New markers of disease activity in children with atopic dermatitis

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Abstract: Atopic dermatitis (AD) is a chronic inflammatory skin disease with 2 phases, acute and chronic.. Recent studies had shown that inducible nitric oxide synthetase (i NOS) is expressed in the atopic skin lesion, suggesting the involvement of nitric oxide (NO) in the skin inflammation of AD. Soluble E-selectin (sE-selectin) adhesion molecules expressed on endothelial cells can regulate leukocyte migration and shed into the circulation. Their level in the serum is known to reflect the degree of systemic inflammation, and therefore be used as a marker of inflammations and activity. Therefore, the aim of this study was to evaluate the role of serum nitrate and sE-selectin concentrations in relation to severity and clinical course of atopic dermatitis in children. Serum Nitrate and sE-selectin levels were assessed in 40 patients with AD (24 males and 16 females) aged 8-84 months and 16 healthy children as controls (9 boys and 7 girls) aged 6-60 months by ELIZA. Serum nitrate and sE-selectin concentrations in patients with AD were significantly increased as compared to non-atopic controls ($P<0.001$) and there were also significant differences between subgroups of AD (mild, moderate and severe) as compared to controls and among subgroups themselves. The levels of both markers were significantly diminished after treatment of severe cases of AD. Significant correlations were present between serum nitrate levels, sE-selectin levels, disease activity and eosinophilic count, but no correlation was found between serum nitrate and sE-selectin levels. Our results indicate that NO and Soluble E-selectin may be involved in the pathogenesis of AD skin and could be used as indicators of disease severity and activity

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Introduction:

Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting about 15% of children, with consequent costs and morbidity. The AD has its onset during the first 6 months of life in 45%, in the first year of life in 60% and before 5 years in at least 85% of affected children (1, 2). The criteria proposed by Hanifin more than 20 years ago, still maintain their validity for diagnosing the disease (3). In addition, the severity score atopic dermatitis (SCORAD) index provides a standardized and reproducible method to quantify the disease's severity (4). Nowadays, the exact role of allergy/atopy in the pathogenesis of AD is still controversial, but it is true that the immunological mechanisms underlying the disease (5) somewhere resemble those of other allergic disorders. It is also well-known that many children with AD are sensitized to inhalant/food allergens, so that AD is called extrinsic. (6)

Nitric oxide (NO) has been found to be involved in inflammation, vasodilatation and oxidative damage to cells and tissues of atopic dermatitis skin.(7,8) The direct measurement of NO is difficult due to its very short half-life in plasma.(9) NO reacts readily with molecular oxygen to yield nitrite and nitrate. NO, like nitrite is oxidized very rapidly to nitrate by oxyhemoglobin.(10) Fortunately, nitrate is stable and detectable in the serum.(11) Recent studies about AD and serum nitrate levels have been reported.(12,13)

Adhesion molecules and their ligands play a major role in endothelial–leukocyte interactions which affect the binding, transmigration and infiltration of lymphocytes and mononuclear cells during inflammation, injury, or immunological stimulation.(14). E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) are such adhesion molecules. After they are expressed on endothelial cells, they are released into the circulation through shedding or by proteolytic mechanisms (15). Consequently, soluble forms of these adhesion molecules can be detected in the systemic circulation. In vitro studies show that the level of soluble adhesion molecules correlates with the concentration of adhesion molecules expressed on the endothelial cells. (16) These data suggested that the level of systemic inflammation could be measured by the level of soluble adhesion molecules in the blood. (17, 18)

This study was aimed to evaluate serum nitrate and sE-selectin levels for assessment of disease severity and clinical course atopic dermatitis in children.

