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Predictive Value Of 14-3-3 Eta Protein as A Novel Biomarker in Juvenile Idiopathic Arthritis (Oligoarticular Type): Relation to Activity and Severity of The Disease

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Abstract

Background: Juvenile idiopathic arthritis (JIA) is a term that encompasses all forms of arthritis that begin before a patient is aged 16 years that persist for more than 6 weeks and are of unknown origin. It is the most common childhood chronic rheumatic disease and causes much disability. We investigated the relation of serum 14-3-3 η (eta) protein in oligoarticular JIA (OJIA) and; the disease activity and severity.

Methods: This study is a case control study including 14 JIA patients and 14 control group. Patients were (6 males and 8 females).14-3-3η was measured for all patients and control volunteers by enzyme-linked immunosorbent assay (ELISA) technique. ANA was measured by indirect immunofluorescence (IIF) technique. Disease activity was assessed by the Juvenile Arthritis Disease Activity Score27 (JADAS-27). Functional ability was assessed by childhood health assessment questionnaire (CHAQ), and disease severity was assessed by juvenile arthritis damage index (JADI). Radiological damage was assessed by Poznanski score.

Results: Elevated 14-3-3 η levels were detected in 5/14 (35.7%) patients. Positivity for 14-3-3 η was significantly related to disease activity, severity and ANA. Positivity for 14-3-3 η had no significant correlation with CHAQ or Posnanski score.

Conclusion: Serum 14-3-3 η can be detected in oligoarticular JIA patients, and appears to correlate with disease activity, severity and (ANA). But no correlation with CHAQ or Poznanski score.

Keywords: Juvenile idiopathic arthritis, oligoarticular JIA, Biomarker, 14-3-3(eta), activity, severity.

1-Introduction

Juvenile idiopathic arthritis (JIA) is inflammation of one or more of joints that begins before the age of 16, It most commonly occurs in pre-school age children or teenagers and the onset of symptoms could be sudden or insidious **[1].** There are different types of JIA and symptoms vary between the different types. JIA may be difficult to control in some people, but most cases will be well controlled with treatment most of the time **[2].**

Joint pain can lead to a poor functional outcome, psychological and physical distress **.**No laboratory test can confirm the diagnosis of JIA so the diagnosis is mainly clinical **[3].**

Oligoarticular JIA (OJIA) is the most common JIA subtype in developed countries, and is generally seen among female patients **[4].** The RF is negative but the ANA is positive in 70-80% of patients and is further subdivided into two subgroups: persistent (no more than four joints affected during the course of the disease) and extended (after the initial 6-month period, the total number of affected joints exceeds four) **[5].**ANA-positive oligoarticular JIA female patients with early disease onset are at an especially high risk of uveitis, the ophthalmological examination should be performed every three months **[6].**

14-3- 3η (eta) protein, is an intracellular chaperone protein belongs to a family of seven isoforms named as; (i) beta (ii) gamma (iii) epsilon (iv) eta (v) tau (vi) and zeta (vii) sigma **[7].** 14-3-3 eta proteins play a role in many intracellular biological functions as cell proliferation, differentiation and apoptosis **[8].** 14-3-3 eta protein had not been studied in a large pediatric population with JIA, except for few studies **[9, 10, 11].** This study aimed to assess the relation between 14-3-3 eta protein and activity, functional ability and severity of oligoarticular JIA patients.

Subjects and Methods;

This study was performed in the Rheumatology Rehabilitation and Physical medicine department of Zagazig University Hospitals, after review and approval by the Institutional Review Board, Faculty of Medicine, Zagazig University. This study included 28 subjects divided into 2 groups; 14 patients with oligoarticular JIA, and 14 apparently healthy children as control during the period between June 2020 and June 2021. Patients were (8 females and 6 males), their age range was 6 to 16 years. Control group was age and sex matched with disease group. Study patients fulfilled International League of Associations for Rheumatology (ILAR) criteria **[12]**. Other causes of childhood chronic arthritis were excluded such as all autoimmune diseases other than JIA, infectious arthritis and neoplastic diseases. Written informed consent was obtained from all participants. This study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Clinical assessment;

All patients were subjected to full history taking, clinical examination, referred to ophthalmologist and assessed for disease activity assessment by juvenile arthritis disease activity score (JADAS 27) with its 4 components; physician global assessment of overall disease activity, patient - parent rating of intensity of the child`s pain, Active joint count, erythrocyte sedimentation rate (ESR) with a total score 0-57 , A higher JADAS-27 indicates higher disease activity and a lower JADAS-27 indicates lower disease activity, 0 = no disease activity and 57 = maximum disease activity [**13]**. Childhood health assessment questionnaire (CHAQ) **[14]** was assessed for all study patients**.** Disease severity was assessed by: juvenile idiopathic arthritis damage index with its two components articular (JADI-A) and extra articular (JADI-E) **[15].** Growth retardation was detected by growth chart; height for age [**16]**

