Evaluation the serum level of the 14-3-3η protein antibody compare with the Anti-Citrullinated peptide antibody and rheumatoid factor antibody in rheumatoid arthritis patients

Evaluación del nivel sérico del anticuerpo proteico 14-3-3η en comparación con el anticuerpo antipéptido citrulinado y el anticuerpo del factor reumatoide en pacientes con artritis reumatoide

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Abstract

Background: Rheumatoid arthritis is an inflammatory disease characterized by chronic inflammation of the synovial membranes. The gold standard antibodies for diagnosis are RF and ACPA. Numerous investigations demonstrated that Anti-14-3-3η can be used as prognostic and diagnostic marker.

Objective: Comparison of serum levels in patients with RA based on disease severity between Anti-14-3-3η with ACPA and RF.

Materials and Methods: Case-control study with 270 participants from October 2022 to April 2023 at Al-Mujtaba Hospital/Rheumatology Unit split into 180 RA patients [90 seropositive and 90 seronegative RA cases] and 90 controls. All patients consented to ACR 2010 rheumatologists' diagnoses; venous blood samples were collected and divided into sodium citrate tubes for Westergren ESR and gel tubes for serological assays, C-RP and RF using Nephelometry, ACPA, and Anti-14-3-3η by ELISA. Statistical analysis was performed with SPSS 26.

Results: RA cases 180, 90 were seropositive and 90 were seronegative, Anti-14-3-3η mean was significantly higher in seronegative than seropositive at *P*.value (0.000), in mild DAS28-ESR at *P*.value (0.032), and in regulars treatment than irregulars at *P*.value (0.044), in contrast, the mean of ACPA and RF were significantly higher in seropositive at *P*.value (0.000), in severe DAS28-ESR at *P*.value (0.01), and in irregulars treatment at *P*.value (0.001).

Conclusions: Anti-14-3-3η concerning to RA patients, particularly seronegative RA, and may be helpful as a diagnostic marker in addition to RF and ACPA, concerning to mild DAS28-ESR and regular treatment in comparison to RF and ACPA, so it may be used as a prognosis marker for the severity of the illness.

Keywords: Rheumatoid arthritis (RA), RF, ACPA, Anti-14-3-3η, DAS28-ESR.

Resumen

Antecedentes: la artritis reumatoide es una enfermedad inflamatoria caracterizada por la inflamación crónica de las membranas sinoviales. Los anticuerpos estándar de oro para el diagnóstico son RF y ACPA. Numerosas investigaciones demostraron que el Anti-14-3-3η puede utilizarse como marcador de pronóstico y diagnóstico.

Objetivo: Comparación de los niveles séricos en pacientes con AR según la gravedad de la enfermedad entre Anti-14-3-3η con ACPA y RF.

Materiales y métodos: estudio de casos y controles con 270 participantes desde octubre de 2022 hasta abril de 2023 en el Hospital/Unidad de Reumatología Al-Mujtaba dividido en 180 pacientes con AR [90 casos de AR seropositivos y 90 seronegativos] y 90 controles. Todos los pacientes dieron su consentimiento para los diagnósticos de reumatólogos ACR 2010; Se recogieron muestras de sangre venosa y se dividieron en tubos de citrato de sodio para Westergren ESR y tubos de gel para ensayos serológicos, C-RP y RF mediante nefelometría, ACPA y Anti-14-3-3η por ELISA. El análisis estadístico se realizó con SPSS 26.

Resultados: 180 casos de AR, 90 fueron seropositivos y 90 seronegativos, la media de Anti-14-3-3η fue significativamente mayor en seronegativos que seropositivos en el valor P (0,000), en DAS28-ESR leve en el valor P (0,032), y en tratamiento regular que en pacientes irregulares con un valor de P (0,044), por el contrario, la media de ACPA y RF fueron significativamente mayores en los seropositivos con un valor de P (0,000), en DAS28-ESR grave con un valor de P (0,01) y en tratamiento irregular a P.valor (0,001).