Subjects and Methods:

This study was conducted in the departments of Pediatrics, Dermatology & Andrology and Clinical Pathology, EL-Minia University Hospital, during the period from January 2010 to December 2010. Fifty six infants and children were included in this study. They were 40 patients with AD (aged 8-84 months: 24 boys and 16 girls) and 16 healthy non-atopic child (aged 6-60 months: 9 boys and 7 girls). The diagnosis was based on the criteria of Hanifin. None of the studied patients was febrile, had an infection or was receiving systemic steroids, anti-inflammatory medications, anti-allergic medications or anti-histamine at the time of sampling of blood (base line). The patients with atopic dermatitis were divided into three subgroups depending upon the severity of the atopic dermatitis according to Rajka's grading (19) and Kiamata scoring system, that is mild (16 patients, skin score 0-8), moderate (14 patients, skin score 9-16), and severe (10 patients, skin score (17-24).(20)

Patients with mild and moderate disease were received topical corticosteroids ointment and oral antihistaminic (citrizine dihydrochloride, 0.5-1mg/kg/day), While severe cases received systemic corticosteroids (prednisone, 1-2mg/kg/day) in addition.

A specimen of peripheral venous blood (3ml) was collected to determine peripheral eosinophilic counts, serum nitrate and sE-selectin levels. After centrifugation, the serum was stored immediately at -70°C until analysis.

The following investigations were done for all patients and controls:

1. Peripheral eosinophilic count: peripheral blood leukocytes were counted in a coulter counter, and the eosinophilic percentage was evaluated after May-Grunwald staining. Data were expressed as range and median ($x10^6$/L).
2. Serum nitrate was measured by an enzymatic one step assay with nitrate reductase (Boehranger Manheim) as described by Bories. (21)
3. Serum sE-selectin was measured by sandwich ELISA according to the instructions provided by the manufacturer (R&D Systems Inc., Minneapolis, Minn.).(22)

Statistical Methods:

After collection of data, they were added and entered into a personal computer. Analysis of the data was done using SPSS (Statistical Package for the Social Sciences). The following statistical tests were used:

1. Mean and standard deviation (SD) to describe quantitative data's
2. Student t test was used to compare between two groups as regards parametric data.
3. Chi-square test was used to compare between two groups as regards non-parametric data.
4. Pearson correlation was used to correlate two quantitative variables.

For all tests, a probability (p) of less than 0.05 was considered significant.

Results:

Fifty- six infants and child were included in the study and the results were shown in the following tables;

Table (I); showed the characteristics of studied subjects. Patients with mild disease (skin score 0-8) were16 (40 %) child, aged from 8-72 months and were 10 males and 6 females), while patients with moderate disease (skin score9-16) were 14 child (35% of patients), age from 10-66 months and were 6 females and 8 males), and 10 patients with severe disease (skin score 17-24) aged from 9-84 months and were 7 females and 3 males. As regard serum Nitrate, sE-selectin and peripheral blood eosinophils levels in the patients were ranged from (14-225 mmol/L), (122-2722 ng/ml) and (0.39-2.78 $x10^6$/L) respectively while that of the control were ranged (8-40mmol/ L), (25-512 ng/L) and (0.05-0.3$x10^6$/L).

Table II; showed a significant increase of serum nitrate and sE-selectin concentrations in patients with AD as compared to non-atopic controls {85.62 $\pm$ 20.12 vs 20.2 $\pm$ 7.61) for serum nitrate and 650.43 $\pm$ 153.1 vs 122.65s $\pm$ 70.16) for sE-selectin}. (P <0.001)

Table III; showed a highly significant increase in serum nitrate and sE-selectin levels especially in moderate and severe cases compared to control group (p value were <0.001 and 0.000) and also among the three grades themselves (P <0.001 for both markers).

Table IV shows a significant decrease of serum nitrate and sE-selectin levels after treatment of 10 cases with severe AD {36.2 $\pm$ 15.6 vs 130.3 $\pm$ 60.7)(P <0.002) and (350.65 $\pm$ 112.4 vs 1180.46 $\pm$ 515.2) (P <0.001) respectively.

