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RESEARCH ARTICLE

Serum Amyloid A in Patients with Juvenile Idiopathic Arthritis and Its Association with Disease Activity.

Hanan A. Amar[1], Ahmed A.I. Elhewala[2]

1.Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University, Egypt

2.Pediatrics Department , Faculty of medicine , Zagazig University, Egypt

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Corresponding Author

Hanan A. Amar

Abstract

Background and aim of work. The serum amyloid A levels (SAA) levels increases intensely during acute inflammation. Previous studies reported that SAA concentrations was increased and correlated with disease activity in RA and in ankylosing spondylitis patients. The aim of the study is to evaluate the correlation between JIA disease activity score and the SSA concentration.

Subjects and Methods. Fifty JIA patients and 30 controls were enrolled in this study. JIA patients were examined and disease activity was determined. SAA, CRP and ESR were assessed for all participants.

Results.SAA was significantly higher in the JIA patients compared to the controls (20.6 ±12.8 versus 5.3 ±2.5 mg/dl respectively, p<0.001). The CRP and ESR levels were also significantly higher in the JIA patients compared to the controls.The SAA concentration was significantly correlated with JIA acitivity, CRP level and ESR level. The SAA level in the patients with systemic JIA was higher than in the patients with polyarticular JIA and was lowest in oligoatircular JIA. Binary regression model revealed that only SAA was significantly associated with JIA activity (OR = 1.149). In ROC analysis, the SAA concentration can discriminate between the patients with active from those with inactive disease (AUC = 0.643).

Conclusions.SAA is significantly increased in patients with JIA and is strongly correlated with JIA disease activity. SAA is better indicator for JIA activity than CRP and ESR.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory autoimmune rheumatic disease in children ***(1)***. The annual incidence and the prevalence of JIA among individuals less than 16 years of age are 3.2/100000 and 19.8/100000, respectively ***(2)***. Despite significant improvements in the management of children with JIA, the likelihood of long-term persistent disease activity remains high ***(3)***. Studies had shown that persistent disease activity results in severe morbidity and may result in permanent articular damage ***(4,5, 6,7)***. Therefore, proper assessment of JIA activity is crucial for the planning the management of JIA.

Among JIA patients, high erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are used as criteria for determining clinical disease activity ***(8,9)***. There are several important disadvantages to the use of CRP and ESR as a marker of JIA, including changes in serum concentrations associated with age and gender and the existence of co-morbid infection ***(10,11)***.

Serum amyloid A (SAA) is a highly conserved acute-phase protein, released in response to inflammation or infection. Production of acute-phase SAA is stimulated by pro-inflammatory cytokines, such as TNF-α, IL-1β and to a lesser extent by IL-6 in fibroblast like synoviocytes of RA patients ***(11)***. As with other acute-phase reactants, the liver is the primary source of circulating SAA. However, it has been reported that several extrahepatic tissues and cell types produce SAA ***(12,13,14)***.

The SAA levels increases intensely during acute inflammation, reaching within 5-6 hours levels that are 1000-fold in regard to normal concentration (***15,16,17)***. If such stimuli persist, as occurs in some chronic diseases, SAA concentration may reach a critical threshold over which it becomes prone to aggregation and hence result in tissue damage. SAA is known to participate to immune cells recruitment at inflammatory sites ***(18,19)***and to induce expression of pro-inflammatory cytokines ***(19,20)***and matrix metalloproteinases ***(19)***. SAA had been found to play an important, pathogenic role in the inflammatory process in the course of RA ***(21)***.Previous studies had shown that SAA concentrations was increased and correlated with disease activity in RA***(22)*** and in ankylosing spondylitis patients***(23)***.
The aim of the study is to evaluate the correlation between JIA disease activity score and the SAA concetration.

Subjects and Methods

In this study, 50 patients (29 females and 21 males) with JIA according to the criteria of the International League of Associations for Rheumatology for classification of JIA***(24)***were enrolled. Patients were consecutively invited to participate in the study from the outpatient clinic of the Rheumatology and rehabilitation Department and from the Pediatric Department , Zagazig University hospital, Zagazing city, Egypt between December 2014 and May 2015. Children with recent infection, trauma, diabetes mellitus, other inflammatory or autoimmune disease other than JIA, neoplastic diseases were excluded from the study as these conditions may affect SAA levels ***(25,26)***. Thirty healthy age and sex matched children were also included in the study as a control group. Written consent was obtained from the legal guardian of each eligible participant in this study after the approval of this study from local Ethical Committee.

