Original Research Article

Level Of 14-3-3 (Eta) Protein in Rheumatoid Arthritis Patients

Abstract

Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder that affects synovial ~~membrane 14~~membrane. 14-3-3η (eta) is a novel protein marker showing potential in predicting radiographic deterioration in early and establishedRA.The goal of this work was to estimateserum level of 14-3-3η (eta) protein in early and established RAcases and its association with disease activity.

Methods: This study was carried out on 50RAcases and 25 apparently healthy volunteers. The RA cases were classified into: group A: 25 cases with early RA (diagnosed within the first three years from the onset of the disease), group B: twenty five cases with established RA (diagnosed for more than three years of the disease) and group (C) control group:25 apparently healthy volunteers. All cases were subjected tomeasurements of.erythrocyte sedimentation rates (ESR) -1st hr -2nd hr, anti-cyclic citrullinated protein antibody (Anti-CCP), and~~of 14~~of 14-3-3 eta protein and itsassociationwith disease activity.Radiologicalassessment by Sharp/Van Der Hedje score~~d~~(SHS),pain was estimated by Visual Analogue Scale (VAS) and disease activity by DAS 28.

Results: There is positive association between serum 14-3-3 eta ~~p~~proteint~~n~~ level and all the clinical data (DAS 28, MHAQ, number of tender joints, number of swollen joints and VAS) in both groups (A and B). There is positive association between 14-3-3η ptn and the radiological score in group (Aand B).

Conclusions: 14-3-3 eta proteinis higher in RA cases' serum than that of healthy controls. Adding this test to either RF or both RF and ACPA improved their diagnostic capability for

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RA. Predicting radiographic deterioration in early and established RA shows promise. Up - regulation of 14-3-3 proteins is linked to adverse consequences. in RAcases.

Keywords:14-3-3 (Eta), Rheumatoid Arthritis, Disease Activity

Introduction:

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory condition that damages the synovial membrane. It is a complex illness with a significant degree of variation amongst cases as the disease progresses. If left untreated, significant joint damage and deformity lead to a decline in physical activity and incapacity in the workplace [1, 2].

It is now generally accepted that early diagnosis of RA, appraisal of disease severity, and execution of an appropriate treatment approach can significantly improve prognosis[3].

Markers such as rheumatoid factor (RF), anti-citrullinated protein anti-bodies, and C-reactive protein (CRP) are responsible only for 32% of the total variance in trying to predict joint destruction, leaving 68% of the variance unaccounted for. 14-3-3 (eta) is a new marker with the ability to forecast radiographic worsening in early and established RA. 14-3-3 proteins consist of seven isoforms that bind to and control the biological activity of several intracellular proteins [4, 5][6].

The higher expression of the 14-3-3 isoform in arthritic cases relative to healthy persons is assumed to be a result of 14-3-3's direct capacity to produce variables associated with inflammation and radiographic damage. 14-3-3 has been demonstrated to produce inflammatory mediators such as interleukin (IL)-1 and -6 and has been connected to the development of joint injury as it also promotes receptor activator of nuclear factor-kB ligand (RANKL) and matrix metalloproteinase (MMP)[7].

Our goalin this work wasto estimateserum level of 14-3-3 (eta) protein in early and established RAcases and itsassociation with disease activity.

Patients and Methods:

This study was carried on fifty RAcasesdiagnosed according to ACR/EULAR 2010 classification criteria for RA, aged above 18 years old, and selected from The Department

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Rheumatology, Rehabilitation and Physical Medicine at Tanta University Hospitals, and twenty-five apparently healthy volunteers.

The study was done after approval from the Ethical CommitteeTanta University Hospitals. An informed written consent was obtained from the patient or relatives of the cases.

Exclusion criteria were viral hepatitis, other autoimmune diseases, and inflammatory arthropathies.

The RA cases were classified according to the duration of the disease into: group A: 25 cases with early RA (recently diagnosed within the first two years of the disease), group B: twenty five cases with established RA(diagnosed for more than three years from the onset of the disease).Group (C) control group included 25 apparently healthy volunteers matched in age and sex with cases' groups.

All cases were subjected tofull medical history taking, general examination, estimation of pain by Visual Analogue Scale (VAS), Modified Health assessment Questionnaire (MHAQ) to measure disease related disability, discomfort and quality of life, estimation of the disease activity, radiological and laboratory(Complete blood picture(CBC), erythrocyte sedimentation rates (ESR) -1st hr -2nd hr, CRP,RF and anti-cyclic citrullinated protein antibody (Anti-CCP) estimation and 14-3-3 eta protein measurement.

Assessment of pain by VAS

The patient was instructed to mark their present degree of discomfort on the line. The distance in millimetres between the 'no pain marker' (zero) and the present pain mark was then measured using a ruler. This delivers a score out of 10 for pain severity.

