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M. Al-Haggar
Department of Pediatrics,
Faculty of Medicine,
Mansoura University,
Mansoura, Egypt

Tel: +96638982022,
+96638982831
Fax: +96638951196

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Soluble Adhesion Molecules in Juvenile Idiopathic Arthritis: Relation to Activity and Clinical Subtype

[1]Mohammad Al-Haggar, [1]Mohamed Ragab, [2]Manal Awad and [3]Rizk Al-Baz

The present research aimed to evaluate serum Adhesion Molecules (AMs) in patients with Juvenile Idiopathic Arthritis (JIA) to correlate their values with disease activity in different clinical subtypes. Serum levels of some soluble AMs (E-selectin, $sICAM_1$ and $sVCAM_1$) were assayed by ELISA in 37 patients with JIA both during activity and after remission. Other activity parameters like sedimentation rate and leukocytic counts were tested as well. Twenty healthy children of matched age and sex were taken as control. Serum E-selectin was found significantly higher in JIA compared to control (in all subtypes across all disease stages), with significant drop after remission, yet not reached the normal values. These changes were more evident in systemic JIA compared to other subtypes. Serum $ICAM_1$ and $VCAM_1$ showed the same changes in relation to control and to the disease activity. We can conclude that systemic JIA is associated with higher levels of soluble AMs thus explaining the perpetual inflammatory process and hence the remissions and exacerbations which are usually associated with higher morbidity in systemic JIA than in the other subtypes. We recommend following JIA patients until laboratory remission (normalization of serum AMs) to correlate AMs levels to clinical course aiming to put forward a therapeutic plan.

Key words: Adhesion molecules, juvenile idiopathic arthritis

[1]Department of Pediatrics, Faculty of Medicine, [2]Department of Rheumatology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[3]Laboratory Section of Pediatric Genetics Unit,
Mansoura University Children's Hospital, Mansoura, Egypt

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INTRODUCTION

Juvenile Idiopathic Arthritis (JIA) is a chronic disease of childhood with a major morbidity; it is characterized by synovitis of the affected joints usually associated with soft tissue swelling and effusion (Rothschild and Masi, 1982). Synovial inflammation is due to cascade of immunologically based reactions in which several Adhesion Molecules (AMs) are involved e.g., Intracellular Adhesion Molecule-1 (ICAM1), Vascular Cell Adhesion Molecule-1 (VCAM1), P-selectin and E-selectin (Pitzalis *et al*., 1994). These AMs play an important role in initiation and progression of disease (Mason *et al*., 1993).

In adult Rheumatoid Arthritis (RA), raised serum E-selectin and $ICAM_1$ have been found (Paleolog, 1996, Koch *et al*., 1993). In contrast to selectins, which are rapidly down regulated after induction, once $ICAM_1$ is up regulated, it remains on the cell surface for more than 48 hours (Vestwever, 1992). Up to our knowledge this is the first work evaluating the levels of some AMs (E-selectin, $sICAM_1$ and $sVCAM_1$) among Egyptian children with JIA in an attempt to put forth an explanation for the different clinical severities.

MATERIALS AND METHODS

The present study was conducted over a period of 2 years from March 2002 to July 2004 on children with JIA from outpatient clinics of Mansoura University Children's Hospital, Egypt. It comprised 37 patients with different disease status (7 systemic, 14 polyarticular and 16 pauciarticular). They were fulfilling criteria of the International League against Rheumatism (Petty *et al*., 1998) and their demographic data are shown in Table 1. All children and/or their parents gave informed written consent to participate in the study.

Patients considered as active polyarticular or pauciarticular disease should have at least one joint swollen, tender with limited mobility. Systemic disease was defined as arthritis with fever≥38.5°C lasting for at least four days a week without definable infection or explainable systemic disease. Patients were considered to be in clinical remission when they had morning stiffness not exceeding 15 min, no fatigue, no joint pain, no joint tenderness, no joint or tendon sheath swelling and ESR_1<20 mm based on the American College of Rheumatology "ACR" criteria for remission in adult RA (Pinalis *et al*., 1981). Patients with local or systemic infections or other systemic diseases expected to affect AMs levels were excluded.

