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Assessment of serum and tissue level of interleukin 33 in patients with atopic dermatitis

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Abstract
Background: Atopic dermatitis (AD) is a common chronic inflammatory skin disease. The pathogenesis of AD involves interplay between skin-resident keratinocytes & endothelial cells, infiltrating immune cells (Th2 cells and later Th1 cells, macrophages, dendritic cells, mast cells, and eosinophils), and activated peripheral sensory nerves. Interleukin (IL)-33 is a new member of the IL-1 family that plays a role in the induction of production of Th2 cytokines. Thus, IL33 may have an important role in the pathogenesis of AD.
Objectives: To assess serum and tissue IL-33 level in patients with AD and to correlate its level with the disease severity.
Methods: This case control study included 30 patients diagnosed clinically as having AD according to Hanifin and Rajka criteria. Further, 10 age and sex matched healthy controls were recruited. Patients were divided into 3 groups based on clinical severity according to Eczema Area and Severity Index (EASI). Blood sample collection and a three mm punch biopsy from lesional skin was taken and homogenized for assessment of IL-33 using ELISA technique in all subjects.
Results: There was a high statistically significant difference between cases and controls regarding both serum & tissue IL-33 levels ($P<0.01$). Also, there was a high statistically significant positive correlation between EASI score and both serum & tissue IL-33 level among cases.
Conclusion: The current study suggests that IL-33 has an important role in AD pathogenesis as well as an indicator of severity of the disease.

Keywords: atopic-dermatitis-interleukin 33

Introduction
The pathogenesis of atopic dermatitis (AD) is a product of complex interactions [1]. The key events in AD includes an interplay among skin-resident keratinocytes & endothelial cells, infiltrating immune cells, and activated peripheral sensory nerves [2].
IL-33 is a member of the IL-1 family [3], it is a nuclear cytokine released by the epithelial cells in various tissues, including endothelial cells, keratinocytes, and immune cells, and also it can be released by necrotic structural cells, as fibroblast or keratinocytes [4]. IL-33 has been described as an epithelial ‘‘alarmin’’ defense system, released upon cellular damage through the activation of various immune cells through its supression of tumorigenicity 2 (ST2) receptor, which leads to the production of various molecules [5, 6, 7, 8]. Once IL-33 is released, it induces the production of Th2 cytokines (IL-4, IL-5 and IL-13) through activating the ST2/IL-1alpha receptor on different type of cells, including mast cells and TH2 cells [9]. Thus IL33 plays a role in the link between the innate and adaptive immune responses in allergic diseases through the production of proinflammatory cytokines [10].
Savinko et al. [11] investigated the expression profiles of IL-33 and ST2 in different mouse models of atopic-like dermatitis. They found that topical application of different allergens to mice, led to the upregulation of IL-33 and ST2 messenger RNA expression. These findings suggest that IL-33 and its receptor may be induced in AD when exposed to triggering factors. *Webb et al.* [12] suggested that IL-33 could be relevant for the diagnosis, staging, and monitoring of the progression of allergic disease.
The aim of the present work was to assess serum and tissue IL-33 level in patients with AD and to correlate its level with the disease severity.

Methods
- This case control study included 30 patients diagnosed clinically as having AD according to Hanifin and Rajka criteria [13]. Further, 20 age and sex matched healthy controls were recruited. The subjects were recruited from the dermatology outpatient clinic of Ain-Shams University Hospitals in the period from April 2017 till February 2018. Patients who are currently on or have received topical or systemic medications for AD in the previous 3 months, patients with any autoimmune disease that may affect IL-33 level and patients with other dermatological diseases known to affect level of IL-33 e.g. psoriasis, vitiligo, and chronic spontaneous urticaria were excluded. An approval from the research ethical committee of faculty of medicine Ain Shams University was taken. A written informed consent was obtained from the study subjects/ children’s guardians.
- **All patients were subjected to:** Full history taking, clinical examination and calculation of Eczema Area

and Severity Index (EASI). Blood sample collection for assessment of IL-33 level using enzyme-linked immunosorbent assay (ELISA) technique (Human IL-33 ELISA kit) (SinoGeneClon Biotech Co., LTD, China), Catalog Number: SG-10295. A three mm punch biopsy was taken from lesional skin and homogenized for assessment of IL-33 using ELISA technique.

