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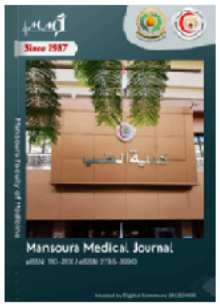

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Zeinab Zeid
Rheumatology and Immunology unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt,
zainabzaid78@gmail.com

Ehab Eltoraby
Rheumatology and Immunology unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt,
ehabeltoraby@yahoo.com

Adel Abdelsalam
Rheumatology and Immunology unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt,
adel_abdelsallam_2013@yahoo.com

Youssef Mosaad
Clinical pathology Department, Faculty of Medicine, Mansoura University, Egypt, youssefmosaad@yahoo.com

Samar Tharwat
Rheumatology and Immunology unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt,
samartharwat2000@yahoo.com

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Serum Level Interleukin-33 and Relation to Systemic Disease Activity in Juvenile Idiopathic Arthritis (JIA)

Zeinab Shaaban Mahmoud Zeid[1], Samar Tharwat[1], Youssef Mosaad[2], Adel Abdelsalam[1], Ehab Eltoraby[1]

[1] Rheumatology and Immunology unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt

[2] Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt

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Abstract

The pathogenesis of juvenile idiopathic arthritis (JIA) is not fully understood. So, this study was conducted to investigate the clinical usefulness of serum IL-33 levels as an indicator of the disease activity in JIA. **Methods.** This cross-sectional study was conducted on 45 JIA patients. All patients were subjected to detailed history taking, complete clinical examination with emphasis on pattern and distribution of articular and extra-articular involvement. Disease activity score (DAS28) was assessed. Venous blood (10 ml) was collected to assess routine laboratory investigations in addition to IL-33. **Results .** Most of patients had sudden arthritis onset (64.4%) and (95.6%) had progressive course. 6 patients (13.3%) of patients had oligoarticular arthritis, 17 patients (37.8%) had polyarticular arthritis and 22 patients (48.9%) had systemic arthritis. 88.9% had emotional aggravation factor, while 86.7% had medication and movement as relieving factors. Only 8.9% of patients' diseases are controlled, 46.7% had relapse, and 6.7% stopped treatment. 66.7% of patients had good response and 33.3% had poor response. JIA patients had a significantly higher serum IL-33 levels compared to IL-33 serum in the control group (p=0.008). There was significant positive correlation between IL-33 with duration of disease (p=0.002, r=0.612), DAS28CRP (p<0.001, r=0.705) and DAS28_ESR (p=0.001, r=0.667), However, serum IL- 33 levels did not significantly correlate with the levels of serum CRP or ESR. **Conclusions.** JIA patients have significantly elevated IL-33 serum concentrations and that considerably correlated with clinical, laboratory and activity parameters of disease suggesting that it could be a valuable marker of JIA disease activity.

Corresponding author: Zeinab Shaaban Mahmoud Zeid, Email : zainabzaid78@gmail.com, Tel: :00201090699046
Address: Faculty of medicine- Mansoura university

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a heterogeneous and multifactorial inflammatory disease characterized by chronic joint inflammation in children with onset ages younger than 16 years. It is the most common chronic rheumatic disease of childhood and an important cause of disability in them. JIA has different subtypes that are defined based on the number of joints involved in the first 6 months of disease and the extra-articular involvement [1].

The pathogenesis of JIA is not fully understood but high numbers of autoreactive T cells are observed in inflamed joints in patients with poly-JIA, indicating an antigen-driven activation of the adaptive immune system might play a role in the pathogenesis of poly-JIA [2].

Interleukin-33 (IL-33) is a novel IL-1 family cytokine that plays a major role in inflammatory, infectious, and autoimmune diseases. IL-33 is a nuclear protein that is constitutively expressed in epithelial and endothelial cells, fibroblast, and activated macrophage [3].

There is increasing evidence in both humans and mouse models that IL-33 plays a role in the development and progression of rheumatoid arthritis (RA). In human RA, IL-33 expression in fibroblast-like synoviocytes increases after stimulation by TNF-a with or without IL-1b [4].

Previous studies revealed that IL-33 is elevated in sera and synovial fluids from RA patients and IL-33 was released from fibroblast-like synoviocytes after stimulation with TNF-a and IL-1b [5]. Furthermore, IL-33 levels in sera and synovial fluids correlated with disease activity [6].

