sclerosis, rheumatoid arthritis and SLE [12,13]. Some studies reported the correlation of IL-33 with rheumatic diseases, and most of them found that the IL-33 expression levels were consistent with disease activity and development [14]. Furthermore, evidence has indicated that IL-33-related treatment may ameliorate the pathogenic conditions and attenuate disease progression of those rheumatic diseases [15]. The aim of this study was to estimate serum levels of IL-33 in SLE and determine whether these levels correlated with disease activity and clinical presentation in SLE patients.

Patients and Methods

This study was carried out in the Dermatology and Venereology, Rheumatology, Nephrology and Clinical Pathology Departments, Zagazig University Hospitals during the period from May 2015 to May 2016 on 42 subjects divided into two groups: Group A included 24 SLE patients diagnosed according to the American College of Rheumatology (ACR) classification criteria for SLE which uses a standard classification scheme requiring 4 of 11 criteria for definite case definition [16] and group B included apparently healthy persons who were age and sex matched and non-relative to patients as control. Patients received no systemic treatment before inclusion into the study and all subjects who had known allergic disease as bronchial asthma or atopic dermatitis were excluded. This study was approved by the Institutional Review Board (IRB) at Faculty of Medicine, Zagazig University. A written informed consent was taken from all subjects before inclusion into the study.

Patients were subjected to: Full history taking, complete clinical examination as pulse, blood pressure, temperature, cardiovascular, chest, renal, vascular, neurological and dermatological examination for skin, hair, nail and mucous membranes.

Laboratory investigations included: Routine laboratory tests as Complete Blood Picture (CBC), Erythrocytic Sedimentation Rate (ESR), C-reactive protein (CRP), urine analysis, kidney function tests and protein in urine within 24hrs.

Specific investigation: Sera were analysed for IL-33 by sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) according to the instructions of Novus Biological USA. Assessment of disease activity in SLE patients by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score: No activity (SLEDAI; 0), mild activity (SLEDAI; 1-5), moderate activity (SLEDAI; 6-10), high activity (SLEDAI; 11-19), very high activity (SLEDAI>20) [17].

Statistical Analysis

Data were checked, entered and analysed using SPSS version 20. Data were expressed as mean ± SD for quantitative variables or numbers and percentage for categorical variables. Chi-Squared (x^2), t test, ANOVA (f test) and correlation coefficient (r) were used when appropriate. P value>0.05 was considered statistically significant.

Results

Group A included 18 females (75.0%) and 6 males (25.0%) and their ages ranged between 22-46 years with a mean ± SD of 31.8 ± 7.4. Family history was positive only in 2 patients. Group B included 18 apparently healthy control subjects who were age and sex matched with group A. They included 12 females (66.7%) and 6 males (33.3%), and their ages ranged from 23-45 years with a mean ± SD of 32.5 ± 6.3 (Table 1). There was no significant difference in age and sex between patients and control.

	Group A	Group B	t	P
Age(years)	N=24	N=18		
$\bar{x}$ ± SD	31.8 ± 7.4	32.5 ± 6.3	0.32	0.74
Range	22-46	23-45		
Gender	N%	N%	x^2	P
Male	6 25.0	6 33.3	0.35	0.55
Female	18 75.0	12 66.7		Ns
Family history			x^2	P
+ve	2 8.3	0 0.0	1.57	0.2 Ns

Table1: Demographic data of patients and control groups (Ns: Non-significant, there was no significant difference in age and sex between patients and control).

Clinical finding of SLE patients was observed that most frequent clinical finding was arthritis (87.5%), followed by photosensitivity (62.5%) and malar rash (54.2%). After examination of hair, 7 patients (29.2%) were complaining of alopecia (Table 2).

