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Estimate of Serum 14-3-3η Levels as a Marker in Rheumatoid Arthritis Iraqi Patients

Aya Yassin Taher[1] , Kamal AL-Deen Hussein Ali [2]
[1] Chemistry and Biochemistry Department, College of Medicine, Al-Iraqia University, Baghdad, Iraq. ORCID: 0009-0009-9440-8587
e-mail: aya.y.taher@aliraqia.edu.iq
[2] Education Department, Directorate General of Salahuddin Education, Ministry of Education, Iraq. ORCID: 0009-0003-1370-4680
e-mail: kamalaldeenali93@gmail.com , Corresponding author

Abstract :

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation, leading to significant morbidity. Identifying reliable biomarkers for early diagnosis, disease progression, and treatment monitoring remains an area of active research. The 14-3-3η protein has emerged as a promising biomarker for RA. **Objectives:** This research aims to explore the serum levels of 14-3-3η in Iraqi patients with rheumatoid arthritis, evaluate its diagnostic and prognostic significance, and compare it with other established biomarkers in RA. **Methods:** This study is a case-control investigation involving 80 participants. They were divided into 2 groups. Group 1: 20 healthy controls matched for age and sex, Group II: includes 60 patients with Rheumatoid arthritis, is divided into 3 subgroups by assessment of disease activity in RA patients, all done by the RA Disease Activity Score using 28 joint counts (DAS 28). Serum 14-3-3η was measured for all participants by Sandwich ELISA. Radiological examinations, in the form of plain X-rays, were performed on all patients for the hands and feet.

Results: Serum levels of 14-3-3η were significantly higher in RA patients compared to the control group ($p < 0.001$). **Conclusion:** 14-3-3η could be a valuable marker for the diagnosis of RA patients, and it may have prognostic value.

Keywords: Anti-CCP, Erythrocyte Sedimentation Rate (ESR), Rheumatoid Factor, Iraq, Disease Activity Score (DAS 28).

تقدير مستويات مصل البروتين 14-3-3η كمؤشر لدى المرضى العراقيين المصابين بالتهاب المفاصل الروماتويدي

اية ياسين طاهر[1] , كمال الدين حسين علي[2]
1. قسم الكيمياء والكيمياء الحياتية، كلية الطب، الجامعة العراقية، بغداد، العراق
2. قسم تربية الاسحاقي، المديرية العامة لتربية صلاح الدين، وزارة التربية، العراق

مستخلص:

الخلفية: يُعد التهاب المفاصل الروماتويدي (RA) مرضًا مناعيًا ذاتيًا مزمنًا يتميز بالتهاب المفاصل، مما يؤدي إلى اعتلال وظيفي كبير. ويُعد تحديد المؤشرات الحيوية الموثوقة للتشخيص المبكر، وتتبع تطور المرض، ومراقبة الاستجابة للعلاج مجالًا نشطًا للبحث. وقد برز البروتين 14-3-3η كمؤشر حيوي واعد لالتهاب المفاصل الروماتويدي.

الأهداف: تهدف هذه الدراسة إلى استقصاء مستويات مصل البروتين 14-3-3η لدى المرضى العراقيين المصابين بالتهاب المفاصل الروماتويدي، وتقييم أهميته التشخيصية والتنبؤية، ومقارنته مع المؤشرات الحيوية الأخرى المعتمدة في هذا المرض. **طريقة العمل**: تُعد هذه الدراسة من نوع الحالات والشواهد، وشملت 80 مشاركًا قُسِّموا إلى مجموعتين: المجموعة الأولى تضمنت 20 شخصًا سليمًا متوافقين مع المرضى في العمر والجنس، بينما شملت المجموعة الثانية 60 مريضًا مصابًا بالتهاب المفاصل الروماتويدي، وقد جرى تقسيمهم إلى ثلاث مجموعات فرعية اعتمادًا على تقييم نشاط المرض باستخدام مقياس نشاط التهاب المفاصل الروماتويدي القائم على 28 مفصلًا (DAS28). تم قياس مستوى مصل البروتين 14-3-3η لدى جميع المشاركين باستخدام تقنية ELISA من نوع الساندويتش. كما أُجريت فحوص شعاعية لجميع المرضى شملت صور الأشعة السينية لليدين والقدمين.