In JIA patients, previous report found that serum IL-33 was elevated in patients with s-JIA [7, 8]. So, this study was conducted to investigate clinical usefulness of serum IL-33 levels as an indicator of disease activity in other subtypes of JIA and determined their correlation with measures of disease activity.

Methods

This cross-sectional study was conducted on 45 JIA patients fulfilling the International League of Associations for Rheumatology (ILAR) classification criteria for JIA from the Rheumatology and Immunology Departments, inpatient and outpatient clinics, Mansours University Hospital from May to December 2018.

Patients were excluded if they age >15 years, had a recent infection, malignancy, other autoimmune disease, DM, IBD or received anti-TNF in the past 3 months.

All JIA patients were subjected to detailed history taking, full clinical examination with emphasis on pattern and distribution of articular involvement, presence of uveitis and other extraarticular findings and current medications.

Juvenile arthritis disease activity score (JADAS28) was assessed in oligoarticular and polyarticular patients as its performance in systemic-onset is unclear. Disease activity was graded to high, moderate and low according to Beukelman et al. [9] and inactive disease was identified using Wallace criteria [10].

Venous blood (10 ml) was collected and separated into two tubes: one on EDTA for Complete blood count (CBC) and IL-33 relative gene expression and the other left to clot for 10 15 min. Serum was centrifuged (2000 rpm for 10 min.) and used for clinical chemistry tests: aminotransferases (AST, ALT), ferritin, C-reactive protein (CRP), rheumatoid factor (RF) and serum IL-33. Another blood sample on citrate was collected to assay the erythrocyte sedimentation rate (ESR). Serum IL-33 level was assessed by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software. Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Chi square test (x2), fisher's exact test and analysis of variance (f) test were used as tests of significance. Kruskal-Wallis tests were used for nonparametric variables. We used Fisher's exact test and Chi-square test (X2) to compare categorical variables. Spearman's correlation coefficient was used to test linear associations between variables.

Results

The mean age of the 45 patients was 11.29±3.43 years ranged from 3.5-16 years; 32 females (71.1%) and 13 males (28.9%) (F: M 2.5:1). Nearly half of them have rural residence. Most of patients (97.8%) are students, 28 (62.2%) had moderate socioeconomic status, 11 (24.4%) low and 6 (13.3%) had high socioeconomic status. Most of patients had negative consanguinity. 21 (46.7%) had disease duration from 1 to 5 years, 15 (33.3%) less than one year and 9 (20.0%) more than five years of disease.

In this study,6 patients (13.3%) of patients had oligoarticular arthritis, 17 patients (37.8%) had polyarticular arthritis and 22 patients (48.9%) had systemic arthritis. 24.4% of patients had three years delayed diagnosis, 22.2% had two years, 17.8% had one year and 20% had six months delayed diagnosis. Most of patients had sudden arthritis onset (64.4%) and (95.6%) had progressive course. Most of patients (64.4%) had general sites of distribution. 88.9% had emotional aggravation factor, while 86.7% had medication and movement as relieving factors. Only 8.9% of patients' diseases are controlled, 46.7% had relapse, and 6.7% stopped treatment. 66.7% of patients had good response and 33.3% had poor response.

Regarding treatment, 95.6% take steroids, 46.7% on methotrexate, 2.2% on

Tocilizumab with 51.1% of patients with duration of medications less than one year, 40% with duration of medications between 1 to 5 years and 8.9% with duration of medications more than 5 years.

Table 1. Associated manifestations of the study patients.

Manifestation	(n=45)
Morning stiffness	
Less 1hour	1 (2.2%)
More than hour	44 (97.8%)
General manifestation	
Fever, fatigue	15 (33.3%)
Anorexia, loss appetite	1 (2.2%)
All	29 (64.4%)
Skin mucus membrane	
Rash	16 (35.6%)
Normal	26 (57.8%)
Psorasis start first then progress to arthritis	3 (6.7%)
Ocular symptoms	
Itching	19 (42.2%)
Redness	7 (15.6%)
Normal	19 (42.2%)
Uveitis	
Present	3 (6.7%)
Absent	42 (93.3%)
Genitourinary manifestation	
Discharge	1 (2.2%)
Normal	44 (97.8%)
CVS manifestation	
Dyspnea	1 (2.2%)
Normal	44 (97.8%)
CNS manifestation	
Headache	1 (2.2%)
Normal	44 (97.8%)
GIT manifestation	
Heart burn	27 (60.0%)
Normal	18 (40.0%)

Table 1 shows that 97.8% had morning stiffness more than one hour, 33.3% had fever and fatigue, 35.6% had skin rash, 42.2% had eye itching, 6.7% had uveitis, 2.2% had genitourinary discharge, 2.2% had dyspnea and 60% had heart burn.

