Original Article

Interleukin-33 (IL-33) in Systemic Sclerosis and its Association with Early Cardiovascular Involvement By Using Magnetic Resonance Imaging.

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Abstract:

The aim of this study: was to determine serum levels of IL-33 in systemic sclerosis patients and evaluate its association with early cardiovascular involvement by using magnetic resonance imaging. Group (A): included 30 adult patients with Systemic Sclerosis (SSc), all diagnosed according to the American college of rheumatology (ACR) criteria for SSc. Group (B): included 20 healthy adult persons (age and sex matched) as a control group. **Methods:** Forced vital capacity (FVC) was measured using a PKM spiroflow spirometer, Cardiac magnetic resonance examination by using delayed enhancement technique, Carotid Doppler to evaluate carotid intima-media thickness (IMT) and plaque stiffness. Computed tomography (CT) of the chest and coronary calcium scoring, Serum IL-33 levels were examined by enzyme-linked immunosorbent assay in 30patients with SSc and 20 healthy individuals, Skin assessment using modified Rodnan Skin Score. **Results:** IL-33 was increased in all SSc patients as compared to controls.IL-33 and clinical findings in diffuse subtype dSSc patients had a positive correlation between the presence of joint contracture. There is statistically significant correlation between patients with CMR findings and modified rodnan skin score Increased level of IL-33 ,positive findings in CMR also increased in both diffuse and limited subtypes ,however statistical significance was only with diffuse one with (p=0.031), Higher level if IL-33 (median =100 pg/ml) was associated with positive findings in HRCT in statistically significance ,IL-33 is highly correlated to the presence of pulmonary fibrosis, skin sclerosis, joint contracture and modified rodnan skin score, So IL-33levels were increased in SSc patients and correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis. Therefore, IL-33 possibly plays a role in cutaneous and pulmonary fibrosis in SSc patients. **Conclusion:** Cardiac MRI is an excellent measure for identifying early cardiovascular involvement in order to provide a more accurate management of SSc patients. IL33 most probably has as significant role in the pathogenesis of Systemic Sclerosis. IL-33 serum levels paralleled the severity of the disease subset. Understanding of IL-33 functions is important for development of new therapeutic approaches including IL-33 and IL-33 receptor as a therapeutic target.

Keywords: IL33, Systemic Sclerosis & CVD, MRI

Introduction:

Systemic sclerosis (SSc) is a complex chronic connective tissue disease. It is characterized by chronic multisystem involvement of skin and internal organs. SSc is a connective tissue disease characterized by cutaneous and visceral fibrosis [(1)],and widespread vascular pathology. Theunderlying cause for SSc remains poorly understood, but it is well established that microvascular endothelial cells are the target tissue in this disease. [(2)]

The ACR and the European League Against Rheumatism (EULAR) established a committee to provide a joint proposal for new classification criteria for SSc.[(3)]

The new classification criteria shows that skin thickening of the fingers extending proximal to the meta carpo phalangeal joints is sufficient for the patient to be classified as having SSc; if that is not present, seven additive items apply, with varying weights for each: skin thickening of the fingers, fingertip lesions, telangiectasia,

abnormal nailfold capillaries, interstitial lung disease or pulmonary arterial hypertension, Raynaud's phenomenon, and SSc-related autoantibodies. [4]

The vascular involvement of SSc has been considered to be mainly micro vascular. Macro vascular involvement is considered rare. However, increased prevalence of macro vascular disease has been reported in recent literature.[5]

Coronary artery disease is not uncommon in SSc patients,[5] but its prevalence is similar and not greater to that expected in individuals without SSc. An increased prevalence of distal peripheral artery disease in the digits has been found in several studies. [6-8]

Epidemiology Similar to other connective tissue diseases, SSc is more frequent in women than men, with the most common age of disease onset in the 25 to 50 years range.

Pathophysiology Endothelial alterations may lead to a cascade of stimulatory changes that involve many cells, including fibroblasts, T lymphocytes, macrophages, and mast cells. In turn, the activated cells secrete a variety of substances, including cytokines and their soluble receptors and enzymes and their inhibitors. These substances lead to changes in the extracellular matrix compounds, including fibronectin; proteoglycans; and collagen types I, III, V, and VII. Increased collagen deposition in tissues is a characteristic feature of systemic sclerosis. Increased collagen production or disturbances in its degradation can cause excessive collagen deposition in tissues.[9]

Pathogenesis Activation of T cells is evident in lesional tissues and in peripheral blood, and seems to play a direct role in tissue injury. Vascular injury and activation are the earliest and possibly primary events in the pathogenesis of SSc.[10]

Clinical Features

Skin Manifestations Cutaneous telangiectasia, dilations of dermal blood vessels, occur in limited cutaneous SSc and diffuse cutaneous SSc, but are more extensive in limited cutaneous SSc, particularly the subgroup previously designated as CREST (calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, and telangiectasia) syndrome.[11]

Gastrointestinal Tract Manifestations Esophageal involvement is frequent with dysmotility and lower esophageal sphincter dysfunction, and consequent gastro esophageal reflux is almost universal in SSc.[12]

Musculoskeletal Involvement The fibrotic process of SSc commonly affects the tendons (causing tendon friction rubs), ligaments, and joint capsules, restricting movement.