Laboratory investigations;

Laboratory tests for oligoarticular JIA group included; complete blood count (CBC), fasting and postprandial blood sugar, serum amyloid A (normal value <6.4 mg/L) by CFX96 Optical Reaction Module for Real-Time PCR Systems with Starter Package. Erythrocyte

sedimentation rate (ESR) by Westergren method **[17].**C-reactive protein (CRP) was carried out by Cobas 6000, c501 module by turbidimetry (Roche Diagnostic, Germany), and anti-nuclear antibody (ANA) was assessed by indirect immunofluorescence (IIF) by Olympus CH40 , Nova lite ANA Hep-2 substrated slide pack (Inova Diagnostics A Werfen Company)**.** 14-3-3 Eta protein was measured in all groups. Serum level of 14-3-3 eta protein of > 16.5 ng/ml was considered positive. 14-3-3 eta protein was assessed by enzyme-linked immunosorbent assay (ELISA) according to the instruction of the manufacturer (SunRed, Shanghi).

Imaging study;

MRI for any joint suspected to have avascular necrosis of bone such as hip or knee especially in those receiving corticosteroids. Plain x-ray on spine, anteroposterior and lateral views for scoliosis, lordosis or vertebral collapse. Radiological assessment of joint damage in JIA by plain x-ray for both hands antero-posterior view for Poznanski score, and the more negative the score the more severe the disease and radiographic damage **[18].**

Statistical analysis;

Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance; difference and association of qualitative variable by Chi square test (X2). Differences between quantitative independent multiple groups by ANOVA or Kruskal Wallis, correlation by Spearman's. P value was set at <0.05 for significant results &<0.001 for high significant result.

Results

Serum 14-3-3 eta protein was detected at levels above the 16.5 ng/ml among studied subjects with a sensitivity 52.0% and specificity 80 % (table 1).

Table (1): Cutoff value, sensitivity and specificity of 14-3-3 eta protein:

Area	Cutoff value	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.57	**>16.**	**0.39**	**0.36**	**0.78**	**52.0%**	**80.0%**

In oligoarticular JIA group we found 5/14 (35.7%) positive for 14-3-3 eta protein with a mean 10.99±6.23 (ng/ml) .Its levels were found to be significant higher in oligoarticular JIA group than control group (P=0.038) (table 2) (figure 1).

Table (2): 14-3-3 Eta protein levels (ng/ml) among studied groups:

Oligoarticular JIA group	Control group	test	P
10.99±6.23	**7.84±2.36**	**-2.07**	**0.038**
12.85 (1.21-23.76)	**6.40 (0.96-15.63)**		

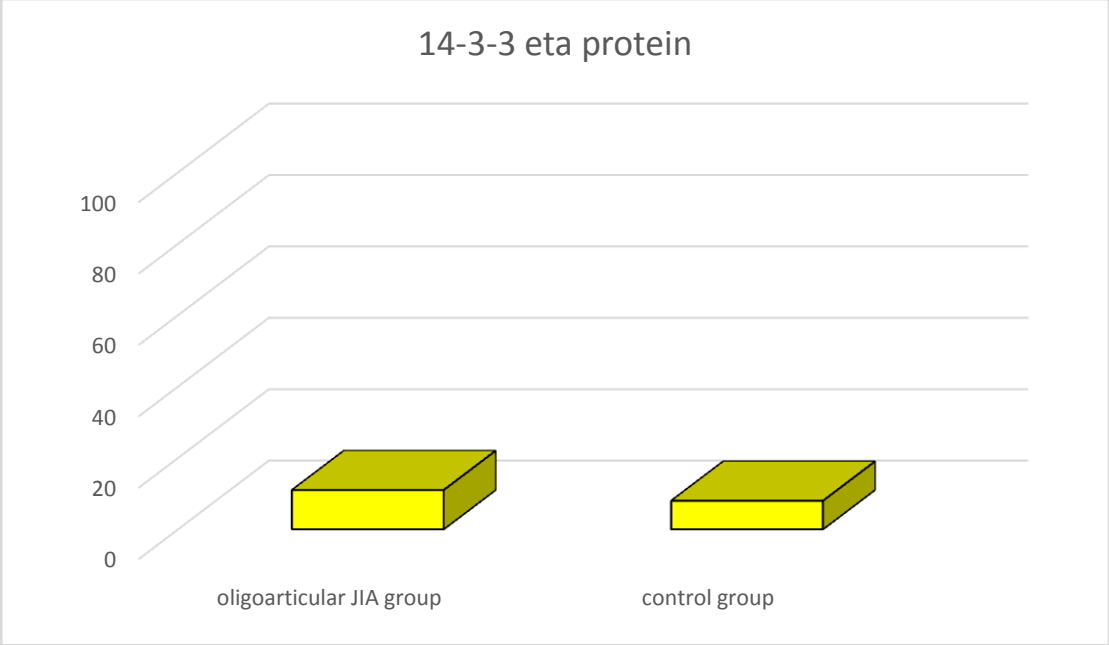

Figure (1): 14_3_3Eta protein level(ng/ml) in oligoarticular JIA group and control group:

As regard Poznanski score, 3 patients had hand manifestations (3/14; 21.4%) with a mean (-0.83±0.00) for right hands and (-0.57±0.0) for left hands. (table 3).

