Study of interleukin 33 in rheumatoid arthritis versus osteoarthritis patients

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Aim of the work
To compare the expression of serum interleukin 33 (IL33), a new member of the interleukin1 (IL-1) cytokine family, in rheumatoid arthritis (RA) versus osteoarthritis (OA) patients and to correlate it with clinical, laboratory and radiographic parameters.
Subjects and methods
20 RA and 20 primary knee OA patients. The levels of serum IL-33 were measured by enzyme-linked immunosorbent assay (ELISA) while anticyclic citrullinated peptide (Anti-CCP), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured by standard laboratory techniques. Plain x-ray of both hands and wrists were evaluated using the modified Larsen score 1995 (MLS) in RA patients. Knee OA grading was performed according to the KellgrenLawrence classification. The correlation of IL-33 level with clinical, laboratory and radiological data of RA and OA was analyzed.
Results
Serum IL-33 level was significantly higher in RA than in OA patients ($P < 0.001$). This level was positively correlated with disease duration, clinical and laboratory markers of disease activity, impaired functional status and radiographic severity in RA while not in OA patients.
Conclusions
These findings support that IL-33 could have an essential proinflammatory role in the pathogenesis of RA and that IL-33 level may be a monitor of disease activity and severity. IL-33 may become therapeutic target for RA.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown etiology that primarily involves the joints but can also cause multiple extra-articular manifestations. RA is the most common autoimmune disease. It is characterized by synovitis and autoantibody formation. The hallmark feature of the disease is persistent symmetric polyarthritis that affects the hands, wrists and feet, although almost all diarthrodial joints may become involved. The severity of RA may fluctuate over time, but chronic RA most commonly results in the progressive development of various degrees of joint destruction, deformity, significant decline in functional status and premature death [1].

Osteoarthritis (OA) is a common condition associated with pain and irreversible progressive joint damage that undermines the quality of life. This multifactorial disease is characterized by destruction of the joint cartilage, subchondral bone thickening and osteophyte formation [2].

Interleukin 33 (IL-33) is a newly found cytokine of the IL-1 family, which also includes IL-1β and IL-18. It is involved in the RA inflammatory status through the IL-1 receptor-related protein sequential control of Toll-like receptor (ST2) [3]. Anti-ST2 antibody that blocks IL-33 signalling could attenuate the severity of experimental arthritis. These studies suggest that IL-33 contributes to the pathogenesis of joint inflammation and destruction. Synovial fibroblasts are believed to be one of the main sources of IL-33 in RA, producing huge amounts of IL-33 in

the presence of tumor necrosis factor-α stimulation *in vitro* [4]. IL-33 locates mainly in the nucleus and may regulate the expression of some genes [5]. The role of IL-33 in bone metabolism and remodelling has been studied with conflicting results [6].

Patients and methods

Study population

This study included 20 RA patients and 20 primary knee OA patients. Informed consent was taken from all participants in the study. The study was approved by the ethics committee of the Faculty of Medicine Tanta University with approval code 2600/06/14. All patients were selected from the Physical Medicine, Rheumatology and Rehabilitation Clinics, Tanta University Hospitals.

(1) RA patients were diagnosed according to the American College of Rheumatology/European League Against Rheumatism 2010 Criteria for RA [7].
(2) Primary knee OA patients of either sex were diagnosed according to the clinical criteria developed by American College of Rheumatology for the diagnosis of OAs of the knee [8].

Exclusion criteria

RA and OA patients who were suffering from the following diseases were excluded from the study: inflammatory skin disorders, inflammatory bowel disease, asthma, cancer, cardiovascular diseases, secondary OA, type 2 diabetes and liver fibrosis (chronic hepatitis C and chronic hepatitis B).

Clinical assessment

Assessment of rheumatoid factor patients

(1) Morning stiffness: Its duration was assessed in minutes.
(2) Assessment of activity using Disease Activity Score (DAS28) [9].
(3) Functional assessment using Modified Health Assessment Questionnaire [10].

Assessment of osteoarthritis patients

(1) Pain was assessed using Visual Analogue Scale [11].
(2) Modified Ritchie Articular Index [12].
(3) Morning stiffness: Its duration was assessed in minutes.
(4) Functional assessment was carried out using Lequesne's Algo functional Index [13].

Laboratory assessment of total serum interleukin 33 level

Venous blood samples were collected from all participants with sterile disposable syringes in a sterile tube, and samples were centrifuged at 1000g for 15 min for serum separation with a dry clean Pasteur pipette. The serum samples were frozen at −70°C until used for assay of total serum IL-33 level using ELISA technique [14].

