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ORIGINAL ARTICLE

The role of IL-33 in Severity of Systemic Sclerosis

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ABSTRACT

Background: *Systemic sclerosis (SSc) is an autoimmune connective tissue disorder characterized by sclerotic changes which affect the skin and internal organs. Interleukin-33 (IL-33) is a newly reported cytokine of the IL-1 family.* ***Objective****: The aim of this work was to determine serum levels of IL-33 in SSc patients and evaluate its association with clinical manifestations and disease subset.* ***Methodology:*** *The study included two groups. Group A included 40 adult patients diagnosed asSSc, these were subdivided into diffuse systemic sclerosis (dSSc) and limited systemic sclerosis (lSSc) groups. All patients were diagnosed according to the ACR criteria for SSc. Group B included 20 healthy adult persons (age and sex matched) as the control group. All patients were selected from the Rheumatology department Benha University Hospital. Serum IL-33 levels were examined by means of enzyme-linked immunosorbent assay. Result: mean serum level of IL-33 were highly significant in SS patients in comparison to control groups [p<0.0001]. The levels of IL-33 were significantly higher in the dSSc subset compared with the lSSc subset. Also there was a statistically significant correlation between disease activity and serum levels of IL33.* ***Conclusion****: IL-33 may have a significant role in the pathogenesis of SSc. IL-33 serum levels paralleled the severity of the disease subset. Understanding of IL-33 functions is important for the development of new therapeutic approaches including IL-33 inhibitors and IL-33 receptor blockers as a therapeutic target.*

INTRODUCTION

Systemic sclerosis (SSc) is a generalized connective tissue disease characterized by sclerotic changes which affect the skin and internal organs. SSc is generally considered as an autoimmune disorder due to the presence of antinuclear antibodies. Although the pathogenesis of SScis still unclear, previous studies have suggested that SSc induction is regulated by some cytokines or growth factors which induce the extracellular matrix components synthesis, causing injury of the endothelial cells and modulating the function of leukocyte.[1]

Systemic sclerosis (SSc) is a complex autoimmune rheumatic disease that is characterised by widespread skin (scleroderma) and internal organ fibrosis, immune system dysregulation, and vascular alteration.[2]

SSc is a rare rheumatological condition and its incidence is higher in females.[3]

There are 2 clinical subsets according to the extent of skin involvement: diffuse SSc (dcSSc) (skin damage proximal to elbows and/or knees or that affects thorax and/or abdomen at any given time) and limited cutaneous SSc (lcSSc) (skin damage distal to elbows and knees without involvement of either thorax or abdomen).[4]

The pathogenesis of SSc is complex including vascular changes, dysregulation of immunity, and aberrant tissue fibrosis.[5]

This disease may lead to major disabilities due to vascular complications, cardiopulmonary involvement, inflammatory myopathy, and arthritis;also, malnutrition can occur as result ofto gastrointestinal tract involvement, in addition, psychological and social impact can occur decreasing quality of life.[4]

Vasculopathy is believed to occur at early stage of the disease. These vascular changes involve defective/decreased/uncontrolled mechanisms of vascular repair.[6]

Stimulation of both innate and adaptive immunity is seen in SSc. For example, there is a rich perivascular infiltrate in the skin of patients having early diffuse cutaneous SSc andmany patients have SSc related antibodies .[7]

The aetiology and pathogenesis of SSc are complex. The activation of microvascular endothelial cells (ECs) and fibroblasts, and the dysfunction of the acquired immune system are the main pathogenic processes of SSc (2-3). Fibroblast dysfunction results in over-expression and accumulation of collagen and other matrix components, which leads to the occurrence and development of SSc.[8]

Interleukin-33 (IL-33), a member of the IL-1 family, plays a key role ininnate and adaptive immunity. Full-length IL-33 (fl-IL-33) is mainly produced by ECs, fibroblasts, smooth muscle cells and epithelial cells, such as, lung and gut epithelial cells.[6] It is stored in the

nucleus and released by necrotic cells in damaged tissues to modulate inflammatoryresponse.[9]

The receptor ofIL-33, also known as ST2, is selectively expressed by a variety of immune cells, including basophils, eosinophils, dendritic cells, mast cells, macrophages, B cells, T helper 2 (Th2) cells, natural killer (NK) cells, CD8+T cells and regulatory T (Treg) cells.[10]

IL-33 has pleiotropic biological functions that facilitate the proliferation, survival and cytokine secretion of ST2+ cells and several studies suggest that IL-33 could be involved in the pathogenesis of tissue fibrosis.[11]

This study aimed to determine serum levels of IL-33 in SSc patients and evaluate its association with clinical manifestations and disease subset.