Table (3): Parameters of activity, severity in oligoarticular JIA group:

parameter	Mean ±SD
JADAS27	**16.71±5.23**
CHAQ	**0.70±0.23**
JADI_A	**0.64±0.55**
JADI_E	**0.57±0.63**
RT hand Poznansk score	**-0.83±0.00**
LF hand Poznansk score	**-0.57±0.0**

Extraarticular manifestations in JADI-E in oligoarticular JIA group was found as; (5/14; 35.7%) had uveitis, (1/14; 7.1%) had ocular surgery and (1/14; 7.1%) had growth failure. None of our patients had diabetes mellitus, secondary amyloidosis or osteoporosis with fracture (table 4). And as regard treatment; 28.6% took corticosteroids, 100% took DMARDs and 21.4% took biological treatment (table 5).

Table (4): Extraarticular manifestations in JADI-E in oligoarticular JIA group:

	Oligoarticular JIA group	
	No	%
Cataract	0	0%
Uveitis	5	35.7%
Ocular surgery	1	7.1%
Ms. Atrophy	0	0%
Osteoporosis with fracture	0	0%

Avascular necrosis of bone	0	0%
Abn. Vertebral curve	0	0%
Leg length discrepancy	0	0%
Striae rubrae	0	0%
Subcutaneous atrophy	0	0%
Growth failure	1	7.1%
Pubertal delay	0	0%
Diabetes mellitus	0	0%
Secondary amyloidosis	0	0%

Table (5): treatment in our studied oligoarticular JIA group:

Treatment			**Oligoarticular JIA group**
Corticosteroids	**No**	**N**	**10**
		%	**71.4%**
	Yes	**N**	**4**
		%	**28.6%**
DMARDs	**No**	**N**	**0**
		%	**0%**
	Yes	**N**	**14**
		%	**100%**
Biological	**No**	**N**	**11**
		%	**78.6%**
	Yes	**N**	**3**
		%	**21.4%**

Parameters associated with disease activity as ESR (p=0.023) was statistically significantly higher in oligoarticular JIA group with positive eta protein as compared to cases with negative eta protein (table 6).

ANA was found in oligoarticular group (5/14; 35.7%) (table 6). 4 of them were positive for 14-3-3 eta protein and complicated with uveitis (4/5; 80%). 14-3-3 eta protein is significant correlated with ANA and uveitis (p=<**0.001**) (table 6).

Table (6): Relation between 14-3-3 eta protein and; demographic and laboratory parameters in oligoarticular JIA group:

		Eta protein				**Test**	**P**
		Negative		**Positive**			
		Mean	**± SD**	**Mean**	**± SD**		
Age (year)		**12.44**	**2.60**	**11.60**	**.55**	**0.704**	**0.495**

disease duration(year)		**3.11**	**3.18**	**2.60**	**.55**	**0.350**	**0.732**
CRP(mg/L)		**6.92**	**8.22**	**80.60**	**64.91**	**3.47**	**0.064**
ESR(mm/hr)		**25.56**	**29.96**	**96.00**	**46.56**	**3.48**	**0.023***
platelets(x$10^3/mm^3$)		**348.00**	**159.69**	**317.60**	**49.84**	**0.408**	**0.690**
WBCs(x$10^3/mm^3$)		**6.90**	**1.26**	**5.10**	**.55**	**1.64**	**0.127**
HB(gm/dl)		**12.72**	**1.17**	**12.20**	**.55**	**0.929**	**0.371**
		No	**%**	**No**	**%**		
Sex	**Male**	**1**	**11.1%**	**0**	**0.0%**	**0.501**	**0.479**
	Female	**8**	**88.9%**	**5**	**100.0%**		
ANA	**Negative**	**8**	**88.9%**	**1**	**20%**	**8.795**	**<0.001***
	positive	**1**	**11.1%**	**4**	**80%**		

There was a significant correlation between 14-3-3 eta protein and; JADAS-27 (p=0.000), and JADI-A (p=0.004). No significant correlation was found between 14-3-3 eta protein and JADI-E (P=0.264), CHAQ (P=0.754) or Poznanski score (table 7).