النتائج: أظهرت النتائج ارتفاعًا معنويًا في مستويات مصل البروتين 14-3-3η لدى مرضى التهاب المفاصل الروماتويدي مقارنةً بمجموعة الشواهد الأصحاء ($p < 0.001$). **الاستنتاج**: يُمكن أن يُشكّل البروتين 14-3-3η مؤشرًا مهمًا لتشخيص مرضى التهاب المفاصل الروماتويدي، كما قد يحمل قيمة تنبؤية لمسار المرض.

الكلمات المفتاحية: الأجسام المضادة لـ CCP، معدل ترسيب كريات الدم الحمراء (ESR)، العامل الروماتويدي، العراق، مقياس نشاط التهاب المفاصل الروماتويدي (DAS28) .

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that primarily affects the synovial joints, leading to pain, swelling, and potentially irreversible joint damage. In RA, the immune system mistakenly targets healthy tissues, leading to inflammation **(Kocer, B., et al. (2019).** Early detection and assessment of disease activity are crucial for effective management. Traditional biomarkers like C-reactive protein (CRP) and rheumatoid factor (RF) provide some diagnostic value but have limitations in identifying early disease or predicting disease severity. Recent studies suggest that the 14-3-3η protein, a member of the 14-3-3 family, could serve as a useful biomarker for RA diagnosis and prognosis. This study explores the serum levels of 14-3-3η in Iraqi RA patients and its potential clinical significance **(Li, Z., et al. (2018), (LabCorp. (2025).**

It affects approximately 1% of the global population, with significant variations in prevalence and severity across different ethnic and geographic populations. In Iraq, the burden of RA is substantial, with many patients experiencing delayed diagnosis and inadequate management due to limited access to advanced diagnostic tools and therapies **(Black, R. J., et al (2023).**

The 14-3-3η protein is a novel biomarker that has gained attention for its role in RA pathogenesis. It is released by synovial fibroblasts and immune cells in response to inflammatory stimuli, contributing to the activation of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-17 **(Trimova, G., et. al (2020).**

Early diagnosis and treatment are crucial to managing RA and preventing irreversible joint damage. Biomarkers play a pivotal role in early detection and monitoring of disease activity. One emerging biomarker is the 14-3-3η protein, a member of the 14-3-3 protein family involved in intracellular signaling, apoptosis, and cell cycle regulation **(Maksymowych et al., 2014).**

The 14-3-3η protein is released from damaged or stressed cells and is found in elevated levels in the serum of patients with RA. Research indicates that 14-3-3η interacts with pro-inflammatory cytokines such as tumor necro-

sis factor-α (TNF-α) and interleukin-6 (IL-6), contributing to joint inflammation and bone erosion **(Kilani et al., 2016).**

Despite global research on 14-3-3η, data specific to Iraqi patients remain limited. Variations in genetic, environmental, and healthcare factors may influence RA presentation and biomarker profiles in this population. Understanding serum 14-3-3η levels among Iraqi RA patients could enhance early diagnosis and inform treatment strategies tailored to regional needs. making this research significant for identifying population-specific trends and improving clinical outcomes **(Al-Saffar et al., 2019).**

Materials &Methods

Subjects of study

A case-control study included 80 participants (20 healthy controls and 60 patients with Rheumatoid arthritis (RA) of both genders (40-69 years, 18 males and 62 females) divided into four groups shown below. Collected during the period between 15 November 2024 and February 2025, the permission to do the research was obtained for outpatients by the consultation unit of the RA consultant in Baghdad Teaching Hospital/Medical City.

Measured parameters: The data collection included anthropometric measurements, such as age, gender, and body mass index (BMI). Approximately 5 ml of blood was collected from each patient. Each blood samples divided into three parts: The first part 3 ml of whole blood retained in EDTA tubes for measuring WBCs, Hb, PLT. The second part 1 ml to measurements of ESR, the third part 3 ml of blood were left for 30 min at room temperature allow samples to clot in plain tube. After coagulation, sera were separated by centrifugation at 3000 rpm for 10 min. Sera were aspirated and divided into two aliquots in Eppendorf tubes for: Aliquot 1: measurements of CRP, RF, and anti-CCP. Aliquot 2: The rest were stored at (< -35 C°) until assayed for serum 14-3-3 theta protein. They were measured using enzyme-linked immunosorbent assay (ELISA).

The study groups

80 the total samples of the study were divided into four groups by using as follows:

Group I: includes 20 persons as a healthy control.

Group II: includes 60 patients with Rheumatoid arthritis (RA) are divided into 3 subgroups by assessment of disease activity in RA patients, all done by the RA Disease Activity Score using 28 joint counts (DAS 28).

