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Original article

High plasma levels of adrenomedullin in collagen diseases

Background: *Adrenomedullin (ADM), a potent vasorelaxant/hypotensive peptide, was shown recently to be over-expressed in inflammatory rheumatic diseases.*

Objectives: *The aim of this study was to investigate the value of ADM as a laboratory marker of disease activity in juvenile rheumatoid arthritis (JRA) and pediatric onset- systemic lupus erythematosus (SLE) and its relation to other markers of disease activity such as clinical scores, the ESR and tumor necrosis factor-α (TNF-α).*

Methods: *The study included 24 patients with JRA, 17 with childhood onset-SLE, as well as, 19 with rheumatic arthritis and twenty clinically healthy age- and sex- matched subjects. Clinical evaluation for disease activity was performed using the clinical activity score index in JRA, and SLE-DAI in SLE. Subjects were investigated to verify the diagnosis and disease activity. Plasma ADM and serum of TNF-α levels were then assayed.*

Results*: Serum TNF-α and plasma ADM levels were significantly higher in JRA and SLE patients than in rheumatic arthritis patients and healthy controls. Though serum TNF-α and plasma ADM levels were both higher in JRA (73.88±11.6 pg/ml and 156.5±22.4 pg/ml, respectively) compared to SLE (48.82±7.5 pg/ml and 85.12±15.7 pg/ml, respectively), the difference was of statistical significance only in ADM. Both serum TNF-α and plasma ADM levels were significantly higher in systemic onset-JRA (139.75±18.5 and 260.25±28.6 pg/ml, respectively) compared to the pauciarticular-onset type (33.8±3.04 and 93.4±9.35 pg/ml, respectively), but comparable to the polyarticular onset cases (69.97±8.45 and 149.87±21.15 pg/ml, respectively). Positive correlations were noticed between plasma ADM and activity score index (r=0.72), ESR (r=0.59) and serum TNF-α (r=0.64) in JRA. The serum TNF- α was not influenced by the site of lupus activity unlike plasma ADM that was higher in subjects suffering from lupus arthritis or cardiovascular manifestations. The afore-mentioned markers correlated positively to the ESR in SLE but not to the SLE-DAI. With a cut-off value of TNF-α = 31 pg/ml and that for ADM = 80 pg/ml calculated from the results of the included rheumatic arthritis patients, ADM appeared to be a more sensitive marker of activity in JRA and SLE compared to TNF-α.*

Conclusion: *Plasma ADM was over-expressed in JRA and SLE. It correlated with the clinical and biochemical activity markers in JRA suggesting that it can be used as an indicator of disease activity. In SLE, ADM levels correlated with ESR and TNF- α levels and it could be of value in identifying patients with arthritis and cardiac involvement.*

Key words: Adrenomedullin, JRA, SLE, TNF-α, arthritis.

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INTRODUCTION

Rheumatic diseases result from abnormally regulated immune response, leading to inflammation of target organs [1]**.** The disease process is usually assessed by the so called markers of disease activity, such as the number of swollen joints, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor, in addition to radiographic evidence in JRA and by systemic lupus erythematosus-disease activity index (SLE-DAI) in SLE [2,3]. However, the role of these nonspecific markers of inflammation, as markers of disease severity in established cases, is limited, so to monitor the disease course, newer markers need to document their superiority over the usual markers [4].

Adrenomedullin (ADM) is a potent vasorelaxant / hypotensive peptide, initially isolated

and purified from the adrenal medulla and human pheochromocytoma [5]. It is a 52 amino acid peptide that shows some homology with calcitonin gene-related peptide (CGRP) and has been therefore added to the calcitonin/CGRP/amylin peptide family[6]. Studies showed that the gene is highly more expressed in endothelial cells, that this peptide has come to be regarded as a secretory peptide of the vascular endothelium, together with nitric oxide and endothelin[7]. In addition, ADM gene expression is seen in studies of other cultured cell lines e.g. leukocytes and macrophages [8].