Conclusiones: Anti-14-3-3η en pacientes con AR, particularmente AR seronegativa, y puede ser útil como marcador de diagnóstico además de RF y ACPA, en relación con DAS28-ESR leve y tratamiento regular en comparación con RF y ACPA, por lo que puede utilizarse como marcador de pronóstico de la gravedad de la enfermedad.

Palabras clave: Artritis reumatoide (AR), RF, ACPA, Anti-14-3-3η, DAS28-ESR.

Introduction

Rheumatoid arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that begins in the tiny joints and progresses to the skin, eyes, heart, kidneys, and lungs[1], the most common symptoms of RA include pain, joint swelling and stiffness, and perhaps cartilage and bone degeneration, which can lead to joint function loss[2].

Rheumatoid arthritis has an estimated incidence of 1% and raises the risk of significant morbidity and premature death[3], The most common age range for RA patients is 30 to 50 years, with ratio three to one female to male[4]. Common risk factors for RA include both non-modifiable characteristics, such as genetics and sex, and modifiable lifestyle-associated variables[5].

The pathogenesis of RA involves both innate immune system cells (monocytes and macrophages) and adaptive immune system cells (B and T lymphocytes)[6]. Autoantibodies are a distinguishing characteristic of RA, Anti-Citrullinated Peptide Antibody (ACPA) and Rheumatoid Factor (RF) antibodies are the two most notable autoantibodies in RA and provide distinct clinical and pathophysiological information, they predict a more severe disease course[7],these autoantibodies are prevalent diagnostic biomarkers included in the 2010 American College of Rheumatology (ACR)/European League of Rheumatism (EULAR) RA classification criteria[8].

In international treatment recommendations, the presence of RF and or ACPA, particularly at high levels is cited as a poor prognostic factor for MTX treatment response. MTX is the most commonly used anti-rheumatic substance in clinical practice[9].

Protein 14-3-3η is an intracellular chaperone (cellular adapter) protein that is released into the extracellular space during the early phases of RA and functions as an inducer of the innate immune system. Through 14-3-3η protein, numerous inflammatory mediators and pathways implicated in the pathogenesis and progression of RA have been upregulated[10].

Anti-14-3-3η has the potential to be used in the early diagnosis of rheumatoid arthritis with greater sensitivity and specificity than conventional diagnostic biomarkers. The addition of anti-14-3-3η as a novel biomarker to RF and ACPA is advantageous for early diagnosis of rheumatoid arthritis and early therapeutic intervention to reduce disease progression and structural damage[11], (71%) of seronegative RA cases demonstrated anti-14-3-3η positivity, anti-14-3-3η appears to be a highly specific and beneficial marker for RA[12].

The objective of this study was to compare the serum level of ACPA, RF, and anti-14-3-3η in all patients and their related with disease activity.

Materials and methods

Patients attended to Imam Al-Hassan Al-Mujtaba Hospital- Rheumatology Unit were included in the case-control study. From October 2022 to April 2023, 270 participants were divided into two main groups, including 180 rheumatoid arthritis (RA) patients and 90 healthy control subjects. The RA cases were further divided into two subgroups, including 90 seropositive and 90 seronegative cases. There were 180 patients with RA (20 males and 160 females), 90 seropositive cases (11 males and 79 females), and 90 seronegative cases (9 males and 81 females). All patients were diagnosed by rheumatologists at the Imam Al-Hassan Al-Mujtaba Hospital in the Karbala rheumatology department. Ethically, permission was obtained from the College of Medicine at Karbala University and the Iraqi Ministry of Health/Karbala Health Directory, Imam Al-Hassan Al-Mujtaba Hospital/Rheumatology Department, and the patients about taking blood samples and using their data for research purposes; all of them met at least six criteria from the 2010 ACR/EULAR, and those with RA disease did not have other rheumatological disorders or other autoimmune diseases and infectious diseases.