METHODOLOGY

The study was done on two groups: Group A included 40 adult patients with SSc, who were subdivided into diffuse systemic sclerosis (dSSc) and limited systemic sclerosis (lSSc) groups. All patients were diagnosed according to the ACR criteria for SSc.[12] Group B included 20 healthy adult persons (age and sex matched) as the control group. All patients were selected from the Rheumatology Department Benha University Hospital. Informed consent was taken from all patients before the beginning of the study. The study protocol was approved by the Local Ethics Committee of the Rheumatology Department Benha University Hospital.

The study population was subjected to the following:

1. Complete history taking
2. clinical examination
3. Skin assessment using the modified Rodnan skin score (mRss)[13]
4. Laboratory investigations, including
   - Complete blood count, routine blood chemistry,
   - Urine analysis,
   - Anti-Scl-70,
   - Anti-centromere, determination of serum levels of IL-33 was measured by human enzyme-linked immunosorbent assay. The procedure was done according to manufacturer's instructions.

Statistical Analysis

The clinical data were recorded on a report form. These data were tabulated and analysed using the computer program SPSS [Statistical package for social science] version 20 to obtain: Descriptive data Descriptive statistics were calculated for the data in the form of: 1) Mean standard deviation (+ SD) Median and inter-quartile range (IQR) for quantitative data. 2) Frequency and distribution for quantitative data.

Analytical statistics in the statistical comparison between the different groups, the significance difference was tested using one of the following tests: 1) Student's t-test and Mann-Whitney test: Used to compare mean of two groups of quantitative data of parametric and nonparametric respectively. 2) ANOVA A test (F value) and Kruskal-Wallis test: Used to compare mean of more than two groups of quantitative data of parametric and non-parametric respectively. 3) Inter-group comparison of categorical data was performed by using Chi square (X 2 -value) and Fisher's exact test (FET). 4) Rho test to measure association between two variables.

P value< 0.05 was considered statistically significant (*) while >0.05 statistically insignificant. P value< 0.01 was considered highly significant (**) in all analyses.

RESULTS

This study was conducted on two groups:

- Group A (40 patients), which was subdivided into dSSc (18 patients) and lSSc (22 patients). They were 37 females and 3 males clinically diagnosed and classified according the ACR criteria for SSc. Their ages ranged between 23 and 70 years with a mean of 45.33±11.79 years
- Group B included 20 healthy persons (age and sex matched) as the control group. They were 18 females and 2 males, their ages ranged between 31 and 65 years with a mean of 50.2±7.67years.

The present study revealed that the mean serum level of IL-33 was significantly higher in SS patients in comparison to control groups [p<0.0001] as shown in table (1).

Table 1:-Serum IL-33 in the studied groups

	Case group (40)		Control group (20)	P value
	dSSc	ISSc		
IL33 level in serum mean value	102.83 ± 35.27 pg/ml	83.02 ± 11.28 pg/ml	65.40 ± 711.26 pg/ml	<0.0001
SD	35.26	10.28	11.27	

Also there was a statistically significant association between disease activity and serum levels of IL33. Table (2)

Table 2: Correlation between activity, serum levels of IL33

	dSSc	ISSc
Mild		
Mean	94.00	79.68
SD	8.50	9.15
Moderate		
Mean	98.4	80.5
SD	8.50	9.15
Severe		
Mean	106.00	85.54
SD	40.83	14.32
P value	< 0.05	< 0.05

