Al-Azhar International Medical Journal

Volume 1 | Issue 10 Article 15

10-1-2020

Correlation between 14-3-3η protein and Muskelosketal ultrasound in early rheumatoid arthritis patients.

Jamal Dammona
Pharmacology departmen,faculty of medicine, Misurata university , Libya, dammona100@gmail.com

Sameh Elzayat
Rheumatology and rehabilitation, faculty of medicine, alzhar university,Cairo, s.alzayt@hotmail.com

Hany Aly
Rheumatology and rehabilitation, faculty of medicine, alzhar university , cairo, hanyaly79@yahoo.com

Abdullah Gaafar
Clinical pathology, faculty of medicine, alzhar university, Cairo, abdullah.gaafar@yahoo.com

How to Cite This Article

Dammona, Jamal; Elzayat, Sameh; Aly, Hany; and Gaafar, Abdullah (2020) "Correlation between 14-3-3η protein and Muskelosketal ultrasound in early rheumatoid arthritis patients.," *Al-Azhar International Medical Journal*: Vol. 1: Iss. 10, Article 15.
DOI: https://doi.org/10.21608/aimj.2020.29538.1219

OPEN AIMJ ORIGINAL ARTICLE

Rheumatology $ Rehabilitation

Correlation between 14-3-3η Protein and Ultrasound Musculoskeletal Early Rheumatoid Arthritis Patients

Jamal M. Dammona[1,*] MSc; Sameh A. El-Zayat[2] MD ; Hany M. Aly[2] MD
Abdullah M. Gaafar[3] MD

**Corresponding Author:*
Jamal M. Dammona
dammona100@gmail.com

Received *for publication May 6, 2020;*
Accepted November 20, 2020;
Published online *November 20, 2020.*

doi: 10.21608/aimj.2020.29538.1219

[1]*Pharmacology Department, faculty of medicine, Misurata University, Misurata, Libya.*

[2]*Rheumatology and Rehabilitation Department, Faculty of Medicine, Al-Azhar University,Cairo, Egypt.*

[3]*Clinical Pathology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.*

ABSTRACT

Background: Despite recent advances in treatment, Rheumatoid arthritis (RA) remains a significant cause of morbidity and premature mortality. 14-3-3η protein was found at higher levels of serum and synovial fluid of patients with arthritis.
Aim of work: to assess serum levels of 14-3-3η protein in early rheumatoid arthritis and correlate it with the disease activity and ultrasound findings.
Patient and Methods: Thirty naïve patients with early RA were subjected for thorough clinical examination, labratory investigations including 14-3-3η protein and musculoskeletal Ultrasonography of both hands. 20 healthy persons matched for age and sex were selected as the control group.
Results: Their mean age was 38.2 ± 10.11years, with mean disease activity (4.4 ± 0.74). Mean ESR, CRP and anti-CCP was (40.0 ± 17.75), (18.6 ± 11.78) and (73.5 ± 81.86) respectively. Serum 14-3-3η protein level for patients was (344.8 ± 121.9) ng\ml while for the control group it was (38.4 ± 15.) ng\ml with a significant statistical difference between both groups ($p < 0.05$). Serum 14-3-3η protein showed significant positive correlation with RF and Anti-CCP (r = 0.36 and 0.52 respectively) while no significant correlation was found with ESR, CRP, or DA28, (r = 0.04, 0.08 and 0.02 respectively).
Also, we didn't find a significant correlation between serum 14-3-3η protein and musculoskeletal ultrasound parameters.
Conclusion: The Serum level of 14-3-3η protein may not be associated with structural damage in RA while it may be positively correlated to RF and Anti-CCP in early rheumatoid arthritis.