Five milliliters of venous blood were extracted from patients and controls for the estimation of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF, ACPA and Anti-14-3-3η using the western green method for ESR-kits, AFCCO, and Jordan. To the ESR tube containing 0.4 ml of sodium citrate anticoagulant, 1.6 ml was added. The remaining volume was added to the gel tube, which was then centrifuged to separate the serum, which was then stored at -20 degrees Celsius in four small Eppendorf containers for serological analysis. CRP test-titer kits, Hipro Biotechnology (China) and RF test-titer kits, Hipro Biotechnology (China), by Nephelometry, ACPA in-direct-ELISA kits, Sun Long Biotech (China) and Anti-14-3-3η Sandwich ELISA kits, Sun Long Biotech (China), disease activity score (DAS) calculator for (the severity of the disease) mild, moderate and severe. The statistical analysis was conducted using SPSS version 26.

Results

Distribution of the sex, age and age categories

The study involved 270 participants, and the total number of RA patients was 180 patients females 160 (88.9%) and males 20 (11.1%), with the mean Standard Error(SE) age 49.82 (0.86), the seropositive RA cases were 90 patients females 79 (87.8%) and males 11 (12.22%) with the mean (SE) age 47.73 (1.208) and the range of age 20–73 years, the seronegative RA cases were 90 patients females 81 (90%) and males 9 (10%) with the mean (SE) of age 51.90 (1.195) and the range of age 21–73 years, both seropositive and seronegative RA patients exhibit the greatest frequency of females compared to males, the healthy control group were 90 subjects females 78(86.7%) and males 12(13.3%) with the mean (SE) of age 49.00 (0.991) and the range of age 21–70 years. The patients in the current study are separated into three groups based on their age: Group one (ages 20–39) has 33 (18.3%) members, Group two (ages 40–59) has 113 (62.8%) members, and Group three (ages 60–79) has 34 (18.9%) members. Group two has the highest percentage.

Comparison between RA patients and Healthy Control Group

Regarding the comparison between RA patients and healthy controls, the variables of the current investigation, disease duration, ESR-level, CRP-level, RF-titer, ACPA, and Anti-14-3-3η, show highly statistically significant differences for the RA patients group compared to the healthy control group at *P*.value (0.000), whereas the mean (SE) of the age variables show no statistically significant differences between the RA patients group and healthy control group, as shown in Table 1.

Table 1: Comparison between RA patients and Healthy Control Group

Parameters	RA Patients N=180	Control N=90	Sig
	Mean (SE)	Mean (SE)	
Age	49.82 (0.86)	49.0 (0.99)	0.508^{NS}
D. Duration	6.30 (0.43) *	0.00 (0.00)	0.000**
ESR-level	38.73 (1.64) *	6.83 (0.21)	0.000**
C-RP-titer	18.61 (2.06) *	0.95 (0.037)	0.000**
RF-titer	43.55 (4.29) *	5.44 (0.23)	0.000**
ACPA	55.81 (4.24) *	3.97 (0.096)	0.000**
Anti-14-3-3η	10.02 (0.84) *	3.94 (0.23)	0.000**

NS: Non-Significant ****: Highly significant *P.* value < 0.01**

Comparison between Seropositive and Seronegative RA patients

The mean (SE) of age, disease duration, ESR level, and CRP titer are not statistically different between

seropositive and seronegative RA patients. Whereas the mean (SE) of the RF-titer and ACPA show highly statistically significant differences for seropositive than seronegative RA patients at *P.*value (0.000), while there are highly statistically significant differences in the mean (SE) of Anti-14-3-3η for seronegative than seropositive RA patients at *P.*value (0.000), as shown in Table 2.

