

LETTER TO THE EDITOR

HMGB1 levels in children with atopic eczema/dermatitis syndrome (AEDS)

To the Editor,

Atopic eczema/dermatitis syndrome (AEDS) is a chronic relapsing-remitting inflammatory skin disorder beginning usually in early childhood, characterized by a skin barrier dysfunction resulting in epidermal damage and altered permeability to allergens and microbes (1). Depending on whether there is an association with or without immunoglobulin (Ig)E sensitization, AEDS may be defined as atopic (aAEDS) or non-atopic (naAEDS), respectively. However, it is currently unclear whether aAEDS and naAEDS are really two different diseases or whether AEDS starts in early infancy as naAEDS phenotype without evidence of IgE sensitization and then progresses through allergic sensitization to the IgE-associated phenotype (aAEDS). Moreover, in infants, the progression from AEDS to other forms of allergic diseases (e.g., rhinitis and asthma), usually referred to as the 'atopic march', as well as their relationships with one other, seems more complicated than a simple evolution of one condition into another and one which does not always follow the classic sequential pattern of these illnesses (1).

Although the exact etiology of AEDS remains unknown, both genetic and environmental factors have been proposed to play crucial pathogenetic roles. However, various factors including immunological abnormalities have been suggested to contribute to the pathogenesis and development of AEDS.

In this regard, we recently studied serum interleukin (IL)-17, IL-23, and IL-10 levels in 181 children with AEDS (99 males, 82 females; mean age: 8.31 ± 3.21 years), and we found significantly higher levels of IL-17 and IL-23 and significantly lower levels of IL-10 than in a healthy control group. Moreover, serum IL-17 and IL-23 levels, but not IL-10 levels, were significantly higher in aAEDS than in naAEDS subtypes. Additionally, this immune biomarker phenotype significantly correlated with score atopic dermatitis (SCORAD) values. Interestingly, in the aAEDS subjects, we found a concomitant association of atopic diseases such as asthma and rhinitis, and higher serum IL-17 and IL-23 levels than in children with non-allergic sensitization (naAEDS) (2).

To add new data on a 'double-face' disease, we, firstly, investigated the levels of high-mobility box 1 (HMGB1), an inflammatory marker belonging to the alarmin family, in the patients' sera frozen of our previous study (2).

High-mobility group box (HMGB) 1, is implicated as a mediator of both infectious and non-infectious inflammatory conditions. Extracellular HMGB1 acts as an endogenous danger signal and binds to Toll-like receptor (TLR) 2, TLR4, receptor for advanced glycation end products (RAGE), and chemokine receptors. Recent studies suggest that the elevated levels of extracellular HMGB1 have been implicated in association with several disease states, includ-

ing sepsis, and autoimmune disease such as arthritis, meningitis, ischemia-reperfusion injury, cancer, psoriasis, and local inflammation.

Furthermore, although several studies have assessed the role of HMGB1 in immune-mediated tissue-damaging responses (3, 4) of some allergic diseases (asthma and rhinitis), to date, no data have been reported in children with AEDS (5, 6) and only a few data in mice (7, 8).

In this report, we analyzed the HMGB1 levels in both patients with naAEDS and patients with aAEDS, the relationship between serum HMGB1 levels and clinical severity of the disease, and, finally, the HMGB1 levels in different allergic phenotypes such as aAEDS plus allergic sensitization, aAEDS plus rhinitis, aAEDS plus asthma, and aAEDS plus rhinitis and asthma. Moreover, we investigated the relationship between HMGB1 and IL-17/IL-23 axis and IL-10.

HMGB1 levels (IBL Shino Test Corporation, Hamburg, Germany) were determined through ELISA according to the manufacturer's instructions.

The data collected were statistically analyzed using a statistical computer software SPSS, version 15.0 (IBM. Statistical Package for the Social Sciences. 2009). A *p* value of <0.05 was considered to be statistically significant. Statistical analysis was performed with ANOVA test and relative *post hoc* vs. control. Mean and standard deviations were calculated for the variables, and *t*-test was employed for the comparisons. For the attributes, percentages were calculated first, and then, χ^2 test was used for comparisons. Correlations between HMGB1 levels and serum total IgE levels and between SCORAD index and serum IL-17, IL-23, and IL-10 levels were calculated using Spearman's correlation coefficient.

Clinical and laboratory findings of all patients are shown in Table 1.

Although no difference was found between aAEDS and naAEDS subtypes (serum HMGB1 levels 19.73 ± 1.68 ng/ml vs. 19.27 ± 3.16 ng/ml, respectively, $p = 0.208$), serum HMGB1 levels were significantly higher in both aAEDS and naAEDS subtypes than in a healthy control group (serum HMGB1 levels 3.34 ± 0.84 ng/ml) ($p < 0.001$).

