Serum Amyloid A Level in Patients with Juvenile Idiopathic Arthritis

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Abstract: *Background and Aim of the Work*: JIA is the commonest rheumatic disease in childhood characterized by inflammatory arthritis lasting more than 6 weeks before the 16th birthday. In addition to routine ESR and CRP, there are other inflammatory biomarkers as SAA. It is one of the major acute phase reactants which was found to be elevated in inflammatory arthritis and a good indicator of disease activity.

This study assessed the value of SAA level in a cohort of patients with JIA.

Subjects and Methods: 45 JIA patients and 40 healthy controls were recruited from the outpatient clinic of Rheumatology and Rehabilitation Department at MUCH. All patients underwent a thorough clinical evaluation. Assessment of JIA patients involved assessment of disease activity, tenderness and functional status. Laboratory tests were done including: CBC, ESR, CRP and SAA.

Results: A significant rise in SAA levels was found in JIA patients compared to control group and it was significantly higher in SJIA subtype. SAA level was positively correlated with JADAS-27, VAS, physician global assessment, C-HAQ, Ritchie articular index score, platelet count and ESR in 1st hour and 2nd hour. The levels of SAA were significantly lower in JIA patients taking methotrexate while it was significantly higher in cyclosporine treated patients.

Conclusion: SAA can be used as additional indicator of JIA disease activity. Moreover, it can help in differentiation between subtypes when combined with clinical features of the disease and it may be considered in assessment of patient, s response to therapy.

Keywords: Juvenile Idiopathic Arthritis, Serum Amyloid A, Acute phase reactants.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) consists of a group of varied disorders of chronic arthritis in childhood. JIA is the commonest pediatric rheumatic disorder in which their patients are usually suffering from long-term morbidity and mortality [1].

According to the International League of Associations for Rheumatology (ILAR); JIA consists of 7 heterogeneous subsets with unique clinical patterns of each subtype [2].

The treatment goals of JIA are to help remission of active disease, normalize the function of the joint, maintain normal growth, prevent long-term joint destruction and prevent patient disability [3].

In addition to ESR and CRP, there are other known inflammatory markers such as serum amyloid A (SAA) protein which is the circulating precursor of amyloid A protein, the fibrillar component of amyloid deposits. It is an acute-phase reactant transported mainly as an apolipoprotein and is primarily synthesized in the liver by activated monocytes and macrophages in response to pro-inflammatory cytokines. Principally, interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor α (TNF α) are involved in the induction of SAA protein synthesis [4].

The value of measuring SAA levels as a good marker of inflammation has been noticed in several diseases like rheumatoid arthritis, psoriatic arthropathy, ankylosing spondylitis and Behçet's disease. It has been recommended that SAA levels should be one of the most valuable measurements of the acute phase reaction [5]. Considering these findings, the measurement of this biomarker can assist in evaluation of disease activity and treatment monitoring.

The objective of this study was to estimate the value of serum level of amyloid A protein in patients with JIA.

SUBJECTS AND METHODS

A. Subjects

This study was conducted on 2 groups of participants

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Group 1

It included 45 JIA patients who fulfilled the classification criteria for JIA according to ILAR [2]. They were 22 female and 23 male with age ranged from 3 to 16 years. Patients were recruited from the outpatient clinic of Rheumatology and Rehabilitation Depatment at Mansoura University Children Hospital (MUCH) during their routine visits from April 2014 to October 2014. All JIA patients were receiving DMARDS in addition to steroid (22 children) and biological treatment (3 cases); 2 of them were treated with etanercept and 1 took tocilizumab.

Exclusion Criteria

Patients who had any medical or surgical condition which may affect SAA level such as recent infection, trauma, neoplastic diseases and other autoimmune diseases.

Group 2

It included 40 children of matching age and gender; some of them were recruited from MUCH coming for health assessment of simple musculoskeletal pain, orthopaedic deformity and others from normal healthy children.

This study was approved by the ethical committee of Faculty of Medicine Mansoura University. Parents of each participant were informed about the nature of the study and they gave their consent during their clinical visits.

