with a molecular weight of 40 kDa that is secreted by some cell types in the arthritic joint but has no enzymatic action.YKL-40 may be a marker for inflammation, tissue remodeling, or degradation. In this study, we evaluated YKL-40 in RA patients and compared them to the control group, as well as the relationship with disease activity score (DAS-28)and Anti-Cyclic citrullinated peptide antibody (ACCPAb)The study design was based on the Case-control study methodology, with 45 patients with Rheumatoid arthritis the diagnosis made in by specialist consultation at the Rheumatoid Unit at Baghdad Teaching Hospital of Iraq and 45 healthy controls.Disease activity score 28 (DAS-28) was calculated. The level of serum YKL-40 and serum Anti-ccpAb were measured using an enzyme-linked immunosorbent assay (ELISA). RA patients had considerably higher serum YKL-40 levels (157.62 62.13 ng/ml) than normal controls (124.51 30.72 ng/ml).(p,0.001). Furthermore, anti-ccpAb levels in RA patients were considerably higher (232.37 U/ml) than in normal controls (4.76 2.04 U/ml). (p=0.000).There were no significant correlations between serum YKL-40 levels and anti-cyclic citrullinated peptide (r,-0.056, P,0.714),and ESR (r,0.008, P,0.957),RF(r, 0.086, P,0.655),CRP(r,0.074, P,0.576).There was significant differentiability in serum YKL-40 levels between remission and server DAS-28 disease activity scores. (p,0.02).on the other hand, there was no significant difference in serum YKL-40 levels among RA patients who were treated with groups DMARDs (p,0.919). Additionally, The level of serum YKL-40 was shown to have a strong positive relationship with disease activity.(r,0.345, P,0.020). In comparison to normal controls, RA patients had elevated serum YKL-40 levels, which were positively associated with disease activity and showed a large variation in serum YKL-40 levels due to disease activity. As a result, YKL-40 is a new biomarker that can be used to measure disease activity in RA patients.

Keywords
Rheumatoid arthritis, glycoprotein YKL-40.

INTRODUCTION

Rheumatoid arthritis (RA) is a persistent inflammatory circumstance characterized by synovial joint irritation. It leads to bone loss and painful cartilage breakdown (1).The syndrome is symmetric chronic synovitis, which mostly affects small peripheral joints but can affect almost every joint with a synovial membrane(2).Local joint inflammation causes deformity and impairment, and it has the potential to propagate across the body, affecting the skin, eyes, heart, lungs, kidneys, nervous system, and gastrointestinal system(3-4-5).Up to 1% of the adult population worldwide suffers from rheumatoid arthritis (6-7). The patient's history, clinical observations (including imaging techniques), and serological laboratory testing are the three

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foundations of rheumatological disease diagnosis.(8-9).Discovering new biomarkers in RA studies that entice scientists to learn about the key roles in different stages of evolution is still a matter of attention for RA researchers. "anti-cycliccitrullinated peptide" (ACCPA) and the "rheumatoid factor" (Rf) is currently used for diagnosing RA.As a result, novel markers that can forecast the clinical course and prognosis of a disease, as well as monitor treatment response, are necessary.