Recently, in animal models, Karuppagounder et al. (7, 8) demonstrated that HMGB1 signaling might play an important role in skin inflammation, and suggested that quercetin treatment could be a promising agent for AEDS by modulating the HMGB1/RAGE/NF κ B signaling. Furthermore, these authors also demonstrated that resveratrol effectively inhibits HMGB1, ERK1/2, NF κ B, IL-1 β , IL-4, IFN- γ , TNF- α , and other pro-inflammatory cytokines. Confirming these findings, the same authors hypothesized that, by modulating the HMGB1 protein expression, this treatment modality could have beneficial effects on atopic dermatitis. Our findings in children with AEDS

Table 1 Clinical and laboratory findings of patients with AEDS

	N	SCORAD	HMGB1 ng/ml
aAEDS	104	38.91 ± 5.26	19.73 ± 1.68
Mild	32	20.31 ± 2.23	17.52 ± 0.56
Moderate	37	36.67 ± 4.52	18.55 ± 0.48
Severe	35	59.76 ± 9.03	23.03 ± 0.64
aAEDS with positive SPT*	54	37.91 ± 5.29	18.47 ± 2.07
aAEDS+ rhinitis	11	39.85 ± 5.42	18.32 ± 0.93
aAEDS+ asthma	13	37.82 ± 4.81	18.84 ± 1.07
aAEDS + rhinitis and asthma	26	40.07 ± 5.52	25.83 ± 1.91
naAEDS	77	37.61 ± 4.29	19.27 ± 3.16
Mild	25	21.33 ± 2.13	16.98 ± 1.02
Moderate	29	34.31 ± 2.11	18.11 ± 1.13
Severe	23	57.21 ± 8.64	22.72 ± 1.01
Control group	93	–	3.34 ± 0.84

*SPT, skin prick test.

support this possibility and suggest that the measurement of HMGB1 might offer a useful predictive biomarker also in AEDS.

We found a direct correlation between serum HMGB1 levels and SCORAD index in patients with AEDS ($r = 0.2861$, $p < 0.001$) and in both subgroups (aAEDS $r = 0.3526$,

$p < 0.001$; naAEDS $r = 0.2703$, $p < 0.05$) (Fig. 1). The results of our studies, in children suffering from both aAEDS and naAEDS, patients with severe SCORAD index points, showed significantly higher HMGB1 levels (serum HMGB1 levels 23.03 ± 0.64 ng/ml and 22.72 ± 1.01 ng/ml, respectively) than in those with moderate (serum HMGB1 levels 18.55 ± 0.48 ng/ml and 18.11 ± 1.13 ng/ml, respectively) or mild (serum HMGB1 levels 17.52 ± 0.56 ng/ml and 16.98 ± 1.02 ng/ml, respectively) SCORAD index point ($p < 0.001$). No significant differences were found between subjects with aAEDS and naAEDS with respect to mild (20.31 ± 2.23 vs. 21.33 ± 2.13 , respectively, $p = 0.094$), moderate (36.67 ± 4.52 vs. 34.31 ± 2.11 , respectively, $p = 0.011$), and severe (59.76 ± 9.03 vs. 57.21 ± 8.64 , respectively, $p = 0.289$) SCORAD index points (Table 1). These results suggest that HMGB1 may not only offer a very sensitive biomarker of AEDS severity and, furthermore, it even seems to indicate that aAEDS and naAEDS phenotypes are similar with regard to this biomarker.

With reference to the characterization of allergic phenotypes in children with aAEDS, we found no significant difference in SCORAD index points in subgroups of children with AEDS plus allergic sensitization compared to children with AEDS plus allergic rhinitis (37.91 ± 5.29 vs. 39.85 ± 5.42 , respec-