Table (7): Correlations between 14-3-3 eta protein and disease activity parameters in oligoarticular JIA group:

Oligoarticular JIA	**14-3-3 Eta protein**	
JADAS27	**r**	**0.872****
	P	**0.000**
CHAQ	**r**	**0.092**
	P	**0.754**
JADI_A	**r**	**0.710****
	P	**0.004**
JADI_E	**r**	**0.320**
	P	**0.264**
Rt hand Poznanski score	**r**	**-0.223**
	P	**0.148**
Left hand Poznanski score	**r**	**-0.230**
	P	**0.142**

Discussion:

Juvenile idiopathic arthritis is the most common, chronic rheumatic disease of childhood, affecting approximately 7-401 per 100,000 children **[19]**.Oligoarticular JIA (formerly called

pauciarthritis or pauciarticular-onset juvenile rheumatoid arthritis) is defined as juvenile idiopathic arthritis involving fewer than five joints and the diagnosis is mainly based on clinical assessment rather than relying on laboratory testing **[20]** 14-3-3eta protein, is an intracellular chaperone (cellular adapter) protein, which released to the extra cellular space during the early stages of the juvenile idiopathic arthritis (JIA), and acts as an inducer of innate immune system. Many inflammatory mediators and pathways that involved in the pathogenesis and progression of JIA have been up-regulated through 14-3-3 eta protein. So, this marker in addition to existing biomarkers can augment the laboratory efficacy in the early identification and progression assessment of the JIA **[21].** Recent work has implicated the eta isoform as having diagnostic potential in inflammatory arthritides **[22].**

The present study was designed to evaluate the utility of 14-3-3 eta protein as a new diagnostic biomarker for oligoarticular JIA and it´s relation with disease activity and severity. 14-3-3 eta was considered positive above 16.5 ng/ml with a sensitivity 52% and specificity 80 %.

In oligoarticular JIA group we found 5/14 (35.7%) positive for 14-3-3 eta protein with a mean (10.99±6.23) ng/ml. This went ahead with the findings of **Reyhan et al., (2021)** who found that among 36 oligoarticular JIA patients there were (22%) positive for 14-3-3 eta protein **[21]**.

There was a significant correlation between 14-3-3 eta protein and JADAS-27 (p=**0.000**) in oligoarticular JIA. This agreed with **Hassan et al., (2019)** who found that 14-3-3 eta protein had a significant correlation with disease activity in JIA **[23].** This finding disagreed with **Reyhan et al., (2021),** who stated thatserum 14–3-3η can be detected in all forms of JIA tested and does not appear to correlate with disease activity in JIA **[21].**

Moreover, this study revealed no correlation between CHAQ and 14-3-3 eta protein in oligoarticular group. This agreed with **Reyhan et al., (2021)** who found no correlation between CHAQ and 14-3-3 eta protein in children with JIA **[21]**.

14-3-3 eta protein was significantly correlated with juvenile arthritis damage index – articular (JADI-A) with (p=0.004), but no correlation with (JADI-E) was found. **Dalrymple et al., (2017)** noted some association between 14-3-3η and erosions in JIA patients **[22].Maksymowychet al., (2014)** found that 14-3-3 eta protein plays a role in upregulating proinflammatory cytokines in the RA joint **[7].** Few studies discussed it's role in juvenile idiopathic arthritis.

As regard Poznanski score, 3 patients had hand manifestations (3/14; 21.4%),with p value (P=0.148) for right hand and (P=0.142) for left hand. No significant relation with 14-3-3 eta protein and Poznanski score. This finding can be explained by **Ogdie & Weiss. (2015)** who found thatoligoarticular JIA predominantly involves lower-extremity joints, such as the knee and ankle joint. The hip joint is rarely affected. Small-joint involvement is pretty rare in this entity **[24].**

ANA was found in oligoarticular group (5/14; 35.7%). 4 of them were females with positive for 14-3-3 eta protein and complicated with uveitis (4/5; 80%). This agreed with **Reyhan et al., (2021)** who found uveitis more common among OJIA patients than in other groups **[21].**

Limitation of the study was small sample size.

Conclusion

Serum 14-3-3 η can have a diagnostic potential in oligoarticular JIA patients, and appears to correlate with disease activity, severity and this could indicate higher risk of aggressive disease. There is a need for further evaluation on larger sample of Egyptian population. Further study is essential to evaluate the presence of this marker prior to diagnosis among patients at the time of diagnosis with longer course of follow up.

Recommendations;

Comparing 14–3-3η level among JIA vs. patients with arthralgia, and healthy patients with follow up over several years.

Measurement of 14–3-3η pretreatment and posttreatment, as well as comparison via imaging may be helpful to assess the value of 14–3-3η as a marker of joint damage and treatment response.

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