OPEN AIMJ ORIGINAL ARTICLE

Rheumatology and Rehabilitation

Evaluation of Serum Protein 14-3-3 Eta as a Novel Biomarker for Juvenile Idiopathic Arthritis

Mahmoud Ibrahim Mohamed Risha [1]*MSc; Khaled Abdelmonaem Zaky [1]MD; Hany Mohamed Aly [1]MD; Abdullah Mustafa Gaafar [2]MD.

Corresponding Author:
Mahmoud Ibrahim Mohamed Risha
dr.m7risha@yahoo.com

Received *for publication December 09, 2021;* ***Accepted*** *May 27, 2022;* ***Published online*** *May 27,2022.*

doi: *10.21608/aimj.2022.109653.1705*

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

[2]Clinical pathology Department, Faculty of Medicine, Al-Azhar University, Cairo , Egypt.

ABSTRACT

Background: Juvenile idiopathic arthritis is considered a significant cause of disability. International League of Associations for Rheumatology (ILAR) classification for JIA has limited diagnostic biomarker consideration.14-3-3 Eta is a novel biomarker could lessen diagnostic delay and ultimately improve outcomes.
Aim of The Work: Assessment of serum 14-3-3 Eta as a potential new biomarker for juvenile idiopathic arthritis diagnosis.
Patients and Methods: : Fifty JIA patients fulfilling the ILAR classification criteria were included in a case-control study, and classified into three groups: polyarticular JIA (PJIA n = 20), oligoarticular JIA (OJIA n = 20), and systemic-onset JIA (SJIA n = 10), and twenty healthy children as a control group. The juvenile arthritis disease activity score (JADAS 27), CHAQ (Childhood Health Assessment Questionnaire), 14-3-3η protein, RF, and Anti-CCP levels were measured for all cases.
Results: JIA patients mean age (11.80±2.75) and for healthy children (5.55±3.38). Elevated 14-3-3 η levels were higher in Polyarticular JIA (median 27.7 ng/ml) followed by OJIA and SOJIA (median 22.03 ng/ml), in which the 14-3-3η level in healthy children (median 0.9 ng/ml) was the lowest. In JIA patients, there was a significant positive relation between serum 14-3-3η protein with RF and Anti-CCP (p values = 0.034 and 0.040, respectively), Also a positive correlation between serum 14-3-3 eta (ng/ml) level and CHAQ with no correlation with JADAS 27.
Conclusion: JIA patients had significantly more serum 14-3-3 η proteins than healthy individuals, and positively linked with RF and Anti-CCP.

Keywords: *Juvenile idiopathic arthritis; Anti-CCP antibody; RF; 14-3-3 η (eta).*

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

JIA represents a heterogeneous range of arthritis that started prior to the age of sixteen and lasted at least six weeks with no known etiology. [1]

JIA is considered a significant cause of acquired impairment and disabilities in children and adolescents. The majority of JIA patients proceed to have joint involvement in adult life. [2]

The prevalence of JIA worldwide is 1-3 per 1,000 children. [3]

JIA is often hard to diagnose since it represents a diverse group of conditions that appear as inflammatory arthritis with no actual testing to verify and validate the diagnosis. Moreover, because several disorders might mimic JIA, clinical evaluation is used to make a diagnosis instead of laboratory testing. [4]

The ILAR classification system for JIA has limited diagnostic usefulness and does not include biomarkers other than rheumatoid factor (RF) and antinuclear antibody (ANA) in the diagnosis. [5]

All eukaryotic cells include 14-3-3η (eta) proteins, which are intracellular chaperonins. There are seven human 14-3-3 isoforms, each with its own name: β, γ, ε, ζ, η, σ, and τ (beta, gamma, epsilon, zeta, eta, sigma, and tau). They are involved in a variety of intracellular biological processes, including cell proliferation, differentiation, and apoptosis. [6]