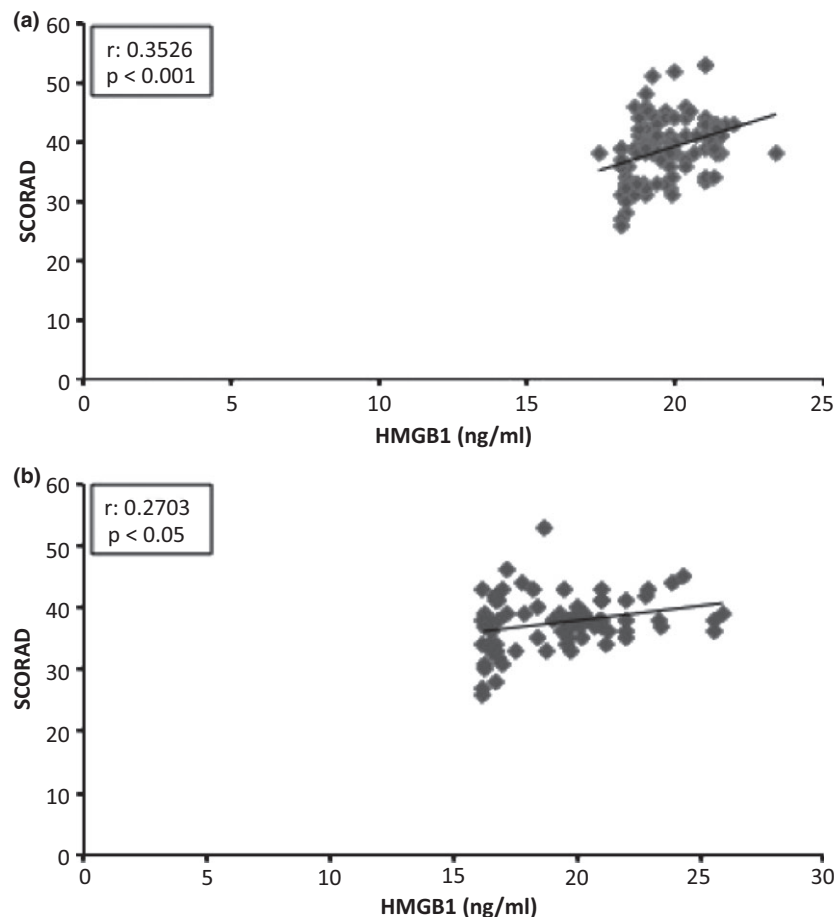


Figure 1 Correlation between serum HMGB1 levels and SCORAD in aAEDS (a) and naAEDS (b) patients.

tively, $p = 0.273$), children with AEDS plus asthma (37.91 ± 5.29 vs. 37.82 ± 4.81 , respectively, $p = 0.955$), and children with AEDS plus rhinitis and asthma (37.91 ± 5.29 vs. 40.07 ± 5.52 , respectively, $p = 0.095$), suggesting that the presence of allergic disorders of airways among children with aAEDS does not influence AEDS severity (Table 1). This clinical observation is confirmed by the HMGB1 levels, which are similar between aAEDS plus only atopic sensitization and aAEDS plus rhinitis (18.47 ± 2.07 ng/ml vs. 18.32 ± 0.93 ng/ml, respectively, $p = 0.815$), between aAEDS plus only atopic sensitization and aAEDS plus asthma (18.47 ± 2.07 ng/ml vs. 18.84 ± 1.07 ng/ml, respectively, $p = 0.536$), and between aAEDS plus rhinitis and aAEDS plus asthma (18.32 ± 0.93 ng/ml vs. 18.84 ± 1.07 ng/ml, respectively, $p = 0.221$). Interestingly, the phenotype characterized by the occurrence of AEDS plus allergic rhinitis and asthma is associated with significantly higher levels of HMGB1 (25.83 ± 1.91 ng/ml) than other phenotypes such as aAEDS plus allergen sensitization (18.47 ± 2.07 ng/ml), aAEDS plus rhinitis (18.32 ± 0.93 ng/ml), and aAEDS plus asthma (18.84 ± 1.07 ng/ml) ($p < 0.001$) (Table 1), but there is no significant difference in SCORAD index points.

Our results demonstrate that HMGB1 represents a suitable marker of AEDS severity. Probably, the higher levels of HMGB1 found in patients with both allergic rhinitis and asthma might reflect a more severe pattern of inflammation and a more extensive systemic disease involvement. On the contrary, as the 'atopic march' is characterized by a sequence

of clinically symptomatic entities, we think that the relevance of HMGB1 as a marker for 'atopic march' is questionable.

Our findings also show a clear correlation between HMGB1 and IL-17/IL-23 axis and IL-10 levels in children with AEDS.

Moreover, contrary to IL-17 and IL-23, we found no difference in IL-10 levels between the aAEDS and naAEDS subtypes. These data suggest that serum HMGB1 levels in children with AEDS might be related to skin inflammation independently by AEDS subtype. Because it is known that HMGB1 influences the inflammatory responses, we hypothesized that increased serum HMGB1 levels in patients with AEDS might also regulate cytokine production in both subtypes of aAEDS and naAEDS.

Furthermore, serum HMGB1 levels were positively related to serum total IgE ($r: 0.1625$; $p < 0.05$), IL-17 ($r: 0.3950$; $p < 0.001$), and IL-23 levels ($r = 0.1791$; $p < 0.05$) and inversely related to IL-10 levels in patients with AEDS ($r = -0.1612$; $p < 0.05$) (Fig. 2).