MHAQ

Quantify disease-related impairment and life quality. The patient was instructed to mark the relevant answers on the whole questionnaire. Normal is a score of less than 0.3, however the

average increases with age. The score is split into three categories: mild (MHAQ) 1.3, moderate (MHAQ) 1.3 to 1.8, and severe (MHAQ) > 1.8.

Assessment of the disease activity by Disease Activity Score (DAS) 28

Number of swollen joints (out of 28), number of sensitive joints (out of 28), draw blood for CRP measurement, and have the patient complete a 'Global assessment of Health' (indicated by marking a 10 cm line between very good and very bad). These values are then input into a sophisticated mathematical process to get the disease activity score as follow: 0.56sqrt (TJC28) + 0.28sqrt (SJC28) + 0.36ln(CRP+1) + 0.014GH + 0.96

(SJC) Swollen Joint Count (0-28)
(TJC) Tender Joint Count (0-28)
(CRP) C- reactive protien (GH) Global Health
VAS disease activity (0-100mm)
The score divided into: Active disease greater than 5.1 Low disease activity less than 3.2 Remission less than 2.6

Radiological assessment:

Radiographic estimation by Sharp/Van Der Heijde Score (SHS)

The scoring is done by SENSE (**THE Simple Erosion Narrowing Score**) method was derived from the Sharp/Van Der Heijde method as an easier, quicker and quite reliable to score joint lesions, especially in the first few years of disease[8, 9].

The examined joints are identical to those tested for the SvH score. For erosions, there are sixteen beneficial places in each hand and six in each foot. There are 15 viable places for joint space reduction in each hand and six in each foot. If at least one joint degradation is seen, SENS awards a score of 1. Similarly, SENS assigns a score of one if there is any joint constriction, regardless of the severity of the lesion observed. The maximum degradation score in both hands is 32, while the maximum score for narrowing OR subluxation is 30. The maximum degradation score in the foot is 12, and the maximum score for narrowing OR subluxation is 12.

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Serum 14-3-3 eta protein:

This (ELISA) technique was used based on the Biotin double antibody sandwich technology to assay the Human 14-3-3 protein eta (YWHAH). Add 14-3-3 protein eta (YWHAH)to the wells, which are pre-coated with 14-3-3 protein eta(YWHAH)monoclonal antibody followed by incubation. After that, biotin-~~labeled~~labelled anti-YWHAH antibodies are combined with streptavidin-HRP to produce an immunological complex. After incubation and washing, remove any free enzymes. Add substrate A and substrate B. As a result, the acid will cause the solution to become blue and then yellow. The hues of the solution and the amount of human 14-3-3 protein eta (YWHAH) are positively correlated.

Statistical analysis

SPSS v27 (IBM, Chicago, IL, USA) was used for statistical analysis. Using the Shapiro-Wilks test and histograms, the normality of the data distribution was determined. The quantitative parametric data were given as mean and standard deviation (SD) and analysed using the ANOVA (F) test with post hoc comparisons (Tukey). Quantitative non-parametric data were given as the median and interquartile range (IQR), and each group was compared using the Kruskal-Wallis test and the Mann Whitney test. The Chi-square test was utilised to analyse qualitative data reported as frequency and percentage (%). A two-tailed P value less than or equal to 0.05 was deemed statistically significant. Pearson coefficient (r) for correlating two quantitative variables with normal distributions.

Results:

Table 1: Comparison between the patient groups according to demographic data and duration of disease

		Group A(n = 25)	Group B(n =25)	p. value
Sex	Male	0(0%)	1(4.0%)	1.000
	Female	25(100%)	24(96%)	
Occupation	Housewife	20(80%)	22(88%)	0.423
	Farmer	5(20%)	2(8.0%)	
	Chef	0(0%)	1(4.0%)	

Age(years)	41.60±6.34	54.28±5.78	<0.011*
Duration of disease	1.28 ±0.85	11.60±3.64	<0.001*

Data are presented as mean ± SD orfrequency (%), *: significant P value.

Table 1 shows that both groups are matched regarding sex and occupation. There is significant difference between the two groups regarding age, duration of thedisease.