- **Statistical Analysis:** The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. Median and interquartile range (IQR) for non-parametric numerical data. Frequency and percentage of non-numerical data. P>0.05: Non significant (NS). P< 0.05: Significant (S). P< 0.01: Highly significant (HS).

Results

The clinical data of the 30 patients included in the present study is shown in Table 1. There was no statistically significant difference between cases and controls as regard age (P= 0.093) and sex (P= 0.714).

Tissue IL-33 level among cases ranged from 1250 to 5450 pg/ml (median= 2275 pg/ml), while among controls, the tissue IL-33 level ranged from 150-1750 pg/ml (median= 250 pg/ml). There was a high statistically significant difference between cases and controls regarding tissue IL-33 levels (P= 0.000) with higher tissue IL-33 levels among cases as compared to controls (Table 2)**.** Serum IL-33 level among cases ranged from 850 to 5400 pg/ml (median= 2250 pg/ml), while among controls, Serum IL-33 level ranged from 125 to 250 pg/ml (median= 150 pg/ml). There was a high statistically significant difference between cases and controls regarding serum IL-33 (P= 0.000) with higher serum IL-33 levels among cases as compared to controls (Table 2)**.**

There was no statistically significant difference between tissue and serum IL- 33 level in comparison with the sex of the patients (P= 0.136, P= 0.075), personal history of atopy (P= 0.605, P= 0.260) and family history of atopy (P= 0.716, P= 0.353) (Table 3, 4).

There was a high statistically significant positive correlation between EASI score and IL-33 levels in both tissue (P= 0.000, r= 0.871) and serum (P= 0.000, r= 0.965) among cases (Table 5). There was a statistically significant positive correlation between age and tissue IL-33 levels among cases (P= 0.044, r= 0.370) **(**Table 5). There was a high statistically significant positive correlation between age and serum IL-33 levels among cases (P= 0.010, r= 0.461) (Table 5). There was no statistically significant correlation between age and tissue IL-33 levels (P= 0.277, r= 0.381) or serum IL-33 levels (P= 0.836, r= 0.075) among controls.

There was a high statistically significant positive correlation between duration of the disease and IL-33 levels in both tissue (P= 0.000, r= 0.606) and serum (P= 0.000, r= 0.629) among cases (Table 5).

Regarding tissue IL-33 levels, it ranged from 1250 to 2700 pg/ml (median= 2150 pg/ml) in patients with mild AD**,** from 2200 to 5450 pg/ml (median= 2750 pg/ml) in patients with moderate AD and from 2700 to 5400 pg/ml (median= 5400 pg/ml) in patients with severe AD (Table 6). There was a high statistically significant difference between mild and moderate cases (P= 0.003), mild and severe cases (P= 0.000) regarding tissue IL-33 levels (Table 6). No statistically significant difference was found between moderate and severe cases regarding tissue IL-33 levels (P= 0.127) (Table 6). Regarding serum IL-33 levels, they ranged from 850 to 2400 pg/ml (median= 1850 pg/ml) in patients with mild AD**,** from 2550 to 3300 pg/ml (median= 2700 pg/ml) in patients with moderate AD and from 2800 to 5400 pg/ml (median= 3775 pg/ml) in patients with severe AD (Table 6). There was a high statistically significant difference between mild and moderate cases (P= 0.000), mild and severe cases (P= 0.000), moderate and severe cases (P= 0.006) regarding serum IL-33 levels (Table 6).