It is known that IL-17 plays a crucial role in the pathophysiology of AEDS (2) as it is highly detectable in acute AEDS and during the relapse of the disease [19]. Th17 cells-related cytokines are even promoted by IL-23, which, in turn, is produced both by the innate immune system and by dendritic cells (DC) in response to trigger factors, favouring the maintenance of chronic skin inflammatory condition (2, 9). Additionally, both IL-17 and IL-23 stimulate further Th17 cells, maintaining the inflammatory response to tissue injury (9). HMGB1, through an increased pro-inflammatory cytokine

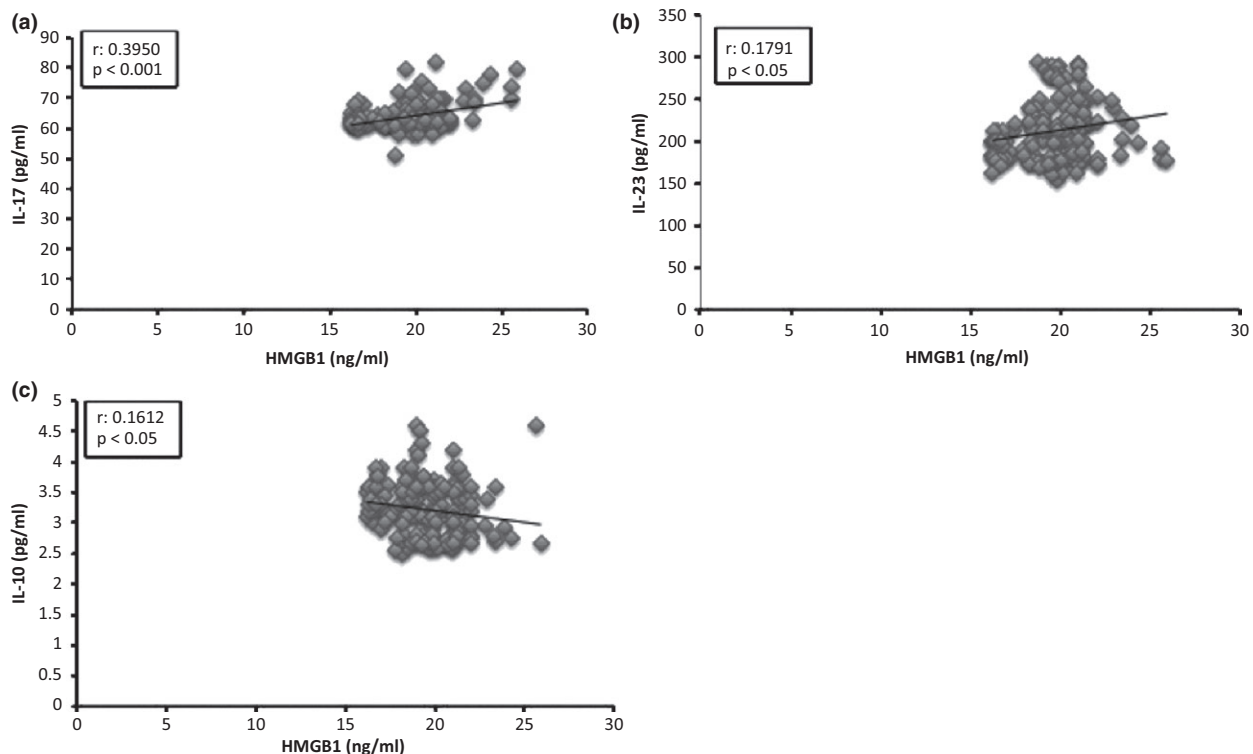


Figure 2 Correlations between serum HMGB1 levels and IL-17 (a), IL-23 (b), and IL-10 (c) levels.

synthesis and/or by a TLR2/TLR4-dependent manner, can also prime the naïve *CD4*⁺ T-cell differentiation into Th17 cells (10). All these pathways might explain the relationships between IL-17/IL-23 axis, IL-10, and HMGB1 as confirmed also in our study.

In conclusion, the results of the present study suggest that HMGB1 may offer a sensitive biomarker for AEDS in children, which is well correlated with the degree of disease severity as well as with more severe comorbidities. We also suggest that although HMGB1 may be relevant in the pathogenesis of the disease, it does not seem to be a suitable biomarker of atopic march. Finally, these findings of the complex interactions between IL-17/IL-23 axis, IL-10, and HMGB1 may provide new insights that could help unravel the

conundrum of the pathological pathways that are involved in this 'double-face' disease of AEDS. Future studies will be needed to confirm whether modulating HMGB1 protein expression could be a suitable therapeutic target for atopic dermatitis.

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