Interpretation of the DAS results was done according to the following classification: 20 low disease activity (DAS28 $\leq$ 3.2), 20 moderate disease activity (3.2 < DAS28 $\leq$ 5.1), and 20 high disease activity (DAS28 > 5.1).

Inclusion and Exclusion Criteria: The study population comprised Iraqi patients who had been diagnosed with Rheumatoid arthritis (RA) with an age range of 40-69 years. Individuals were excluded if they were pregnant, had acute infections, were diabetic, had liver disease, kidney disease or had tumors.

Ethical Considerations

The study received ethical approval from the institutional review board and followed as per ethical studies requirements. All participants received written informed consent prior to participation in the study. Participants were debriefed about the purpose, methods and potential risks and confidentiality was kept throughout the research. All procedures were performed in accordance with ethical standards as outlined in the Declaration of Helsinki.

Statistical Analysis

Data collected through the online form were automatically transferred into a computerized database, facilitating efficient data entry and minimizing the risk of errors. Prior to analysis, the data underwent thorough review to identify and address missing values and outliers. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 29, and the data were described using the mean, standard deviation, and percentages. The difference in the mean level of numerical data between more than two variables was assessed using the ANOVA test. The mean of the biomarkers that were investigated across the groups was displayed using histogram graphics. To examine the relationship between qualitative vari-

ables, utilize the Chi-square test.

Results

Patients and healthy controls: included 80 subjects from both sexes (female and male), who is divided into four groups according to their radiological examination. showed the clinical characteristics of the four study groups significant differences as shown in the Table 1.

Table 1: Anthropometry of clinical characteristic, about the investigation parameters in all studied groups.

Clinical Variables		Group I (N=20) (Control)	Rheumatoid Arthritis Patient (N=60)			P-value*
			Group I (N=20) (RA Stage I)	Group II (N=20) (RA Stage II)	Group III (N=20) (RA Stage III)	
Gender	Female	12 (60%)	17 (85%)	15 (75%)	18 (90%)	62 (77.5%)
	Male	8 (40%)	3 (15%)	5 (25%)	2 (10%)	18 (22.5%)
Age (years)	M±SD	45.3 ± 6.8	44.2 ± 8.5	52.2 ± 6.7	55.2 ± 7.7	0.001
BMI (Kg/m2)	M±SD	25.154 ± 2.344	27.010 ± 2.775	28.300 ± 2.641	28.027 ± 4.024	0.001
WBCs (thousands/mm3)	M±SD	8.07 ± 2.3	11.8 ± 2.4	9.3 ± 1.8	7.5 ± 2.1	0.001
Hb (g/dl)	M±SD	12.5 ± 2.7	11.7 ± 1.9	10.2 ± 1.7	8.6 ± 1.4	0.001
PLT (thousands/mm3)	M±SD	265 ± 85	258 ± 73	235 ±68	233 ± 76	0.001
ESR 1st hour (mm/h)	M±SD	12 ± 3	32 ± 6	38 ± 7	43 ± 7	0.001
CRP (mg/L)	M±SD	3.2 ± 1	10.5 ± 2.1	17.3 ± 3.6	24.8 ± 4.3	0.001
RF (IU/mL)	M±SD	13 ± 2	27 ± 4	52 ± 8	58 ± 9	0.001
Anti-CCP (IU/mL)	M±SD	16.3 ± 2.3	23.4 ± 3.5	27 ± 4.8	30.2 ± 6.7	0.001
14-3-3η (pg/ml)	M±SD	46.36 ± 8.36	52.23 ± 9.87	63.67 ± 11.56	75.37 ± 13.24	0.001

Patients were divided into three groups according to radiological examination and a control group. Compared the mean ± SD level of 14-3-3η and Anti-CCP were significantly ($p < 0.001$) between the groups, as presented in (Fig. 1). Compared the mean ± SD level of 14-3-3η and RF were significantly between the groups, as presented in (Fig. 2). Compared the mean ± SD level of Anti-CCP and RF were statistically significantly ($p < 0.001$); as presented in (Fig. 3).