Radiographic assessment

Radiological assessment of rheumatoid factor patients

Plain x-ray of both hands and wrists was obtained and radiological assessment was carried out using the modified Larsen score 1995 [15].

Radiological assessment of osteoarthritis patients

OA severity was determined using weight-bearing anteroposterior radiographs of both knees. It was evaluated according to the Kellgren and Lawrence (KL) grading system [16].

Statistical analysis [17]

Statistical analysis was carried out using the statistical package for social sciences (SPSS) software, version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Demographic data between patients were compared using the χ^2 and unpaired Student's *t*-tests when appropriate. Pearson's correlation coefficient (r) was used to determine the correlation between concentrations of IL-33 in plasma and clinical and radiographic parameters. Data are expressed as a mean±SD. *P* values of less than 0.05 were considered statistically significant for differences and correlation.

Results

There was no statistically significant difference in demographic data between RA and OA patients, but there was a significant difference between RA and OA patients as regards erythrocyte sedimentation rate (ESR) first hour, C-reactive protein (CRP), haemoglobin levels, rheumatoid factor titre, anticyclic citrullinated peptide (CCP) titre and total serum IL-33 levels, as demonstrated in Table 1.

Serum IL-33 levels were not correlated with demographic data (age and sex) in studied RA patients. However, there was a significant correlation between serum IL-33 levels and disease duration, morning stiffness, number of tender joints, number of swollen joints, Visual Analogue Scale of pain, DAS28 score, Modified Health Assessment Questionnaire, ESR first hour,

Table 1 Demographic and laboratory data in rheumatoid arthritis and osteoarthritis patients

	RA patients (*n*=20)	OA patients (*n*=20)	*P*-value
Demographic data			
Age	45.7±11.9	50.05±5.37	0.148
Sex (male/female)	2/18	2/18	0.165
Laboratory			
Serum IL-33 level (pg/ml)	44.63±27.4	3.78±2.13	<0.001*
ESR (first hour) (mm/h)	39.7±24.07	11±3.84	<0.001*
CRP (mg/dl)	25.5±12.1	4.8±1.54	<0.001*
Haemoglobin levels (g/dl)	10.8±1.26	11.96±1.06	0.003*
RF titre (IU)	60.3±50.2	9.7±6.6	<0.001*
Anti-CCP titre (IU)	95.6±56.4	13±4.6	<0.001*

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; OA, osteoarthritis; RA, rheumatoid arthritis; RF, Rheumatoid factor. * means significant difference between the 2 groups *P*<0.05.

Table 2 Correlation between serum interleukin 33 levels and demographic, clinical, laboratory and radiological data in rheumatoid arthritis

	Serum IL-33	
	R	*P*
Age	0.119	0.617
Sex	0.196	0.409
Disease duration (years)	0.51	0.02*
Morning stiffness (min)	0.83	0.001*
Number of tender joints	0.79	<0.001*
Number of swollen joints	0.85	0.001*
VAS of pain	0.58	0.007*
DAS28 score	0.8	<0.001*
MHAQ	0.59	0.006*
ESR (first hour) (mm/h)	0.8	<0.001*
CRP (mg/dl)	0.72	0.001*
Haemoglobin levels (g/dl)	−0.06	0.79
Rheumatoid factor (IU)	0.78	0.001*
Anti-CCP (IU)	0.53	0.016*
Modified Larsen grades	0.61	0.004*

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; IL, interleukin; MHAQ, Modified Health Assessment Questionnaire; VAS, Visual Analogue Scale.

Table 3 Correlation between serum interleukin 33 levels and demographic, clinical, laboratory and radiological data in osteoarthritis

	Serum IL-33	
	R	*P*
Age	0.286	0.221
Sex	0.196	0.41
Duration of illness (years)	0.075	0.752
Morning stiffness (min)	−0.055	0.82
Tenderness	0.093	0.69
VAS of pain	0.14	0.56
Lequesne Algo functional index (range: 0–24)	0.078	0.74
ESR (mm/h)	−0.037	0.88
CRP (mg/dl)	−0.303	0.19
Rheumatoid factor (IU)	0.15	0.51
Anti-CCP (IU)	0.12	0.56
KL grades	0.34	0.15

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; KL, Kellgren and Lawrence; VAS, Visual Analogue Scale.

CRP, rheumatoid factor titer, anti-CCP titer and modified Larsen score, whereas there was no significant correlation between serum IL-33 levels and haemoglobin level in RA patients studied, as demonstrated in Table 2.

Serum IL-33 levels were not correlated with demographic data (age and sex), clinical data, Lequesne Algo functional index, ESR first hour, CRP, rheumatoid factor titre, anti-CCP titre and KL grades in studied OA patients, as demonstrated in Table 3.