Contraction of the fingers is a hallmark of SSc, may develop rapidly, and has a significant impact on hand function.[13]

Pulmonary Involvement The most common forms of interstitial lung disease in SSc are histologically classified as usual interstitial pneumonia and nonspecific interstitial pneumonitis.

PAH, defined as an elevation in the mean pulmonary artery pressure greater than 25 mm Hg at rest, occurs in limited and diffuse cutaneous forms of SSc and is a leading cause of mortality.[14]

Renal Manifestations Scleroderma renal crisis occurs in 10% to 15% of patients with diffuse cutaneous SSc and only vary rarely (1% to 2%) in limited cutaneous SSc.[15]

Neurologic Manifestations In early-stage diffuse cutaneous SSc, patients commonly report symptoms of median nerve compression, and many patients undergo surgical treatment for carpal tunnel syndrome before the diagnosis of SSc is established.[15]

Interleukin 33 IL-33 is a newly reported cytokine of IL-1 family. Recent evidence suggests a role for IL-33/ST2 in several rheumatological diseases, including systemic sclerosis, rheumatoid arthritis (RA), osteoarthritis (OA), psoriatic arthritis (PsA) and systemic lupus erythematosus (SLE).[16,17] In SSc, the combination of vascular abnormalities, collagen deposition, and autoimmunity leads to widespread tissue and organ fibrosis.[18] It has been found that, compared to healthy controls, IL-33 expression was significantly increased in SSc patients. Meanwhile, the serum level of IL-33 was correlated with

early disease stage and micro vascular involvement.[19] Moreover, some other investigators reported the same observations recently.[20] These data prompted us that IL-33 should be involved in the SSc pathogenesis, and the mechanism may be correlated with the role of IL-33 in promoting fibrosis.[21]

Subjects:

The study included 2 groups:

Group (A): included 30 adult patients with Systemic Sclerosis (SSc), all diagnosed according to the American college of rheumatology (ACR) criteria for SSc.[3]

Group (B): included 20 healthy adult persons (age and sex matched) as a control group.

All patients were selected from Internal medicine department, Rheumatology unit, Main Alexandria University hospital.

An informed consent was taken from all patients before the beginning of the study.

Methods:

This study was conducted on 30 patients with Systemic Sclerosis and 15 healthy adult persons as a control group admitted to rheumatology unit at Alexandria Main University Hospital.

The study population was subjected to:

A. Complete History taking including:

- Demographic data.
- History of the present complaints.

B. Thorough clinical examination included the following

- Vital signs
- Head and neck examination
- Cardiovascular examination
- Chest examination
- Abdominal examination
- Skin and extremities

C. Skin assessment using modified Rodnan Skin Score:[22]

Using a semi-quantitative estimation of the degree of skin thickening.

D. Laboratory investigations including:

1- Complete Blood Count, routine blood chemistry.

2- Urine analysis.

3- Anti Scl-70.

4- Anti-centromere.

5- Determination of Serum levels of IL-33 measured by human ELISA.

6- Computed tomography(CT) of the chest and coronary calcium scoring

7- Carotid Doppler: to evaluate carotid intima – media thickness

8- Cardiac magnetic resonance examination to evaluate cardiac anatomy, myocardial perfusion and viability

9- Pulmonary function test using spirometer

RESULTS:

Table (I) shows IL-33 in the studied groups. IL-33 in dSSc patients ranged 80-140 pg/ml with mean value 97.21±13.43 pg/ml, in lSSc ranged 88-125 pg/ml with mean value 103.33±15.56 pg/ml and in control persons ranged 50-84pg/ml with mean value 66.20±10.72 pg/ml values. There was statistical significant difference regarding IL-33 in studied groups (P =0.001). Serum levels of IL-33 were significantly higher in SSc patients than in healthy controls with no significant difference between diffuse and limited subtype regarding serum levels of IL-33 (p=0.363) table (I)

There is statistically significant correlation between patients with CMR findings and modified rodnan skin score with median 30.0 versus 23.0 in patients without CMR CHANGES, So CMR changes correlate significantly with severity of mRSS in patients subtypes.

Table (III**) shows:** With Increased level of IL-33, positive findings in CMR also increased in both diffuse and limited subtypes ,however statistical significance was only with diffuse one with (p=0.031)

Table (IV): shows Higher level if IL-33 (median =100 pg/ml) was associated with positive findings in HRCT in statistically significance with (p=0.031) versus level of IL-33 (median =90 pg/ml) with normal HRCT imaging.