Table V shows a positive correlation between serum nitrate levels and grades of skin scores (disease activity) (r=0.69, P<0.05), and also with eosinophilic count (r=0.71, P<0.001). There was a positive correlation between sE-selectin levels and disease activity (r=0.73, P<0.01), and also with eosinophilic count (r=0.63, P<0.05). A positive correlation was reported between eosinophilic count and disease activity (r=0.721, P<0.01), but no correlation was reported between serum nitrate and sE-selectin levels (r=0.353, P>0.05).

Table I: Characteristics of studied subjects

	Number	**Age** (months)	**Sex** (F/M)	**Serum nitrate** (mmol/L)	**Serum E-selectin** (ng/ml)	**Eosinophils** ($x10^6$/L)
1-Controls	16	6 – 60*	7/9	8-40* (26)**	25-512* (123)**	0.05-0.3* (0.12)**
2- Cases of AD	40	(18)**	24/16	14-225 (95)	122-2722 (816)	0.39-2.78 (0.97)
a-Mild AD (score 0-8)	16(40%)	8-84 (17)	6/10	14-72 (46)	122-826 (495)	0.39-1.85 (0.84)
b-Moderate AD **(score 9-16)**	14(35%)	8-72 (16)	6/8	28-195 (93)	136-1050 (635)	0.43-2.20 (0.96)
c-Severe AD (score 17-24)	10(25%)	10-66 (18)	7/3	67-225 (146)	216-2722 (1317)	0.49-2.78 (1.12)
		9-84 (20)				

** Range ** Median*

Table II: comparison of serum nitrate & s E-selectin concentrations among cases and controls

Parameter	**Controls** (n =16)	**Cases of AD** (n =40)	*P-value**
1. Nitrate (mmol/L)**:**			
Mean ± SD	20.02 ± 7.61	85.62 ± 20.12	<0.001**
2. sE-selectin (ng/ml)	122.65 ± 70.16	650.43 ± 153.1	<0.001**
Mean ± SD			

**** Highly significant**

Table III: comparison of serum nitrate and sE-selectin and eosinophil levels according to severity of disease

	Mild cases N=16	Moderate cases N=14	Severe cases n=10	Controls N=16	P value				
					Mild vs control	Moderate vs control	Severe vs control	Mild vs moderate	Moderate vs severe
Nitrate (mmol/L) **Mean ± S.D**	46.62 ± 9.16	85.62 ± 6.41	130.3 ± 60.7	20.02 ±7.61	*<0.05**	*<0.001***	*0.000***	*<0.05**	*<0.01**
sE-selectin (ng/ml) **Mean ± ssS.D**	426.1 ±210.3	755.14 ± 362.35	1180.46 ± 515.2	122.56 ± 70.16	*<0.05**	*<0.001***	*0.000***	*<0.05**	*<0.002***
Eosinophils ($x10^6$/L) **Mean ± S.D**	0.39-1.85	0.43-2.20	0.49-2.78	0.05-0.3	*<0.05**	*<0.001***	*<0.0001***	*>0.05*	*<0.05**

** P<0.05: significant ** highly significant p>0.5 non significant*

Table IV: comparison of serum nitrate and sE-selectin levels in severe cases of AD before and after treatment

Parameter	**Severe AD before treatment**	**Severe AD after treatment**	P-value*
1. Nitrate (mmol/L) **Mean ± S.D**	130.3 ± 60.87	36.2 ± 15.64	<0.002**
2. sE-selectin (ng/ml) **Mean ± S.D**	1180.46 ± 515.2	350.65± 112.4	<0.001**

*** highly significant*

Table V: Correlations between serum nitrate levels, sE-selectin levels, disease activity and peripheral eosinophil count

Variables correlated	**R**	**P**
1. Serum nitrate and sE-selectin levels	0.345	>0.05
2. Serum nitrate and disease activity	0.68	<0.05*
3. sE-selectin and disease activity	0.73	<0.01*
4. Serum nitrate and eosinophilic count	0.71	<0.01*
5. sE-selectin and eosinophilic count	0.63	<0.05*
6. Eosinophilic count and disease activity	0.72	<0.01*

** P<0.05: significant*

Grades of r: 0.00 to 0.24(weak or no association), 0.25 to 0.49 (fair association), 0.50 to 0.74 (moderate association), 0.75 and more (strong association).

