Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 3 Vol 8 No 1 2023

Article

Evaluation of IL-32 and IL-37 levels in Rheumatoid Arthritis

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.03.66

Abstract
The present study aimed to evaluate the serum level of interleukins 32 and 37 (IL-32 and IL-37) in rheumatoid arthritis (RA) patients. An ELISA was used to measure the levels of cytokines in the blood of 56 RA patients and 44 healthy volunteers who were enrolled in the study from November 2021 to March 2022. Serum levels of IL-32 and IL-37 in the RA patients were significantly higher compared to the control groups (IL32, p = 0.035; IL37, p = 0.011). In addition, cytokine concentration levels were higher in RA patients under therapy than in RA patients with a first diagnosis and without therapy, with significant differences in IL-37(141.389 ± 24.133 vs. 58.852 ± 7.806 ng/L). In comparison, there were no significant differences in IL-32 (93.740 ± 10.835 vs. 76.722 ± 9.321 ng/L). The results suggest that pro-inflammatory and anti-inflammatory cytokines may play an essential role in the progression of RA.

Keywords: Autoimmunity, IL-32, IL-37, Rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA), the most common type of arthritis, is a chronic autoimmune inflammatory disease in which immune and inflammatory cells infiltrate the synovial membrane, bone, and cartilage tissue, causing progressive destruction of bone and cartilage. Synovial tissue proliferation and joint erosion result from several inflammatory cytokines involved in RA[1]. IL-32 is one of the pro-inflammatory cytokines that can induce other inflammation-related cytokines, including tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6. Recent research indicates that IL-32 is essential for both the pathophysiology of chronic inflammation and defense against infections. Several autoimmune disorders, including RA and inflammatory bowel diseases, have been linked to abnormal IL-32 expression. However, although there is more evidence, many properties of this cytokine are still mostly unknown[2].IL-37, a recently discovered member of the interleukin-1 family, is a crucial anti-inflammatory cytokine involved in the control of inflammation. In macrophages and epithelial cells, IL-37 expression almost wholly suppresses the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, IL-12, and TNF-, whereas IL-37 silencing increases pro-inflammatory cytokine synthesis[3]. The study aims to highlight the evaluation. Of interleukin levels, which may significantly impact host immune responses.

Materials and Methods

Subjects

A total of 56 patients with RA were referred to the Rheumatology Consultation Clinic/Baghdad Teaching Hospital in Baghdad for diagnosis and treatment from

November 2021 to March 2022 and were enrolled in this study (approval was obtained from the Ministry of Health No. 37776 on 25/10/2021). Also, 44 healthy volunteers were included.

Assessment of Cytokine Serum Levels

The levels of IL-32 and IL-37 were measured in the blood of RA patients and healthy controls using sandwich ELISA kits from the Bioassay Technology Laboratory in China. These kits were designed to measure human cytokines based on the same principles quantitatively.

Statistical analysis

Statistical analysis was performed using SPSS (version 14), and all data was reported as the mean ± standard error (SE). ANOVA (one-way analysis of variance) was used to evaluate differences between groups, and a T-test was used to compare cytokine levels between groups. $P<0.05$ was used to indicate a statistically significant difference.

Results

One hundred samples (56 patients and 44 controls) were collected from November 2021 to March 2022. All laboratory tests were performed for patients and healthy volunteers, including CRP, ESR, RF, and anti-CCP, in addition to clinical signs and symptoms that were evaluated by the consultant medical staff in the Rheumatology Consultation Clinic in Baghdad for diagnosis and confirmation of RA. The results of this study showed a significant increase in the concentration of IL-32 and IL-37 in RA patients compared with the control group, IL-32 (90.646 ± 9.038 vs. 66.407 ± 4.545 ng/L) and IL-37 (125.816 ± 20.092 vs. 64.157± 5.311 ng/L), as shown in Table 1. Regarding therapy status, the results showed elevated serum levels of all cytokines in patients under therapy when compared to those not under therapy, with significant differences in IL-37(141.389 ± 24.133 vs. 58.852 ± 7.806 ng/L). At the same time, there were no significant differences in IL-32 (93.740 ± 10.835 vs. 76.722 ± 9.321 ng/L).