Table 1: Demographic data of patients with JIA; age at time of diagnosis (in years), sex, duration of symptoms before diagnosis (in weeks) and duration required to achieve clinical remission (in weeks)

	Systemic (n = 7)	Polyarticular (n = 14)	Polyarticular (n = 16)
Age (years)*	8.8±3.6	11.3±4.2	10.6± 3.2
Sex			
Males	4	3	7
Females	3	11	9
Duration of illness (weeks)*	13.2±5.8	14.2±5.2	12.8±4.7
Duration until remission (weeks)*	18.8±7.8	20.6±6.2	17.5±5.6

* Mean±SD

Initially all patients were treated uniformly with salicylates or diclophenac sodium alone then dosage schedule was adjusted and other drugs were added according to the subsequent clinical responses and laboratory findings. At the moment of clinical remission, patients were using the controlling medicine (salicylates and/or diclophenac) with one or more additional drug (corticosteroids or immunosuppressive drugs like methotrexate or cyclosporine).

Serum blood samples were assayed for AMs including E-selectin, sICAM1 and sVCAM1 during the acute phase (activity) and after treatment (control of inflammation) in all patients. Serial blood sampling was done every six weeks for follow up of the disease activity by ESR, CR protein and WBCs.

Control serum samples were simultaneously obtained and analyzed from 20 healthy children aged 3-16 years, attending the hospital for irrelevant causes.

Measurement of serum E-selectin: By MESEACUP methodology, E-selectin was measured using a special kit (monoclonal antibodies for 2 epitopes) provided from Immunotech. Optical density is measured at 492 nm using a microplate reader and then serum level of E-selectin is calculated from standard curve performed in the same assay.

Measurement of serum $sICAM_1$ and $sVCAM_1$: By utilizing the same principle (EASIA, enzyme amplified sensitivity immunoassay) using different kits from the same agent (Medgenix). This assay is based on oligoclonal system in which several monoclonal antibodies directed against epitopes of AMs. The amount is measured calorimetrically; the absorbencies were read at 450 nm, plotting the optical density against the standard using linear graph, then concentration calculated from the standard curve.

Statistical methods: Serum AMs values were normalized by log transformation, analysis was done by SPSS version 10.0 (SPSS, 1999). Statistical differences were

Table 2: Serum AMs in the 3 clinical subtypes of JIA during disease activity and after remission, data expressed are mean values

	Systemic (n = 7)		Polyarticular (n = 14)		Pauciarticular (n = 16)	
	Active	Remission	Active	Remission	Active	Remission
E selectin	313	255	267	209	250	230
P_1	0.004		0.02		0.04	
P_2	<0.001	<0.001	<0.001	<0.01	<0.01	<0.05
P_{3a}			0.005			
P_{3r}			0.02			
$sICAM_1$	2020	1590	1900	1750	1670	1600
P_1	0.01	0.02	0.85			
P_2	<0.001	<0.001	<0.001	<0.01	<0.01	<0.05
P_{3a}			<0.001			
P_{3r}			0.21			
$sVCAM_1$	1690	1020	1460	1400	1440	1400
P_1	0.008		0.04		0.54	
P_2	<0.01	0.04	<0.01	0.02	<0.01	<0.01
P_{3a}			0.03			
P_{3r}			0.04			

P_1 is paired t-test for difference between activity and remission in each clinical subtype, P_2 unpaired t-test for difference between each subtype (during activity and after remission) and control, However, P_{3a} and P_{3r} are one way ANOVA between the 3 clinical subtypes during activity and after remission, respectively.

carried out by paired t-test for difference between activity and remission in the same subtype, unpaired t-test for difference between a disease stage of one subtype and control. ANOVA analyzes difference between the 3 clinical subtypes at each disease stage. Pearson correlation was done between serum AMs and disease parameters.