During the early stages of JIA, the 14-3-3 eta protein is liberated into the extracellular space as a cellular adapter protein. It works as an innate immune system inducer. As a result, additionally to current biomarkers, 14-3-3 eta protein has the potential to improve laboratory efficiency in the early diagnosis as well as prognosis of JIA. Evaluation of 14-3-3 Eta protein role was estimated in many rheumatic diseases, but its role in JIA diagnosis is still uprising and under consideration. [7]

This study aimed to evaluate serum 14-3-3 Eta as a potential new biomarker for JIA diagnosis as well as its relation with other biomarkers like RF and Anti-CCP.

PATIENTS AND METHODS

The case-control study included fifty patients, subdivided into 20 PJIA patients, 20 OJIA patients, and 10 SJIA patients, and all of them fulfilled ILAR classification criteria. [8] They were selected from AL Hussein and Bab El Sharia University Hospitals 'Rheumatology Departments' outpatient clinics and inpatients, with twenty healthy children serving as a control group, between March 2020 and June 2021. Other reasons of childhood arthritis were excluded, such as Infection, malignancy, rheumatic fever, and patients with endocrinal diseases like DM. obtained informed consent from all participants.

All participants were subjected to

Disease activity assessment :
Juvenile arthritis disease activity score (JADAS 27) [9], CHAQ (Childhood Health Assessment Questionnaire) [10] for all participants.
laboratory assessment:

Included C - reactive protein (CRP), Erythrocyte sedimentation rate (ESR), complete blood count (CBC), RF, Anti-CCP and Serum 14-3-3 Eta by ELISA.

Statistical analysis:

The statistical package for the social sciences, version 23.0, has been employed to collect, code, revise, and enter data (SPSS Inc., Chicago, Illinois, USA). The quantitative data has been presented as mean, standard deviations when the parametric distribution is normal. In contrast, non-normally distributed variables (non-parametric data) have been shown to have a median with an inter-quartile range (IQR). Numbers and percentages have also been employed to represent qualitative variables. Data was examined for normalcy employing the Kolmogorov-Smirnov and Shapiro-Wilk tests, as well as the relationship between qualitative variables employing the Chi-square test (X2). ANOVA or Kruskal-Wallis, Spearman's correlation to compare differences between quantitatively independent multiple groups. The margin of error acceptable was 5%, with a confidence interval of 95%. As a result, the significance of the p-value was determined as follows: p-values > 0.05 indicate non-significant (NS), p-values < 0.05 indicate significance (S), and p-values < 0.01 indicate highly significant (HS).

RESULTS

Fifty JIA patients have been categorized into three groups: polyarticular JIA (PJIA n = 20), oligoarticular JIA (OJIA n = 20), systemic-onset JIA (SJIA n = 10), and twenty healthy children (control group n= 20).The mean age of JIA patients (11.80±2.75) and for healthy children (5.55±3.38).

The serum 14-3-3 eta protein receiver operating curve (ROC) has been discovered at levels greater than1.45 ng/ml among all JIA subgroup patients with 84 % sensitivity, 55 % specificity, 65.6% positive predictive value (PPV), and 69.2% negative predictive value (NPV). (Table 1) (figure1).

Cutoff	Sensitivity	Specificity	PPV	NPV	AUC [95% C.I.]
≥1.45	**84%**	**55 %**	65.6%	69.2%	0.671 [0.462-0.775]

Table 1: The 14-3-3 eta protein's cutoff value, sensitivity, and specificity.

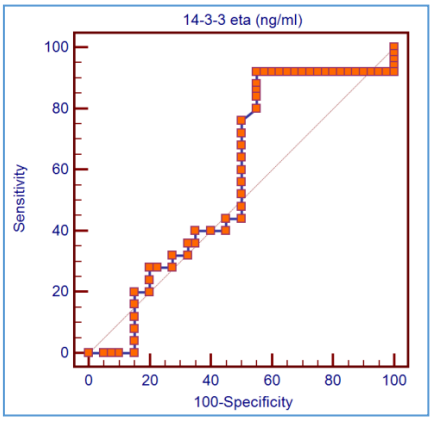

Fig. 1: ROC curve for diagnosis and prediction of JIA using Serum Protein 14-3-3 Eta.