ADM has been shown to have a remarkable range of actions, from regulating cellular growth and differentiation, through modulating hormone secretion, to antimicrobial effect[6]. ADM production in vascular smooth muscle cells is highly augmented by TNF-α, IL-1 and lipopolysaccharides (LPS) suggesting that adrenomedullin participates in local regulation of vascular smooth muscle cell function, especially in cases of endotoxin shock, atherosclerosis and inflammation [9].

Increased plasma adrenomedullin expression has been associated with a wide array of diseases including essential hypertension, acute myocardial infarction, chronic obstructive pulmonary diseases, chronic renal failure, glomerulonephritis, sepsis, Raynaud's disease and Wegner granulomatosis[6]. Recent studies have shown that the production of ADM is elevated in inflammatory rheumatic diseases. Studies reported a correlation of ADM plasma levels with clinical and biochemical markers of activity in patients with rheumatoid arthritis[10].

We sought to investigate whether or not plasma level of ADM could be considered as a laboratory indicator of disease activity in juvenile rheumatoid arthritis (JRA) and childhood onset- systemic lupus erythematosus (SLE) and its relation to clinical and other laboratory markers of activity as ESR and TNF-α.

METHODS

This study was carried out at the Pediatric Allergy and Immunology Clinic at Children's Hospital of Ain Shams University in Cairo. The study was approved by the local ethical committee and informed consents were taken from the older children or the parents before enrollment.

The study included:

- *Patients with JRA*: Twenty-four patients with JRA, fulfilling the 1987 American Rheumatism Association Revised Criteria for the diagnosis of rheumatoid arthritis (11), were included in the study. They were 6 males (25%) and 18 females (75%) with a mean age of 11.88± 0.0915 years (range 4-18 years).
- *Patients with SLE*: This group included 17 patients with SLE fulfilling the 1982 American Rheumatism Association Revised Criteria for diagnosis of SLE (12). They were 1 male (6%) and 16 females (94%) with a mean age of 13.47±1.09 years (range 5-18 years).

For comparison of our results, two groups were included:

- Patients with rheumatic arthritis: This group included 19 patients with rheumatic arthritis (mean age 9.74±0.63 years) who were diagnosed according to the modified Jones criteria[13].
- Twenty clinically healthy children, whose ages and sex were matched with the patients, served as a control group.

Exclusion criteria:

Patients with renal impairment, congestive heart failure, hypertension, diabetes mellitus or acute infections were excluded from this study.

The patients were subjected to:

1. Clinical evaluation:

A. For patients with JRA (Group I), stress was laid on the duration of disease, type of disease onset, number of affected joints, activity of arthritis, systemic manifestations and medication received. Patients were assessed for clinical parameters of joint inflammation using the summed joint index score[14,15]. For each of the clinical indices (the joint swelling, the pain on motion/joint tenderness and limitation of motion), the total articular activity for each patient was calculated, considering the affected joints only, as follows:

Activity score = -Sum of the clinical indices for each of the affected joints = x

$$\frac{\text{Sum of } x \text{ of all examined joints.}}{\text{Number of affected joints.}}$$

B. Patients with SLE (group II): Stress was laid on age, sex, signs and symptoms of SLE, duration of disease, different system involvement e.g. cardiovascular and renal affection and received medications received[12]. Renal involvement: was diagnosed by persistent proteinuria of 0.5g/day or cellular cast (red blood casts, granular, tubular or mixed). Cutaneous vasculitis was diagnosed if any of the following skin lesions were present: purpura, ulcers, subcutaneous nodules or livedo reticularis. Arthritis: was diagnosed if two or more joints showed signs of inflammation. Disease activity was measured by systemic lupus erythematosus disease activity index (SLE-DAI) score[3].

