

Updates from medicine

Serum angiopoietin-2 level as a novel biomarker in atopic dermatitis

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Abstract

Background Atopic dermatitis (AD) is a chronic, pruritic inflammatory skin disease. Angiopoietin is an intrinsic mediator induced by T helper 2 cytokines and has been reported to play a critical role in the pathogenesis of AD.

Objectives To determine the relationship between circulating angiopoietin-2 (Ang-2) in AD patients and to determine its correlation with the severity and chronicity of the disease.

Methods The present study included 38 AD patients and 38 healthy controls, age- and sex-matched. We collected 3 mm of venous blood from each subject. Enzyme-linked immunosorbent assay (ELISA) determined serum angiopoietin. We determined the correlation between serum Ang-2 level, duration of disease, severity, type of atopy, and scoring atopic dermatitis (SCORAD) score.

Results There were highly significant differences between patients and controls with regard to serum Ang-2 levels ($P < 0.001$). There were significant differences between severe AD patients with serum Ang-2 levels with an increasing level of degree severity ($P < 0.001$). There were significant differences in serum Ang-2 levels among widespread type ($P < 0.03$). There was a positive significant correlation between Ang-2 levels and both duration and SCORAD score of the patient group.

Conclusion Serum Ang-2 levels may correlate with disease severity, chronicity in patients with AD, and serve as a potential biomarker of the disease severity.

Introduction

Atopic dermatitis (AD) is a chronic, inflammatory skin disease characterized by the defective skin barrier and allergic skin inflammation with dominant T helper-2 (Th2) type immune response. The etiology of AD is not fully understood, but evidence has revealed a complex involvement of both innate, adaptive immune responses, and environmental factors with the activation of keratinocytes.¹

Altered angiogenesis is under evaluation in common inflammatory skin disorders as an AD. It is possible that specific inhibitors of various mediators and receptors (Tie-2, etc.) controlling angiogenesis may offer novel strategies for dealing with the treatment of microvascular changes in AD.²

Angiopoietin is part of a family of vascular growth factors. They are a family of seven secret glycoprotein ligands, angiopoietin 1–7, originally identified as important with blood vessel formation. The best characterizations of these ligands are angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). Ang-2 is expressed by endothelial cells and acts as an antagonist for receptor Tie-2. Ang-2 has been reported to completely disrupt protective Tie-2 signaling.^{3,4}

Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) were identified as ligands of the Tie-2 (Tek) receptor, a novel endothelial-specific receptor tyrosine kinase.⁵ Ang-1 induces Tie-2 autophosphorylation as a receptor activator, whereas Ang-2 blocks Tie-2 signaling as a naturally occurring antagonist of Ang-1.⁶

The aim of this study was to estimate the serum Ang-2 level in AD patients and to determine the correlation between this level and severity and chronicity of AD.

Patients and methods

Patients

The study was a case-control study. It included 38 patients (16 male and 22 female) with AD ranging in age from 3 to 18 years, and 38 healthy subjects, age- and sex-matched, enrolled as controls. We recruited patients from the dermatology outpatient clinics at Zagazig University Hospital. The diagnosis was determined on each patient based on history and Hanifin and Rajka criteria.

Patients took no systemic treatment when recruited. A written informed consent was obtained from patients and controls. Ethical clearance was obtained before the commencement of this study.

Inclusion criteria were: patients had AD through at least 2 years before the start of the study, any age and any gender.

Exclusion criteria were patients: with active or history of asthma or allergic rhinitis, with other chronic dermatological diseases, with AD less than 2 years before the start of the study, with treatment within the previous 3 months of the study, with a bleeding tendency, with fever, and with active viral, bacterial, or fungal infections.