Keywords: *Rheumatoid arthritis; 14-3-3η protein; musculoskeletal ultrasound.*

Disclosure: *The authors have no financial interest to declare in relation to the content of this article. The Article Processing Charge was paid for by the authors.*
Authorship: *All authors have a substantial contribution to the article.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic destructive inflammatory arthropathy that considerably affecting personal, social, and economic status.[1] RA affects about 1.5% of the population and if left untreated it leads to joint destruction, decreased workability and decreased quality of life.[2] Early diagnosis and treatment of RA with disease-modifying antirheumatic drugs (DMARDs) in the first 12 weeks after symptoms start significantly improves the patient prognosis and outcome[3]. The inability of currently available biomarkers to identify many RA patients in early stage [4] in addition to their inability to determine and expect the outcome at the individual level hinders the immediate adjustment of treatment according to the severity of the disease.[5] Also lack of sensitivity of available serological markers in diagnosis and prognosis of the disease lead to the necessity for the search of new biomarkers to improve diagnostic sensitivity for early diagnosis.[6] In 2007, Kilani, et al demonstrated by Western blot analysis that a novel soluble biomarker, 14-3-3η protein, was found in a significantly higher levels in the synovial fluid and serum of arthritic patients compared to healthy individuals and its serum levels was strongly correlated with the matrix metalloproteinases (MMP) MMP-1 and MMP-3.[7]

Detecting a high level of extracellular 14-3-3η protein in RA patient serum acts as a cell damage signal that potently induces pro-inflammatory cytokines and bone degrading enzymes.[8] The 14-3-3 family of conserved regulatory protein consist of 7 isoforms of intracellular chaperone proteins (α/β, γ, δ/ζ, ε, η, θ/τ, σ)[7] the only 14-3-3η isoform was present in synovial fluid with levels of at least 5-fold higher than those found in matched sera, implicating the joint as the likely source of 14-3-3η[9]. Soluble 14-3-3η protein works via signaling cascades like the extracellular signal-regulated kinase and p38 pathway to increase expression of proinflammatory

cytokines, including interleukin 1β (IL-1β), IL-6, tumor necrosis factor- α (TNF- α), and factors that are involved in joint degradation such as MMP-9 and receptor activator of nuclear factor-KB ligand (RANKL).[10]This study aim to assess serum 14-3-3η protein level in early RA patients, and correlate it with clinical activities, serological markers and musculoskeletal ultrasound (MSK US) finding.

PATIENT AND MATERIALS

The study was conducted between November 2019 and February 2020 on thirty naïve Egyptian patients with early RA attending to Rheumatology and Rehabilitation outpatient clinic at Al-Hussein university hospital during the time of the study. The study included patients with symptom duration for more than six weeks and less than one year since onset[11], all fulfilled American College of Rheumatology (ACR) 2010 criteria for the diagnosis of RA[12]. Written consent was signed by all patients and controls after explaining the study procedure for both groups. Patients included in the study were subjected to the following: Full medical history with focusing on morning stiffness and hand swelling. Full clinical examination with assessment of disease activity for rheumatoid arthritis using disease activity score 28 (DAS28). Laboratory investigations including complete blood count (CBC) was done on fully automated cell counter Sysmex KX-21NTM,erythrocyte sedimentation rate (ESR) by Westergren method , serum levels of Rheumatoid factor (RF), C-reactive protein (CRP)were assessed using MISPA- I2, anticitrullinated protein (Anti-CCP) was conducted by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique, estimation of 14.3.3 eta protein by Enzyme-linked immune sorbent assay noncompetitive sandwich method.Radiological investigations including plain X-ray both hands and feet and MSK US scanning was conducted for all subjects. The scaning was done by commercially available equipment using 8-12 MHz linear phased array transducer (APLIO 400 Model, Toshiba ultrasound machine) grayscale, and power Doppler device. Radiocarpal, metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints were examined. Evaluation of synovial thickening, effusion and doppler flow by semiquantitative score. Synovial thickening was defined as a non-compressible hypoechoic intracapsular area [grade 0 = no synovial thickening; grade I = minimal synovial thickening filling the angle between the periarticular bones, without elevation of the line linking tops of the bones; grade II = synovial thickening elevates the line linking tops of the periarticular bones but without extension along the bone diaphysis; grade III = synovial thickening elevating the line linking tops of the periarticular bones and expanding to one of the bone diaphysis][13]. Power Doppler signal was used to assess flow signal in the synovium [grade 0 = no fluid signal in the synovium; grade I = single vessel signal in the synovium; grade II = convergent vessel signals in less than half of the area of the synovium; grade III = vessel signals detected in more than half of the area of the synovium].[14]