Methods

All Participants in this study were subjected to detailed history taking: personal history, present history, past history and family history.

Disease activity was assessed using Juvenile arthritis disease activity score (JADAS) [6] and Physician rated global assessment [7]. Assessment of tenderness was done using articular index of Ritchie, [8]. Functional assessment was assessed using Child-hood-health assessment questionnaire (C-HAQ) [9].

Investigations

Complete blood count (CBC) using Haematology Auto analyser, ESR in mm/hr was estimated by westergren method [10] and CRP was tested by latex agglutination expressed in mg/dl [11].

SAA level was tested using Enzyme Linked Immunosorbent Assay (ELISA) supplied by assay pro based on sandwich technique [12].

Statistical Analysis

Statistical analysis was performed using SPSS for windows version 20.0 (SPSS, Chicago, IL). Continuous data were expressed as mean ±standard deviation (SD), while categorical data were expressed in number and percentage. All continuous data were tested for normal distribution prior to any statistical calculations.

The differences between variables with continuous data were determined by independent samples t test or one way ANOVA test as appropriate. Pearson Correlation test was used to determine how one continuous variable changes in response to another continuous variable. Multivariable regressions were modelled to examine the association between SAA, ESR, CRP and the presence of JIA activity. Statistical significance was set at $p \leq 0.05$.

RESULTS

The number of JIA patients with polyarticular subtype was 19 (42.2%) 5 of them were RF –ve (11.1%) and 14 were RF +ve (31.1%), while those with oligoarticular subtype were 14 (31.1%) and SJIA was found in 12 patients (26.7%) (Table **1**).

Table 1: Frequency of Disease Subtypes among JIA Patients

	N	%
Polyarticular subtype	19	42.2
RF –ve	5	11.1
RF +ve	14	31.1
Oligoarticular subtype	14	31.1
SJIA (Still's disease)	12	26.7

RF=rheumatoid factor, SJIA=systemic juvenile idiopathic arthritis.

SAA levels were significantly higher in JIA patients when compared with control group ($p < 0.001$) (Table **2**).

Table 2: Comparison of Serum Concentrations of SAA among JIA Patients and Controls

	JIA patients (mean + SD)	Controls (mean+SD)	Student's t test	
			t	p
SAA (µg/ml)	1.8 ±0.9	0.5 ±0.4	7.875	<0.001*

SAA=serum amyloid A.
*P ≤0.05 is significant.

Table **3** shows a significant difference in SAA concentration among JIA subtypes (p=0.049) with

Table 3: Comparison of SAA Levels, ESR, CRP, Disease Activity Parameters and Functional Status among JIA Subtypes

	Subtypes of JIA				
	Oligoarticular	Polyarticular	SJIA	ANOVA test	
	Mean ±SD	Mean ±SD	Mean ±SD	F	P
SAA	1.8 ±1.1	1.4 ±0.3	2.3 ±1.1	3.245	0.049*
ESR 1st hour	51.6 ±29	41.8 ±29.6	47.3 ±30.4	0.449	0.641
CRP	35.2 ±33.6	29.4 ±29.3	39.3 ±33.2	0.379	0.687
JADAs-27	15.8 ±10.3	11.7 ±9.5	16.9 ±1	1.002	0.376
VAS physician global assessment	54.3 ±35.9	30.8 ±32	44.2 ±44.8	1.670	.201
C-HAQ	1.2 ±1	1.1 ±0.9	1.5 ±1	.684	.510
Ritchie index score	6.7 ±4.9	4.3 ±3.6	9.3 ±12.8	1.670	.201

SJIA=systemic juvenile idiopathic arthritis; SAA=serum amyloid A; ESR=erythrocyte sedimentation rate; CRP=c reactive protein; JADAS-27=Juvenile Arthritis Disease Activity Score with 27 joints count; VAS=Visual Analogue scale; C-HAQ=Childhood- Health Assessment Questionnaire.*P is significant when <0.05.

higher concentration found in SJIA than oligoarticular or polyarticular forms.