RESULTS

The three clinical subtypes of JIA were of matched age and sex (Table 1). Serum AMs during disease activity and after clinical remission were shown in Table 2 in comparison with the controls. Serum E selectin in all JIA subtypes was significantly higher during activity compared to remission (313 vs. 255 ng mL^{-1} in systemic, 267 vs. 209 ng mL^{-1} in polyarticular and 250 vs. 230 ng mL^{-1} in pauciarticular) and higher than the control (92±28 ng mL^{-1}) (Table 2 and Fig. 1A). Serum $ICAM_1$ and $VCAM_1$ are nearly parallel and showed a marked drop with the control of inflammation in systemic (2020 vs. 1590 ng mL^{-1} for sICAM1 and 1690 vs. 1020 ng mL^{-1} for sVCAM1) and polyarticular JIA (1900 vs. 1750 ng mL^{-1} for sICAM1 and 1460 vs. 1400 ng mL^{-1} for sVCAM1) (Table 2, Fig. 1B and C). In pauciarticular JIA values of sICAM1 and sVCAM1 were not significantly different during activity and remission (1670 vs. 1600 ng mL^{-1} for sICAM1 and 1440 vs. 1400 ng mL^{-1} for sVCAM1), but still significantly higher than controls (mean±SEM 548±136 and 630±148 ng mL^{-1} for $sICAM_1$ and sVCAM1, respectively).

The highest values of serum AMs were noted among cases with systemic JIA as determined from the significance of ANOVA during activity (P_{3a} = 0.005, <0.001 and 0.03 for E selectin, $sICAM_1$ and $sVCAM_1$, respectively) and after remission (P_{3r} = 0.02, 0.21 and 0.04 for E selectin, $sICAM_1$ and $sVCAM_1$, respectively) (Table 2).

Table 3: Pearson's correlation between acute phase reactants and AMs in JIA patients (all subtypes are merged together)

	E-selectin		$sICAM_1$		$sVCAM_1$	
	r	p-values	R	p-values	r	p-values
ESR_1	0.48	0.02*	0.39	0.04*	0.42	0.03*
ESR_2	0.65	0.001**	0.48	0.02*	0.43	0.04*
CRP	0.43	0.04*	0.39	0.04*	0.44	0.04*
WBCs	0.56	0.007**	0.42	0.03*	0.48	0.02*

* p is significant if <0.05, ** highly significant if <0.01

Acute phase reactants were positively correlated to all tested AMs with maximum strength of significance with E-selectin (Table 3).

DISCUSSION

JIA is a systemic inflammatory disease characterized by immunologic dysfunction leading to chronic inflammation and marked tissue damage. Synovial thickening is one of the pathologic hallmarks of JIA; it was found to be caused mainly by vascular endothelial growth factor which increases the inflammatory cells infiltrate (Scola *et al*., 2001). Since chemotaxis and penetration of leucocytes to the site of inflammation were proved to be dependant upon cell-cell or cell-endothelial interaction, so AMs are believed to play a crucial role in pacing and perpetuating such autoimmune disease (Mojcik and Shevach, 1997).

Serum AMs are strongly correlated with synovial AMs; thus suggesting the synovial origin of serum AMs. Many studies had been correlated between soluble AMs in serum and synovial fluid as well as parameters of disease activity especially ESR (Dolezalova *et al*., 2002; Bloom *et al*., 2002). A strong evidence of the synovial origin of E selectin and $ICAM_1$ was provided