The best cut off point for tissue IL-33 to differentiate between mild and moderate cases of AD was found to be > 2650 pg/ml with sensitivity of 62.5%, specificity of 93.7% and AUC 78.9. While the best cut off point for serum IL-33 to differentiate between mild and moderate cases of AD was found to be > 2400 pg/ml with sensitivity of 87.5%, specificity of 100% and AUC of 88.7%. The ROC curve showed that serum IL-33 was found to be better in prediction of moderate cases than tissue IL-33 (Table 7) (Fig. 1). The best cut off point for serum IL-33 to differentiate between moderate and severe cases of AD was found to be > 2700 pg/ml with sensitivity of 100%, specificity of 71.43% and AUC 95.2% (Table 8) (Fig. 2).

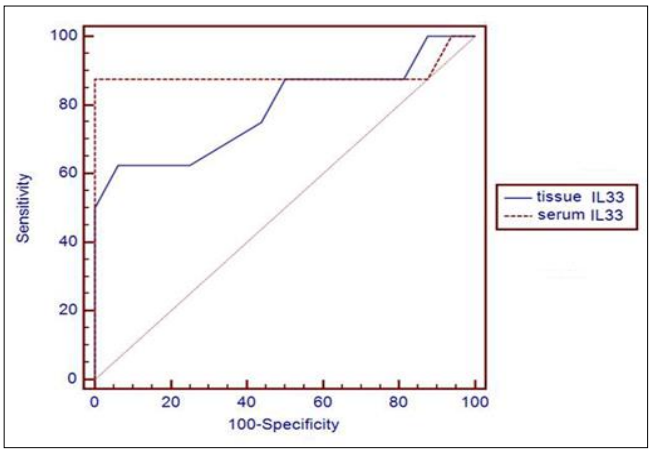

Fig 1: ROC curve for serum and tissue IL-33 in differentiation between mild and moderate cases of AD.

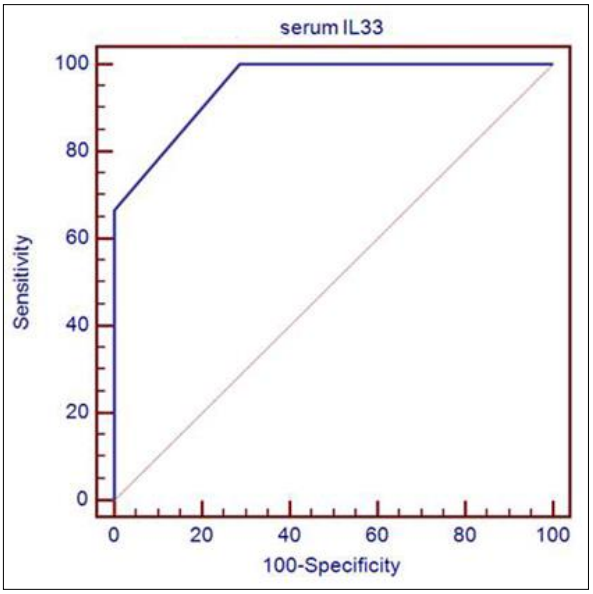

Fig 2: ROC curve for serum IL-33 in differentiation between moderate and severe cases of AD.

Table 1: Clinical data of selected cases

		Total no. = 30
Age	Median (IQR)	11 (9 – 14)
	Range	2 – 42
Sex	Male	13 (43.3%)
	Female	17 (56.7%)
History of atopy	Yes	22 (73.3%)
	No	8 (26.7%)
Smoking	Yes	0 (0.0%)
	No	30 (100.0%)
Addiction	Yes	0 (0.0%)
	No	30 (100.0%)
Family history	Yes	21 (70.0%)
	No	9 (30.0%)
Past history	Yes	21 (70.0%)
	No	9 (30.0%)
EASI	Median (IQR)	9.2 (4.8 – 15.3)
	Range	1 – 45.6
EASI severity	Mild	17 (56.7%)
	Moderate	7 (23.3%)
	Severe	6 (20.0%)

