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Validity of 14-3-3η Protein in Diagnosis of Rheumatoid Arthritis among Iraqi Patients

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Abstract: ***Objective: to assess serum 14-3-3η protein in rheumatoid arthritis (RA) patients and its association with other basic immunologic markers, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA). Methods: The study included 50 RA patients and 35 healthy controls. Demographic and clinical characteristics of patients and controls were recorded. Serum 14-3-3η proteinwas measured in patients and controls. highly sensitive C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), RF, and ACPA were evaluated. Results: Age and BMI were significantly higher in RA patients compared to control, the percentage of female was significantly higher in RA compared to control (female to male ratio was 5.25:1 in RA, and 1.7:1 in control). Serum levels of 14,3,3η protein, ACPA and RF were significantly higher in RA patients compared to control, hsCRP was higher in RA patients compared to controls however it was not statistically significant. RF had an excellent ability to discriminate between RA and control (AUC ≥0.9), serum 14-3-3ηprotein had a good ability to discriminate (AUC between 0.800 – 0.899), the ACPA and hsCRP had poor ability to discriminate between RA and controls. Conclusion: The study demonstrates that the 14-3-3η protein in combination with RF and ACPA identified in most patients with RA and had a good ability to discriminate between patients and control.***

Keywords: rheumatoid arthritis, 14-3-3η protein

1. Introduction

Rheumatoid arthritis (RA) is a progressive inflammatory disorder characterized by proliferation of the synovial membrane and persistent uncontrolled inflammation resulting in chronic destructive polyarthritis. Typically, RA manifests as a symmetric arthritis involving numerous small and large joints. Articular symptoms may be accompanied by systemic inflammatory symptoms such as fatigue, articular stiffness, anorexia, and fever (1). Underlying the initial complaint of pain and limited lifestyle, inflammatory events within the synovium become chronic and potentially destructive. These immune-mediated inflammatory changes give rise to many of the clinical findings and destructive articular changes that characterize this disorder. Without treatment, rheumatoid inflammation may have catastrophic consequences in the months and years to come (2).

Many autoantibodies have been described in RA, but anti-cyclic citrullinated peptide antibodies (anti-CCP) have been proven to be more specific and sensitive in the diagnosis of RA, and therefore appear to be better predictors of the progressive disease (3).

ACPAs, being detected in 60–80% of RA sera, are more specific for RA, as they are rare in other diseases and only present in approximately 2% of the healthy population (4). ACPAs as well as RFs, have been detected in the serum of RA patients years before the onset of RA (5), suggesting that the development of RA occurs long before the appearance of symptoms.

Anticitrullinated peptide antibodies (ACPA) are the most specific autoantibodies known as markers of rheumatoid arthritis (RA). In cohorts of early RA, ACPA are associated with increased clinical disease activity and progression of structural damage. ACPA have high weight in the final scoring system of the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) RA classification criteria for RA (6).

Serum 14-3-3η (eta) is a novel protein biomarker showing potential in predicting radiographic deterioration in early and advanced RA. 14-3-3 proteins belong to a family of seven isoforms known to bind to and regulate the biologic activity of various intracellular proteins (7). Overexpression of 14-3-3 proteins is associated with worse outcomes in various diseases, such as cancers, neurodegenerative diseases and Creutzfeldt-Jakob's disease (8, 17). The 14-3-3η isoform is expressed at higher levels in patients with arthritis compared with healthy individuals, which is thought to be related to 14-3-3η's direct ability to induce factors linked to inflammation and radiographic damage. 14-3-3η has been shown to induce inflammatory factors such as interleukin (IL)-1 and -6, and is linked to the process of joint damage as it also induces factors such as receptor activator of nuclear factor-kB ligand (RANKL) and matrix metalloproteinase (MMP) 1(18).

2. Materials and Methods

The study included 50 Irheumatoid arthritis patients and 35 healthy controls. Thepatients fulfilled the American college of Rheumatology criteria (ACR) for RA (19).These RA patients were form the Rheumatology consultation clinic / Baghdad Teaching Hospital from March2017to July 2017.

The evaluation of subjects included physical examination, with particular focus on the pattern of joint involvement, the presence of nodules and other extra –articular features and laboratory features such as rheumatoid factor (RF). Disease activity has been determined on the basis of defined

parameters [the number of swollen and tender joints, ESR, Health Assessment Questionnaire (HAQ), C – reactive protein (CRP)] and a global physician's assessment.

Eight to ten ml of venous blood were drawn from each individual of the two groups under complete aseptic condition.

The blood samples were collected in anticoagulant – free tubes used for separation of serum to detect RF, CRP, and ACPA. Two ml was collected in ESR tube. Immunological assays done in the medical laboratories of Nursing Home Hospital. Quantitative measurement of rheumatoid factor (RF), screen rheumatoid factor, anti – cyclic citrullinated peptide (ACPA), highly sensitive C – reactive protein (hs – CRP), and 14-3-3ŋ protein were measured.

3. Results

Age, and BMI were significantly higher in RA patients compared to controls, the percentage of females was significantly higher in RA compared to control (female to male ratio was 5.25:1 in RA, and 1.7:1 in control) as shown in table 1.

Table 1: demographic, clinical, and Lab features of patients and controls

	Control	RA	P value
Number	35	50	-
Age (years)	27.54 ± 4.54	47.62 ± 10.43	<0.001
BMI kg/m^2	26.47 ± 3.61	29.86 ± 5.44	0.001
Gender, no.(%)			0.026
Female	22 (62.86%)	42 (84.00%)	
Male	13 (37.14%)	8 (16.00%)	
Smoking, no.(%)			0.354
Non-smoker	30 (85.70%)	46 (92.00%)	
Smoker	5 (14.30%)	4 (8.00%)	

BMI, body mass index

Serum levels 14,3,3ŋ protein, ACPA and RF were significantly higher in RA patients compared to control, CRP had higher serum levels in RA patients however it was not statistically significant, as shown in table 2.