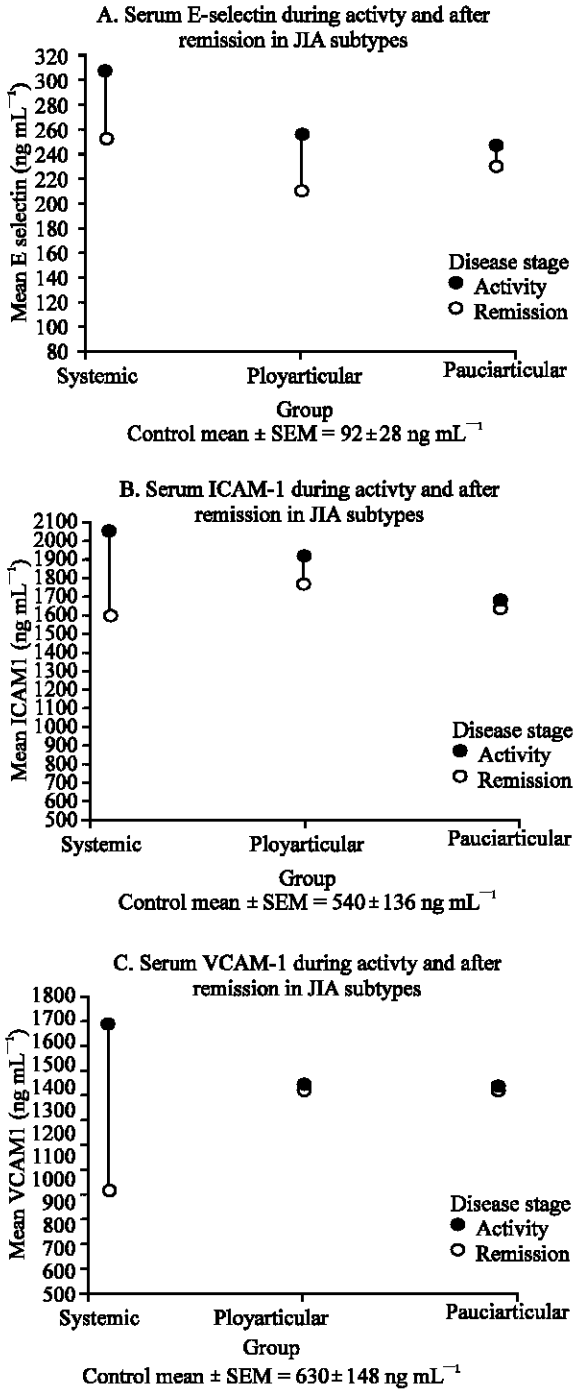

Fig. 1: Drop lines for levels of AMs in the 3 subtypes of JIA during activity and after remission; A. E selectin, B. $sICAM_1$ and C. $sVCAM_1$.

(Dolezalova *et al.*, 2002), however, synovial E-selectin was found to accurately reflect intra-synovial inflammation but serum $ICAM_1$ reflects the effects of disease-modifying drugs (Bloom *et al.*, 2002). As JIA is characterized by clinical, laboratory and histologic heterogeneity, serum and synovial AMs are higher in follicular synovitis which tends to run a more severe course compared to diffuse synovitis (Klimiuk *et al.*, 2002).

In the present work, we have found that serum E selectin in all JIA subtypes was significantly higher during activity compared to control (P_2 between control and active stage of systemic, poly- and pauci-articular JIA were <0.001, <0.001 and <0.01, respectively) with a significant drop after remission (P_1 0.004, 0.02 and 0.04, respectively) but not reaching to normal values as there was still significant difference between control and remission stage of systemic, poly- and pauci-articular JIA (P_2 were <0.001, <0.01 and <0.05, respectively). So, in all subtypes both during activity and after remission, serum E selectin was significantly higher than control (Table 2 and Fig. 1A). This might be explained by the smoldering subclinical inflammation during stage of remission (evidenced by the persistently increased soluble AMs) resulting in tendency towards recurrence.

ANOVA for difference between the 3 subtypes as regards serum E selectin during activity and after remission were statistically significant (Table 2; P_{3a} 0.005 and P_{3r} 0.02) being highest in systemic compared to the other 2 subtypes (Fig. 1A). This finding could partly explain the greater morbidity and resistance to treatment observed in systemic JIA. In agreement to our results, it has been found that serum E-selectin to be strongly correlated to synovial E selectin and could serve as a marker of aggressiveness of disease especially in active systemic disease (Dolezalova *et al.*, 2002, Bloom *et al.*, 2005).