SAA levels displayed a significant positive correlation with the following parameters: JADAS-27 (r=0.457, p=0.002), VAS physician global assessment (r=0.499, p <0.001), C-HAQ (r=0.367, P=0.013), Ritchie articular index score (r=0.557, p <0.001) (Table **4**).

Table 4: Correlation of SAA Levels with Parameters of JIA Activity and Functional Status

	Serum amyloid A level	
	r	p
JADAs 27	0.457	0.002*
VAS physician global assessment	0.499	<0.001*
C-HAQ	0.367	0.013*
Ritchie articular index score	0.557	<0.001*

JADAS-27=Juvenile Arthritis Disease Activity Score with 27 joints count; VAS=Visual Analogue scale; C-HAQ=Childhood- Health Assessment Questionnaire.*P is significant when ≤0.05.

SAA was positively correlated with ESR in1st hour and 2nd hour (p=0.017, p=0.019) respectively, platelet count (p=0.047) and CRP (p=0.015) (Table **5**).

The levels of SAA were significantly decreased in JIA patients using methotrexate therapy (p=0.030) than those not receiving it. However, SAA levels in cyclosporine treated patients were significantly higher (p <0.001) than non-cyclosporine treated patients (Table **6**).

DISCUSSION

Inflammation plays a prominent role in JIA. So, the determination of the extent of inflammation is important to design the treatment plan. The advancement in discovery of biological therapy, which enhances the management of JIA, has augmented the need for newer biomarkers of inflammation that may help in monitoring disease course and detection of relapse as SAA [13].

Table 5: Correlation between SAA Levels and Laboratory Findings in JIA Patients

	R	P
ESR 1st hour	0.355	0.017*
ESR 2nd hour	0.348	0.019*
RBCs count	0.288	0.055
Hb concentration	-0.268	0.075
WBCs count	0.267	0.076
Platelet count	0.297	0.047*
CRP	0.359	0.015*

ESR=erythrocyte sedimentation rate; CRP=c reactive protein.
*P is significant when <0.05.

As regard the frequency of JIA subtypes in the current study; the most common subtype was polyaticular JIA (19 of 45 JIA patients) representing 42.2%. This finding runs with the data reported by other authors who concluded that the polyarticular JIA was the commonest subtype in their observational studies about the prevalence of JIA [14, 15]. In contrast to this finding, the oligoarticular subtype was the commonest in a retrospective study conducted in Cairo University by Salah *et al*., 2009 who studied the characteristics of JIA over 196 Egyptian children [20]. This difference could be attributed to the larger number of patients they had collected and the long duration of the study which was carried out from 1990 to 2006.

Table 6: Association of SAA Levels with the Medications Used in JIA Patients

		SAA	Student's t test	
		Mean ±SD	t	P
Steroids	No	1.6 ±0.9	1.152	0.256
	Yes	1.9 ±0.9		
Methotrexate	No	2.3 ±1.2	2.238	0.030*
	Yes	1.6 ±0.8		
Leflunomide	No	1.8 ±1	0.907	0.369
	Yes	1.5 ±0.3		
Anti-malarials	No	1.8 ±0.9	0.880	0.384
	Yes	1.4 ±0.3		
Sulfasalazine	No	1.7 ±0.9	1.258	0.215
	Yes	2.6 ±0.2		
Cyclosporine	No	1.7 ±0.7	3.782	<0.001*
	Yes	3.4 ±1.4		
Etanercept	No	1.8 ±0.9	0.763	0.450
	Yes	2.3 ±0.2		
Tocilizumab	No	1.7 ±0.9	1.285	0.206
	Yes	2.4 ±0.3		

SAA=serum amyloid A, No=patients not receiving the medication, yes=patients receiving the medication.
*P is significant when <0.05.

While in western studies, the most common subtypes were SJIA and oligoarticular as stated by other researchers. This variability in the frequency of subtypes among studies may be related to the environmental and genetic factors in different countries [16, 17].

In our study we found that the levels of SAA was significantly increased in JIA patients when compared to controls (P<0.001). We confirmed the previous studies conducted by Cantarini *et al*., 2012 and Amar and Elhewala, 2015 who also found that SAA was significantly rised in JIA patients [18,19].