Table 2: shows comparison serum levels of different immunological markers between patients and controls

Variables	Control	RA	P value
Number	35	50	-
14-3-3 η protein	254.74 ± 54.80	357.58 ± 132.71	<0.001
hsCRP	7.09 ± 2.15	7.81 ± 1.59	0.097
ACPA	1.79 (1.47 – 2.50)	2.32 (1.64 – 3.98)	0.024
RF	1.9 (4.9 – 12.1)	104.2 (25.9 – 485)	<0.001

hsCRP, highly sensitive C reactive protein, RF, rheumatoid factor; ACPA, anticitrolinated peptide antibody

RF had an excellent ability to discriminate between RA and control (AUC ≥0.9), 14-3-3 η protein had a good ability to discriminate (AUC 0.811), the rest of the variables had poor ability to discriminate between RA and control as illustrate in table 3

Table 3: ROC curve for different immunological markers between to Dxx patients and controls

Variable	*AUC*	*95%CI AUC*	*P value*
14-3-3 η protein	0.811	0.711 to 0.888	<0.001
ACPA	0.644	0.533 to 0.745	0.018
hsCRP	0.533	0.421 to 0.642	0.664
RF	0.955	0.887 to 0.988	<0.001

hsCRP, highly sensitive C reactive protein, RF, rheumatoid factor; ACPA, anticitrolinated peptide antibody

The serum level of14-3-3 η protein has good accuracy and positive predictive value (PPV) to differentiate between patients and controls. Other immunological markers are shown in table4.

Table 4: validity parameters for different immunological markers

Variable	Cut point	Sensitivity	Specificity	Accuracy	PPV	NPV
14-3-3 η protein	>279.73	78%	80%	78.8%	84.8%	71.8%
ACPA	>2.05	66%	65.7%	66.4%	73.3%	57.5%
hsCRP	≤8.53	92%	42.9%	67.5%	56.4%	39.1%
RF	>18.398	86%	100%	93.0%	100%	83.3%

hsCRP, highly sensitive C reactive protein, RF, rheumatoid factor; ACPA, anticitrolinated peptide antibody

In RA patients there was direct significant correlation between 14-3-3 η protein with number of swollen joints, DAS-28, ESR, and RF as illustrated in table 5

Table 5: Correlation between demographic features, clinical features, and Lab with Eta protein 14, 3,3ŋ in RA patients

Variables	14-3-3 η protein	
	β	P value
Age	0.148	0.304
BMI	0.055	0.704
Disease Duration	-0.097	0.501
Number of tender joints	0.041	0.779
Number of swollen joints	**0.363**	**0.010**
Patient Global assessment	0.149	0.301
Evaluator global assessment	0.220	0.126
CDAI	0.204	0.155
SDAI	0.223	0.120
DAS-28ESR	**0.353**	**0.012**
ESR	**0.556**	**<0.001**
ACPA	0.066	0.649
RF	**0.338**	**0.016**
CRP	0.167	0.248

4. Discussion

In recent years several potential biomarkers were described such as for examples anti-carbamylated protein antibodies (anti-carp) (9), anti-MCV (10) and peptidyl arginine deiminase type 4(anti-pad-4)(11) such biomarkers may help to improve prediction, but also offer new insights into the course of events leading to clinical arthritis.

Serum 14-3-3ŋ (eta) protein is the novel protein biomarker which shows potential in predicting radiographic deterioration in early and advanced RA (12).

In this study, we aimed to analyzed the level of serum 14-3-3ŋ in RA patients and association with other basic

immunologic markers, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA).

The study showed that age, BMI were significantly higher in RA patients compared to controls and the percentage of female was significantly higher in RA compared to control. Also the serum levels of 14-3-3ŋ proteins, ACPA and RF was significantly higher in RA patients compared to control, CRP had higher serum levels in RA but it was not statistically significant.

The study also show that RF had an excellent ability to discriminate between RA and control (since its AUC ≥0.9), eta protein had a good ability to discriminate (since its AUC between 0.800-0.899).
In RA patients there was direct significant correlation between 14-3-3ŋ protein with number of swollen joints, DAS-28, ESR and RF.

The 14-3-3ŋ is an inflammatory mediator, for clinical practice it would be very useful if 14-3-3ŋ positivity could enhance the perdition of rheumatoid arthritis when combined with ACPA and RF (13).

In a study which has shown that 14-3-3ŋ is often present in arthralgia subjects positive for ACPA and RF prior to the development of arthritis and was associated with the development of arthritis (14).

In another study the levels of 14-3-3ŋ protein ≥0.50ng/ml predict poorer clinical and radiographic outcomes (15).

Another study shows that 14-3-3ŋ is a modifiable marker in identifying patients with RA in a high disease state. Patients who achieve a negative 14-3-3ŋ status following 1 year of treatment do better clinically with pretreatment 14-3-3ŋ in forming response (16).

Limitation of the study was small number of patients and short duration. However This was the first pilot study in Iraq that assessed 14-3-3 η proteinin RA and controls and this drawback can be solved by a larger and longer duration of a case control study.

In conclusion the study demonstrates that the 14-3-3ŋ protein in combination with RF and ACPA identified in most patients with RA and had a good ability to discriminate between patients and control.

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