Discussion:

Atopic dermatitis is a multifactorial genetically based disease that affects 10 to 15% of children in many parts of the world and constitutes a significant burden to patients and their families. The pathogenesis of AD is considered a combination of both Ig-E mediated and delayed type hypersensitivities (23).

In this study, we measured serum level of nitrate and sE-selectin in 40 infants and children with AD and 16 healthy controls in order to evaluate their roles in the pathogenesis, assessment of severity and clinical course of atopic patients.

The study showed that serum level of nitrate in AD children were significantly increased as compared to controls and also there were significant

differences among subgroups of AD tab.(II and III). Our data are in accordance with the previous recent studies by Taniuchi et al (24) However, Omata,(13), reported that nitrate levels were significantly lower in patients with AD than in the controls. The intrinsic sources of nitrate could be derived from activated macrophages, leukocytes, and endothelial cells.(25) Therefore, vascular injury and/or stimulated endothelial cells may yield the endothelial nitrate.(26) (27) Inducible nitric oxide synthetase (iNOS) immunoreactivity was found to be closely associated with upper dermal microvasculature in all inflammatory skin lesions of AD patients, but not in the non inflammatory area.(28) The increased nitrate levels of AD patients' serum suggest that iNOS of endothelial cells in the atopic skin are stimulated and produce nitric oxide, which causes vasodilatation associated with skin erythema or edema.(24) Decreased nitrate levels after treatment of severe AD in our study and previous reports ,may support this hypothesis(29).

In the present study, there were a significant correlations between serum nitrate levels and both the disease activity and eosinophilic count. tab V. These results are in agreement with those of Ererugi (12) and Taniuchi et al.,(24), who reported that serum nitrate levels of patients with AD were significantly correlated with skin scores(disease activity) as well as peripheral eosinophil counts. These results suggest that nitric oxide product bears an important function in the allergic inflammation, which is concerned with the lesion expansion of the skin and correlated well to skin scores of AD patient.

As regards soluble E-selectin, there was a significant increase in their levels in AD patients as compared to control group. There were also significant differences among subgroups of AD tab.(II and III). Our findings are in agreement with previous recent studies by Loan et al.,(30) they reported that during inflammation of the skin, membrane expression of adhesion molecules and TNF-receptors are increased, and soluble forms of these molecules are released into circulation as a result of shedding leading to increase of their levels in the serum. The soluble E-selectin levels seemed to be related to the clinical severity and represent the clinical features of AD.

It has been noted that E-selectin is important for the homing activity of skin-specific memory T cells. (31) furthermore, E-selectin transcripts of human skin are said to contain a stable type of alternative messenger RNA splicing, suggesting a prolonged expression of E-selectin and an abundant formation of sE-selectin at the skin site.(32) Thus it may be that this elevation of sE-selectin levels in cases of atopic dermatitis has a pathologic interpretation. In this regard different soluble adhesion molecules bind to their respective receptors, thereby resulting in a competitive response of leukocyte adhesion. It has also been reported that soluble E-selectin has the ability to activate neutrophils and acts as a chemoattractant.(33) Taken together, these findings suggest that sE-selectin by itself has the ability to regulate leukocyte migration.

Topical and systemic corticosteroid agents have been the mainstay of therapy for atopic dermatitis because of their broad immuno-modulatory effects also they inhibit the inflammatory mediators, and processes known to be related to AD pathogenesis, Furne et al.,(34) and Kagi,(35) in agreement with our results tab (IV), demonstrated a significant reduction of soluble E-selectin levels after treatment.