Table 2. Radiological assessment of the study patients.

	(n=45)
X-ray	
Wrist, erosion	2 (4.4%)
Wrist, elbow erosion	2 (4.4%)
Wrist, elbow sclerosis	3 (6.7%)
Knee erosion	1 (2.2%)
Normal	37 (82.2%)
MRI	
Fat infiltration	1 (2.2%)
Bone erosion, edema, fat infiltration	2 (4.4%)
Normal	42 (93.3%)

Regarding radiological evaluation, 4.4% of patients had wrist and elbow erosion, 6.7% had wrist and elbow sclerosis, 2.2% had knee erosion by x-ray. 2.2% of patients had fat infiltration and 4.4% had bone erosion and edema by MRI as shown in table 2

Table 3. Laboratory results among the study patients.

	(n=45)
Urine analysis (Normal)	45 (100%)
RF (negative)	45 (100%)
ACCP (negative)	45 (100%)
HLAB27	2 (4.4%)
• **Positive**	43 (95.6%)
• **Negative**	
ANA (negative)	45 (100%)
DAS28_CRP	
• **Moderate activity (3.2 to 5.1)**	6 (13.3%)
• **High activity (>5.1)**	39 (86.7%)
DAS28_ESR	
• **Moderate activity**	4 (8.9%)
• **High activity**	41 (91.1%)
WBCs$_{(103/ul3)}$	7.98±3.26
HB$_{(g/dL)}$	10.39±1.44
Platelet$_{(103/ul3)}$	284 (133-3257)
Creatinine$_{(mg/dl)}$	0.76±0.13
ALT$_{(Iu/l)}$	30 (16-85)
AST$_{(Iu/l)}$	25 (17-80)
Albumin$_{(g)}$	3.98±0.53
Bilirubin$_{(mg/dl)}$	0.678±0.15
Ferritin$_{(mg/dl)}$	50 (15-2000)
CRP$_{(mg/dl)}$	30 (5-100)
ESR$_{(mm/1sth)}$	25 (10-80)
IL33$_{(ng/l)}$	11.5 (4.3-40.8)

RF; rheumatoid factor, ACCP; anti-Cyclic Citrullinated Peptide, ANA; antinuclear antibody, WBCs; white blood cells, HB; hemoglobin, ALT; Alanine aminotransferase, AST; aspartate aminotransferase, CRP; C reactive protein, ESR; erythrocyte sedimentation rate, IL; interleukin.

As shown in table 3, all patients had normal urine analysis, negative RF, ACCP and ANA. 86.7% had high disease activity by DAS28CRP and 91.1% had high disease activity by DASESR. Patients had median IL-33 level of 6.5 ng/l, ranged from 2.3-40.8.

Table 4. Serum IL-33 among patients regarding mode of distribution.

	Oligoarticular (n=6)	**Polyarticular (n=17)**	**Systemic (n=22)**	**Control group (n=45)**	**P value**
$IL33_{(ng/l)}$	7.6(4.3-40.8)	32.4(23.5-40.8)	13.8 (8.5-21.5)	1.8(0.7-2.5)	**0.008***

Kruskal wallis test used.

*Statistically significant as p<0.05.

As illustrated in table 4, JIA patients had a significantly higher serum IL-33 levels compared to IL-33 serum in the control group (p=0.008).

Table 5. Correlation between serum IL-33 level and disease criteria.

	IL-33	
	P value	r
Duration of disease	**0.002***	0.612
CRP	0.109	0.218
ESR	0.223	0.110
DAS28CRP	**<0.001***	0.705
DAS28ESR	**0.001***	0.667

Pearson correlation test used.

*Statistically significant as p<0.05.

As shown in table 5, there are significant positive correlation between IL-33 with duration of disease (p=0.002, r=0.612), DAS28CRP (p<0.001, r=0.705) and **DAS28**ESR (p=0.001, r=0.667), However, serum IL-33 levels did not significantly correlate with the levels of serum CRP or ESR.