Demographic and JIA related data was obtained from the eligible patients through interview for history taking and physical examination. The medical records of all patients were reviewed. JIA categories were classified according to the ILAR criteria ***(24)***. JIA duration and type of JIA were also reported for each patients.

Juvenile Arthritis Disease Activity Score (JADAS)

JADAS consists of 4 components: physician global assessment of disease activity on a 10-cm VAS (where 0 = no activity and 10 = maximum activity), parent/patient global assessment of well-being on a 10-cm VAS (where 0 = very well and 10 = very poor), number of joints with active disease, and CRP as an inflammatory marker ***(27)***. In this study the 27-joint count form of JADAS is used (JADAS27) due to its greater feasibility. This count has been found to be a valid surrogate for the whole joint count in JIA ***(28)***. The 27-joint counted in JADAS27 includes: cervical spine, elbows, wrists, metacarpophalangeal joints (from the first to third), proximal interphalangeal joints, hips, knees, and ankles.CRP level was truncated to a 0–10 scale according to the following formula: [CRP (mg/L) – 10]/10. Before calculation, CRP values <10 mg/liter were converted to 0 and CRP values >110 mg/liter were converted to 10 ***(27)***.The cut-off score for classifying a patient as having "inactive disease" as $\leq$1 for all JIA categories ***(28)***.

Laboratory assessment

At the same day of clinical evaluation, 10 ml of blood was withdrawn from every participants at 9 a.m. after over-night fast. To assess SAA and CRP, serum samples were collected, frozen, and stored at the temperature of -80^{o} C until analyses were performed. Serum concentration of SAA was determined by a commercial enzyme-linked immunosorbent assay (ELISA), with detection limit 0.005 mg/L (Human SAA; BioSource Europe S.A., Belgium). According to literature, SAA normal reference range is under 10 mg/L ***(29)***. ESR was measured using the Westergren method, and expressed in mm/hour. An ESR of <15 mm/hour was considered to be normal for males and an ESR of <20 mm/ hour was considered to be normal for females***(Westergren, 30)***. Quantitative assay of CRP kit was supplied by Turbox® CRP, Orion Diagnostica. Turbox assay for CRP is a liquid-phase immunoprecipitation

assay with nephelometric detection. Antiserum to CRP is diluted and added to patient serum. The light scattering caused by antigen antibody complexes is measured after incubation. The resulting light scattering is directly proportional to the CRP concentration in the sample ***(31)***. Serum CRP concentrations were measured using a nephelometric immunoassay, and expressed in mg/dL. A CRP of <0.5 mg/dL was considered to be normal.

Results

The characteristics of the JIA patients and controls were shown and compared in Table 1. JIA patients and controls were age and sex matched and the BMI did not differ significantly between JIA patients and controls. Of the patients with JIA included in the current study, 30% (n=15) had oligoarticular JIA, 42% (n=21) patients had polyarticular JIA while 28% (n=14 had systemic JIA. The current drug intake of the JIA patients is demonstrated in Table 1.

SAA was significantly higher in the JIA patients compared to the controls (20.6 ±12.8 versus 5.3 ±2.5 mg/dl respectively, p<0.001). The CRP and ESR levels were also significantly higher in the JIA patients compared to the controls (Table 1).

The correlations of the clinical and laboratory data were shown in Table 2. The SAA concentration was significantly correlated with JADAS27 (p=0.033) (Figure 1), serum concentration of CRP (p=0.002) (Figure 2) and ESR level (p=0.012) (Figure 3). The SAA level in the patients with systemic JIA was 27.2 ±16.6 mg/dl, in the patients with polyarticular JIA was 20 ±10.7 mg/dl while in the oligoatircular JIA was 15.4 ±8.9 mg/dl. These differences were significant (p=0.040) (Table 3). On the other hand, no significant association had been found between the SAA and the current drug use among the JIA patients (Table 3).

Binary regression model was performed to evaluate the association of the SAA, CRP and ESR with the presence of JIA activity and revealed that only SAA was significantly associated with JIA activity (OR = 1.149). In this study, we conducted ROC analysis to estimate the diagnostic ability of the SAA concentration in discrimination between the patients with active from those with inactive disease. As shown in Figure 4, the AUC was found to be 0.643.