Our results showed significant correlations between sE-selectin concentrations and disease activity as well as eosinophilic count.(tab. V) These findings are in accordance with Wolberstofer et al.,(36) and Loan et al.,(31) reported that disease activity which is measured by objective SCORAD (scoring Atopic Dermatitis), as well as eosinophilic count were significantly correlated with the serum levels of s E-selectin. The expression of adhesion molecules on endothelial cells regulates leukocyte migration. The level of soluble adhesion molecules which are shed into the circulation is known to reflect the degree of inflammation, and this level can therefore be used as an indicator of disease activity.

Lewis (37) suggested that the atopic's monocytes/T cell interaction enhances development of eosinophil infiltration in skin lesions of AD and also in the peripheral circulation, which is related to the skin expansion and disease activity. This supports our results which demonstrated a positive correlation between eosinophil count and disease activity.

Conclusion:

From this work we can conclude that nitric oxide and sE selectin are involved in the pathogenesis of atopic dermatitis in children and their level was highly increased in acute and severe disease compared to those in remission ,therefore NO and sE-selectin are good and useful markers for assessment of severity and clinical course of atopic dermatitis New drugs that selectively inhibit NO and selectin production may help in treatment and maintenance of remission of the disease instead of long term corticosteroid therapy which has serious side effects.

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References:

1. Schultz-Larsen F, Diepgen T, Svensson A;. The occurrence of atopic dermatitis in north Europe: an international questionnaire study. J Am Acad Dermatol 1996;34:760–764.
2. Girolomoni G, Abeni D, Masini C, Sera F, Ayala F, Belloni-Fortina A et al; The epidemiology of atopic dermatitis in Italian schoolchildren. Allergy 2003;58:420–425.
3. Hanifin JM; Atopic dermatitis. J Am Acad Dermatol 1982;70:452–457.
4. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A; Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on atopic dermatitis. Dermatology 1997;195:10–19.
5. Gelmetti E, Colonna C.; The value of SCORAD and beyond. Towards a standardized evaluation of severity? Allergy 2004;59 (Suppl. 78):61–65.
6. Leung DYM;. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 2000;105:860–876
7. Anggard E; Nitric oxide: mediator,murderer and medicine. Lancet 1994; 343: 1199-1206.
8. Wel X, Charles IG, Smith A et al; Altered immune responses in mice lacking inducible nitric synthase. Nature 1995; 375: 408-11.
9. Griffith OW, Stuehr DJ; Nitric oxide synthase: properties and catalytic mechanism. Annu Rev Physiol 1995; 57: 707-36.
10. Mocada S, Palmer RMJ, Higgs EA; The biological significance of nitric oxide formation from L-arginine. Biochem Soc Trans 1989; 17: 642-4.
11. Takahashi H, Takanishi T, Nishimura M et al ; Measurement of serum levels of nitrate ions in men and women: implications of endothelium-derived relaxing factor in blood pressure regulation and atherosclerosis. J Cardioavasc Pharmacol 1992; 20 Suppl 12: S214-16.
12. Ererugi M; Serum levels of NO products ,IL-8 in infants with atopic dermatits. J Allergy 2000; 49(7): 572–84.
13. Omata N; Increased oxidative stress in childhood atopic dermatitis. Life Sci 2001; 69 (2): 223-8.
14. Lobb RR, Chi-Rosso G, Leone DR, Rosa MD, et al**;** Expression and functional characterization of a soluble form of ELAM**-1.** *J Immunol*. 1991;147:124–129
15. Gearing AJH, and Newman W; Circulating adhesion molecules in disease. *Immunol Today*. 1993;14:506–512
16. Pigott R, Dillon LP, Hemingway LH, and Gearing AJH**;** Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in tShe supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun*. 1992;187:584–589
17 Spronk PE, Bootsma H, Huitema MG, Limburg PC, and Kallenberg CGM.; Level of soluble VCAM-1, soluble ICAM-1, and soluble E-selectin during disease exacerbation in patients with systemic lupus erythematosus (SLE): a long term prospective study**.** *Clin Exp Immunol*. 1994;97:439–444
18. Newman W, Beall LD, Carson CW, Hunder GG, Graben N, et al; Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock**.** *J Immunol*. 1993; 150:644–654
19-Rajka G and Longeland H,; Grading the severity of atopic dermatitis. Acta Derm Venerol 1989;suppl 144: 13-4.
20. Kimata H and Lindley I; Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology*. 1993;186:23–31
21-Bories P, and Bories C.; Nitrate determination in biological fluid by an enzymatic one step assay with nitrate reductase. Clin Chem 1995; 41 (6): 904-7.
22. Stucki A et al; Determination of soluble adhesion molecules in cell culture supernate, serum and plasma. Lancet. 1995; 345: 286-9.
23. Bernice R.; Atopic dermatitis. *Allergy Immunol* 2006; 1: 7 - 9.
24. Taniuchi S, Kojina T, Haro K, Yananata A et al ; Increased serum nitrate levels in infants with atopic dermatitis. Allergy 2001; 26: 693-5.
25. Stuehr DJ, and Marletta MA;. Mammalian nitrate biosynthesis,mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. Proc Natl Acad Sci USA 1985; 82: 7738–42.
26. Takahashi H, Takanishi T, Nishimura M et al ; Measurement of serum levels of nitrate ions in men and women: implications of endothelium-derived relaxing factor in blood pressure regulation and atherosclerosis. J Cardioavasc Pharmacol 1992; 20 Suppl 12: S214-16.
27. Spack L, Havens RMJ, and Griffith OW. Measurements of total plasma nitrite and nitrate in pediatric patients with systemic inflammatory response syndrome. Crit Care Med 1997; 25: 1071-8.
28. Rome A, Farrell AM, and Burnker CB; Constituitive endothelial and inducible nitric oxide synthase in inflammatory dermatosis. Br J Dermatol 1997; 136: 18-23.
29. Rosenkranz–Weiss P, Sessa W, Milstien S et al; Regulation of nitric oxide synthesis by proinflammatory cytokines in human umblical vein endothelial cells: elevation in tetrahydrobiopterin levels enhances endothelial nitric oxide synthase specific activity. J Clin Invest 1994; 93: 2236-43.
30. Loan MP, Koning H, Baert MR et al; Levels of soluble intercellular adhesion-1, soluble E-selectin, TNF-α in atopic dermatitis. Allergy 1998; 53(1): 51-8.
31. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, and Butcher EC.; ELAM-1 is an adhesion molecule for skin-homing T cells**.** *Nature*. 1991;349:796–799
32. Chu W, Presky DH, Swerlick RA, and Burns DK; Alternatively processed human E-selectin transcripts linked to chronic expression of E-selectin in viv**o.** *J Immunol*. 1994;153:4179–4189
33. Lo SK, Lee S, Ramos RA, Lobb R, Rosa M, Chi-Rosso G, et al;. Endothelial-leukocyte adhesion molecule 1 stimulates the adhesive activity of leukocytes integrin CR3 (CD11b/CD18, Mac-1, $\alpha_m\beta_2$) on human neutrophils. *J Exp Med*. 1991;173:1493–1500
34. Furne M, Koga T, and Yamashita N;. Soluble E–selectin and eosinophil cationic protein are distinict serum markers that differentially represent clinical features of atopic dermatitis. J Dermatol 1999; 140(1): 67-72.
35. Kagi MK.; Soluble E-selectin correlates with disease activity in cyclosporin A treated patients with atopic dermatitis. Allergy 1999; 54(1): 57-63.
36. Wolberstorfer A, Loan MP, Savelkoul HF et al.; Soluble E-selectin, other markers of inflammation and the disease severity in children with atopic dermatitis. Br J Dermatol 1998; 138 (3): 431-5.
37. Lewis JC; Eosinophilia-related syndrome. In Charles Worth EN (ed): Cutaneous Allergy. Cambridge, Blackwell Science 1996: 35-40