Table (I): Comparison between the studied groups according to readings of IL-33.

	Cases			Control (n=20)	^{KW}p
	Total (n=30)	Diffuse type (n=24)	Limited type (n=6)		
IL-33 pg/ml					
Min. – Max.	80.0 – 140.0	80.0 – 140.0	88.0 – 125.0	50.0 – 84.0	
Mean ± SD.	98.43 ± 13.82	97.21 ± 13.43	103.33 ± 15.56	66.20 ± 10.72	<0.001*
Median	96.0	95.0	98.50	66.0	
Sig.bet.Grps	p_1<0.001*,p_2<0.001*, p_3<0.001*, p_4=0.362				

Table (II**)**: Relation between cardiac MRI findings and modified rodnan skin score in total sample (n=30)

	Cardiac MRI findings		T	P
	No (n =14)	Yes (n =16)		
Modified rodnan skin score				
Min. – Max.	18.0 – 29.0	22.0 – 35.0		
Mean ±SD.	23.64 ± 3.52	29.88 ± 3.81	4.629*	<0.001*
Median	23.0	30.0		

Table (III): Relation between cardiac MRI findings and IL.33 levelin SSc.

Cardiac MRI Findings	N	IL.33 level			Z	P
		Min. – Max.	Mean ± SD.	Median		
Diffuse type						
No	**10**	80.0 – 98.0	90.70 ± 5.98	91.0	2.180*	0.029*
Yes	**14**	85.0 – 140.0	100.79 ± 14.1	100.0		
Limited type						
No	**4**	88.0 - 95.0	92.0 ± 3.56	92.50	1.879	0.060
Yes	**2**	100.0 – 125.0	112.50 ± 17.7	112.50		

Table (IV): Relation between HRCT findings and IL33 level in SSc patients

	HRCT findings		Z	P
	absent (n =11)	present (n =19)		
Il.33 level				
Min. – Max.	80. – 125.0	90.0 – 140.0		
Mean ± SD.	95.16 ± 12.70	104.09 ± 14.42	2.163*	0.041*
Median	90.0	100.0		

Discussion:

One of the proinflamatory cytokines believed to be involved in the pathology of SSC is IL-33.

Recent evidence suggests a role for IL-33/ST2 in several rheumatological diseases, including systemic sclerosis, rheumatoid arthritis (RA), osteoarthritis (OA), psoriatic arthritis (PsA) and systemic lupus erythematosus. (SLE). The present study was conducted on 30 patients with systemic sclerosis fulfilling the ACR criteria for diagnosis of the disease and 15 age- sex matched healthy individual control group.

IL-33 in patients Group A ranged 75-220, Group B ranged 62-98 and in control group ranged 50-84. There was statistical significant difference regarding IL-33 in three studied groups ($P < 0.5$).

In the present study IL-33 was increased in all SSc patients as compared to controls.

Furthermore the levels of IL-33 were significantly higher in the dSSc subset compared to the lSSc subset. Thus IL-33 serum levels paralleled the severity of the disease subset. Similar results have been reported by several studies including Wagner A, et al in 2015.[23] Wagner A, et al suggested that an endothelial, T cell and fibroplast activation can be present in patients with early SSc, suggesting that new routes of investigation of cell-cell dynamics in target tissues predating overt disease manifestations, thus opening new therapeutic approaches.

Furthermore YanabaK, et al in 2011[24] also reported that Serum IL-33 levels were elevated in SSc patients compared with healthy individuals. Patients with diffuse cutaneous SSc had higher levels of IL-33 than those with limited cutaneous SSc.

As in our study, patients with diffuse SSc had higher levels of IL-33 than those with limited cutaneous SSc.

We also studied the correlation of IL-33 levels in SSc patients with clinical manifestations.

We found a significant positive correlation between IL-33 levels and the presence of pulmonary fibrosis, skin sclerosis, Raynaud's phenomenon, pitting scares and ulcers, pulmonary hypertension, Joint contracture and modified rodnan skin score.

Similar results are reported by YanabaK, et al in 2011[24] who reported that IL-33 levels corrected positively with extent of skin fibrosis and pulmonary fibrosis.

Thus suggesting that IL-33 may possibly play a role in organ fibrosis in SSc patients.

Conclusion:

IL-33 was increased in all SSc patients as compared to controls. So we can conclude that IL33 most probably has as significant role in the pathogenesis of Systemic Sclerosis. Furthermore the levels of IL-33 were significantly higher in the dSSc subset compared to the lSSc subset.

Thus IL-33 serum levels paralleled the severity of the disease subset.

IL-33 is highly correlated to the presence of pulmonary fibrosis,skin sclerosis, Raynaud's phenomenon, pitting scares and ulcers, pulmonary hypertension, Joint contracture and modified rodnan skin score.

So understanding of IL-33 functions is important for development of new therapeutic approaches including IL-33 and IL-33 receptor as a therapeutic target.

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