The levels of 14-3-3η eta (ng/ml) in JIA subgroups and control group normal children showed statistically significant differences (P-value <0.05). It has been discovered that polyarticular JIA (median 27.7 ng/ml) had significantly greater levels followed by OJIA and SOJIA (median 22.03 ng/ml) (Table 2) (figure2).

14-3-3η eta (ng/ml)	Group I: Polyarticular JIA patients	Group II: Oligoarticular JIA patients	Group III: Systemic onset JIA patients	Control Group Normal Children	Test value	p-value
Median (IQR)	27.7 (13.3-61.9)	22.03 (10.4-36.15)	22.3 (10.0075-29.5225)	1.005 (0.8725-1.135)	13.682	0.003*
Range	1.33–66.8	0.38–70.3	0.56–46.18	0.64–148.65		

Table 2: Comparison of 14-3-3η eta (ng/ml) levels in sub-group JIA patients and control group (normal children).

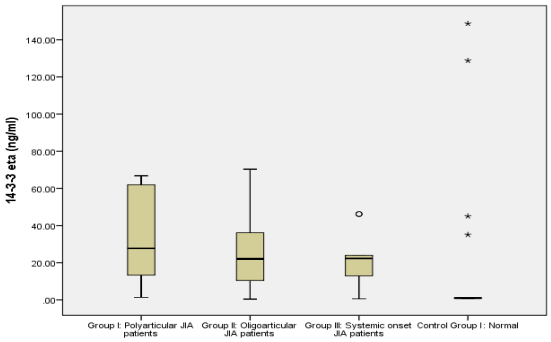

Fig. 2: Eta protein levels (ng/ml) in JIA subgroups and normal children according to 14-3-3η eta (ng/ml).

There was a statistically significant (p-value < 0.05) relationship between 14-3-3 eta level with RF and Anti-CCP, In which 14-3-3η eta levels being higher in positive RF and Anti-CCP JIA cases compared to negative cases (p values = 0.034 and 0.040 respectively) (Table 3).

	Negative	22.3 (13.75 – 46.18)			

		14-3-3 eta (ng/ml)	Test value	P-value	Sig.
		Median (IQR)			
RF	Negative	23.13 (13.75 – 45.12)	-2.116 ≠	**0.034**	S
	Positive	47.14 (35.7 – 64.22)			

	Positive	36.15 (35.7 – 58.14)			

Table 3: Relation between RF, Anti-CCP and 14-3-3η eta (ng/ml) in JIA patients.

In JIA patients, serum 14-3-3 eta (ng/ml) levels exhibited a statistically significant positive association with CHAQ (Table 4).

Parameters	14-3-3 eta (ng/ml)	
	R-value	p-value
ESR	0.111	0.445
CRP	0.127	0.378
CHAQ	**0.360***	**0.026**
JADAS 27	0.149	0.303

Table 4: Correlation between 14-3-3 eta (ng/ml) with all parameters, using Spearman's rho in JIA patients.

DISCUSSION

The most frequent rheumatic illness in children is JIA, with occurrence rates ranging from 0.038 to 4 per 1000. [11]

The JIA clinical classification has a limited diagnostic value and does not include biomarkers other than RF and ANA .[12]

For juvenile idiopathic arthritis, there are no accurate, well-studied, and publicized biomarkers for diagnosis or surveillance. [13]

A biomarker unique to JIA disease activity may reduce diagnostic delays, consequences and improve outcomes. [14]

The goal of this study was to evaluate if 14-3-3 eta protein might be used as a potential new biomarker for JIA diagnosis.