Table 2: Comparison between cases and controls regarding IL-33 level

		Control group No. = 20	**Patients group No. = 30**	**Test value**	**P-value**	**Sig.**
Tissue IL-33 level	Median (IQR)	250 (150 – 300)	2275 (2150 – 2750)	-4.507ǂ	0.000	HS
	Range	150 – 1750	1250 – 5450			
Serum IL-33 level	Median (IQR)	150 (150 – 200)	2250 (1850 – 2800)	-4.697ǂ	0.000	HS
	Range	125 – 250	850 – 5400			

‡: Mann-Whitney test

Table 3: Relation between sex, personal history of atopy and family history of atopy and tissue IL33 level among cases

		Tissue IL33		**Test value‡**	**P-value**	**Sig.**
		Median (IQR)	**Range**			
Sex	Male	2250 (2100 – 2300)	1300 – 5400	-1.491	0.136	NS
	Female	2700 (2200 – 4500)	1250 – 5450			
History of atopy	Yes	2475 (2150 – 4500)	1250 – 5450	-0.518	0.605	NS
	No	2250 (2125 – 2675)	1400 – 5400			
Family history	Yes	2300 (2200 – 2750)	1250 – 5450	-0.363	0.716	NS
	No	2250 (2100 – 2750)	1300 – 5400			

‡: Mann Whitney test

Table 4: Relation between sex, personal history of atopy and family history of atopy and serum IL33 level among cases

		Serum IL33		**Test value ‡**	**P-value**	**Sig.**
		Median (IQR)	**Range**			
Sex	Male	1850 (1350 – 2400)	900 – 3900	-1.782	0.075	NS
	Female	2550 (2100 – 2800)	850 – 5400			
History of atopy	Yes	2550 (1850 – 2800)	850 – 5400	-1.128	0.260	NS
	No	2000 (1825 – 2100)	1350 – 3650			
Family history	Yes	2400 (1850 – 2800)	850 – 5400	-0.929	0.353	NS
	No	2000 (1350 – 2550)	900 – 3650			

‡: Mann Whitney test

Table 5: Correlation between EASI score, age, and duration of the disease and IL-33 level among cases

	Tissue IL33		**Serum IL33**	
	r	**P-value**	**r**	**P-value**
EASI	0.871	0.000	0.965	0.000
Age	0.370	0.044	0.461	0.010
Duration of the disease	0.606	0.000	0.629	0.000

Spearman correlation coefficients

Table 6: Relation between serum & tissue IL-33 and disease severity

		EASI severity			Test value‡	P-value
		Mild	Moderate	Severe		
Tissue IL-33	Median (IQR)	2150 (1400 – 2250)	2750 (2250 – 5350)	5400 (4500 – 5400)	17.510	0.000
	Range	1250 – 2700	2200 – 5450	2700 – 5400		
Serum IL-33	Median (IQR)	1850 (1350 – 2100)	2700 (2550 – 2800)	3775 (3300 – 5400)	22.910	0.000
	Range	850 – 2400	2550 – 3300	2800 – 5400		

‡: Kruskal Wallis test

		Post Hoc analysis by LSD		
		Mild vs Moderate	Mild vs Severe	Moderate vs Severe
Tissue IL-33	P-value=	0.003	0.000	0.127
Serum IL-33	P-value=	0.000	0.000	0.006

Table 7: Mild cases vs moderate cases regarding tissue & serum IL-33 level (ROC analysis)

Parameter	Cut off Point	AUC	Sensitivity	Specificity	PPV	NPV
Tissue IL-33	>2650	0.789	62.50	93.75	83.3	83.3
Serum IL-33	>2400	0.887	87.50	100.00	100.0	94.1

AUC: area under curve; **PPV:** positive predictive value; **NPV:** negative predictive value

Table 8: Moderate cases vs severe cases regarding serum IL-33 (ROC analysis)

Parameter	Cut off Point	AUC	Sensitivity	Specificity	PPV	NPV
Serum IL-33	>2700	0.952	100.00	71.43	75.0	100.00

AUC: area under curve; **PPV:** positive predictive value; **NPV:** negative predictive value

Discussion

In the present study, the possible role of IL 33 in the pathogenesis of AD was evaluated by measuring its level in both serum and tissue of AD patients and comparing it with IL33 level in controls. Also, IL 33 level in serum and tissue of AD patients was correlated with the disease severity. Different scoring methods (e.g: EASI score [14], SCORAD [15], The Three Item Severity score [16] and The Leicester Score [17]) are used in grading AD severity, however in our study we used EASI score to measure the extent and severity of atopic eczema (Eczema Area and Severity Index). It is an easy method that takes few minutes and don't require much experience to calculate it accurately.