All patients were subjected to complete history taking, a general examination of body systems, detailed dermatological examination, measurement of AD severity by scoring atopic dermatitis (SCORAD) score (<25 mild, 25–50 moderate, >50 severe), and measurement of serum Ang-2 level by specific enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Enzyme-linked immunosorbent assays

We collected venous blood samples into pyrogen-free tubes at the time of clinical assessment. They could clot at 4 °C for 1 h and centrifuged at 2000 *g* for 10 min. They stored the samples of sera at 70 °C. The serum was randomly coded, and we carried out testing without the knowledge of the clinical status of the subject or any related laboratory data.

ELISA sets were performed according to the manufacturer's protocols. The ELISA Duo Sets (R&D Systems, Minneapolis, MN, USA) were used for the measurement of Ang-2. We did all the measurements in duplicates. The measurement of absorption was done at 492 nm. Assay sensitivity was 9.0 pg/ml for Ang-2

Statistical analysis

After data collection, the data were tabled and analyzed using SPSS-19 (IBM Corp, Armonk, NY, USA). The following tests were used: a Chi-squared (χ^2), Student *t*-test, Spearman's correlation coefficient (*r*), and one-way ANOVA. Differences were considered statistically significant (S) when $P < 0.05$, highly significant (HS) when $P < 0.001$, and nonsignificant (NS) when $P > 0.05$. Pearson correlation coefficient was used to calculate the correlation between quantitative variables. A $P < 0.05$ was being signed.

Results

The present study included 38 AD patients with a mean age (9.64 ± 6.24). We divided the patients into 24 females (61.1%) and 14 males (38.9%). Controls included 38 people with a mean age (7.03 ± 3.33). We divided controls into 19 females (50%) and 19 males (50%). There were no significant differences between patient and control in age or sex distribution (Table 1).

The mean duration of the disease among the patient group was (7.36 ± 3.67), and mean SCORAD score was 52.1 ± 18.24 (Table 2). The localized type was in 29 patients (80.6%), while the widespread type was in seven (19.4%).

Mean serum Ang-2 level of the patients was (170.14 ± 38.23), while in controls was (60.39 ± 13.76). There was a highly significant difference between patients and controls in serum Ang-2 level ($P < 0.001$) (Table 3). There was no significant difference between males and females of the patient group in the serum Ang-2 level ($P = 0.27$). According to the SCORAD score of disease severity, there were highly significant differences between disease severity, with an increased level among patients with severe disease ($P < 0.001$) (Table 4).

With regard to the extent of the disease (localized in certain part, such as back, chest, face, head, and limbs, and spread in more than one area), there were significant differences between spread and localized AD patients with serum Ang-2 levels with increasing level among widespread patients ($P = 0.03$). There was no significant difference between localized types ($P = 0.71$) (Table 5).

There was a negative correlation between serum Ang-2 level and age, while there was a highly positive significant correlation

Table 1 Demographic data of the two studied groups

Variable	Patients (n = 38)		Controls (n = 38)		MW	P
Age: (year)						
Mean $\pm$ SD	9.64	$\pm$ 6.24	7.03	$\pm$ 3.33	498.5	0.09
Range	3–18		3–15			NS
Variable	No	%	No	%	χ^2	P
Sex						
Female	24	61.1	19	50		0.34
Male	14	38.9	19	50	0.90	NS

Table 2 Clinical data of the patients group

Variable	n = 38
Duration: (year)	
Mean $\pm$ SD	7.36 $\pm$ 3.67
Range	2–15
SCORAD score	
Mean $\pm$ SD	52.1 $\pm$ 18.24
Range	15–92

Table 3 Serum angiopoietin-2 level of the two studied groups

Variable	Patients (n = 38)	Controls (n = 38)	t	P
Angiopoietin-2				
Mean $\pm$ SD	170.14 $\pm$ 38.23	60.39 $\pm$ 13.76	16.21	<0.001**
Range	105–251	42–93		HS

**HS, highly significant; t, Student's t-test

Table 4 Relation between serum angiopoietin-2 level and severity of atopic dermatitis of the patient group