RESULTS

Our study was conducted on 30 adult patients 27 females and 3 males with early RA diagnosed according to 2010 RA classification criteria with disease duration ranging from 3-10 months (Mean 5.6 ± 2.24 months). Their age was from 19 - 57 years (Mean 38.2 ± 10.11) with average disease activity assessed by using DAS28 was 2.3 – 5.6 (Mean 4.4 ± 0.74). ESR range was 15-117 (Mean 40.0 ± 17.75), CRP range was 6-48 (Mean 18.6 ± 11.78), RF range was 4 – 68 (Mean 27.9 ± 19.6) and Anti CCP range was 2 - 330 (Mean 73.5 ± 81.86). Serum 14.3.3 eta protein was significantly correlated to RF and Anti-CCP while no significant correlation was found with ESR, CRP, and DAS28 as shown in table 2.Serum 14.3.3η protein levels: As presented in Table 1, serum 14.3.3η protein levels showed a high statistically significant difference between patients and the control group ($p < 0.05$). The Receiver operating curve (ROC) of serum 14.3.3 eta protein levels in the RA group is shown in figure 1.Using ROC, it was shown that eta protein can be used to discriminate between patients and controls at a cutoff level of >105.5, with 100% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 100% negative predictive value (NPV).MSK US findings; As regards Synovitis and Power Doppler signal was show no significant correlation between serum 14.3.3ηprotein, and grayscale of synovitis and grading of power Doppler (P-value > 0.05) as shown in tables 3,4 and 5.

	Patients (N = 30)	Control (N = 30)	MW test	
			MW	p-value
14.3.3 eta	344.8 ± 121.9	38.4 ± 15.3	0.0	< 0.05

Table 1: Relation between patients and control groups in regard of 14.3.3 eta protein.

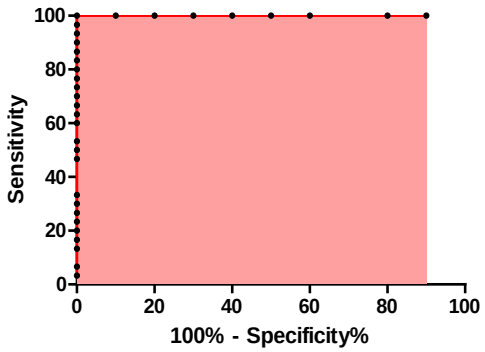


Fig. 1: Serum 14.3.3 eta levels in the RA group. Sens = sensitivity; Spec = specificity.

Variables	(r)	p-value
14.3.3η vs ESR	0.04	p > 0.05
14.3.3ηvs CRP	0.08	p > 0.05
14.3.3η vs RF	0.36	p < 0.05
14.3.3η vs Anti-CCP	0.52	p < 0.05
14.3. η vs DAS28	0.02	p > 0.05

Table 2: Correlation of 14.3.3 eta protein with laboratory parameters.

		Studied patients (N = 30)	
Synovitis	Grade I	5	16.7%
	Grade II	14	46.7%
	Grade III	11	36.6%
Doppler signal	Negative	18	60%
	Grade I	9	30%
	Grade II	3	10%

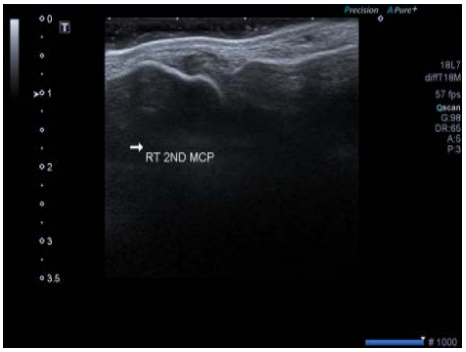
Table 3: Description of musculoskeletal ultrasound findings in all patients.

	Synovitis			ANOVA	
	Grade I (N = 5)	Grade II (N = 14)	Grade III (N = 11)	F	p-value
Serum 14.3.3ηprotein	251.6 ± 54.04	364.7 ± 137.7	361.9 ± 109.9	1.86	p > 0.05

Table 4: Relation of serum 14.3.3 eta protein with ultrasound synovial grading.

	Doppler signal			ANOVA	
	Negative (N = 18)	Grade I (N = 9)	Grade II (N = 3)	F	p-value
Serum 14.3.3ηprotein	351.7± 126.8	347.6 ± 122.6	295.3 ± 121.9	0.26	p > 0.05

Table 5: Relation between serum 14.3.3 eta protein with Power Doppler signal grading.