Study measurements:

Sampling:

About 5 ml of venous blood were withdrawn from each of the control groups and patients and divided as follows:

1. Clotted samples, the obtained serum (by centrifugation for 10 min at 1500 rpm) was divided into three portions, one for the assay of ANA, anti-DNA, C3 and rheumatoid factor, the second portion for estimation of kidney function test and the third one was stored at -20°C until assayed for TNF-α.
2. A blood sample, on EDTA was taken for ESR estimation; another 3ml were also withdrawn using chilled syringe and transferred into a polypropylene tube containing EDTA (1 mg/ml of blood) and aprotinin (500 KIU/ml). Centrifugation was done at 1500 rpm for 15 minutes at 4°C. The obtained plasma was stored at -70°C until assay of ADM.

Analytical Methods:

- Measurement of ESR by Westergren method.
- Serum creatinine using a modified rate Jaffe method on Synchrone CX5 system (Beckman Inst., Brea, California, USA).
- Serum ANA and anti-DNA for SLE patients detected by indirect immunofluorescent technique (IMMCO diagnosis, NY, USA)
- Rheumatoid factor by Rose Waaler test.
- TNF-α:

  Accucyte human TNF-α (CytImmune Science Inc., College park, Maryland, USA) is a competitive enzyme immunoassay (EIA) which measures the natural and recombinant forms of the cytokine [16]. Goat anti-rabbit antibodies are used to capture a specific TNF-α antibody, biotinylated TNF-α and sample/standard. The biotinylated TNF-α (competitive ligand) and samples or standard compete for TNF-α specific antibody binding sites. The assay was visualized by streptavidin alkaline phosphatase conjugate and chromogenic substrate reaction. Results were deducted from standard curve plotted on semilog paper.
- Adrenomedullin (ADM):

  Plasma samples were acidified with equal volumes of 1% trifuoroacetic acid (TFA), followed by centrifugation for 20 minutes at 1500 rpm at 4°C. At the same time equilibration of the C18 column was carried out by washing with 60% acetonitrite in 1% TFA using 1 ml once followed by 1% TFA using 3.0 ml 3 times. Plasma solution was then added. After washing the column with TFA 1% using 3.0 ml twice, the peptide was eluted slowly by the 60% acetonitrite in 10% TFA using 3.0 ml once. The eluent was collected in polypropylene tubes, then it was evaporated to dryness. Prior to assay, the residue was dissolved in RIA buffer.

  Adrenomedullin peptide was measured by radio- immunoassay technique a kit product of Peninsula laboratories (611 Talor wat, Belmont, California). The assay was based upon the competition of labeled I^{125} peptide and unlabeled peptide (either standard or unknown) binding to a limited amount of specific antibodies. As the concentration of standard or unknown in the reaction increased. The amount of the labeled peptide able to bind to the antibody decreased. The separation of free and bound antigen was achieved via a double antibody precipitation followed by decanting. By measuring amount of I^{125} peptide bound as a function of the concentration of the unlabeled peptide in standard reaction mixtures, it was possible to construct a standard curve from which the concentration of peptide in unknown sample were determined [17].

Statistical Methods:

Statistical analysis was done using a software package (SPSS) version 9.05. Quantitative variables were presented as mean (x) ± standard error of the mean (SEM). Comparison between non-parametric variables was done using Mann-Whitney U test. Correlation of different variables was done using Spearman r Correlation test. p values less than 0.05 were considered significant. To assess the diagnostic performance of both the adrenomedullin and TNF-α in cases of JRA and pediatric-onset SLE, calculation of the diagnostic sensitivity of both markers was done at cut-off levels corresponding to the 95th percentile of both markers in rheumatic patients, as they also had arthritic complains.