Variable	Angiopoietin (n = 38)		t	P
	Mean	SD		
Severity				
Mild	115.6	12.31	F	
Moderate	153.12	20.28		<0.001**
Severe	206.47	19.06	54.66	HS

**HS, highly significant; SD, standard deviation; t, Student's t-test

Table 5 Relation between serum angiopoietin-2 level and atopic dermatitis type in the patient group

Variable	Angiopoietin (n = 38)		t	P
	Mean	SD		
Type				
Widespread (n = 7)	198	9.09	T	0.03*
Localized (n = 29)	163.41	39.61	2.27	S
Back	170	24.27		
Chest	130	35.36		
Face	134.50	13.134		0.71
Head	148	15.47	F	NS
Limbs	169.75	48.56	0.59	
Prurigo	167.20			

F, analysis of variance (ANOVA); NS, nonsignificant; *S, Significant; SD, standard deviation; t, student's t-test.

between serum Ang-2 levels and SCORAD score ($P < 0.001$). There was a positive significant correlation between serum Ang-2 levels and duration of disease ($P = 0.04$) (Figs. 1a, b, and 2).

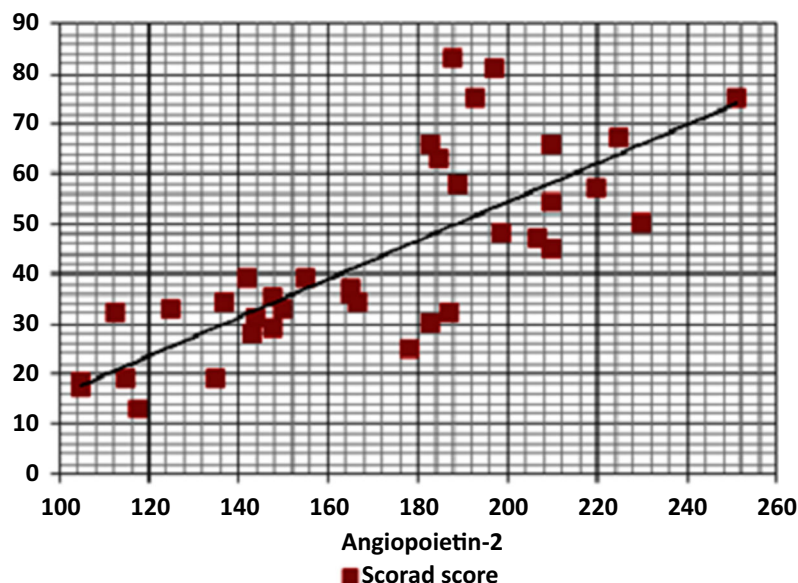
Discussion

Atopic dermatitis is a chronic inflammatory skin disease and reported as persistence of allergic skin inflammation resulting from Th2 cytokine, which induces Ang-2.^{7,8} Ang-2 is an intrinsic mediator that has a role in keratinocyte activation and amplifying the inflammation. It was discovered as a novel mediator in allergic diseases.⁹

To our knowledge, this is the first study done to determine the relationship between circulating Ang-2 in AD patients and its correlation with the severity and chronicity of the disease.

In the present study, the mean serum Ang-2 level of the patients was (170.14 ± 38.23) compared to (60.39 ± 13.76) in the controls. There was a highly statistically significant difference between patients and controls in serum Ang-2 level ($P < 0.001$).