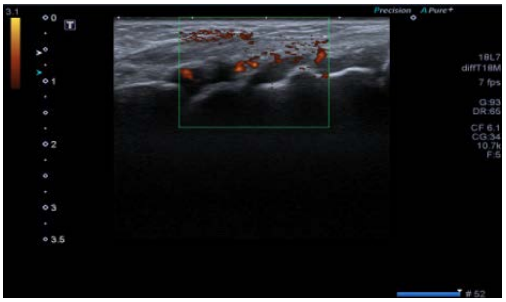


Fig. 2: Ultrasonographic longitudinal scan of the Rt. 2nd MCP showing grade II synovial thickening (Lt. photo) and of wrist joint showing grade 3 synovial thickening and grade 2doppler signal (Rt. photo).

DISCUSSION

Early diagnosis in addition to the accurate assessment of prognostic factors at the time of presentation is crucial for effective management of RA[3]. Low sensitivity of the major serum serological biomarkers RF and Anti-CCP necessitates the research for more sensitive biomarkers[15]. MSK US can detect inflammation of synovial tissue with better diagnostic accuracy[16]and more accurate evaluation of disease activity.[17] In our study, we found a significant difference in serum 14.3.3 eta protein levels (ng/ml) between the patient (344.8 ± 121.9 ng/ml) and control group (38.4 ± 15.3 ng/ml). These findings are in concordance with the results of Walter et al Specificity of 14-3-3η protein for established RA who found a significant difference in serum 14.3.3 eta protein levels between patient and healthy control group (p > 0.05).[18]

In our study, we found a significant positive correlation between serum 14.3.3 eta proteins and both RF and Anti-CCP (p < 0.05) making sense that combination of those biological biomarkers may increase the accuracy of early diagnosis of rheumatoid arthritis. This positive correlation was also confirmed by Walter et al who found the same finding between serum 14.3.3η protein level, RF, and Anti-CCP. Walter et al also reported that when assessing the cohort with early RA, adding RF to ACPA increased diagnostic detection from 59% to 72%. Adding 14.3.3η protein to ACPA also resulted in an identification rate of 72%. However, when combined with RF, 14.3.3η protein increased the diagnostic detection from 57% to 75%. All 3 markers together identified 78% of this patient population with early RA. [18] Also agrees with Carrier et al who reported a moderate positive correlation between serum 14.3.3 eta protein and RF, anti-CCP2 or anti-Sa/citrullinated vimentin antibodies.[19] Sahar et al found higher serum levels of 143.3.3η protein in both seropositive and seronegative RF patients as compared with healthy subjects and the same results as regards anti-ccp.[20]

In our study, we did not find any correlation between CRP, ESR, and DAS28 which agrees with Walter et al and Carrier et al who both reported the same results.[18,19] The absence of correlation suggests that serum 14.3.3 eta protein may play an independent role in the diagnosis of early RA. And its role should

be more investigated taking into consideration the small sample size of patients.

In our study we did not find any correlation with MSK US findings as regards synovial proliferation or power Doppler signal although it is reported that levels of 14-3-3η protein correlate with MMP levels found in serum and synovial fluid of patients with RA, suggesting that it may have a role in the joint degradation process.[10]. This finding may necessitate more investigation on a larger sample size to assess the role of14-3-3η protein in a radiographic and structural progression in RA. Previous studies detected the role of the protein in radiographic progression such as Carrier et al who found that, elevated 14-3-3 eta protein interacted with positive antibodies, elevated CRP and older age to predict subsequent radiographic progression.[19]

CONCLUSION

Serum 14-3-3eta protein may be associated with RF and anti-CCP positivity in early RA which may increase the sensitivity of for early diagnosis of RA. Its association with radiological progression and MSK US findings should be more investigated.

REFERENCES

1- Nogueira E, Gomes A, Preto A, et al.Update on therapeutic approaches for rheumatoid arthritis. *Current medicinal chemistry*.2016;23(21): 2190-203.

2- Tanikawa C, Ueda K, Nakagawa H, et al.Regulation of protein Citrullination through p53/PADI4 network in DNA damage response. *Cancer Res.*2009; 69:8761–9.