RESULTS

JRA patients:

The present study included 24 patients with JRA, 15 (62%) of them had a polyarticular onset, 5 (21%) had a pauciarticular onset and 4 (17%) had a systemic onset- type of JRA. The duration of the disease ranged from 1-13 years (mean 4.61±4.7 years). According to the summed joint index score, the disease activity score in our patients ranged from 0-6.25. The ESR in the studied JRA patients ranged between 5 and 100 mm/hr (mean 37.92±5.8 mm/hr). The mean value of TNF-α in the sera of JRA patients (73.88±11.60 pg/ml) was significantly

increased compared to rheumatic arthritis patients (13.42±1.8 pg/ml) and healthy controls (4.35± 0.30 pg/ml), but was not significantly different from that of SLE patients (48.82±7.5 pg/ml) (Table 1). On the other hand, the mean value of ADM in the plasma of JRA patients (mean 156.50±22.4 pg/ml) was significantly increased compared to SLE (95.12± 15.7 pg/ml), rheumatic arthritis patients (45.21± 5.98 pg/ml) and controls (17.11± 3.28pg/ml) (Table 2, Fig1).

Both serum TNF-α and plasma ADM levels were significantly higher in systemic onset-JRA (139.75±18.5 and 260.25±28.6 pg/ml, respectively) compared to the pauciarticular-onset type (33.8±3.04 and 93.4±9.35 pg/ml, respectively), but not significantly different from the polyarticular onset cases (69.97±8.45 and 149.87±21.15 pg/ml, respectively). Similar to TNF-α, the mean ADM level was not significantly different in pauciarticular onset as compared to polyarticular onset cases (Tables 3 and 4).

Plasma ADM could be positively correlated to the activity score index, and ESR in JRA patients was found. Furthermore, our study demonstrated a highly significant positive correlation between plasma ADM and serum TNF-α (Fig 2 and 3).

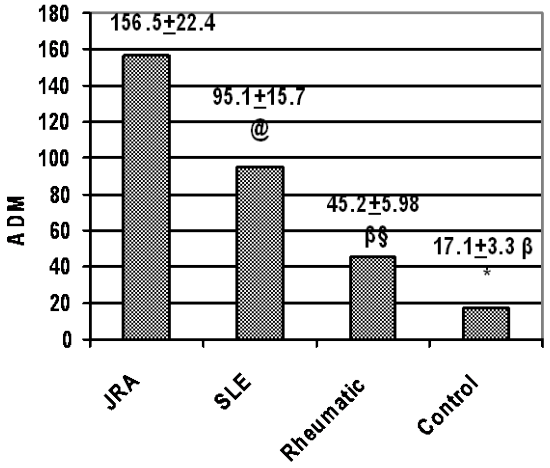
Pediatric-onset SLE patients:

Seventeen patients with pediatric-onset SLE were included in the present study; the duration of the disease ranged from 2-9 years (mean 4.94±5.1 years). Six of our patients were diagnosed as having lupus nephritis. Eight patients had cardiovascular disorders; four had pericardial affection, one had wall motion abnormality and 2 patients showed endocardial lesions, all of which were detected by echocardiogram, also one patient had ectasia of the left coronary system detected by coronary angiography. Six patients had cutaneous vasculitis in the form of purpura, ulcers and livedo reticularis

According to the SLE-DAI, the indices of our patients ranged from 2 to 16. Their ESR values ranged between 5 and 100 mm/hr (mean 38.82±7.54 mm/hr), their TNF-α between 8 and 124 pg/ml (mean 48.82±7.43 pg/ml) and their ADM ranged between 13 and 250 pg/ml (mean 95.12±15.76 pg/ml).