Bourdeau *et al*¹⁰ hypothesized that atopic reaction to a mouse model was driven by deregulation of the Tie-2 signaling pathway and that increased Ang-2 levels can aggravate it and reverse by Ang-1 overexpression. Increased Ang-2 seems to be a crucial proangiogenic cytokine in patients with asthma characterized by repeated exacerbations and Ang-2 serum levels (4.9 ± 0.35 vs. 1.38 ± 0.14 ng/ml; $P < 0.0001$), and can serve as a biomarker of severe asthma.¹¹ Also, Lee *et al*¹² found that plasma Ang-2 levels were 23.96–1.38 pg/mg in patients with bronchial asthma compared with 36.8–4.46 pg/mg in healthy controls ($P = 0.010$), and circulating angiopoietin could be a

**Figure 1** Serum angiopoietin 2 levels of the two studied groups

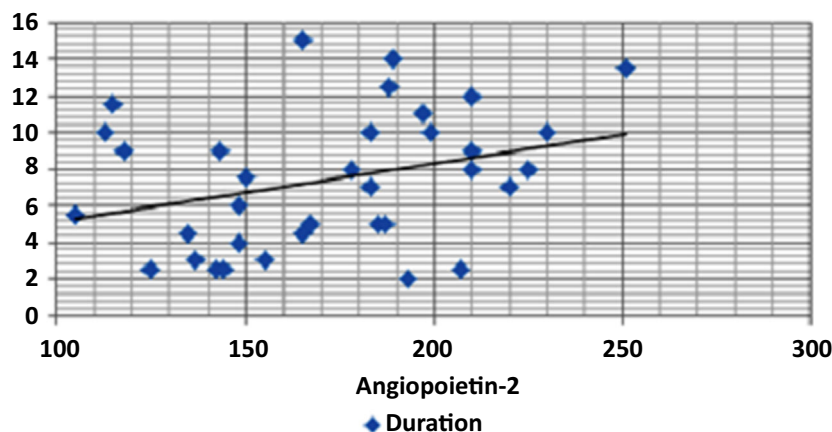


Figure 2 Relation between serum angiopoietin 2 level and severity of atopic dermatitis of the patients' group

useful marker for asthma exacerbation. In agreement with these results, Samochocki *et al*¹³ studied serum levels of vascular endothelial growth factor (VEGF), which is an angiogenic growth factor, as they strongly expressed Ang-2 in the AD more so than in the control group. VEGF concentrations correlated with the severity of AD as expressed by SCORAD score and objective SCORAD.

Takahashi *et al*¹⁴ showed that Ang-2 is unregulated, not only in the local skin lesions but also in the systemic circulation, and that its serum levels correlate well with disease severity in psoriasis vulgaris patients.

In the current study, mean of serum Ang-2 level in mild patients was (153.12 ± 20.28) ng/ml, while in severe AD patients it was (206.47 ± 19.06) ng/ml. There were highly significant differences in serum Ang-2 level between mild, moderate, and severe patients ($P < 0.001$).

In the present study, mean serum Ang-2 level in widespread type was 198 ng/ml compared to 163.41 in localized type; there were significant differences between widespread and localized patients at a serum Ang-2 level ($P < 0.03$) with an increased level among widespread patients.

In the present study, there was a highly positive significant correlation between serum Ang-2 levels and SCORAD score ($P < 0.001$). There was a positive significant correlation between serum on two levels and duration of the disease ($P = 0.04$).

Discovering the role of Ang-2 in the mechanisms underlying tissue remodeling may lead to novel therapeutic strategies against chronic allergic inflammatory diseases soon.

Serum Ang-2 levels appear to be a novel potential clinical biomarker that can predict the efficacy of treatment for asthma patients with inadequately controlled disease despite glucocorticoid therapy.¹⁵ The small number of patients limited the present study. We could not determine how our findings may be useful for patients with intractable AD. Over 77% of the AD subjects had a mild AD, and the overall severity of AD was mild; thus,

we only had a few patients with severe AD. Further larger studies are recommended to confirm the use of serum Ang-2 level as a biomarker in the pathogenesis of AD.

Conclusion

In conclusion, we correlate serum Ang-2 level with disease severity and chronicity in patients with AD. Our results provide suggestions for mechanisms involving Ang-2 in the pathogenesis of AD. Furthermore, we require studies with a large number to definitively ascertain the role of Ang-2 in AD and its role in the treatment.

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