3- Vermeer M, Kuper HH, Hoekstra M, et al. Implementation of a treat-to-target strategy in very early rheumatoid arthritis: results of the Dutch Rheumatoid Arthritis Monitoring remission induction cohort study. *Arthritis Rheum.*2011; 63:2865–72.

4- Tavares R, Pope JE, Tremblay JL, et al. Time to disease modifying anti-rheumatic drug treatment in rheumatoid arthritis and its predictors: A national, multi-center, retrospective cohort. *J Rheumatol.* 2012; 39:2088–97.

5- De Rooy DP, van der Linden MP, Knevel R, etal. Predicting arthritis outcomes—What can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford).*2011 50:93–100.

6- Burr ML, Viatte S, Bukhari M, et al.Long-term stability of anti-cyclic citrullinated peptide antibody status in patients with early inflammatory polyarthritis. *Arthritis Res Ther.* 2012; 14:R109.

7- Kilani RT, Maksymowych WP, Aitken A, et al. Detection of high levels of 2 specific isoforms of 14-3-3 proteins in synovial fluid from patients with joint inflammation. *J Rheumatol.* 2007; 34:1650–7.

8- Maksymowych WP and Marotta A.14-3-3eta: a novel biomarker platform for rheumatoid arthritis. *Clin Exp Rheumatol.* 2014; 32 Suppl 85:S35–9. Review.

9- Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol.* 2009; 9:581–93.

10- Maksymowych WP, van der Heijde DM, Allaart CF, et al.14-3-3eta is a novel mediator associated with the pathogenesis of rheumatoid arthritis and joint damage. *Arthritis Res Ther*. 2014; 16:R99.

11- Karine R, Marcelo M, Giovanna S, et al. A new musculoskeletal ultrasound scoring system (US10) of the hands and wrist joints for evaluation of early rheumatoid arthritis patients. *Rev bras reumatol.* 2016;5 6(5):421–31

12 - Aletaha D, Neogi T, Silman AJ, et al. Rheumatoid arthritis classification criteria.American College of Rheumatology/ European League against Rheumatism, collaborative initiative. *Arthritis Rheum*. 2010; 62:2569e81.

13 -Szkudlarek M, Court-Payen M, Jacobsen S, et al. Interobserver agreement in ultrasonography of the finger and toe joints in rheumatoid arthritis. *Arthritis Rheum* 48:955–962.

14 - Naredo E, Bonilla G, Gamero F, et al. Assessment of inflammatory activity in rheumatoid arthritis: a comparative study of clinical evaluation with grey scale and power Doppler ultrasonography. *Ann Rheum Dis.* 2005; 64:375-781.

15 -Smolen JS, Aletaha D and Mcinnes IB. Rheumatoid arthritis. *Lancet*.2016; 388(10055): 2023–38.

16 - Nakagomi D, Ikeda K, Okubo A, et al. Ultrasound can improve the accuracy of the 2010 American College of Rheumatology /European League Against Rheumatism classification criteria for rheumatoid arthritis to predict the requirement for methotrexate treatment. *Arthritis Rheum.*2013; 65:890–8.

17 - Ikeda K, Nakagomi D, Sanayama Y, et al. Correlation of radiographic progression with the cumulative activity of synovitis estimated by power Doppler ultrasound in rheumatoid arthritis: difference between patients treated with methotrexate and those treated with biological agents. *J Rheumatol*.2013; 40: 1967–76.

18 - Walter P, Stanley J, Vivian A, et al. Serum 14-3-3η is a Novel Marker that Complements Current Serological Measurements to Enhance Detection of Patients with Rheumatoid Arthritis. *J Rheumatol*. 2014; 41:(11)2091-3.

19 – Carrier N, Marotta A, Artur J, et al. Serum levels of 14-3-3η protein supplement C-reactive protein and rheumatoid arthritis-associated antibodies to predict clinical and radiographic outcomes in a prospective cohort of patients with recent-onset inflammatory polyarthritis. *Arthritis Research & Therapy*. 2016; 18:37.

20 – Alwan SA, Iqbal MN, Al-Hafidh AH. plasma levels of 14-3-3η protein and its correlation with avtivity of rheumatoid arthritis patients. *World Journal of Pharmaceutical Research.*2019;8:(2)1485-15.