In the present study, a significantly higher SAA concentration was found in SJIA compared to oligoarticular and polyarticular subtypes (P=0.049). This is in concurrence with results of other authors who also found that SAA values were higher in systemic forms followed by polyarticular form [18, 21]. This may be explained by the fact that SJIA is a special subtype of JIA in which arthritis is accompanied with systemic manifestation. Unlike other forms of JIA, innate immunity plays a major role with increase the secretion of inflammatory cytokines especially IL-6 and IL-1 which in turn increase the induction of acute phase reactants including SAA.

In this study, we reported a positively significant correlation between SAA level and ESR in 1st hour, 2nd hour (p=0.017, p=0.019 respectively) and CRP (p=0.015) in JIA patients. These findings support the studies of other researchers who also detected a positive connection between SAA and both ESR and CRP [18, 19].

One of the remarkable finding of this study is the positive correlation between SAA, activity and functional parameters including: JADAS-27 (P=0.002), VAS physician global assessment (P<0.001), C-HAQ (P=0.013) and Ritchie articular index score (P<0.001). When speaking about SAA and JIA activity nearly all authors had validated SAA as an important marker of active disease like Takako *et al*., 2005 who stated that SAA was strongly correlated to JIA exacerbation [22].

Another study performed on 117 JIA patients for assessment the value of immunoglobulin light chain in JIA. They observed that SAA was statistically raised in active group than inactive group. In the same JIA child, SAA level differs with respect to disease flare. Similarly, ESR and CRP were elevated in the active group [23].

Recently, it has been reported that SAA level was parallel to JADAS-27 (P=0.033) in evaluating the ability

of the SAA to differentiate between active and stable groups [19].

In the current study as regard the relation between SAA and laboratory findings, SAA was positively correlated to platelet count (P=0.047). This finding goes with other authors who studied the correlation between serum levels of IL-6 and both joint involvement and thrombocytosis in 70 SJIA child. They reported that there was a positive correlation between platelet count and IL-6 which plays the major role in induction of SAA synthesis [24].

Another study was performed in 2014 over 40 children with JIA to evaluate the mean platelet volume in those children. They observed that platelets have an important role in inflammatory process and a significant positive relation between acute phase reactant and platelet count and volume was detected [25]. A possible explanation of these results is that active disease is accompanied with increase formation of pro-inflammatory cytokines which stimulate bone marrow to increase the production of platelets. Platelets play important role in joint destruction in JIA and its proteins increase secretion of inflammatory molecules and chemokines [26].

In our study as regard the medications, SAA level was lower in JIA patients receiving methorexate than those not receiving it (P=0.030). On contrast, patients treated with cyclosporine showed a higher level of SAA than others (P<0.001). Similar to our results, another authors stated that SAA was lower in patients taking methotrexate in follow up visits than the basal level and by inducing disease remission; it decreases the production of both SAA and CRP [27].

The lower levels of SAA in patients receiving methorexate may be explained by the well known anti-inflammatory properties of this agent and the acute phase reactant behaviour of SAA which decreases by drugs induce disease remission.

While, Amar and Elhewala, 2015 demonstrated that no difference in SAA level between JIA patients receiving methotrexate and those not treated with it [19]. This dissimilarity may be due to the different doses of methotrexate used and the patient's responsiveness to therapy.

In explaining why SAA concentration was elevated despite cyclosporine therapy; it was mentioned that cyclosporine in high concentration equal to plasma level of 200 ng/ml was needed to suppress IL-1 and IL-6 which are the main stimulators of SAA production [28]. Therefore, the cyclosporine treated JIA patients in our study who were SJIA subtype and had a high disease activity score may had not received a sufficient dose of cyclosporine to suppress the inflammation and to decrease the secretion of pro-inflammatory cytokines or due to cyclosporine treatment failure.

CONCLUSION

Reviewing our study we concluded that SAA can be used as a valuable marker of JIA disease activity. Moreover, it can help in differentiation between subtypes when combined with clinical aspects of the disease. Also SAA may be considered in assessment of patient response to therapy as its level is remarkably decreased with methorexate.

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