GLYCOPROTEINE YKL-40

YKL-40 is a mammalian chitinase-like protein The CHI3L1 gene is responsible for encoding this protein(10). Chitinases are enzymes that help fungi, helminths, insects, and crustaceans maintain homeostasis by digesting chitin and providing cellular and tissue remodeling(11). Mammalian chitinases and chitinase-like proteins are both enzymatically active and inactive. The exact biologic function of chitinaselike proteins, such as the YKL40 protein in humans, is unknown. The most abundant protein secreted by the MG63 human osteosarcoma cell line was discovered to be YKL40 in 1989. (12). Human cartilage glycoprotein39 is another name for it(10), chitinase 3-like-1 protein (13),the chondrex(14), and breast degeneration protein 39 (15). It has a molecular weight of 40 kDa and a single polypeptide chain of 383 amino acids, with Y (tyrosine), K (lysine), and L (leucine) as the three N-terminal amino acids (leucine)(10).The loss of hydrolase activity of YKL40 is caused by two mutations of the catalytic glutamic and aspartic acids to leucine and alanine, respectively(16). Elevated levels of YKL-40 were shown to be consistent with metastasis and poor survival in a variety of human carcinomas, including breast cancer, in much independent research(17). colorectal malignancy(18), ovarian tumor(19), Glioma at a high grade (20), lymphoma (21), YKL-40 may thus be used as a diagnostic, risk assessment, and prognostic biomarker. YKL40 levels have also been linked to tissue remodeling and deterioration in inflammatory disorders, according to other studies(22).

MATERIALS AND METHODS

A case-control study was used in this study. In the present report, 45 people with Rheumatoid Arthritis were involved (10 males and 35 females) additionally to forty-five healthy controls (11 males and 34 females) compatible with the Rheumatoid arthritis patients in age, sex, and body mass index (BMI). The mean age for RA males and females was 46.82 ± 11.85 and the mean age for healthy control males and females was 45.34 ± 13.00. A specialist rheumatologist diagnosed all of the RA patients in the present report. The current research was carried out at the Rheumatology Consultation Clinic/Baghdad Teaching Hospital from the 1st of September 2020 until the 1st of December 2020. a complete history was taken from all patients that include: smoking, age, residence, family history, type therapy(DMARD), and disease duration. The following disorders were excluded: renal failure, liver syndrome, thyroid or parathyroid complication, Cardiovascular disorder, stroke, cancer, and breastfeeding, as well as some other autoimmune disorders or overlapping syndromes.The following factors affect ethical issues: Approval by the Biochemistry Department and a research committee of Babylon Medical College (University of Babylon, Iraq). Both participants in the current study were told about the study's goals and methods, and they verbally agreed to participate. All study groups collected five ml of venous blood. Blood was separated for 10-15 minutes at 14000 revolutions per minute, then the serum was kept at -70C until examination. Serum YKL-40 (Elabscience, China) and anti-cyclic citrullinated peptide (anti-CCP) (AESKULISA, Germany) A sandwich enzyme-linked immunosorbent assay were used to determine the results (ELISA).For qualitative and semi-quantitative measures, the rheumatoid factor (RF) was measured using the latex agglutination test.

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whereas latex-enhanced nephelometry was used to assess C-reactive protein (CRP). Wintrobe methods were used to determine the erythrocyte sedimentation rate (ESR). The SPSS statistical software for Social Sciences was used to do the statistical analyses (version 26.0 for Windows-SPSS-Chicago-IL-USA). information normality distribution was examined using the Shapiro-Wilk test. YKL and ACCP distribution was considered normal. The mean and standard deviation are used to describe quantitative statistics. Count and percentage are used to describe qualitative statistics. The independent sample Ttest and one-way ANOVA study, as well as Student's t-test, were used to compare groups. Pearson correlation test study was used to check the correlation between variables.ROC analysis (Receiver Operating Characteristic) was used as an indicator for YKL, and ACCP for discrimination of patients from control.The test's accuracy was assessed using the district under the curve (AUC) value. Statistical significance was described as a P-value of less than 0.05.

CONSEQUENCES

The general, physiological, and biochemical characteristics of the study participants as shown in Table. There was no significant difference in age (P,0.211) and BMI (P,0.089) between patients and control; while there was a significant difference in ESR, CRP, and RF p-value was (0.005-0.000-0.000) respectively between patients and control. Student’s t-test and Chi-square test. Gender distribution was similar in patients and control Chi-square test.