Moreover, in our study serum $sICAM_1$ and $sVCAM_1$ showed the same changes noticed with E selectin compared to control, but no significant change in pauciarticular group after remission (Table 2; P_1 for $sICAM_1$ and $sVCAM_1$ are 0.85, 0.54, respectively); this could be explained by the poor role of these AMs in the milder forms of pauciarticular JIA. ANOVA between the 3 subtypes as regards $sICAM_1$ and $sVCAM_1$ during activity was highly significant being highest in systemic (P_{3a}<0.001, 0.03, respectively, Fig. 1B and C) with no significant difference in $ICAM_1$ after remission (P_{3r} 0.21) but $VCAM_1$ showed a marked drop after remission in systemic compared to the other 2 types (P_{3r} 0.04, Fig. 1C).

The association between clinical subtypes and certain AMs necessitates a large population and a prolonged prospective study. In a study of 16 cases of JIA, E selectin was found higher in systemic compared to the other subtypes (similar to our current results), but they did not include a control group (Bloom *et al.*, 1999). In addition, $sICAM_1$ was found higher in systemic and

polyarticular JIA compared to pauciarticular group, however there was no follow up of patients until remission (Laucella *et al*., 1999). In a six year cohort study, serum $sVCAM_1$ showed a persistent elevation in adult RA compared to control subjects. Recently, serum $ICAM_1$ was found elevated in all subtypes of JIA but serum E-selectin was elevated only in children with active systemic disease (Kolopp-Sarda *et al*., 2001).

Correlation of soluble AMs to parameters of inflammation (ESR, CRP and WBCs) revealed positive coefficients with different strength of significance being highest with E selectin than with $sICAM_1$ and $sVCAM_1$ (Table 3). Many authors reported different positive correlations between AMs and inflammatory markers thus suggesting the possibility to consider AMs as additional markers for disease activity (Dolezalova *et al*., 2002; Bloom *et al*., 2002, 2005, 1999; Aoki *et al*., 1993; Wellicome *et al*., 1993; Dolezalova *et al*., 2003). However, no association was found between serum $sICAM_1$ and disease parameters or between E selectin and sensitive markers of inflammation like ESR and CRP (Chen *et al*., 2002).

Up to our knowledge no clear solid criteria for definition of JIA remission; in our study we adopted the criteria of adult RA remission formulated by ACR (Pinalis *et al*., 1981). Thus in light of present results, ACR criteria should not be considered conclusive for JIA remission but just as a preliminary index of clinical remission. Furthermore, although AMs were correlated with some markers of disease activity like ESR, CRP and WBC, yet not correlated well with most disease variables i.e., they were not parallel to the disease course, so on cost-benefit basis these AMs could not be used in the meantime as a routine clinical biomarkers for disease activity.

We can conclude that systemic JIA showed significantly higher values of AMs during activity, thus explaining the more aggressive presentation compared to polyarticular and pauciarticular subtypes and could suggest different pathogenetic mechanisms in the 3 subtypes. The persistently elevated AMs after the apparent clinical remission (they were significantly dropped after remission but not to the normal values i.e., still higher than control), may suggest the perpetuation of mild inflammatory process thus explaining the preponderance towards recurrence in JIA.

We recommended a long standing prospective study until laboratory remission is achieved (normalized serum AMs) and to correlate changes in AMs to the clinical course. This study may add to solve the issue of using AMs as makers of disease activity and may have a therapeutic implication in using drugs modulating inflammation (Kavanaugh *et al*., 1994). Moreover, we can predict cases refractory to the conventional therapeutic modalities and offer them pulses therapy with IV cyclophosphamide with methyl prednisolone monthly for 6 months; a line which is proved beneficial (Chen *et al*., 2004).

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