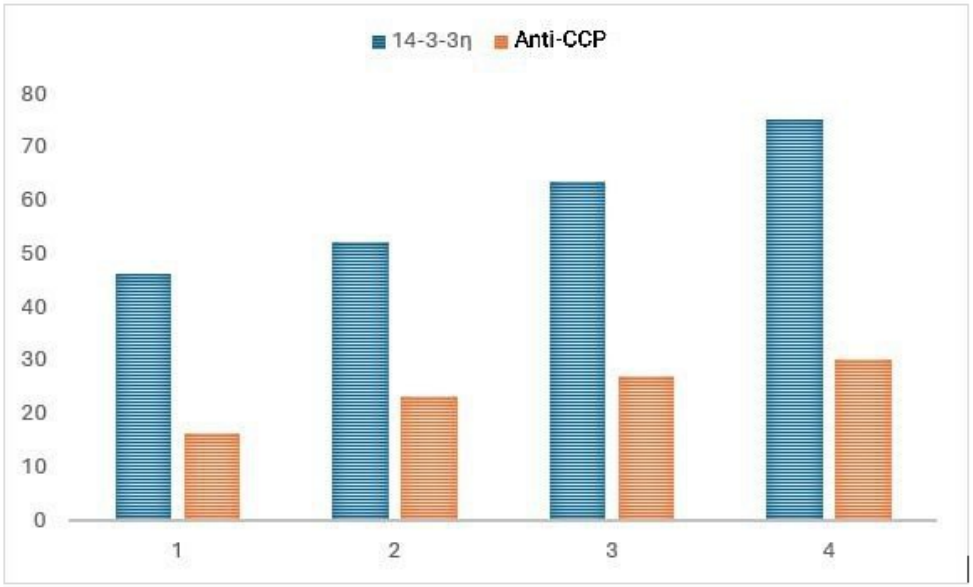

Figure 1. Compare the mean of serum14-3-3 η and Anti-CCP between the groups

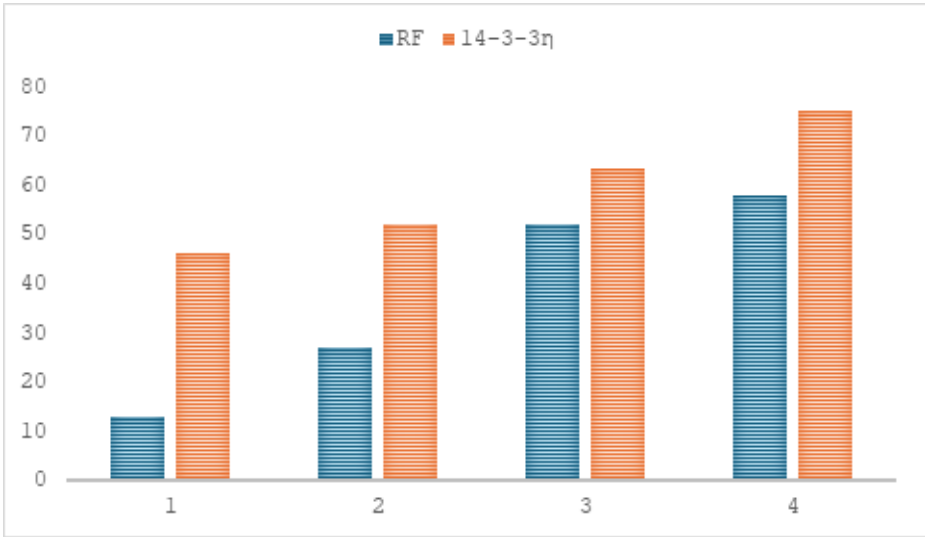

Figure 2. Compare the mean of serum14-3-3 η and RA between the groups

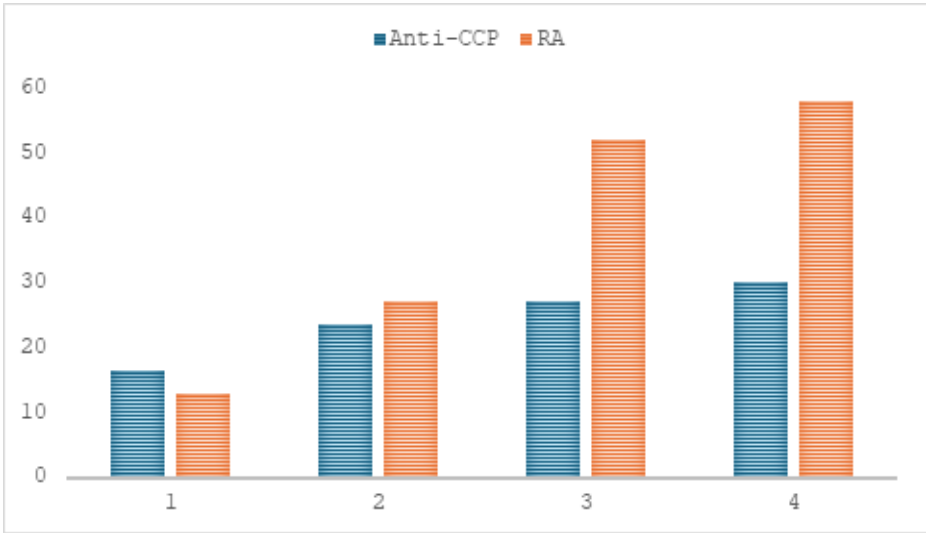

Figure 3. The mean of serum Anti-CCP and RA between the groups

Discussion

The data obtained from our study showed that the serum level of 14-3-3η was significantly higher in RA patients ($p < 0.001$). This finding coincides with that of **Hitchon et al. (2015) and Carrier et al (2016),** who also found that serum levels of 14-3-3η were significantly higher in RA patients in comparison to controls.

Soluble 14-3-3η at levels similar to those in RA patients exhibits ligand activity, particularly stimulating cells of the innate immune system **(Kilani et al., 2007)**. In addition, (**Maksymowych et al. (2014))** reported that soluble 14-3-3η acts through signaling cascades such as the ERK and JNK pathway, leading to upregulation of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, and joint degradation factors such as MMP-9 and RANKL. This action may explain its role in the inflammatory state in RA.

Our laboratory findings are in concordance with those of **Maksymowych et al. (2014), Hitchon et al. (2015), and Carrier et al. (2016).**

This finding indicates that14-3-3η may act independently of these acute phase reactants, which is one of the key requirements suggested by the OMERACT soluble biomarker working group for a biomarker pointing to joint damage **Maksymowych, et. al (2009).**

Naides, S. J., & Marotta, A. (2015) found that of 124 subjects with established RA, 10 of 15 seronegative for RF and Anti-CCP (67%) were14-3-3η positive. Accordingly, 14-3-3η has a clinical use beyond RF and Anti-CCP in RA patients, as 14-3-3η confirms inflammatory joint disease in the absence of conventional serological measures.

Based on the fact that 14-3-3η is an inflammatory mediator, the mechanism through which it is involved in arthritis development might not be the same as that of auto-antibodies such as RF and Anti-CCP. This may be due to the ability of 14-3- 3η to induce inflammatory factors or may be related to the dynamic nature of this biomarker (**Van Beers-Tas, et. al, 2016).**