Table 2: Comparison between the patient groups according toclinical parameters and laboratory investigations

Clinical parameters			
VAS	6.56 ± 1.08	5.84 ±0.85	0.012*
DAS28	4.57 ±0.60	4.96 ±0.61	0.028*
MHAQ	0.35(0.25 – 0.50)	0.50(0.38 – 0.75)	0.010*
Durationof morning stiffness(min)	30.0(15.0 – 45.0)	30.0(15.0 – 60.0)	0.248
NumbersofTender Joints	8.48 ± 2.60	11.60±3.52	0.001*
NumbersofSwollen Joints	3.76 ±1.33	4.76 ±1.69	0.007*
Laboratory investigations			
ESR	48.68 ± 14.88	70.92 ± 25.07	<0.011*
CRP (mg/L)	1.28 ±0.85	11.60±3.64	0.047*
Anti CCP (U/ml)	30.0(8.0 – 44.10)	44.0(25.70 – 66.0)	0.793
DAS28	4.57 ±0.60	4.96 ±0.61	0.028*
RF (IU/ml)	64.0(32.0 – 88.0)	71.90 (50.80 –103.1)	0.252

Data are presented as mean ± SD, frequency (%) or Median (IQR),Group A: Early rheumatoid arthritis; Group B: Established rheumatoid arthritis, *: significant P value, VAS: Visual Analogue Scale, MHAQ: Modified Health assessment Questionnaire, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rates, RF: Rheumatoid factor

Table 2 shows that there is significant difference between the two groups regardingDAS28, VAS, MHAQ & numbers of tender joints, numbers of swollen joints, ESR, and CRP. There is no significance difference between both groups regarding morning stiffness, Anti CCP & RF.

Table 3: Comparison between the three studied groups according to serum level of 14-3-3η ptn

	Group A(n = 25)	**Group B(n = 25)**	**Group C(n =25)**	**P value**
Serum 14-3-3ηptn(ng/ml)	0.77 ±0.23	0.86 ±0.26	0.62 ±0.22	0.002*
Sig. bet. Grps	p_1=0.174,p_2=0.022*,p_3=0.001*			

Data are presented as mean ± SD, *: significant P value,p1: p value for comparing between early rheumatoid arthritis and established rheumatoid arthritis, p2: p value for comparing between early rheumatoid arthritis and control, p3: p value for comparing between established rheumatoid arthritis and control, Group A: Early rheumatoid arthritis Group B: Established rheumatoid arthritis Group C: Control group.

Table 3 shows thatthere is high significance difference between the three studied groups. Regarding 14-3-3 eta ptn, there is significant difference between the group A&C and there is

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significance difference between groups B &C while there is no significance difference between group A &Bptn.

Table 4: Comparison between the patient groups regarding the radiologic score by SENSE method

	Group A(n = 25)	Group B(n =25)	p. value
Numberoferosions	4.92 ±1.91	7.16 ±2.61	<0.001*
Number of Joints withjoint narrowing	9.12 ±2.32	11.36±2.96	0.004*

Data are presented as mean ± SD, *: significant P value, SENSE: simple erosion narrowing score

Table 4 shows thatthereishighsignificantdifferencebetweenthetwogroups regardingradiologicalscoreincludingnumbersoferosionsandnumbersofjointnarrowing.

Table 5:Association between Serum 14-3-3η ptn and clinical data and between Serum 14-3-3η ptn and radiological score by SENSE method in patient groups (A&B)

	Serum14-3-3ηptn(ng/ml)			
	GroupA		GroupB	
	r	p	r	P
DAS 28	0.942	<0.001*	0.945	<0.001*
MHAQ	0.733	<0.001*	0.458	0.021*
Number ofTenderjoints	0.665	<0.001*	0.790	<0.001*
Number ofSwollenjoints	0.657	<0.001*	0.743	<0.001*
VAS	0.451	0.024*	0.481	0.015*
Radiological score by SENSE method				
forerosion	0.881	<0.001*	0.440	0.028*
Joint narrowing	0.747	<0.001*	0.403	0.046*

Table 5 shows that there is positive association between serum 14-3-3 eta ptn level and all the clinical data (DAS 28, MHAQ, number of tender joints, number of swollen joints and VAS) in both groups (A and B). There is positive associationbetween 14-3-3η ptn and the radiological score in group (A&B).

Discussion

Rheumatoid arthritis (RA) is a chronic systemic disabling auto immune inflammatory arthritis associated with progressive joint destructions account for the disabilities and higher mortality[10].

Regarding to duration of the disease in the two studied groups, both groups differed significantly with a P value <0.001. Regarding the clinical parameters, a significant difference in VAS between patient groups (A&B) with a P value of 0.012. Also,regarding MHAQ, patient groups differed significantly.

Going with this, Lyudmila Sizova et al,[11] by use of QOL concluded that cases with early RA gave the worst scores for arthritis and joint pain, and cases with established RA for gave the worst score of "health" including MHAQ, but at any duration of the disease, RA cases had satisfaction regarding friends and family support.

In this study, both groups differed significantlyregarding DAS28. Going with our study, Mohamed HA et al., [12] has demonstrated a significant difference in DAS28 and ESR between early and established RA.

Regarding duration of morning stiffness, both groups were matched with a P value of 0.248.

