Short Communication

Estimation of Some Pro- and Anti-Inflammatory Interleukins in Rheumatoid Arthritis of Iraqi Patients

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Abstract

Background: Rheumatoid arthritis (RA) is an inflammatory condition that mostly affects synovial joints. It produces severe swelling and ongoing inflammation, and as it worsens, the cartilage and bone begin to erode, resulting in deformed joints and bone erosion. **Objectives:** The purpose of this study is to evaluate the levels of anti-cyclic citrullinated peptide (anti-CCP) antibodies and determine the role of interleukin-1 beta (IL-1β), interleukin-18 (IL-18), interleukin-38 (IL-38), and transforming growth factor-beta2 (TGF-β2) in studied groups (RA patients and apparently healthy control). **Materials and Methods:** The study included collecting blood samples from a group of patients infected with RA involving 60 patients (21 male and 39 female), and the healthy group included 50 individuals as control group (21 males and 29 females). **Results:** The outcomes showed of anti-CCP a highly significant difference for RA patients compared to the control group by 0.603 ± 0.02 and 0.274 ± 0.01, respectively, and some immunological parameters that involve inflammation-promoting interleukins (IL-1β and IL-18), the results showed the significant differences at the level ($P < 0.01$) of patients was 126.79 ± 4.18 and 194.37 ± 12.71 compared with control 91.85 ± 2.11 and 92.27 ± 2.08, respectively. The identical results to measure anti-inflammatory interleukins (IL-38, TGF-β2) represented of patients were 190.43 ± 9.82 and 403.23 ± 21.20 compared with control group was 88.39 ± 1.56 and 115.59 ± 5.63, respectively. **Conclusion:** The immunological parameters represented high expression of pro-inflammatory interleukins (IL-1β and IL-18), so, elevation levels of anti-inflammatory interleukins (IL-38 and TGF-β2) of RA patients.

Keywords: Enzyme-linked immunosorbent assay (ELISA) technique, interleukin-1β, interleukin-18, interleukin-38, rheumatoid arthritis, TGF-β2

Introduction

Rheumatoid arthritis (RA) is a persistent inflammation disorder that mostly adversely affects synovial joints and causes recurrent inflammations of the synovial membrane, which ultimately results in the deterioration of the joints, malformation, and incapacity.[1-3] RA has many symptoms such as eye, cutaneous, cardiac, pulmonary, and neurological problems in addition to articular involvement, the true mechanism results in the immune system invading the joints, these immune system reactions lead to joint inflammation, so, cartilage and bone damage.[4,5] Despite the attempts to find biomarkers for RA diagnosis there is still a deficiency of diagnostic and prognostic biomarkers for improved patient classification.[6]

Patients with RA often have autoantibodies referred to as anti-citrullinated protein antibodies (ACPAs). Anti-cyclic citrullinated peptides (anti-CCPs) that belong to ACPAs and have a specificity of 88%–98% are thought to be a reliable marker to diagnose RA, the majority of the ACPAs mechanisms production also bone loss are currently both properly, but are still some undiscovered mechanisms.[7]

Interleukin (IL-1β) is an important driver of B-cell destruction and has been identified as the cytokine that promotes inflammation IL-1β, the primary producers of IL-1β are cell macrophages, as a powerful pro-inflammatory driving cytokine, IL-1β levels are tightly controlled by IL-1Ra, an antagonist of the IL-1 receptor,

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the primary source of IL-1β/IL-1Ra during inflammation is the macrophage, which produces both an auto-regulatory feedback involving cytokines mechanism.[8]

Interleukin-18 (IL-18) is a powerful inflammation-promoting cytokine that plays a role when the host is defending against infections, controls the innate immune system and acquired immunity, cells of both the hematopoietic and non-hematopoietic types, such as keratinocytes, macrophages, monocytes, macrophages, and mesenchymal cells, generate IL-18, autoimmunity may be brought on by IL-18 capacity to stimulate inflammatory and cytotoxic immune cell activity patients who have certain immune-related disorders, such as RA, have reported having its increased levels in their blood.[9]

Interleukin-38 (IL-38) is a novel member of the IL-1 family that showed anti-inflammatory action in many autoimmunity illnesses.[10,11] Whereas its pro-inflammatory properties are a source of disputation[11,12].

It can bind to a variety of receptors and control inflammatory cytokine production and activity via downstream signaling pathways, numerous tissues, including the placenta, heart, and brain express IL-38, which plays a role in a range of illnesses, including chronic inflammatory diseases.[8]

Transforming growth factor-beta2 (TGF-β2) is one of the most well-known cytokines that are essential for controlling joint development, homeostasis, and illnesses, but the molecular mechanism underlying its action is significantly not well understood.[13] So, TGF-β2 further demonstrates its powerful ability to repair cartilage abnormalities, by enlisting utilizing one's own mesenchymal stem cells encouraging the release of additional groups of growth factors. As well as the physiologic processes of chondrocytes, including migration, differentiation, proliferation, and death including the pathological development of cartilage, such as RA and osteoarthritis (OA).[13] The purpose of the current study is to evaluate the levels of anti-CCP antibodies in studied groups (RA patients and control) and determine the role of IL-1β, IL-18, IL-38, and TGF-β2 for these groups.

