

## **Higher Circulating Concentration of Interleukin-38 in Patients with Knee Osteoarthritis: Its Association with Disease Severity**

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### **ABSTRACT**

Evidence showed that chronic inflammatory and immunopathological responses play a pivotal role in the development of osteoarthritis (OA). Interleukin-38 (IL-38) as a novel anti-inflammatory cytokine with influential modulatory properties on both innate and adaptive immune responses can be involved in the pathogenesis of OA. Therefore, this study aimed to measure the serum level of IL-38 in OA patients to clarify the positive or negative association with disease and its severity.

Blood specimens were collected from two groups including 23 newly-diagnosed OA patients and 22 healthy sex and age-matched subjects as a control group. Serum IL-38 quantities were measured using enzyme-linked immunosorbent assay (ELISA).

Significantly higher IL-38 levels were detected in OA patients in comparison with the healthy group ( $265.78 \pm 41.27$  pg/mL vs  $44.23 \pm 6.04$  pg/mL,  $p=0.0001$ ). The IL-38 concentration in OA patients with Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores  $>40$  and in OA patients with visual analog scale (VAS) scores  $>5$  were higher than those with WOMAC scores  $<40$ , and VAS scores  $<5$  ( $p=0.026$  and  $p=0.035$ , respectively). The IL-38 levels in OA patients with body mass index (BMI)  $<25$  were also significantly higher than in patients with BMI  $>25$  ( $p=0.05$ ).

According to our findings, WOMAC, VAS, and BMI indices may influence the IL-38 serum levels in OA patients and it may be elevated in OA patients to modulate inflammatory responses in a compensatory manner. The patients with OA, especially those with more severe disease express higher serum amounts of IL-38. Accordingly, IL-38 may be considered as a valuable marker for OA.

**Keywords:** Articular cartilage; IL-38 protein; Inflammation mediators; Joint diseases; Osteoarthritis

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## INTRODUCTION

Osteoarthritis (OA) is known as the most common form of arthritis which frequently affects joints of the hip, knee, hand, foot, and spine.<sup>1</sup> Previous studies revealed that inflammation plays a pivotal role in the OA pathogenesis and various inflammatory mediators were altered in OA patients that strongly associated with disease progression, pain, and disability.<sup>2</sup> Leukocytes infiltration particularly, macrophages and T cells could be observed in the synovium of OA patients that may play a fundamental role in the disease's pathogenesis.<sup>3,4</sup>

The possible mechanisms by which interleukin (IL)-38 inhibits inflammatory responses were exhibited in Figure 1. IL-38 is an anti-inflammatory cytokine that is mainly expressed in some organs such as the heart, placenta, fetal liver, skin, spleen, thymus, and tonsil.<sup>5</sup> IL-38 exerts its anti-inflammatory properties partly through binding to the interleukin 1 receptor accessory protein-like 1 (IL-1RAPL1) or IL-36 receptor and prevent the downstream signaling pathways, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B) which is involved in the expression of inflammatory cytokines including IL-1 $\beta$ , IL-6, and tumor necrosis factor-alpha (TNF-  $\alpha$ ).<sup>6</sup> A remarkable association has been reported between IL-38 levels and inflammatory diseases, such as rheumatoid arthritis which is very similar to OA.<sup>7,8</sup> However, the relationship between IL-38 levels with OA activity indexes and its clinical manifestations are still obscure. Due to the increased expression of inflammatory cytokines in diseases such as OA, in a compensatory mechanism, anti-inflammatory cytokines such as IL-38 have begun to increase, although its levels can be affected by a variety of factors, including injury, obesity, heredity, as well as overuse. Accordingly, this study aimed to determine the serum level of IL-38 in OA patients to clarify the positive or negative association with disease and its severity. The results can help to better understand the role of IL-38 in modulating inflammatory responses in OA patients.

## MATERIALS AND METHODS

### Subjects

Totally 45 participants, including 23 newly-diagnosed OA patients and 22 healthy subjects were enrolled in this study. The Ethics Committee of Rafsanjan University of Medical Sciences approved the

protocol of the study (IR.RUMS.REC.1397.230) and also the oral and written informed consent was also obtained from each participant. The OA group was selected among patients who were referred to the Ali-Ibn Abi-Talib (Alayh-e Salam) Hospital, Rafsanjan, Iran. The diagnosis of knee OA was done based on the clinical and radiologic criteria of the American college of rheumatology (ACR).<sup>9</sup> The severity of the symptomatic OA was estimated based on the Western Ontario McMaster University Osteoarthritis Index (WOMAC).<sup>10</sup> The patients with OA respond to the WOMAC questionnaire for the estimation of daily pain and function<sup>10</sup> and the higher WOMAC scores indicate greater symptom severity.<sup>10</sup> Additionally, a visual analog scale (VAS) was also used for the estimation of pain and was scored from zero (without pain) to ten.<sup>11</sup> The clinical examinations were performed by expert rheumatologists.