In contrast, **Maksymowych and colleagues (2014) and Carrier et al. (2016)** found that there is a modest correlation between 14- 3-3η serum level with RF and Anti-CCP in a cohort of early RA population. However, Carrier et al. explained this by suggesting that B cells might be activated by extracellular 14-3-3η, resulting in stimulation of RA-associated auto-antibodies production.

This study was in agreement with **Maksymowych et al. (2014) and Maksymowych et al. (2014)** who also reported the same finding. Also, **Hitchon et al. (2015)** pointed to the absence of correlation between 14-3-3η serum level and RA activity indices, and that 14-3-3η positivity usually occurred at the disease onset, making14-3-3η a possible predictor of imminent RA rather than being a disease activity marker.

Conclusion

14-3-3η is present in higher levels in serum of RA patients compared to healthy controls. Adding this test to either RF alone or to both RF and Anti-CCP enhanced their diagnostic capacity for RA. Moreover, high serum levels of 14-3-3η may predict RA in apparently healthy persons as we demonstrated by univariate and multivariate analysis. Serum 14-3-3η did not correlate with any clinical or laboratory features of RA activity; therefore, it has no value in assessing disease activity. However, it may be of good prognostic value for disease severity as it significantly correlates with Larsen's

radiological score.

Recommendations

This is the first study conducted on serum 14-3-3η in the Iraqi population. However, this research has some limitations; one of them is the small sample size and lack of follow-up. So, we recommend further studies on serum 14-3-3η in a large number of patients for more evaluation of its diagnostic utility, and to reveal the relation between serum 14-3-3η and its auto-antibody level in RA and their involvement in the pathogenesis of the disease.

Study Limitations: A notable limitation of this research is the small sample size, which affects the generalizability of the results. Future studies with larger cohorts are needed to confirm these preliminary findings.

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Conflict of interest: None.

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