Table 1. Characteristics of the JIA patients and controls

	JIA patients	Controls	p
Age (years)	10.3 ±2.7	9.9 ±2.9	0.482
Females/Males	35/15	18/12	0.360
BMI (kg/m^2)	17.1 ±2.4	16.8 ±2.4	0.587
Disease duration (years)	3.3 ±1.7		
JADAS27	14.1 ±11.7		
SAA (mg/dl)	20.6 ±12.8	5.3 ±2.5	<0.001
CRP (mg/dl)	24.3 ±13.6	3.4 ±1.6	<0.001
ESR 1^{st} hour (mm)	39.1 ±18	13.6 ±5.8	<0.001
Steroids	25, 50%		
MTX	38, 76%		
Leflunomide	9, 18%		
HCQ	8, 16%		
Biological therapy	3, 6%		
Combination therapy	31, 62%		

Table 2. correlation between the SAA and demographic characteristics and the JIA related clinical and laboratory features

	R	p
Age	0.005	0.970
BMI	0.208	0.147
Disease duration	0.016	0.914
JADAS27	0.322	0.023
CRP	0.433	0.002
ESR 1st hour	0.351	0.012

Table 3. The association of SAA with type of JIA and the drug intake

	SAA mean ±SD	p
Type of JIA		
Oligoarticular	15.4 ±8.9	0.040
Polyarticular	20 ±10.7	
Systemic	27.2 ±16.6	
Current use of Steroids		
No	21 ±9.6	0.826
Yes	20.2 ±15.7	
Current use of methotrexate		
No	15.6 ±10.1	0.122
Yes	22.2 ±13.3	
Current use of leflunomide		
No	20.9 ±11.5	0.694
Yes	19.1 ±18.5	
Current use of HCQ		
No	19.4 ±12.4	0.130
Yes	26.9 ±14	
Current use of biological therapy		
No	21.1 ±13	0.346
Yes	13.8 ±7.3	
Current use of combination therapy		
No	21.1 ±9.5	0.851
Yes	20.4 ±14.6	

Figure 1. Correlation between the SAA and the JADAS27 in the patients with JIA.

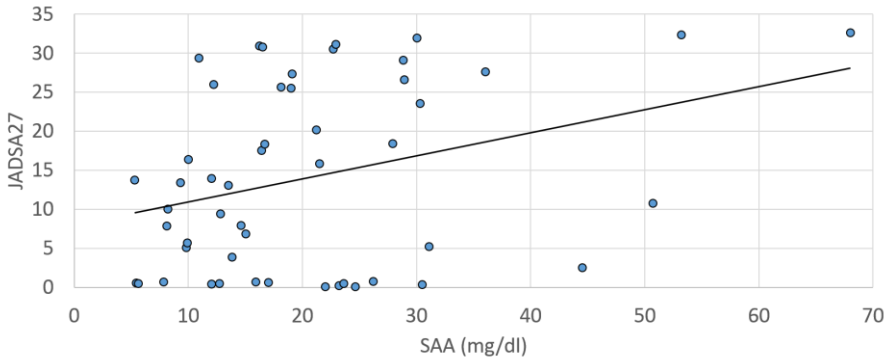


Figure 2. Correlation between the SAA and the CRP in the patients with JIA.

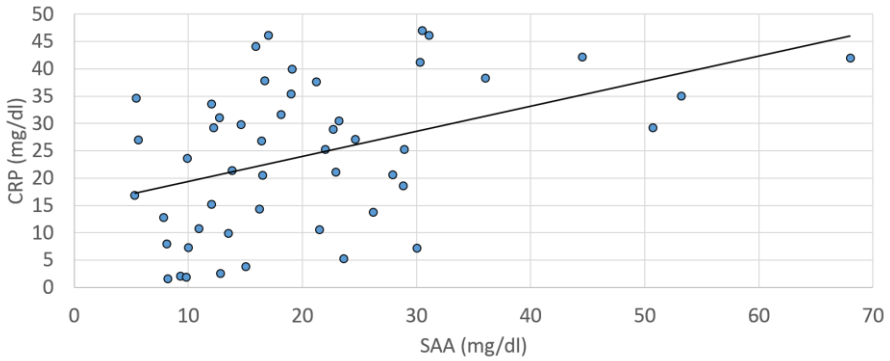


Figure 3. Correlation between the SAA and the ESR in the patients with JIA.