### Inclusion and Exclusion Criteria

Patients with knee OA based on ACR criteria grade I and II OA based on the Kellgren and Lawrence system were enrolled. Also, subjects with secondary OA, other inflammatory diseases and arthritis, use of anti-inflammatory drugs one month before sampling, and uncontrolled hypertension, history of knee trauma, joint infection, smoking, recurrent infections, cardiovascular disorders, diabetes mellitus, pulmonary dysfunction, allergic disorders, renal dysfunction, and neoplasia were excluded from the study.

### Measurement of the IL-38 Concentrations

A peripheral blood sample (5 mL) was obtained from each person and the serum samples were separated and kept at  $-20^{\circ}\text{C}$  until analysis.<sup>12</sup> The serum IL-38 levels measured using human IL-38 enzyme-linked immunosorbent assay (ELISA) kits (EK1662, BOSTER, Pleasanton, USA). According to the manufacturer's information, the sensitivity of the kit was reported  $<10$  pg/mL.

### Statistical Analysis

The data were presented as mean $\pm$ SD and analyzed by a statistical SPSS software (version 22, Chicago, IL, USA), and the suitable statistical tests, including ANOVA, Student t, or  $\chi^2$  were used to analyze differences between groups. A *p* value of less than 0.05 was considered significant.

## RESULTS

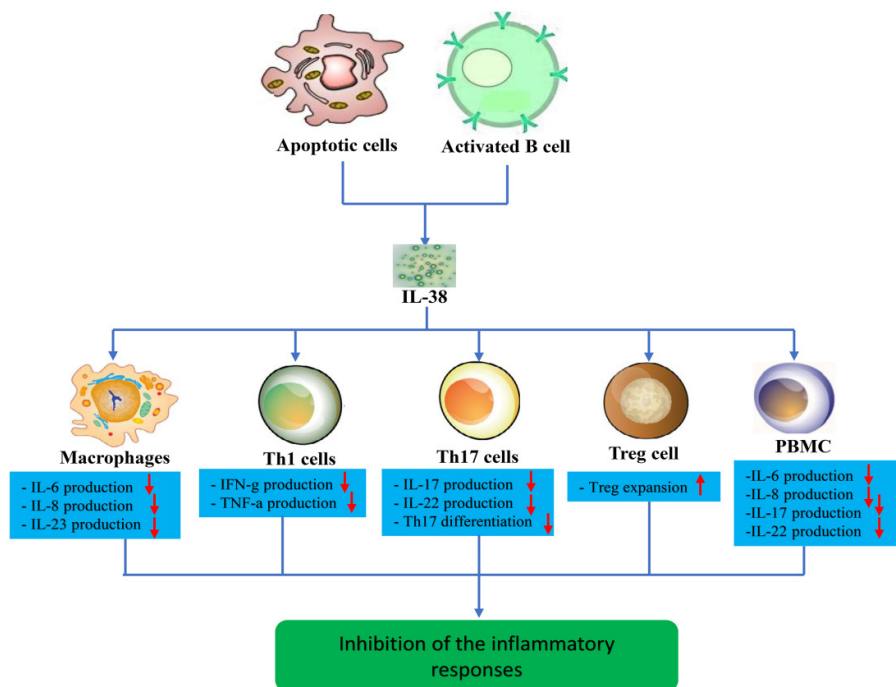
### General Characteristics of Participants

The means of age were  $49.65 \pm 7.27$  years in OA patients and  $47.40 \pm 8.39$  years in the controls ( $p=0.34$ ). According to the WOMAC and VAS gradings systems, the OA patients were arbitrarily categorized into two subgroups including the patients with WOMAC

scores  $<40$  and the patients with WOMAC scores  $>40$ ; the patients with VAS scores  $<5$  and the patients with VAS  $>5$  (Table 1).

### Serum Levels of IL-38 in OA Patients and Controls

Significantly higher IL-38 quantities were detected in OA patients in comparison with the healthy group ( $p=0.0001$ ).