Table 2: Comparison between Seropositive and Seronegative RA patients

Parameters	Seropositive mean(SE)	Seronegative mean(SE)	*P.* Value
Age	47.73 (1)	51.9 (1)	0.182^{NS}
D. Duration	5.94 (0.6)	6.69 (0.6)	0.173 ^{NS}
ESR-level	36.66 (2)	40.81 (2)	0.809 ^{NS}
C-RP-titer	17.8 (2.62)	19.42 (3.2)	0.986 ^{NS}
RF-titer	75.53(7.08)*	11.56 (1.1)	0.000**
ACPA	94.4 (6.22) *	17.21(0.43)	0.000**
Anti-14-3-3η	7.76 (0.67)	12.28(1.5)*	0.000**

NS: Non-Significant **: Highly significant *P.* value <0.01

Comparative Analysis of Study Variables Regarding DAS28-ESR

The mean (SE) of the age variables among the three activity groups (mild, moderate, and severe) is not statistically significant. Whereas the mean (SE) of the disease duration, ESR-level, CRP-titer show highly statistically significant differences at *P.*value less than (0.01) for severe than mild and moderate activity group, RF-titer and ACPA show statistically significant differences at *P.*value less than (0.05) for severe than mild and moderate activity group, but the mean (SE) of the Anti-14-3-3η show statistically significant differences at *P.*value less than (0.05) for mild than moderate and severe activity group, as shown in the Table 3.

Table 3: Comparative Analysis of Study Variables Regarding DAS28-ESR

Parameters	Mild Mean (SE)	Moderate Mean (SE)	Severe Mean (SE)	Sig.
Age	46(3)	49(1)	52(1)	0.204 ^{NS}
D. Duration	3.0(0.6)	5.7(0.5)	8.0(0.8) *	0.001**
ESR-level	22(3)	30(1)	55(3) *	0.001**
C-RP-titer	6.29(1.76)	8.97(0.79)	34.8 (4.7) *	0.001**
RF-titer	36.6(7.94)	34.8 (4.72)	57.13(8.8) *	0.015*
ACPA	83.1(19.4)	48.6(4.9)	58.42(7.1) *	0.018*
Anti-14-3-3η	15.92(6.94) *	9.73(0.87)	8.88(0.37)	0.032*

NS: Non-Significant *: Significant *P.* value < 0.05 **: Highly significant *P.* value <0.01

Comparison of Study Variables Regarding Regularity of Treatment

The mean (SE) of age has no statistically significant difference between regulars and irregulars treatment intake among RA patients, whereas the mean (SE) of disease duration, ESR-level, CRP-titer, RF-titer, and ACPA have highly statistically significant differences at *P.*value (0.001) for irregulars treatment intake than regulars, but the mean (SE) of Anti-14-3-3η has a statistically significant difference at *P.*value (0.04) for regulars treatment intake than irregular, as shown in the Table 4.

Table 4: Comparison of Study Variables Regarding Regularity of Treatment

Treatment Status	Regulars mean (SE)	Irregulars mean (SE)	*P.* Value
Age	50 (1)	50(1)	0.902 ^{NS}
D. Duration	5.4 (0.5)	7.9 (0.8) *	0.001**
ESR-level	32 (2)	50 (3) *	0.001**
C-RP-titer	12.12 (1.55)	29.57 (4.62) *	0.001**
RF-titer	31.46 (3.61)	63.93 (9.35) *	0.001**
ACPA	51.21 (4.83)	63.56 (7.92) *	0.001**
Anti-14-3-3η	10.46 (1.29) *	9.28 (0.60)	0.044*

NS: Non-Significant *: Significant *P.* Value < 0.05 **: Highly significant *P.* Value <0.01

Discussion

Distribution of the Sex, Age and Age categories

Participants 270 were split into case-control groups in this investigation. Case and control group members mean age 49.8 and 49.0 years, respectively. Participants ranged in age from 20 to 73. In addition, serological tests categorized RA patients into seropositive with mean of age 47.73 and seronegative with mean of age 51.9. The study found sufficient mean age and age range similarity between study groups. This is consistent with previous studies by Abbood, 2019 in Iraq[13] which reported mean of age 48.46 for RA cases and 48.85 for control, Hussein, 2019 in Iraq[14] which found mean of age 47.82 for RA patients and 46.82 for controls, and Kolarz et al., 2021 in Poland[15], which found mean of age 52.1 and the age range18–70.