There was a statistically high significant positive correlation between serum levels of IL33and disease duration trunk or abdomen skin changes, ESR, CRP, and significant positive correlation between anti-Scl-70, and anti-centromere and serum IL33 level as shown in table 3

Table 3: Correlation between IL33 levels and other variables

	Rho	P value
Disease duration	0.471	0.003**
Proximal skin changes	0.156	0.337
CRP	0.483	0.002**
ESR	0.649	<0.001*
anti-Scl-70	0.38	0.015
anti-centromere	0.332	0.037*

DISCUSSION

One of the proinflamatory cytokines thought to be involved in the pathology of SSc is IL-33. Recent evidence suggests a role for IL-33 in several rheumatological diseases, including SSc, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and systemic lupus erythematosus**.** [14]

This study was conducted on 40 patients diagnosed as SSc according to the ACR criteria and 20 age-matched and sex-matched healthy individuals.

This study revealed that the mean serum level of IL33 was a highly significant increase of the serum IL33 in SS patients compared with control groups ($p<0.0001$. Furthermore the levels of IL-33 were significantly higher in the dSSc subgroup compared with the lSSc subgroup. so, IL-33 serum levels paralleled the severity of the disease.

This is in agreement with the result of Yanaba et al.[15] They found that serum IL-33levels were significantly higher in SSc patients than in healthy individuals. IL-33 levels in dSSc patients were significantly higher than those in lSSc patients or healthy individuals. There was no significant difference in serum IL-33 levels between lSSc patients and healthy individuals.

Yanaba et al. [15] reported that serum samples were taken from 69 Japanese patients diagnosed as SSc They were 56 females and 1 3 males. Their ages ranged between 13 and 73 years with a mean of 47 years. All patients were clinically diagnosed and classified according the ACR criteria for SSc. They were subdivided into dSSc (42 patients) and lSSc (27 patients). Serum IL-33 levels were measured using specific ELISA kits.

The result was further confirmed by Terras et al.[16] in a German SSc cohort, Manetti et al. [17] in an Italian cohort and Zhang et al [18] in a Chinese cohort **.** Their studies also found that the high serum levels of IL-33 were accompanied with peripheral vascular in-volvement, such as digital ulcers and the severity of skin sclerosis and pulmonary fibrosis [19]

A recent work by MacDonald *et al*. [20] showed that the high tissue-localised expression of IL-33 caused the differentiation of Treg cells into Th2-like cells in SSc lesion skin. They also found that a significantly higher percentage of skin FOXP3+Treg cells co-expressed ST2 compared with FOXP3-Tconv cells Furthermore, in bleomycin-treated *Fli1*+/− mice model for SSc, dermal fibroblast-produced IL-33 contributed to Th2-like Treg trans-differentiation [21]**.** These data suggest that the pres-ence and accumulating Th2-like Treg cells in localised skin might increase fibrosis in patients with SSc and therefore provides new insight into the role of IL-33 in SSc.

Multicentric preliminary study was done on 300 Turkish SSc patients and 280 healthy control individuals, ,showed that rs7044343polymorphism of *IL-33* gene was associated with elevated susceptibility to SSc.[22] However, a study in a Chinese population involving 58 patients with SSc and 113 healthy control individuals failed to find any association between *IL-33* rs7044343 polymorphism and SSc susceptibility [23]. Different ethnic backgrounds and the small number of patients may partly explain this discrepancy. More studies are warranted to undercover the possible active role of *IL-33* gene polymorphism in SSc.

Aberrant expression of IL-33 in tisuses is associated with a variety of fibrotic diseases, and the critical role of IL-33 in SSc pathogenesis has begun to be clear.

CONCLUSION

The level of IL33 is correlated with disease activity, and disease progression. IL33 could be used as anew biomarker to monitor the activity and severity of

disease. However, present studies are not enough to sufficiently understand the precise function of IL-33 in the process of fibrosis. Larger sets are needed to confirm the prognostic and diagnostic efficacy of IL33 in SS. Understanding of IL-33 functions is needed for the development of new treatment approaches including IL-33 inhibitors and IL-33 receptor blockers as a therapeutic target. The authors declare that they have no financial or non financial conflicts of interest.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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