Discussion

IL-33 possesses dual roles both as a traditional extracellular cytokine and as an intracellular nuclear factor with transcriptional regulatory properties [5].

IL-33 acts through the Toll/IL-1 receptor domain of IL-1 receptor accessory protein, which is shared by other IL-1 family members, to transduce the IL-33/ST2 signal. The IL-33/ST2 pathway may contribute to the pathogenesis of joint inflammation and destruction [3].

Our study demonstrated that the serum IL-33 levels in RA patients were significantly higher than that in OA patients. These results are in agreement with Xiangyang *et al.* [18], Talabot-Ayer *et al.* [19], Matsuyama *et al.* [20], Tang *et al.* [21], Mu *et al.* [22] and Ali *et al.* [23], who found that serum IL-33 levels were significantly higher in RA than in OA. This might point to differences in disease mechanism involved in these arthropathy pathogenesis, suggesting that induction of IL-33 expression by fibroblasts might be commonly observed in inflamed or damaged tissues.

However, Hong *et al.* [24] had found that serum level of IL-33 was higher in patients with RA as well as OA;

these results support the belief that OA is an inflammatory disease. Although OA is still generally considered a degenerative disorder, the development and progression of OA are now believed to involve inflammation even in the early stages of the disease. Secreted inflammatory factors such as proinflammatory cytokines are critical mediators of the disturbed metabolism and enhanced catabolism of joint tissue involved in OA.

In agreement with our observations, Matsuyama et al. [20], Tang et al. [21], Ali et al. [23] and Hong et al. [24] found that serum IL-33 was positively correlated with RA disease activity. Xiangyang et al. [18], Tang et al. [21] and Mu et al. [22] found a significant positive correlation between IL-33 and RA-associated autoantibodies, which supported the hypothesis that IL-33 plays an important role in the pathogenesis of RA and that IL-33 may contribute to abnormal B-cell autoimmunity and antibody overproduction by inducing mast cell activation. Both human and animal studies have shown that extracellular IL-33 could stimulate the maturation and activation of mast cells. Mast cells have been confirmed to be a cellular link between autoantibodies and inflammatory arthritis, giving new evidence on the involvement of IL-33 in abnormal humoral immunity with profound proinflammatory effect and may help in understanding the complex issue of autoimmunity in RA; thus, IL-33 is considered a risk factor for poor prognosis in RA [25].

Xu et al. [26] showed that administration of IL-33 in-vivo exacerbates experimental arthritis and elevates the production of proinflammatory cytokines and anticollagen antibodies. Therefore, IL-33 contributes to the antibody response and the severity of inflammation in a serum-induced arthritis mouse model that is mast cell-dependent. However, against our observations, Xiangyang et al. [20] found a nonsignificant correlation between IL-33 and disease activity in RA, which was scored with the DAS28-CRP.

However, in our OA patients there was a nonsignificant correlation between clinical, laboratory and radiological data (KL grades) with total serum IL-33 levels ($P > 0.05$). Our results are in agreement with the results of Xiangyang et al. [18], Tang et al. [21] and Ali et al. [23].

Our study also showed that the serum IL-33 levels were significantly higher in erosive RA patients when compared with nonerosive RA patients ($P < 0.05$). This observation provides further evidence that IL-33 may be a mediator of joint damage in RA patients and an indicator for disease severity. In agreement with our observation, Xiangyang et al. [18] found significant correlation between serum IL-33 levels and radiographic joint damage in RA patients (evaluated by modified Sharp Score). This finding supported the hypothesis that IL-33 may partly contribute to the bone erosion in RA patients which demonstrate that the mechanism by which IL-33 plays a destructive role in joints of RA patients is through activation of the transcription factors NF-kB induction of IL-6 in RA synovial fibroblasts which are responsible for cartilage destruction and joint damage as well as IL-6 and tumor necrosis factor-α in monocytes of these patients.

Conclusion

The serum level of IL-33 was significantly increased in RA patients than in OA patients. This level was positively correlated with disease activity, disease duration, bone erosions, joint damage, and impaired functional status in RA patients, whereas IL-33 level was correlated neither with clinical and laboratory findings nor with radiological findings in OA patients.

These findings suggest that IL-33 could be a useful biochemical parameter to reflect disease activity and severity in RA. Further studies are needed to explore the role of IL-33 in the pathogenesis, disease activity, bone erosions and joint damage, and the possibility of being a novel therapeutic target for RA, as well as to explore its role in OA and other autoimmune and inflammatory diseases.

Conflicts of interest
There are no conflicts of interest.

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