In the current study, group two has the maximum number of participants, with 113 individuals representing 62.8% of the age range of 40 to 59 years. The above frequencies are in agreement with Abbood's, 2019 results from Iraq[13], which showed that patients' ages ranged from 40 to 59 years, with 112 subjects representing 62.22 percent of the study group, and in disagreement with Deraj's, 2021 results from Iraq[16], which showed that patients' ages ranged from 40 to 59 years, with 46 percent of the study group representing this age group. This variation may have resulted from the inclusion and exclusion criteria used to select RA patients for the study.

Concerning to the sex, both seropositive and seronegative cases of RA (87.8%–90%) were found to be female, suggesting that females make up the majority of RA patients in the present study. This result is consistent with the work done in the United States by Favalli et al., 2019[17], who found that females are more common than males in a number of different research areas. The results are consistent with those of an Italian study conducted by Iannone in 2017[18], which found that 77 (or 80.68%) of all RA patients were females. The study's findings contrast to those of Jawaheer et al., 2012 in North America[19], who found that 2263 (75%) of the total 3017 patients with RA were females at the onset of the disease, also contrasted to those of Nilsson et al., 2021 in Sweden[20], who showed female sex represented 68% of RA patients. The largest sample size possible was acquired through a cohort study design, which may explain the discrepancy.

Comparison between RA patients and Healthy Control Group

At *P.*value of (0.5), there are no statistically significant differences in age between RA patients and controls, as shown in Table1. These results are consistent with those of Sveinsson et al., 2020 in Sweden[21], who suggest that in case-control studies, age and sex should be matched or semi-matched, and with those of Hussein, 2019 in Iraq[22], who found no statistically significant differences between patient and control at *P.*value of (0.58).

These results correspond with Hussein[22], Abbood[13], Flyeh[23] in 2019, and Deraj, 2021 in Iraq[16] in terms of disease duration, ESR, CRP, RF, and ACPA statistically significant differences with *P.*values less than (0.01) for RA patients compared to the healthy control group. All of these studies confirmed that all of the previously mentioned variables had a significant *P.*value less than (0.01).

The anti-14-3-3η statistically difference at P. value less than 0.01, and this finding is consistent with El-Sherif et al., 2019 in Egypt[24] these studies showed that the 14-3-3η Abs were higher in early RA than controls, and Bonifacio et al., 2019 in Italy[25] that demonstrated the 14-3-3η Abs are a new proinflammatory mediator implicated in RA than controls.

Comparison between Seropositive and Seronegative RA patients

Regarding the findings presented in Table 2, which indicate that there is no statistically significant difference between seropositive and seronegative RA patients in terms of age, disease duration, ESR, and CRP, this result is consistent with the findings of Kotecki et al., 2021 in Poland[26], which also found no significant difference in RA patient age, disease duration, ESR, and CRP at *P.* value less than (0.05). Oweis et al., 2020 in Jordan[27] also found no statistically significant difference in the mean of ESR among RA patients at *P.* value (0.96).

The findings in Table 2, also show that the RF and ACPA are statistically significant at *P.*value (0.000) for seropositive RA patients in comparison with seronegative RA patients, and it is rationally agreed that RA patients are classified based on these markers, so these findings agree with Kronzer et al., 2021 in Sweden[28], who showed that the ACPA and RF were specific for seropositive RA patients, and Reed et al., 2020 in Sweden[29], which showed that RA patients were classified as seropositive or seronegative based on the presence or absence of ACPA and RF.