Cytokines	Mean ± Stander Error (SE.)		t-test	*P*-values	95% CI.
	Controls n=44	Patients N= 56			
IL-32 (ng/L)	66.407 ± 4.545	90.646 ± 9.038	2.145	0.035	1.803 - 46.673
IL-37 (ng/L)	64.157 ± 5.311	125.816 ± 20.092	2.611	0.011	14.755 -108.562

Table 1: Serum levels of IL-32 and IL-37 in RA patients and healthy controls.

Discussion

IL-32 is a pro-inflammatory cytokine produced by T cells, natural killer cells (NK), and monocytes and also by non-immune cells, including fibroblasts, keratinocytes, epithelial cells, and endothelial cells, upon stimulation by inflammatory cytokines like IFN-γ, TNF, and IL-1β. In addition, RA patients' synovial tissue and fibroblast-like synoviocytes exhibit elevated levels of IL-32 expression[4, 5]. The current study revealed a rise in the levels of IL-32 in RA patients than in controls, which may indicate the potential role of IL-32 in inflammation in RA patients. To support these findings, a significantly increased serum IL-32 level has been reported in type 2 diabetes mellitus patients[6], which also agrees with the data from China for the study of the clinical significance of IL-32 in RA. IL-32 is closely correlated with the development of RA and is highly expressed in RA synovial tissues. It is also associated with ESR levels and local IL-1β, IL-18, and TNF-α levels in RA synovial tissues[7]. In RA,

macrophages, T cells, and B cells are critical immune cells in pathogenesis because they produce pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6. TNFα can stimulate other pro-inflammatory mediators, like chemokines and cytokines (including IL-6, IL-1β, and IL-32). Recent studies have found that IL-32 expression levels in the synovium are correlated with the severity of inflammation in RA[8]. In addition, a previous study in Korea observed a slight elevation in IL-32 levels after treatment of patients with autoimmune diseases[9], and another study in Japan observed that serum levels of IL-32 decreased after treatment with cyclosporine in patients with atopic dermatitis[10]. Anti-inflammatory cytokine, IL-37, is an interleukin that suppresses inflammation by inhibiting pro-inflammatory cytokine production and stimulating macrophages and dendritic cells (DCs)[11]. IL-37 is expressed in monocytes, macrophages, DCs, tonsillar B cells, and plasma cells [12]. In this study, a significant elevation of the serum level of IL-37 was observed in RA patients. A significantly increased IL-37 level has also been reported in patients with psoriasis and multiple sclerosis [13, 14]. It was observed that the expression of IL-37 was increased in the $CD3^+$ and $CD4^+$ T cells because of the activation of T cells but not B cells [15]. IL-37 is a potent inhibitor of innate immunity by shifting cytokine equilibrium away from excessive inflammation. Increases in IL-37 restrict the production of pro-inflammatory cytokines in various inflammation-related diseases, and these suggest an immunosuppressive role for IL-37 in autoimmune inflammation by down-regulating pro-inflammatory cytokines [16]. Zhao (2014)[33] indicated decreased IL-37 concentrations in drug responders after treatment with DMARD but observed IL-37 expression in RA during DMARD treatment. Therefore, there is a strong relationship between plasma levels of IL-37 and disease activity in RA patients. The increase of anti-inflammatory cytokines like IL-37 could be a way to make joint inflammation less severe [17].

Conclusion

Based on the present study's findings, the levels of inflammatory cytokines (IL-32 and IL-37) are augmented during RA disease and represent a crucial immune regulation for exacerbating or alleviating RA.

Acknowledgments

We want to express our deepest thanks to the consultant medical staff, patients, and volunteers for their cooperation during sample collection.

Conflicts of interest

The author declares no conflicts of interest.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023

Citation: Omran, R.H.; Ahmed , Z.A. Evaluation of IL-32 and IL-37 levels in Rheumatoid Arthritis. Revis Bionatura 2023;8 (3) 66. http://dx.doi.org/10.21931/RB/CSS/2023.08.03.66