Materials and Methods

The current study incorporated 110, a complete of 60 patients (39 females and 21 males) suffering from RA, between the ages of 20–69 years, during the period from October to the end of December 2022, from Baghdad Teaching Hospital, Medical City, Rheumatology Unit. All samples were taken with the consent of the patients before being included in the study. Fifty specimens were collected from healthy people for comparison (5 mL), placed into a gel tube, and allowed to stand for 30 min for clot formation at room temperature. To collect the serum, the tubes were centrifuged at 3000 rpm for 10 min to separate the serum from other blood components, using micropipettes, the serum was then transferred to Eppendorf test tubes and maintained in deep freeze at −20 °C until use. For the purpose of immunological and serological assays (anti-CCP, IL-1β, IL-18, IL-38, and TGF-β2. When determining the amounts of cytokines using the Sandwich-Enzyme Linked Immunosorbent Assay (ELISA) technique, the resultant serum was chilled by freezing at –20 °C, and the optical density employed was 450 nm.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number (22/5873 on November 13, 2022) to get this approval.

Results

Anti-CCP test

The study results showed that the concentration of anti-CCPs in patients with RA for both sexes was highly significant at the level of probability ($P < 0.01$) and was (0.603 ± 0.02) compared with the control group (0.274 ± 0.01) according to Table 1.

Serum level of cytokines

Pro-inflammatory cytokines in RA (IL-1 β, IL-18)

The current findings were a high significant difference level ($P < 0.01$) for IL-1β of patients (126.79 ± 4.18) compared with control (91.85 ± 2.11). Also, the same result of highly significant differences to measure of IL-18 between RA patients compared with control was 194.37 ± 12.71 and 92.27 ± 2.08, respectively, according to Table 2.

Anti-inflammatory cytokines in RA (IL-38, TGF –B2)

The present data showed an increased level ($P < 0.0$) of both cytokines IL-38 and TGFβ2 measured in the serum of the patients was 190.43 ± 9.82 and 403.23 ± 21.20 compared with the control was 88.39 ± 1.56 and 115.59 ± 5.63, respectively, according to Table 3.

Table 1: Comparison between patients and control groups according to anti-CCP

Group	Mean ± SE of anti-CCP
Patients	0.603 ± 0.02
Control	0.274 ± 0.01
t test	0.0356**
P value	0.0001

** $P \leq 0.01$

Table 2: Comparison between patients and control groups according to level of IL1β and IL-18

Group	Mean ± SE	
	IL-1β (pg/mL)	IL-18 (pg/mL)
Patients	126.79 ± 4.18	194.37 ± 12.71
Control	91.85 ± 2.11	92.27 ± 2.08
t test	9.860**	27.884**
P value	0.0001	0.0001

** $P \leq 0.01$

Table 3: Comparison between patients and control groups according to level of IL-38 and IL-TGF-β2

Group	Mean ± SE	
	IL-38 (pg/mL)	TGF-β2 (pg/mL)
Patients	190.43 ± 9.82	403.23 ± 21.20
Control	88.39 ± 1.56	115.59 ± 5.63
t test	2.537**	47.192**
P value	0.0001	0.0001

** $P \leq 0.01$

Discussion

Anti-citrulline antibodies test is an alternative measure of the rheumatoid factor, and it determines the severity of RA disease.[14] So, the current study result is compatible with Abd-Ali *et al.*,[15] which explained the anti-CCP result was positive by 58% in patients with an early stages of the RA disease, and the RF was positive by 60% of the patients.[16,17]

The reason for the excess level of IL-1β may be that it plays a significant causal role in auto-inflammatory disorders, chronic inflammatory and degenerative illnesses, so, an increase in IL-1β and IL-18 production which correlates with the onset of the disease, disease severity, and contributed to the symptoms appearance.[17]

This current data of IL-1β compatible with[18,19] the study, that noted significant differences between amounts of IL-1β within the serum of RA patients compared to the control group.

As for IL-18, the present result is approved with previous results done by Subhi and Zgair[20] and Al-Quraishi.[21] Showed increasing amounts of IL-18 in the serum of RA patients than the control group. The high values of interleukin levels in patients may be due to the RA which is a chronic disease and may be the treatments used are limited, which do not reduce their inflammation competence.

The results correspond with the study[10] that revealed RA patients had abnormal excess levels of IL-38 in the serum. The reason for the high percentage may be due to the interleukin (IL-38) have anti-inflammatory effects, mostly by preventing the synthesis of pro-inflammatory cytokines, and macrophages in addition to reduce Th17 maturation. So, this cytokine is remarkable for the treatment of a variety of chronic inflammatory diseases, particularly rheumatic autoimmune diseases.[10]

Additionally, RA patients had higher levels of expression of IL-38 in their plasma, synovium, and synovial fluid compared with OA patients and psoriatic arthritis (PsA).[22,23]

As for the high percentages observed for TGF-β2 in the serum of rheumatoid arthritis patients, for it have an important role in the pathological changes associated with this disease, specially increase its levels at the cartilage interface because it help facilitated cartilage tissue healing by increasing the production of Additionally to fibronectin and collagen suppressing the production those proteases.[24]

Also, TGF-β2 is prevented lymphoid cells from penetrating arthritic joints in RA, so, can cause fibroblast-like synoviocytes (FLSs) to release other interleukins such as IL6, IL-8, and MMPs, which can break down bone tissue and cleave cartilage extracellular matrix (CEM) collagen.[25-28]

Conclusions

There was a significant difference in the percentage of anti-CCPs. The immunological parameters represented high expression of pro-inflammatory interleukins (IL-1β and IL-18), so, elevation levels of anti-inflammatory interleukins (IL-38 and TGF-β2) of RA patients.

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Conflicts of interest

There are no conflicts of interest.

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