Table 1: general characteristics of RA patients and control group

parameters	RA patients	Control	P-value
Age (years) mean ± SD	46.82±11.85	45.60±12.96	0.211
BMI(kg/m^2) Mean± SD	27.4 ±3.72	26.3 ± 2.67	0.089
ESR Mean± SD	42.78±32.03	12.64±3.14	0.005**
DAS-28 ESR Mean± SD	4.69±1.32	-	-
Male **Female N(%)**	10(22.22%) 35(77.78%)	11(24.44%) 34(75.56%)	0.803
RF +ve **RF-ve N(%)**	27 (60%) 18 (40%)	- 45 (100%)	0.000**
CRP + ve **CRP –veN(%)**	25(55.6 %) 20 (40.4%)	9 (20%) 36(80%)	0.000**

RA: rheumatoid arthritis, ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, RF= rheumatoid factor, DAS-28= disease activity score-28.

consistent with the DAS-28, disease severity was Remission in 5 (11.1%) patients, moderate in 19 (42.2%) patients, and Severe in 21 (46.7%) patientsThere were no RA patients with low disease activity in this review.The serum Anti-ccp level was substantially higher in RA patients, as seen in Figure 1 (232.37 ± 254.17 U/mL) compared to healthy controls (4.76 ± 2.08 pg/mL) (p<0.000). also Figure 1 shows that serum YKL-40 levels in RA patients were significantly higher (157.62± 62.13 ng/mL) than in normal controls (124.51 30.72 pg/mL)(p0.001) Student’s t-test.

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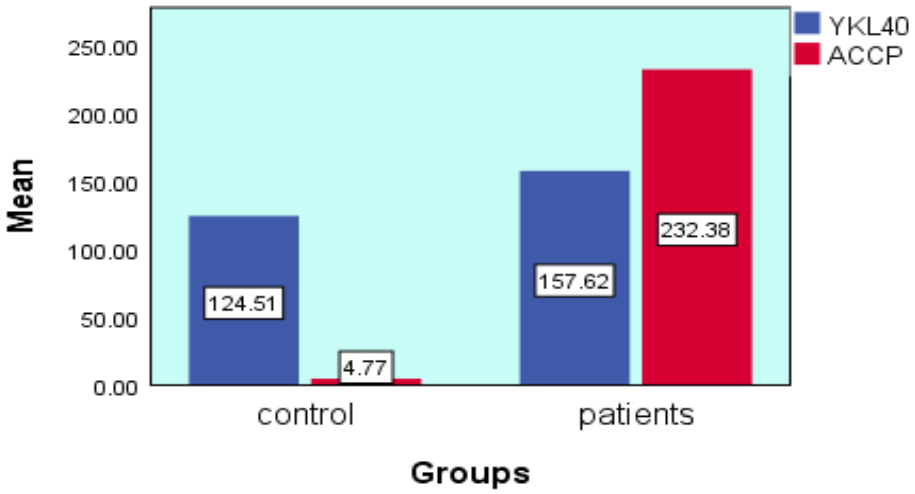

Figure 1.YKL-40 serum concentrations in the RA and healthy controls.

Dependent on the DAS-28 score, Figure.2 shows serum YKL-40 and Anti-ccp levels in RA groups. There was a significantly greater serum concentration of YKL-40 between severe(175.93± 64.58ng/mL) than remission(105.94 ± 28.66ng/mL) based on the type of DAS(P,0.028).however, no significant difference between remissionas well as moderate for YKL-40 (P,0.116).alternatively, serum concentration of ACCP was significantly higher between Severe(302.73± 242.48 U/mL), moderate(211.85± 270.41U/mL) than remission (14.89± 22.15U/mL) depended on disease activity score (p=0.005-0.000) correspondingly.Independent sample T-test for comparison between the groups.