**Figure 1.** Possible mechanisms by which interleukin-38 (IL-38) inhibits inflammatory responses. IL-38 is produced by various cell types such as apoptotic cells and activated B cells. IL-38 exerts inhibitory effects on the secretion of pro-inflammatory cytokines from macrophages, peripheral blood mononuclear cells (PBMCs), T helper type 1 (Th1) cells, and Th17 cells. However, IL-38 enhances the proliferation of regulatory T cells (Treg).

The serum IL-38 concentrations in patients with WOMAC scores  $>40$  were higher than patients with WOMAC scores  $<40$  ( $p=0.026$ ) (Table 1). The patients with VAS scores  $>5$  exhibit higher amounts of IL-38 compared with patients with VAS scores  $<5$  ( $p=0.035$ ). The serum IL-38 levels in both subgroups of OA patients with VAS scores  $>5$  and with VAS scores  $<5$  were significantly higher than the healthy group ( $p=0.001$  and  $p=0.05$ , respectively) (Table 1).

The IL-38 levels in OA patients with body mass

index (BMI)  $<25$  were higher than in patients with BMI  $>25$  significant ( $p=0.05$ ). The IL-38 quantities in both subgroups of patients with BMI  $>25$  and BMI  $<25$  were significantly higher than the healthy group ( $p=0.001$  and  $p=0.023$ , respectively). IL-38 is similarly expressed in healthy subjects with BMI  $<25$  and those with BMI  $>25$ . In total participants (OA patients+ healthy controls), the IL-38 levels in subjects with BMI  $<25$  were significantly higher than in individuals with BMI  $>25$  ( $p=0.005$ ) (Table 2).

## IL-38 in Osteoarthritis Patients

**Table 1. Comparison of the serum interleukin-38 levels according to Western Ontario McMaster University Osteoarthritis Index (WOMAC)**

Groups	Variables	Number	Serum levels of IL-38 (pg/mL)*	Median (Min-Max) IL-38 levels (pg/mL)	<i>p</i>
OA	WOMAC <40	9	154.05 ±45.94	141.2 (25.9-462.7)	0.026
	WOMAC >40	14	337.60 ±53.72	353.0 (85.80-760.8)	
	VAS<5	8	148.93 ±47.81	108.3 (25.9-421.4)	0.035
	VAS>5	15	328.10 ±51.93	327.2 (85.8-760.8)	
	Total patients	23	265.78 ±41.27	200.3 (25.9-760.8)	

\* The levels of interleukin-38 (IL-38) were expressed as mean±standard error of the mean (SEM). The serum IL-38 concentrations in patients with Western Ontario McMaster University Osteoarthritis Index (WOMAC) scores >40 and VAS>5 were higher than patients with WOMAC scores<40 and VAS<5 ( $p=0.026$  and  $p=0.035$ , respectively). The serum IL-38 levels in both subgroups of patients with WOMAC scores>40 and WOMAC scores <40 were significantly higher than the healthy group ( $p=0.001$  and  $p=0.01$ , respectively). The serum IL-38 levels in patients with visual analog scale (VAS) scores >5 and with VAS<5 were significantly higher than the healthy group ( $p=0.001$  and  $p=0.05$ , respectively).

**Table 2. Comparison of the serum interleukin-38 (IL-38) levels according to body mass index (BMI)**

Groups	BMI	Number	Serum levels of IL-38 (pg/mL)*	Median (Min-Max) IL-38 levels (pg/mL)	<i>p</i>
OA	<25	10	356.02 ± 73.68	389.8 (44.80-760.8)	0.05
	>25	13	196.36 ± 38.32	141.7 (25.9-431.2)	
	Total	23	265.78 ± 41.27	200.3 (25.9-760.8)	
Health	<25	4	43.85 ± 15.4	42.1 (8.2-83.0)	0.97
	>25	18	44.3 1± 6.77	39.3 (5.8-119.20)	
	Total	22	44.23± 6.04	39.3 (5.8-119.2)	
All subjects	<25	14	266.82 ± 65.03	174.0 (8.2-760.8)	0.005
	>25	31	108.08 ± 21.19	58.4 (5.8-431.2)	
	Total	45	157.46 ± 26.88	75.5 (5.8-760.8)	

\*The levels of interleukin-38 (IL-38) were expressed as mean±standard error of the mean (SEM).The IL-38 levels in osteoarthritis (OA) patients with body mass index (BMI)<25 were higher than in patients with BMI >25 significant ( $p=0.05$ ). The IL-38 quantities in both subgroups of patients with BMI >25 and BMI<25 were significantly higher than the healthy group ( $p=0.001$  and  $p=0.023$ , respectively).