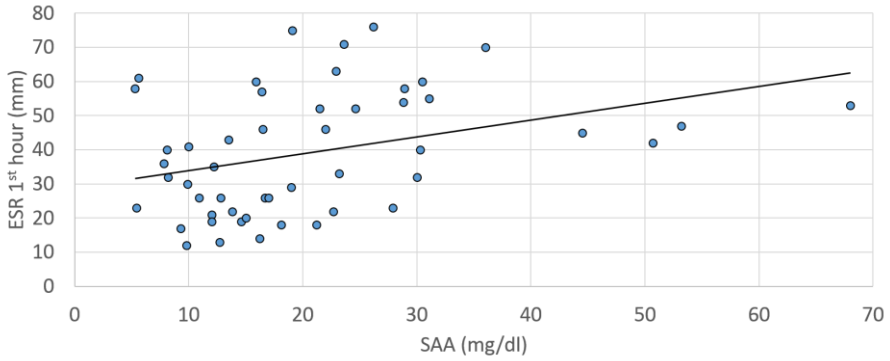
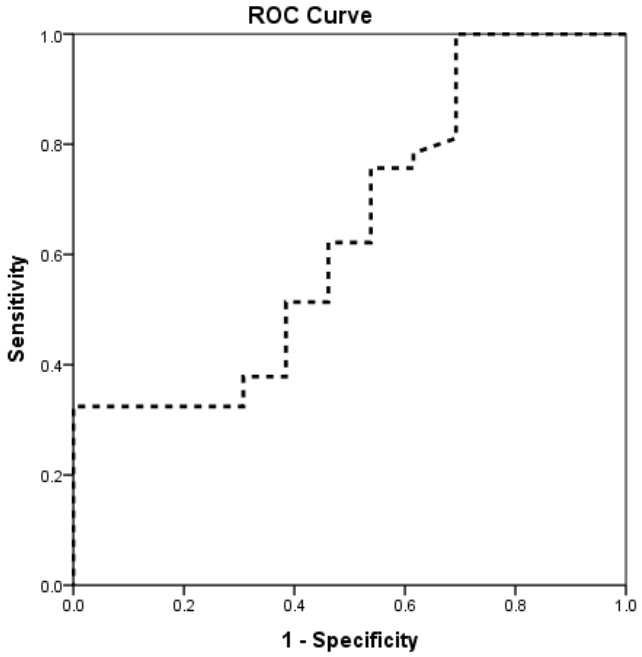


Table 4. Linear regression analysis for the association of SAA, CRP and ESR with the presence of JIA activity

	β co-efficient	SE	p	OR
SAA	0.139	0.058	0.016	1.149
CRP	0.054	0.031	0.082	0.947
ESR	0.046	0.024	0.050	0.955

Figure 4. The Receiver operating characteristics curve to evaluate the ability of SAA to discriminate the JIA patients with active from patients with inactive disease (AUC=0.643).



Discussion

The main finding in this study is that SAA concentrations were significantly higher in the JIA patients compared to the controls and the SAA concentrations were significantly correlated with JIA disease activity score and with the inflammatory markers ESR and CRP. Another major finding in the current study is that patients with systemic JIA were found to have the highest SAA concentrations, the polyarticular JIA comes second while the oligoarticular JIA had the lowest SAA concentration. These differences in the SAA among the JIA types were significant. Moreover, the ROC curve analysis showed that the SAA concentration had the capacity to discriminate the patients with active from those with inactive disease with an AUC =0.643.

In agreement with our findings,***Cantarini et al. (32)***reported a significant increase in SAA levels in JIA patients, compared to controls, and a strong positive correlation between SAA level and JIA disease activity as measured by the presence of active joints and the number of active joints. ***Cantarini et al. (31)*** also reported a positive correlation between the SAA concentrations and the CRP and ESR levels in the patients with JIA. ***Scheinberg, et al. (33)*** had previously demonstrated that SAA levels are elevated in JIA patients, and that they correlate well with disease activity. Moreover, according to ***Scheinberg, et al. (1980)*** higher SAA levels in children with juvenile rheumatoid arthritis were associated with the polyarticular and systemic forms of the disease than patients with oligoarticular type of JIA. This finding in consistent with the results of the present study.

The SAA concentrations had been found to increase intensely during acute inflammation in response to inflammatory mediators, including IL-1, IL-6 and TNF-α. This fact explains the relationship between SAA concentration and disease activity that have been reported in several rheumatic conditions such as rheumatoid arthritis ***(11 , 22)***, polymyalgia rheumatica ***(34)*** and ankylosing spondylitis ***(23, 35, 36)***.

Binary regression analysis in the current study had shown that the correlation of SAA with diseases activity was superior to CRP and ESR. The ROC analysis showed moderate capacity of the SAA concentration in discrimination between the patients with active from those with inactive disease (AUC= 0.643). In agreement with our results, SAA was shown to be more reliable indicator of disease activity than CRP and ESR in JIA ***(29)*** and in other rheumatic diseases ***(34,37)***.

Conclusion

SAA is significantly increased in patients with JIA and is strongly correlated with JIA disease activity. SAA is better indicator for JIA activity than CRP and ESR.

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