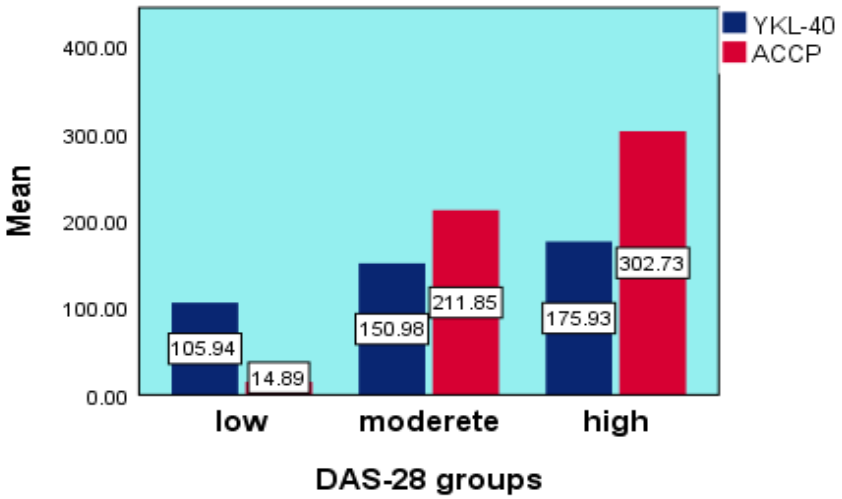

Figure 2.YKL-40 and Anti-ccpconcentration in RA subject based on DAS-28 score.

Figure 3. depicts serum YKL-40 and Anti-ccp levels in RA patients depending on the type of DMARD used.Based on the DMARD category, there was no statistically significant variation in serum YKL-40 values(p,0.919). However, there was a majorvariation in Anti-ccp serum concentration in RA patients depending on the mode of DMARD therapy (p=0.039). One-way ANOVAfor comparison between the groups.

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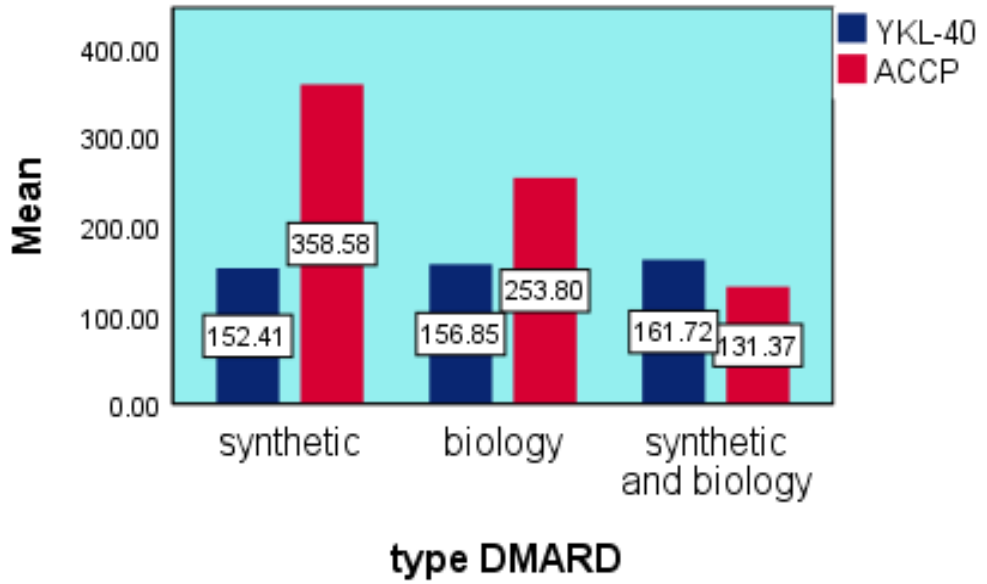

Figure 3. Comparison of serum YKL-40 and Anti-ccp levels based method of DMARD in RA patients

The results found significant positive associations among serum YKL-40 and Anti-ccp concentrations through disease activity (r,0.343, P=0.021), (r,0.341, P=0.022), respectively, as seen in Figures 4.