Items	N	%
Malar rash	13	54.2%
Photosensitivity	15	62.5%
Oral ulcers	6	25.0%
Alopecia	7	29.2%
Nail disorders	1	4.2%
Vasculitis	2	8.3%
Arthritis	21	87.5%
Myositis	0	0.0%
Pleurisy	2	8.3%
CVD (cardiovascular diseases)	3	12.5%
Nephritis	6	25.0%
Seizures	4	16.7%
Psychosis	1	4.2%
Visual disorder	4	16.7%
Lupus headache	2	8.3%

Table 2: Clinical findings of SLE patients.

Laboratory findings of studied groups: The most frequent laboratory abnormalities were ANA positivity found in all patients (100%) and Anti-ds DNA positivity found in 83.3% of patients. In group B, ANA and Anti-ds DNA were negative. Active lupus nephritis was detected by the presence of proteinuria (<0.5gm/day) in 17 patients (70.8%),

Pyuria (<5WBCs/HPF) in 13patients (54.2%), heamaturia (<5RBCs/HPF) in 11patients (45.8%) and casts in 1 patient (4.2%).

Items	No	%
Renal parameters		
Protenuria	17	70,8%
Heamaturia	11	45.8%
Pyuria	13	54.2%
Heamatological parameters		
Leucopenia	1	4.2%
Thrombocytopenia	3	12.5%
Iacreased E S R level	20	83.3%
Iacreased C R P level	20	83.3%
Positive ANA	24	100%
Positive Anti–ds DNA	20	83.3%

Table 3: Laboratory investigations among SLE patients (ESR: Erythrocyte Sedimentation Rate; CRP: C Reactive Protein; ANA: Anti-Nuclear Antibodies; Anti–ds DNA: Anti Double Stranded DNA).

Items	Group A N=24	Group B N=18	t	p
Creatinine in serum (mg/dl)				
x̄ ± SD	1.9 ± 1.97	0.9 ± 0.2	2.19	0.03*
Range	0.5-8	0.6-1.3		
Median	1.2	0.9		
Serum Urea Nitrogen (mg/dl)				
x̄ ± SD	39.5 ± 36.9	12.7 ± 2.8	3.07	0.004*
Range	16-121	8-17		
Median	19.5	12		

Table 4: Differences of kidney function tests between both groups (Serum urea and creatinine were significantly higher in SLE patients more than the control group).

Abnormalities in blood picture were evident with leukopenia (<3000/mm^3) found in one patient (4.2%) and thrombocytopenia (<100 000/mm^3) was found in 3 patients (12.5%). Elevated ESR level was found in 20 patients (83.3%) and high CRP was found in 20 patients (83.3%) (Table 3). In SLE patients, serum urea and creatinine were significantly higher in SLE patients more than the control group (Table 4). The activity categories: Mild, moderate, high and very high activity categories was 4.2%, 16.7%, 33.3% and 45.8% respectively. The majority of patients had very high activity of disease (Table 5).

Estimation of IL-33: In group A; serum level of IL-33 was at a median of 137 with range from 81-625 pg/ml with a mean ± SD of 164.1 ± 110. In group B; it was at a median of 21 with range from 10-42 pg/ml with mean ± SD of 23.3 ± 8.7.

Items	N	%
SLEDAI		
x̄ ± SD	18.2 ± 8.5	
Range	4-40	
Mild activity (1-5)	1	4.2%
Moderate activity (6-10)	4	16.7%
High activity (11-19)	8	33.3%
Very high activity (≥ 20)	11	45.8%

Table 5: Grades of activity in the SLE group (SLEDAI: Systemic Lupus Erythematosus Disease Activity Index).

The serum level of IL-33 in group A was significantly higher when compared to group B (Table 6). There is highly significant positive correlation between IL-33 and ESR, CRP, serum creatinine & SLEDAI where r is 0.58, 0.5, 0.81 and 0.55 respectively and p<0.001. There is no significant +ve or –ve correlation between IL-33 and RBCS, WBCS, platelet count, HB and serum urea nitrogen where r is -0.3, 0.4, -0.06, -0.15 and 0.32 respectively and p<0.05 (Table 7).