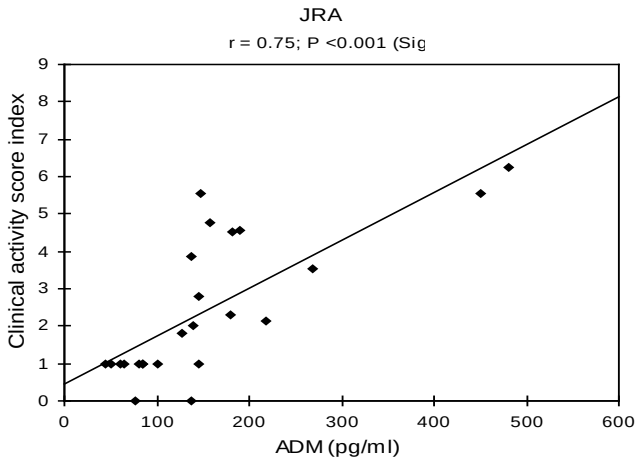
The mean values of serum TNF-α did not vary significantly in SLE with arthritis, CVS disorders or lupus nephritis (mean= 57.5±9.2, 54.0±8.6, and 63.0±7.5 pg/ml, respectively) in comparison to cases not suffering from these disorders (mean of 44.09±6.4, 44.22±6.4, and 39.0±6.9 pg/ml, respectively). On the other hand, the mean values of plasma ADM were significantly higher in SLE patients with arthritis and CVS disorders (mean= 149.5±15.8 and 128.63±16 pg/ml, respectively) compared to SLE patients without such involvement (mean= 65.45±10.5 and 65.33±12.08 pg/ml, respectively) (Figure 4). Though serum TNF-α and ADM levels were found to correlate positively to ESR in SLE patients (r=0.52 and 0.62, respectively), and to one another (r=0.74), no such correlation could link it to the SLE-DAI.

Based on results of the rheumatic patients, cut-off values of 31 pg/ml for TNF-α and 80 pg/ml for ADM were chosen corresponding to 95th percentile. The calculated diagnostic sensitivity was 62.5% for TNF-α compared to 75% for ADM in JRA. While the diagnostic sensitivity was 100% in systemic-onset JRA and 73.3% in polyarticular-onset for ADM, it was 75% and 66.6%, respectively for TNF-α. In SLE patients, the diagnostic sensitivity was 52.9% for TNF-α, and 70.6% for ADM. Furthermore, the diagnostic sensitivity of ADM was more than 80% in SLE patients who had nephritis, arthritis or cardiovascular system involvement (Table 5).



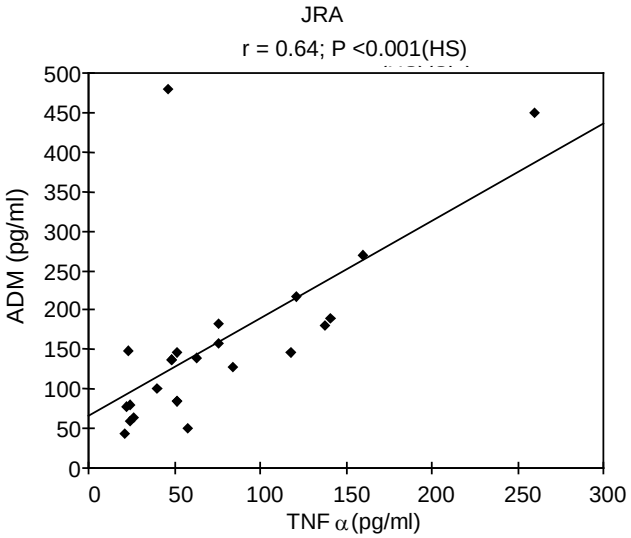
ADM: adrenomedullin; JRA: juvenile rheumatoid arthritis; SLE : systemic lupus erythematosus.
@ p< 0.05 in comparison to the mean value of JRA patients
β p< 0.01 in comparison to the mean value of JRA patients
§ p< 0.05 in comparison to the mean value of SLE patients
* p< 0.001 in comparison to the mean value of SLE patients.