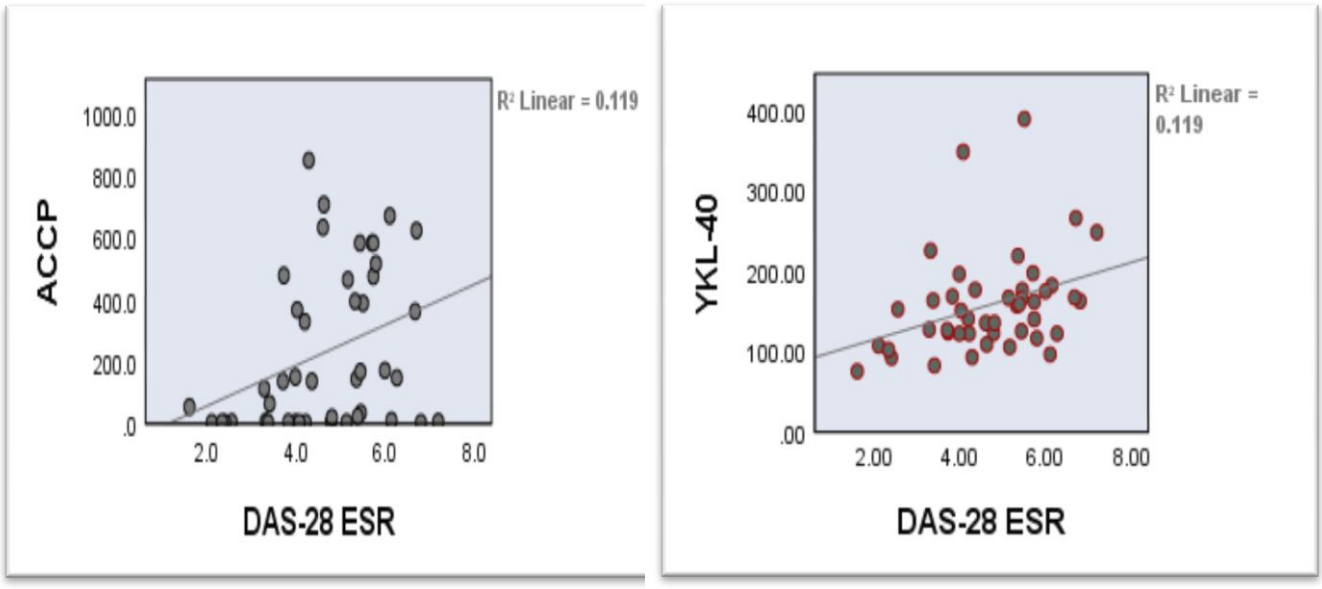

Figure4.the relationship between serumthe levels of YKL-40, Anti-ccp, with DAS-28 in RA groups

There was no significant correlation between serum YKL-40 levels and biochemical measures such as serum RF, C-reactive protein, ESR, and anti-CCP levels (P value more than 0.05)

The ability of plasma YKL-40 amounts to distinguish patients with RA from those without was assessed using ROC curve analysis Figure 5. The test revealed that the area under the curve

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(AUC) was 0. 684 ±0.059 (standard error), 95%CI, 0. 574-0. 794, p,0.004. The test's sensitivity and specificity were 71% and 60%, respectively, indicating a good discriminative value.

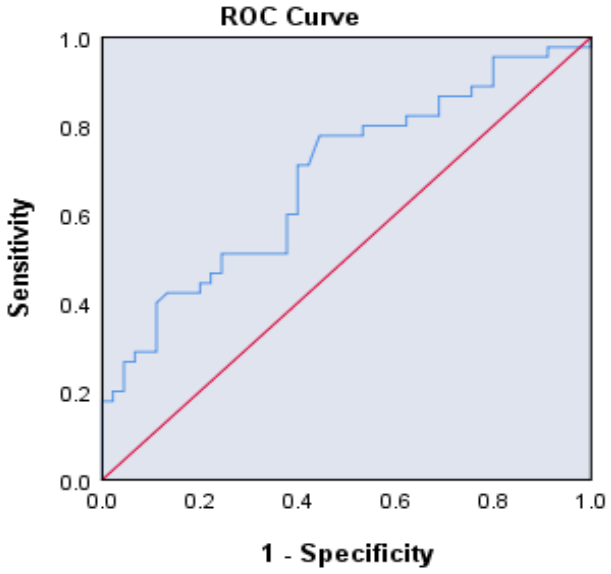

Figure 5.ROC curve study for predicting serum YKL-40 concentrations in RA.