14-3-3 eta protein has been deemed positive over 1.45 ng/ml with a sensitivity of 84% and a specificity of 55%.

In our study, we discovered statistically significant differences (P-value < 0.05) between the JIA subgroups and the control group (Normal children) as regards the 14-3-3η eta (ng/ml) level. This is consistent with the findings of Dalrymple et al.,[١٤] who found that the highest level of 14-3-3 eta positivity has been noted in JIA subtypes and the lowest in healthy controls, and children with JIA had a higher odds ratio than healthy controls (OR 7.1)

It also agreed with the study of Reyhan et al.,[١٥] who found levels of serum 14-3-3η over the 0.2 ng/mL cutoff in all JIA subtypes, which included the OJIA group.

While in a study conducted by Hassan et al.,[١٦] among 20 JIA cases (group I) and 40 healthy controls showed insignificant statistical difference between the two groups for 14-3-3 eta protein level (P-value 0.175) , might be explained by small sample size.

Polyarticular JIA had the highest incidence of 14-3-3η (median 27.7 ng/ml) in our study, followed by OJIA (median 22.03 ng/ml). This finding has been agreed with the findings of Dalrymple et al.,[١٤] who found positive percentages of 14-3-3η were higher in polyarticular JIA patients, with rates of 14-3-3 η positivity (0.5 ng/mL) 28% in RF positive polyarticular JIA, 28% in RF negative polyarticular, and 28% in combined positive and negative versus 5% among healthy control subjects. They stated that this could point to a probable correlation between 14-3-3η and polyarticular JIA.

In our study, we discovered a statistically significant (p-value < 0.05) relationship between 14-3-3 eta level with RF and Anti-CCP, in which 14-3-3η eta levels being higher in positive RF and Anti-CCP JIA cases compared to negative cases (p values = 0.034 and 0.040 respectively) .

This is in line with the results of Reyhan et al.,[١٥] who observed high 14-3-3η levels in 34/151 (23 %) of all participants tested. The majority of 14-3-3η positive individuals also tested positive for RF or Anti-CCP antibodies. Patients with PJIA RF+ had the greatest incidence of 14-3-3η (49%) followed by OJIA (22%).

Also, Dalrymple et al.,[١٤] observed that the contribution of 14-3-3-eta was higher in patients with anti-CCP positivity.

Our serum 14.3.3 eta protein cut off for diagnosis and prediction of JIA is ≥1.45 ng/ml, with 84% sensitivity, 55% specificity, 65.6% PPV, and 69.2% NPV. The titer of serum 14-3-3η was also 10 times greater than the cutoff limit (>1.45 ng/ml). This is in agreement with Reyhan et al.,[١٥] who discovered that the serum 14-3-3η titer was twice as high (> 0.4 ng/ml) in 20% of all cases. The majority of cases who tested positive for 14-3-3η had titers four times higher than the cutoff value.

In JIA subgroups, we found no statistically significant relationship between 14-3-3 eta protein and JADAS-27 (p=0.3). This result agreed with Reyhan et al.,[١٥] who claimed that serum 14–3–3 was found in all types of JIA studied and was unrelated to JIA activity, but disagreed with Hassan et al.,[١٦] who discovered that 14-3-3 eta protein had a significant relation with disease activity in JIA.

Moreover, this study found a statistically significant (p-value < 0.05) positive relation between serum 14-3-3 eta (ng/ml) levels and CHAQ. This finding disagrees with Abo Elsoud et al.,[٧] who stated there was no link between CHAQ and 14-3-3 eta protein in the oligoarticular group.

CONCLUSION

Serum 14-3-3 eta protein may be associated with RF and anti-CCP positivity in early JIA which may increase the sensitivity for early diagnosis of JIA. It is worth investigating a 14-3-3η role with erosive changes in JIA with the evaluation of 14-3-3η as a modifiable biomarker before and after JIA treatment.

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