Fig. (1): Comparison of mean values of plasma ADM in the studied groups



r= 0.75; p< 0.001 (HS)

Fig. (2): Positive correlation between plasma ADM levels and the activity score index in JRA



r= 0.64; p< 0.001 (HS)

Fig. (3): Positive correlation between serum TNF-α and plasma ADM levels in patients with JRA

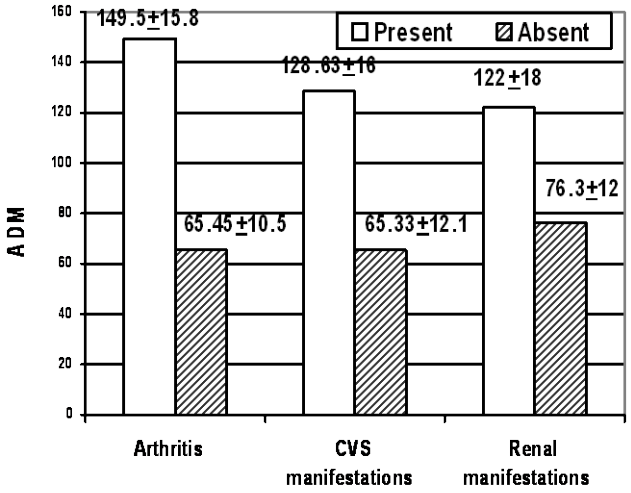


Fig. (4): Variation of mean values of plasma ADM in SLE patients according to systemic involvement

Table (1) : Comparison of mean values of serum TNF-α in the patients and controls.

TNF-α (pg/ml)	**Mean±SEM**	**Z***	**P***	**Z****	**P****
JRA	73.88±11.60				
SLE	48.82±7.5	1.44	>0.05 (NS)		
Rheumatic arthritis	13.42±1.8	5.15	<0.001 (HS)	4.15	<0.001
Controls	4.35±0.30	5.66	<0.001 (HS)	5.18	<0.001

JRA: juvenile rheumatoid arthritis; SLE : systemic lupus erythematosus; TNF-α: tumor necrosis factor-α. * In comparison to JRA patients. ** In comparison to SLE patients.

Table (2): Comparison of mean values of plasma ADM in the patients and controls.

ADM (pg/ml)	Mean±SEM	Z*	P*	Z**	P**
JRA	156.50±22.4				
SLE	95.12±15.7	2.25	<0.05 (Sig.)		
Rheumatic arthritis	45.21±5.98	4.73	<0.01 (HS)	2.47	<0.05
Controls	17.11±3.28	5.56	<0.01 (HS)	4.53	<0.001

ADM: adrenomedullin; JRA: juvenile rheumatoid arthritis; SLE : systemic lupus erythematosus. * In comparison to JRA patients. ** In comparison to SLE patients.

Table (3): Comparison of mean values of serum TNF-α in patients with different JRA subtypes.

TNF-α (pg/ml)	Mean±SEM	Z value*	p*	Z value**	p**
Systemic-onset	139.75±18.5				
Polyarticular-onset	69.97±8.45	1.85	>0.05		
Pauciarticular-onset	33.80±3.04	2.45	<0.05	1.83	>0.05

JRA: juvenile rheumatoid arthritis; TNF-α: tumor necrosis factor-α. * in comparison to systemic-onset JRA. ** in comparison to polyarticular- onset JRA.

Table (4): Comparison of mean values of plasma ADM in patients with different JRA subtypes.

ADM (pg/ml)	Mean±SEM	Z value*	p*	Z value**	p**
Systemic-onset	260.25±28.6				
Polyarticular-onset	149.87±21.15	1.80	>0.05		
Pauciarticular-onset	93.40±9.35	2.20	<0.05	1.57	>0.05

ADM: adrenomedullin; JRA: juvenile rheumatoid arthritis.* in comparison to systemic-onset JRA. ** in comparison to polyarticular- onset JRA.

Table (5): The diagnostic sensitivity of TNF-α and ADM in JRA and SLE patients at the chosen cut off values (95% specificity).