Items	Group A N=24	Group B N=18	t	P
IL-33 (pg/ml)				
x̄ ± SD	164.1 ± 110	23.3 ± 8.7	5.36	>0.001
Range	81-625	10-42		Hs
Median	137	21		

Table 6: IL-33 among studied groups (Hs: Highly-significant, there is a highly significant difference in the serum level of IL-33 in SLE patients compared to controls).

Items	r	p	sig
ESR	0.58	>0.001	Hs
CRP	0.5	>0.001	Hs
RBCS	-0.3	<0.05	Ns
WBCs	0.04	<0.05	Ns
Platelet count	-0.06	<0.05	Ns
HB	-0.15	<0.05	Ns
Serum creatinine	0.81	>0.001	Hs
Serum Urea nitrogen	0.32	<0.05	Ns
SLEDAI	0.55	>0.001	Hs

Table 7: The correlation between IL-33 and laboratory parameters (Hs: Highly-significant; Ns: Non-significant; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; RBCS: Red Blood Cells;

WBCs: White Blood Cells; HB: Haemoglobin. There is highly significant positive correlation between IL-33 and ESR, CRP, serum creatinine & SLEDAI and there is no significant +ve or –ve correlation between IL-33 and RBCS, WBCS, platelet count, HB and serum urea nitrogen).

Items	r	p	sig
Arthritis	0.55	>0.001	Hs
Photosensitivity	0.2	<0.05	NS
Oral ulcers	0.51	>0.001	Hs
Alopecia	-0.33	<0.05	Ns
Visual disorder	0.042	<0.05	Ns
Malar rash	-0.06	<0.05	Ns
Lupus headache	-0.15	<0.05	Ns
Vasculitis	0.81	>0.001	Hs
Seizures	0.32	<0.05	Ns
Nephritis	0.55	>0.001	Hs
CVD	0.58	>0.001	Hs

Table 8: The correlation between IL-33 and clinical finding of SLE patients (There was a highly significant correlation between IL 33 serum levels and arthritis, oral ulcer, vasculitis, CVD (cardiovascular diseases) and nephritis. There were no correlations with other clinical findings).

Discussion

Interleukin-33 is a cytokine that belongs to the IL-1 family. It has been found to be involved in the pathogenesis of chronic inflammatory arthritis like its other family members, IL-1 and IL-18. It has also been described as a modulator of inflammation, mediating Th2 immune responses. Interleukin-33 has been shown to induce production of IL-5, IL-13 and hypergammaglobulinaemia. It is also recognized to possess a chemo attractant effect for human Th2 cells [18]. Interleukin-33 is also a nuclear protein that is also released into the extracellular space, and thus acts as a dual-function molecule, as does IL-1α. Extracellular IL-33 binds to the cell-surface receptor ST2, leading to the activation of intracellular signalling pathways similar to those used by IL-1. Unlike conventional cytokines, IL-33 might be secreted *via* unconventional pathways, and can be released upon cell injury as an alarmin. Interleukin-33 has been implicated in a wide range of immune-mediated diseases, including allergic, cardiovascular, and autoimmune conditions, which, depending on the disease setting, may elicit beneficial or detrimental effects [19].

Based on the insufficient information related to serum IL-33 levels in SLE patients, this comparative study between serum IL-33 in SLE patients and healthy controls was done to evaluate differences between serum IL-33 in patients with SLE and in controls, and its relation with disease activity and clinical presentation in SLE patients. SLE patients were diagnosed according to ACR classification criteria of SLE, and the percentage of criteria in the studied SLE patients were: positive ANA found in all patients (100%), arthritis which was the most frequent clinical finding; it affected about 87.5% of patients and anti-dsDNA antibodies in 83.3% of patients, malar rash affected 54.2% of patients, oral ulcers affected 25% of patients, renal manifestations affected about 25% of patients and neurological manifestations affected 16.7% of patients. These percentages were comparable to the percentages in the study of Wislowska et al. which were arthritis (80%), malar rash (45%), renal manifestations (30%), oral ulcers (20%) and neurological manifestations (20%) (Table 8) [20]. On the contrary, the percentage of the remaining criteria were different, in this work they were photosensitivity affected 62.5% of patients and haematologic manifestations affected 12.5% of patients. In the study of Wislowska et al. they were photosenstivity (90%) and haematologic manifestations (50%) [20].