To the best of our knowledge, the present study is the first to investigate the level of IL 33 in both serum and tissue of patients with AD.

In the present study, all AD patients showed a highly statistically significant elevation in both serum and tissue IL 33 levels compared with healthy controls. These findings highlight the potential participation of IL-33 in the pathogenesis of AD which might be through provoking and/or maintenance of inflammation that influences the disease activity. Our findings are consistent with *Tamagawa-Mineoka et al.* [18] who reported an increased serum IL-33 levels and with *Hay et al.* [19] who reported an increased tissue IL 33 level in patients with AD compared to controls. They suggested that IL-33 is released by skin structural cells in response to various triggering factors and they play an important role in the development of inflammatory reactions and fibrotic remodeling in AD via various mechanisms.

A statistically significant positive correlation was found between IL33 levels in both tissue and serum with the EASI score. This finding highlights the possibility of using IL33 level as an indicator for severity of AD. Our findings are consistent with *Tamagawa-Mineoka et al.* [18] who showed that the EASI score accounted for elevation of serum IL-33 levels among patients with AD. Also EASI score had decrease with consequent decrease in the level of IL-33 after treatment and complete improvement of skin lesions.

Also in our study there was a positive correlation between serum and tissue IL-33 level with age of patients of AD. Our findings are different from *Tamagawa-Mineoka et al.* [18]*; Hay et al.* [19] who showed that there was no statistically significant relation between serum and tissue IL-33 level and age of the patients. This may be attributed to the difference in the age as our study included children (>2years) while the fore mentioned studies were carried in patients (>16 years) in *Tamagawa-Mineoka et al.* [18] and in patients (>10 years) in *Hay et al.* [19] and there may be a correlation between the IL-33 level and the body surface area which varies in different age groups.

Also in our study there was positive correlation between serum and tissue IL-33 level and duration of AD. Our finding is constant with *Metwalli et al.* [20] who found that there is a high statistically significant correlation between serum IL-33 and duration of AD in the patients.

In the current work we tried to detect a possible cut off point for both serum and tissue IL 33 level that can be used later for differentiating between mild, moderate and severe cases of AD. Our study showed that serum IL33 was a good prediction of moderate and severe cases. Our finding are consistent with *Tamagawa-Mineoka et al.* [18] in which the ROC curve establishing cut-off levels between patients with moderate and severe AD for serum IL-33 level was found to be 68.18 pg/mL, giving the maximum efficiency in prediction of moderate to severe AD. The difference in values may be due to different types of kits used in both studies.

No statistically significant difference was found regarding IL-33 levels according to sex, personal history of atopy or family history of atopy which is constant with *Tamagawa-Mineoka et al.* [18].

As many studies suggests an important role for IL33 in the pathogenesis of AD and its relation to disease severity, a 2017 small phase 2a study using a therapeutic anti-IL-33 antibody (ANB020 is humanized IgG1 anti-human IL-33 monoclonal antibody) demonstrated promising results in terms of EASI as early as at day 15 in AD patients [21]. However, larger randomized trials are required to fully determine the efficacy of this drug.

Our conclusion was that IL-33 has an important role in AD pathogenesis regardless of the sex, personal history and family history of atopy. A positive correlation was found between serum and tissue IL-33 level and age of patients, duration of AD and severity of AD. It can be used as an indicator of severity of the disease. Also new drugs targeting IL-33 might be helpful in treating AD.

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