Cut-off value : TNF-α = 31 pg/ml		**ADM = 80 pg/ml**	
JRA = 62.5% (15/24)		**JRA : 75% (18/24)**	
Systemic – onset JRA	75% (3/4)	Systemic– onset JRA	100% (4/4)
Polyarticular–onset JRA	66.6 (10/15)	Polyarticular–onset JRA	73.3%(11/15)
Pauciarticular – onset JRA	40% (2/5)	Pauciarticular– onset JRA	60% (3/5)
SLE = 52.9% (9/17)		**SLE = 70.6% (12/17)**	
Arthritis	66.67% (4/6)	Arthritis	83.3% (5/6)
Nephritis	71.4% (5/7)	Nephritis	85.7% (6/7)
CVS	50% (4/8)	CVS	87.5% (7/8)

DISCUSSION

TNF-α serum levels in JRA patients were significantly increased compared to those in rheumatic patients and normal healthy controls. Our results support the previously reported role of TNF-α as an important mediator of immunity and inflammation. Because of its biological activities (activation of neutrophils, release of arachidonic acid metabolites from synovial cells, induction of cartilage resorption and inhibition of proteoglycan release in cartilage), it is considered one of the potential mediators of chronic inflammation in rheumatoid arthritis [18]. Borzi et al. [18], evaluated the serum levels of TNF-α in patients with RA, SLE and progressive systemic sclerosis (PSS) and reported much higher TNF-α serum levels in patients with RA when compared to normal controls and to other connective tissue disease patients. They stated that TNF-α is one of the potential mediators of chronic inflammation in RA patients, and suggested that TNF-α played a central role in the pathogenesis of RA. Similarly, a study done by Altomonte and his coworkers[19] revealed high levels of serum TNF-α in RA patients compared to matched healthy controls.

Although the mean TNF-α level seemed higher in our JRA patients compared to SLE patients, the difference did not reach statistical significance. In agreement with our results, Maury and Teppo[20], in their study, reported that serum TNF-α levels in SLE patients were lower than in RA patients, but the difference was not statistically significant. On comparing the TNF-α expression in different JRA subtypes, the highest levels of serum TNF-α were observed in systemic onset-JRA followed by the polyarticular- onset and pauciarticular onset-types

with significantly higher levels in systemic- onset compared to pauciarticular onset-JRA. Our findings are in agreement with the results of Mangge et al.,[21] who studied IL-1 beta, IL-2 and soluble IL-2 receptors and reported that systemic JRA showed the most significant elevations of plasma cytokines, followed by the polyarticular and pauciarticular varieties. Similar results were reported by Altomonte et al.[19], who stated that serum IL-1 beta and TNF-α appear to correlate with systemic inflammation, and systemic features of RA may result from dissemination of cytokines produced in the synovium

These findings were explained by Moore [22] who evaluated the interrelation of Th1/Th2 immune response in the immunopathogenesis of JRA and their effect on cytokine release. They indicated a proinflammatory response in systemic onset-JRA manifested by increased secretion of IL-6, while an anti-inflammatory response has been noted in the pauciarticular-onset type evident by the increase of IL-4 mRNA and IL-10 mRNA.

In the current study, the ADM plasma levels were highest in patients with JRA followed by SLE, rheumatic arthritis and healthy controls. Yudoh et al.[10], demonstrated that plasma ADM expression was significantly higher in patients with RA than SLE, polymyositis/dermatomyositis, mixed connective tissue disease, osteoarthritis and normal controls. A recent study by Matsushita et al.[23], reported the presence of ADM mRNA in synovial cells from rheumatoid patients. Similar to our findings in TNF-α, the highest mean value of plasma ADM was seen in systemic onset-JRA followed by polyarticular onset-JRA with a statistically significant difference between systemic onset and pauciarticular onset-types. Our data come in accordance to the conclusions of Sugo et al.[24] , and Dilorio et al.[25], who noted that ADM production in vascular smooth muscle cells is highly augmented by TNF-α, IL-1 and lipopolysaccharide.