Discussion

Juvenile idiopathic arthritis (JIA) refers to a series of unexplained etiology inflammatory arthritis that occur before 16 years and with a minimum of six weeks duration. Although, the exact etiology of JIA is still not fully clear, recent advances in molecular biology in the last

decade led to better understanding of the disease [11].

Current insight into the immunopathogenesis of JIA in Egyptian patients emphasis a key role of cytokines and other immune mediators such as tumor necrosis factor-alpha (TNF-a), interleukin (IL)-1, markers of apoptosis and gene polymorphism as well as in other studies implicating IL- 6, chemokines, calgranulins and toll-like receptors in the initiation and propagation of inflammatory process and many of them have been considered as possible therapeutic targets [12].

Interleukin-33 has many regulatory functions in both physiological and pathological conditions as it is involved in the maintenance of tissue homeostasis besides its role in the immune reaction against various infectious and inflammatory diseases [13].

It is released following tissue injury and act as ‘‘alarmin” to activate many immune cells that express ST2 receptor such as T helper 2 (Th2) cells, regulatory T cells (Tregs), mast cells, eosinophils and macrophages [14].

So, this study aimed to investigate clinical usefulness of serum IL-33 levels as an indicator of disease activity in other subtypes of JIA and determined their correlation with measures of disease activity.

In this study, IL-33 serum levels were significantly higher than their levels in controls. IL-33 serum levels were significantly higher in polyarticular patients compared to oligoarticular and systemic onset. This is in agreement with another recent study [15].

This is in the same line with Matsuyama et al. suggested that increased IL-33 levels represent an enhanced inflammatory process at the joint level rather than systemic inflammation [16].

Similarly, Ishikawa et al. reported higher serum IL-33 in seropositive polyarticular subtype than JIA patients with systemic-onset. IL-33 serum level was higher in seropositive polyarticular JIA patients compared to its serum concentrations in seronegative polyarticular and oligoarticular JIA patients [15].

SF IL-33 was significantly higher than in the serum concentrations and both significantly correlated. This can be attributed to local IL-33 synthesis in the synovium reported by others [15].

Carriere et al. considered inflamed synovium as a main source of IL-33 as they reported increased expression of IL-33 in endothelial cells and synovial tissues [6].

Furthermore, Palmer et al. described increased IL-33 expression by cultured synovial fibroblasts following stimulation with TNF-a [17].

In the present study, there were significant positive correlation between IL-33 with duration of disease ($p=0.002$, $r=0.612$), DAS28CRP ($p<0.001$, $r=0.705$) and **DAS28**ESR ($p=0.001$, $r=0.667$), However, serum IL-33 levels did not significantly correlate with the levels of serum CRP or ESR.

In agreement with another study, IL-33 serum and SF levels had a significant positive correlation with different disease activity parameters such as tender and swollen joint counts, ESR, CRP, JADAS27 as well as ultrasonographic synovitis activity score [15].

Binding of IL-33 to ST2 receptor leads to activation of many intracellular pathways that enhance inflammation through many mechanisms as degranulation of mast cells, the release of several pro-inflammatory cytokines and enhancement of neutrophils migration to the synovial tissue (18).

Ishikawa1 et al. found soluble ST2 receptors serum levels to be correlated with clinical disease activity parameters in systemic-onset JIA patients and observed normalizations of these levels during the remission stage [8].

Noor-eldeen et al. study found that serum IL-33 had a higher sensitivity in predicting PD synovitis activity than JDAS27 [15]. This can be explained by the superiority of MSUS to clinical evaluation in synovitis detection and its ability to identify subclinical synovial inflammation has been established in Egyptian JIA patients [19] .Schmitz et al. suggested that IL-33 can enhance the production of autoantibodies by B cells through enhancement of the secretion of IL-5 and IL-13 which are known Th2 cytokines [20].

To best of our knowledge, only 2 studies investigated IL-33 serum concentrations in JIA patients [7, 8]. However, none of them measured IL-33 mRNA relative expression or measured its level in the SF. Also, the relation between IL-33 and JIA disease activity was evaluated using MSUS in addition to the clinical and laboratory parameter.

A limitation of this work lies in the relative low number JIA patients and the cross-sectional study design. Many of the patients were on medications thus the exact effect of medications on IL-33 was not assessed.

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