DISCUSSION

Rheumatoid arthritis (RA) is a progressive, inflammatory joint condition that affects approximately 5 out of every 1000 adults worldwide. Women are 2 to 3 times more likely than men to develop the disorder, which may happen at all ages.Prevalence is increasing in the sixth decade(23).As a result, is also possible to develop novel biomarkers of synovial inflammation and disease development. CHI3L1 or YKL-40 expression is increased in a variety of inflammatory and chronic diseases such as rheumatoid arthritis(24). NF-B is a well-known transcription factor that plays an important role in the progression and progression of inflammation by regulating as well asthe development of cytokines and prostaglandins. Furthermore, YKL-40 secreted by cells in response to inflammation activates NF-B, which is required for the expression of inflammatory proteins. YKL-40 can also activate pro-inflammatory mediators. and maybe involved in the inflammation caused by type 2 helper cells. As a result, these outcomes suggest that YKL-40 is involved in inflammatory-related diseases(25). YKL-40 is produced by articular chondrocytes, synovial cells, infiltrated macrophages, and neutrophils in RA joints. Furthermore, proinflammatory mediators such as IL-6, IFN-, and TNF- were found to be correlated with YKL-40 levels in RA patients, with anti-rheumatic treatment lowering YKL-40 levels(26). We observed that serum YKL-40 levels were higher in RA in comparison to healthy people and that it was associated with disease activity, that corresponded to prior research(27,28). The disease activity in RA was shown to affect YKL-40 concentration during this research. This result was also confirmed by a beforeresearch that identified that YKL-40 levels were elevated in RA patients by high disease activity(29). Also, This study was supported by a previous study that found There are hardly any major interactions between serum YKL- 40, CRP, or ESR(24,27). Our results on the relationship between YKL- 40 plus Rheumatoid factor and CRP in RA subjects differed from those of Lee and Song (28)who revealed a positive significant relationship between glycoprotein YKL- 40 Rheumatoid factor and CRP in RA groups. The disparities in our results and those of beforeresearch perhaps attributed to variations in the examined people, syndrome

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period, baseline condition symptoms, and YKL-40 circumstance, as well as the larger sample size. Disease-modifying antirheumatic drugs must be used to treat patients with RA (DMARDs). A disease-modifying antirheumatic drug (DMARD) is a medication that reduces the signs and effects of RA, increases physical function, and slows the progression of joint damage.Synthetic (small chemical molecules given orally) and biologic (proteins given parenterally) DMARDs are the 2 kinds (23). Patients that do not respond to two or more traditional synthetic DMARDs are unable to reach their recovery goals. European League Against Rheumatism suggests the addition of some biological DMARD or a synthetic DMARD, in addition to the methotrexate, based on its long-term experience with the effectiveness and protection of biological DMARDs(30). In combination with methotrexate or other traditional synthetic DMARD both biological DMARDs and synthetic DMARDs have increased potency as compared to medication alone(31).Depending on the DMARD category in the present study, there was no statistically relevant difference in serum level YK - 40. There was a major variation for Anti-ccp serum level in RA patients based on the type of DMARD treatment they received.

CONCLUSION

Finally, by comparing RA patients to healthy controls, the current research reported that RA patients had greater serum YKL-40 values, which were correlated to disease activity. As a result, YKL-40 may be considered a new marker for determining the severity of RA disorder.

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