Plasma ADM was found to correlate with clinical (summed activity score index) and biochemical (ESR, TNF-α) markers of activity in our JRA patients, which suggests that it can be an important indicator of disease activity. Similarly, Yudoh et al.[10] reported a positive significant correlation between plasma ADM level and joint activity score, serum TNF-α and other laboratory measures of inflammation (fibrinogen, haptoglobin, C-reactive protein, α-1-antitrypsin) in JRA patients. Hence, it may be concluded that serum ADM level can be used as a marker of disease activity in RA patients.

Serum TNF-α in our SLE patients showed no significant difference in subjects with or those without arthritis, CVS disorders or lupus nephritis. Similarly, Al-Jandi et al.[26], studied TNF-α levels in SLE patients with nephritic involvement and found no significant difference compared to the rest of patients. Concerning the plasma ADM levels in our series, the highest mean plasma level of ADM was observed in SLE patients with arthritis followed by SLE with cardiovascular disorders then SLE with lupus nephritis. Also, SLE patients with CVS and arthritis were found to have significantly higher ADM levels as compared to SLE patients without these disorders. This may be explained by the fact that ADM is directly involved in the pathogenesis of these two conditions. ADM secretion, especially in cardiovascular tissues, is regulated by mechanical stressors such as shear stress, and lipopolysaccharide, hormones as angiotensin II and metabolic factors as hypoxia or ischemia. Elevation of ADM due to overproduction in response to any of these stimuli in pathological conditions may explain the rise in their levels in cardiovascular diseases[27,28]. ADM was not significantly different in SLE patients with lupus nephritis compared to those without. However, in 1998, Kubo and colleagues demonstrated that chronic glomerulonephritis was associated with high levels of ADM[8]. Cheung et al.[29], noted that ADM, lowers systemic vascular resistance and acts as a natriuretic and diuretic peptide. These properties resemble those of atrial natriuretic peptide which has an important role in the progression of renal disease. Larger scale studies are needed to verify the findings.

The plasma ADM levels were found to correlate significantly with ESR and TNF-α levels, but not with SLE-DAI. In accordance to our results, Yudoh and his colleagues[10] and Cheung et al.[29], revealed a significant correlation between ADM and TNF-α levels in SLE patients and a poor correlation between ADM and SLE-DAI and biochemical markers (fibrinogen, IL-6, C-reactive protein, α-1-antitrypsin). While the rise of ADM could be secondary to the rise in inflammatory cytokines as TNF-α[29], it is unclear why ADM levels correlated with disease activity only in JRA.

TNF-α is a well established marker for disease activity in RA as suggested previously by many researchers [22, 30], so trying to evaluate the usefulness of ADM in comparison to TNF-α, we chose a cut-off value depending on the values of the rheumatic arthritis patients involved, and compared the

diagnostic sensitivity for both. With the cut off values of 31 pg/ml for TNF-α and 80 pg/ml for ADM, the diagnostic sensitivity in JRA was 62.5% for the former compared to 75% for the latter. While the diagnostic sensitivity of ADM was 100% in systemic-onset JRA and 73.3% in polyarticular-onset, it was 75% and 66.6%, respectively for TNF-α. In SLE patients, the diagnostic sensitivity was 52.9% for TNF-α and 70.6% for ADM. Furthermore, the diagnostic sensitivity of ADM was more than 80% in SLE patients with nephritis, arthritis or cardiovascular system involvement. No similar data could be traced in the literature for comparison .

In conclusion, plasma ADM levels were significantly higher in patients with JRA and SLE compared to rheumatic patients and healthy controls. It is suggested that ADM may be directly involved in arthritic changes among those patients. Plasma ADM levels correlated with the summed clinical activity score index and biochemical activity markers in JRA suggesting that it can be used as an indicator of disease activity in JRA. In SLE, ADM expression correlated with ESR and TNF- α and it might be useful in the evaluation of patients with systemic involvement such as those with arthritis and cardiac disorders. The prognostic value of ADM levels in rheumatologic diseases needs to be assessed in larger scale prospective studies.

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