### DISCUSSION

Since the increase in serum levels of IL-38 has been reported in some inflammatory disorders, the levels of this cytokine have not yet been measured in patients with OA, and its association with clinical manifestation and the stage of the disease is still unclear. In this study, we provide a detailed analysis of IL-38 plasma levels in patients with OA and its association with disease severity. Our findings indicated that serum

levels of IL-38 in OA patients were higher than in the control group. In agreement with our results, Takenaka et al and Boutet et al detected elevated IL-38 expression in the joints of mice with collagen-induced arthritis (CIA) and in the synovial tissue of patients with RA, which is associated with major inflammatory parameters.<sup>13,14</sup> Takenaka et al also indicated that the administration of IL-38 to the CIA mice leads to the reduction in the clinical symptom of disease, represent that IL-38 may perform a protective role against the

development of arthritis.<sup>13</sup> Elevated serum IL-38 concentrations were also detected in patients with SLE, especially in those with active disease, which is reduced after treatment.<sup>15,16</sup> Chu et al also observed in the mouse models of lupus that the treatment with IL-38 reduces the serum amounts of IL-6, IL-17, IL-22, CXCL10, IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ , while increases the amounts of IL-10 that were accompanied with remission of vessel infiltrate, reduction in skin lesions severity, reduction in proteinuria score, decreasing in glomerulonephritis scores when compared to the control mice.<sup>17</sup> Treatment of the lupus mice with IL-38 also diminishes the splenic Th17 cells and reduced spleen weight.<sup>17</sup> Boutet et al reported that the IL-38 expression in colonic biopsy samples from patients with Crohn's disease was higher than non-affected biopsies from the same patients that are related to the expression of IL-1 $\beta$ , IL-17A, and IL-6.<sup>14</sup> In a mouse model of colitis elevated IL-38 expression in the colon was detected.<sup>14</sup> Li et al also indicate that psoriasis patients also display higher serum IL-38 levels.<sup>18</sup>

Thus, IL-38 can act as an anti-inflammatory cytokine by inhibiting inflammatory signaling pathways resulting in the reduction of inflammatory mediators and prevent destructive inflammatory responses. Together, higher IL-38 expression was indicated in the aforementioned inflammatory and autoimmune diseases. However, the mechanisms by which IL-38 negatively controls the pathogenesis of the diseases remain to be explained in future studies. The IL-38 up-regulation may indicate a negative feedback mechanism to inhibit the excessive immunopathological responses and to limit vigorous inflammation in OA subjects. Therefore, IL-38 may exert a protective role to restrict the OA development, similar to that demonstrated in some aforementioned inflammatory diseases.

The serum IL-38 concentrations in patients with WOMAC scores >40 and VAS scores >5 were higher than patients with WOMAC scores <40 and VAS scores <5, respectively. The association of the WOMAC scores with the levels of pro-inflammatory cytokines has been evaluated in several studies. A positive association was reported between IL-16, IL-17A and IL-23, IL-34, eotaxin-1, and WOMAC score.<sup>19-22</sup> More investigations need to clarify the association of IL-38 with pain severity in OA patients. Additionally, the IL-38 levels in patients with BMI <25 were higher than in patients with a BMI >25. These results represent a

negative association between BMI and IL-38 levels. It was indicated that IL-37 (another anti-inflammatory cytokine) can reduce the mice's BMI through decreasing food intake<sup>23</sup> and maybe similar effects attributed to IL-38.

This study has several limitations. We showed an association between OA and serum IL-38 levels but not a cause-effect relationship. In addition, our lower sample size encourages the conduction of more studies with a greater sample size on this cytokine.

In conclusion, the results of this study revealed that patients with OA, especially those with more severe disease express higher serum amounts of IL-38. Accordingly, IL-38 may be considered as a valuable marker for OA. Increasing the IL-38 levels could be due to the initiation of homeostatic mechanisms of the immune system to modulate destructive responses. However, further study is needed to better understand this immunomodulatory and protective role of IL-38 by examining the balance between inflammatory and anti-inflammatory cytokines in both plasma and synovial fluid of OA patients.

#### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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