The severity of disease was measured using SLEDAI according to Bombardier et al. [17]. It was observed that SLEDAI of the SLE patients ranged between 4-40 with a mean ± SD 18.2 ± 8.5, mild, moderate, high and very high activity categories was 4.2%, 16.7%, 33.3% and 45.8% respectively, which indicated that the majority of patients had very high activity of disease. In the present study, we detected higher levels of IL-33 in patients with SLE, compared to control. In addition, serum IL-33 levels were correlated with disease activity index (SLEDAI), which was consistant with previous results of [21,22]. On the other hand Mok et al. [23] reported that majority of SLE patients and healthy controls had very low IL-33 levels, even below the lowest detection limit of the assay and no difference for serum IL-33 level was found between SLE patients and control. The reason of this discrepancy remained unclear, but it may be related to different assay kit or different population studied. In the study of Yang et al. [21], most clinical and laboratory characteristics of SLE patients did not correlate with serum IL-33 levels, with the exception of thrombocytopenia, erytherocytopenia, ESR, CRP and IgA. In our present study we found that IL-33 levels correlated significantly with ESR, CRP, serum cereatinine and SLEDAI. As ESR and CRP are diagnostic indicators of SLE patients being in acute inflammatory phase, these results suggest that IL-33 may be involved in the acute inflammatory phase of the disease and acts as an alarmin and early inducer of inflammation.

Yang et al. [21] also reported that haematologic disorders were one of the most frequent disorders of patients with SLE, including leukopenia (43%), thrombocytopenia (30%) or erythrocytopenia (44%). However in the present study haematologic disorders were leukopenia (<3000/mm^3) in one patient (4.2%) and thrombocytopenia (<100000/mm^3) in 3 patients (12.5%). Interestingly, Yang et al. [21] results demonstrated that IL-33 was not correlated with leukopenia in patients with SLE, which suggested that IL-33 may not influence production of the whole leukocytes although it can regulate some functions of leukocytes.

In an another study, Mock's et al. [23] demonstrated that soluble ST2 levels were higher in SLE patients with active disease, including renal and non-renal manifestations compared with those of lesser disease activity and normal controls. They suggested that soluble ST2 may be a potential surrogate marker for disease activity in SLE patients. Increasing evidence has shown that IL-33 and its receptor ST2 contribute to renal fibrosis, which is the end stage of lupus nephritis [18]. In an animal study, Li et al. [22] reported that anti IL-33 antibodies treatment provided therapeutic and survival benefit for lupus prone mice. They reported the therapeutic effect of IL-33 blockade might be due to systemic blunting of auto-immunity and pro inflammatory responses as reflected by reduced levels of auto antibodies, C3, and pro inflammatory cytokines in kidney deposits and

serum as well as renal immune complex deposition. In our study, we detected significant correlation between IL-33 levels and serum creatinine in SLE patients. Although the role of IL-33 in the development of SLE still to be declared, this provide indirect evidence for its role in lupus nephritis. Rheumatoid arthritis (RA) is an another autoimmune disease closely related to SLE. Pei et al. [14] reported that the levels of IL-33 and ST2 in sera and synovial fluid samples are significantly increased in RA patients, particularly those with active disease, compared with healthy controls, osteoarthritis and psoriatic arthritis patients.

Furthermore, higher levels of IL-33 are observed in synovial fluid than serum samples of the same RA patients. These findings could explain that arthritis was the most frequent clinical finding in the present study; it affected about 87.5% of patients.

Conclusion

The serum IL-33 level was significantly higher in SLE patients than control group and its level was significantly related to disease activity. IL-33 may play a role in the inflammatory phase of SLE and may have a role in local inflammation of tissues as arthritis and lupus nephritis.

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