~~in~~In this study, both groups differed significantly (T~~t~~able 4) regarding the number of tender joint and swollen joints which was more in group B (the established one). According to ESR, ~~,~~both groups differed significantly with a p value <0.001, Also, the two studied groups differed significantlyregarding CRP with a p value of 0.047 which may be due to uncontrolled disease or may be due to the small study sample.

T~~t~~he Anti~~NTI~~ CCP and RF were matched in both group, and these were in agreement with Mohamed et al [12] , who showed that t~~T~~here were insignificant difference in RF and ACPA levels between the early and established RA groups.

Also in our study, group A (early) and group B (established) were matched regarding 14-3-3 eta protein level with a p value of 0.174. But There was a significant difference between group A (early) and group C (control) regarding level of 14-3-3 eta ptn with a p value of 0.022 (table 3) and between group B (established) and C (control) regarding level of 14-3-3

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eta ptn with a p value of 0.001 , also,regarding 14-3-3 eta pt., the three groups differed significantly with a P of 0.002.

Our study was in agreement with, Mohamed et al., [12]who stated that there were insignificant difference in14-3-3η levels in-between early and established RA.

While in 2017, Xun Gong et al [13]demonstrated that 14-3-3η level in early RA group was significantly elevated than that in those with established RA.

But in both of them there were a significant difference between the RA cases' weather early or established and different control groups support that 14-3-3 eta ptn may be helpful in diagnosis of RA.

Although, Maksymowych et al.,[4] statedt~~T~~he addition of RF to ACPA elevated the identification rate from 59% to 72%; the addition of 14-3-3 to ACPA likewise elevated the identification rate to 72%. However, when 14-3-3 was paired with RF, the identification rate elevated to 75% from 57% with RF alone. Together, the three indicators indicated 78% of early RA cases.

In contrast, Vasconcellos et al. [15] discovered that 14-3-3 provided a small improvement in diagnostic value in comparison to RF and ACPA. Furthermore, 14-3-3 added little value to the distinction of RA from other RF-positive inflammatory arthropathies.

Regarding the radiologic score by SENSE method by its two parameters (numbers of erosions and numbers of joint narrowing), groups A and B differed significantly with higher level in established group than the early RA, ~~With~~with a p value of 0.001 and 0.004 for number of erosions and numbers of joint narrowing respectively

Our study was in agreement with, Lehtinen JT et al.[16] who revealed that ~~P~~progression of the destructive process and fibrosis leading to joint narrowing, this symptom indicates an established stage of RA, so it is difficult to find joint narrowing early in the disease process and may need high resonance imaging or ultrasound to be detected.

In this study, regarding the associationbetween 14-3-3 eta ptn and DAS28~~.~~ there was a positive association in both group A and B.

Our result were i~~I~~n agreement with Hirata et al.[17]about normalization of 14-3-3 levels which was found to be associated with improved clinical outcomes. Furthermore, cases who were 14-3-3-positive at the beginning of treatment and turned negative at the one-year follow-up had substantially decreased DAS28- ESR scores than cases who remained positive or became positive.

Despite this, Maksymowych et al. [4] revealed that soluble 14-3-3 functions via signalling cascades that result in the overexpression of pro-inflammatory cytokines, such as IL-1, IL6, and TNF, and joint degradation factors, such as MMP and RANKL.

Also,in this study there was a positive association between MHAQ and 14-3-3 eta ptn with a P value of in group A<0.001 and 0.021 in group B.

Going with our study, Maksymowych et al.[4]demonstrated that RA patient's positive for 14-3-3η had more severe disease and their median baseline DAS 28 and HAQ was significantly higher than RA cases with negative 14-3-3η. Although, Carrier et al. [18]found insignificantrelation ~~between 14~~between 14-3-3η and HAQ score.

In this study, there was a positive association between number of tender joints as well as the number of swollen joints ~~with level~~with level of14-3-3 eta ptn with a p value<0.001 in group A and in group B. Going with our study, i~~I~~n 2016, van Beers-Tas et al. [19]demonstrated that 14-3-3η is often present in ACPA- and/ or RF-positive cases with arthralgia before development of arthritis.

A positive association between VAS and 14-3-3 eta ~~ptn level~~ptn level with a p value 0.024 in group A and 0.015 in group B.A positive association~~between 14~~between 14-3-3 and radiological score was also found by SENSE method by its two parameters (joint erosions and joint narrowing) in both groups with a p value <0.001 in group A and 0.028 in group B.

Going with our results, Maksymowych et al. [4]concluded that the cases who already had joint damage and those who developed damage later, both had high 14-3-3η levels.

Conclusions:

14-3-3 eta protein RA cases' serum contains a greater concentration of than that of healthy individuals. Adding this test to either RF or both RF and ACPA improved their diagnostic capability for RA. Predicting radiographic deterioration in early and established RA shows promise. Overexpression of 14-3-3 proteins is related with worst outcomes in individuals with RA.

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