The anti-14-3-3η results in Table 2 are statistically significant at *P.*value (0.000) for seronegative RA patients versus seropositive RA patients. The anti-14-3-3η antibody result agrees with Salman et al., 2019 in in Turkey[30], who demonstrated the importance of anti-14-3-3η Abs in seronegative RA patients at *P.* value (0.001), as well as Chawla and Jain, 2023 in India[31], who stated that anti-14-3-3η Abs levels are significantly elevated in RA patients and may be used as an additional diagnostic test for RA. Furthermore, the above findings correspond with Zhang et al., 2020 in China[32], who discovered the efficacy of anti-14-3-3η in increasing RA diagnosis in comparison to gold standard markers.

Comparative Analysis of Study Variables Regarding DAS28-ESR

The results presented in Table 3, revealed that there were no statistically significant differences between the mean age and DAS28-ESR Sparks et al., 2019 in USA[33], whereas there were statistically significant differences between the disease duration and severe disease progression over time. These findings agree with Vadell et al., 2020 in Sweden[34], who demonstrated that ESR, CRP, RF, and ACPA are significantly different among DAS28 groups, particularly the severe group, as shown in Table 3, also these results are consistent with the study of Hussein, 2019 in Iraq[22], which showed that ESR, CRP, RF, and ACPA were related to a high disease activity score group rather than other groups, also agree with Madan et al., 2019 in India, which demonstrated the association between disease severity and elevated levels of ESR, CRP, RF, and ACPA.

Regarding DAS28-ESR groups, anti-14-3-3η results were statistically significant at *P.* value (0.032) for mild DAS28-ESR among RA patients compared to other groups. The results of anti-14-3-3η agree with El-Sherif et al., 2019 in Egypt[24], that reported the use of this marker for preventing disease progression, particularly before RF and ACPA become positive, agree with Dammona et al., 2020 in Egypt[35], which determined the anti-14-3-3η was associated with non-erosive damaging outcomes in RA, and disagree with Raft et al., 2022 in Denmark[36], that demonstrated 14-3-3η Abs was associated with disease severity.

Comparison of Study Variables Regarding Regularity of Treatment

The irregular group had noticeably longer disease duration, according to the findings of Table 4, which compares the research characteristics between the regular and irregular groups according to treatment intake. ESR, CRP, RF, and ACPA were considerably higher in the irregular group than in the regular group. These results are in agreement with those of the following studies: Ghaseminasab-Parizi, 2022 in Iran[37], who showed that regular specific treatment received by RA patients resulted in a significantly lower disease activity score, Abbood, 2019[13] and Hussein, 2019[22] in Iraq, who both reported significant mean differences for patients getting irregular treatment regarding the ESR, CRP, RF, and ACPA level becoming high in the irregular group.

The anti-14-3-3η Abs results in Table 4 show significant differences with the regular group treatment intake of RA patients at P. value less than (0.05) These results are in agreement with the results of the Zeng et al., 2020 study in China[38], this study demonstrated the high level of anti-14-3-3η Abs with regular treatment intake and can be used as prognostic marker for RA patient follow-up.

Conclusions

Anti-14-3-3 antibodies can aid in the diagnosis of RA patients, particularly seronegative RA, and can be combined with the gold standard antibodies ACPA and RF. Anti-14-3-3 is also associated with mild disease activity and regular treatment of RA patients, so can be used as a prognostic marker when compared to ACPA and RF, which are associated with severe disease activity and irregular treatment of RA patients.

Recommendation

- More investigation into other antibodies in individuals who have seronegative RA.
- Larger sample sizes for further research are required to evaluate anti-14-3-3 serum levels in RA patients.
- Further investigation is required to ascertain the potential significance of anti-14-3-3 in the etiology of RA in order to further clarify their importance in disease activity, therapy monitoring, and disease progression, especially in patients with seronegative RA.

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