Evaluation of IL-35 and IL-39 in Rheumatoid Arthritis

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Abstract:

Background: Cytokines have an essential contribution to the inflammatory response and the development of chronic inflammation. Therefore, they have a pivotal role in the pathogenesis of rheumatoid arthritis. Interleukins are closely related to rheumatoid arthritis, and the exact role of some interleukins in the pathogenesis of rheumatoid arthritis is not yet known, such as IL-35, which has suppressive activity, particularly in cancer and autoimmune diseases. As well as IL-39, which promotes inflammatory responses and the activation of immune cells. Therefore, the aim of current study was to evaluate the levels of interleukins 35 and 39 and their ratio in rheumatoid arthritis patients in Iraq.

Methods: ELISA was used to measure the levels of interleukins in the blood of 56 patients with rheumatoid arthritis and 44 healthy volunteers who were enrolled in the study from November 2021 to March 2022. The level of interleukins was statistically analyzed using the computer program Statistical Package for Social Sciences (version 14).

Results: The serum levels of IL-39 in the rheumatoid arthritis patient groups were significantly higher than in the control group (p = 0.043). In contrast, the level of IL-35 was slightly higher in rheumatoid arthritis patients but not by significantly different values (p = 0.055). The cytokine ratio, IL-39/IL-35, was the same for the groups, and there were no significant differences when comparing patients to controls (14.30 ± 1.47 vs. 13.18 ± 0.71). In addition, IL-39 concentration levels were significantly higher in rheumatoid arthritis patients under therapy than in rheumatoid arthritis patients with a first diagnosis and without therapy.

Conclusion: Rheumatoid arthritis has been associated with an increased level of IL-35 and IL-39, so assessment of the levels of these cytokines may be helpful in confirming rheumatoid arthritis activity.

Keywords: Autoimmunity, Cytokines, IL-35, IL-39, Rheumatoid arthritis.

Introduction:

RA is a chronic autoimmune inflammatory disease that causes progressive destruction of bone and cartilage. The development of autoimmune diseases such as RA and systemic lupus erythematosus (SLE) can be caused by either a decrease in anti-inflammatory cytokines or an increase in pro-inflammatory cytokines[1] [2]. This imbalance between pro- and anti-inflammatory cytokines may be an underlying element in disease progression via inflammation and the loss of articular cartilage [3]. Members of the IL-12 cytokine family have a crucial role in regulating innate and adaptive immunity and also in the management of inflammatory diseases [4]. Interleukin-35 and interleukin-39 (IL-35 and IL-39) are members of the IL-12 family and play significant roles in several autoimmune diseases. In addition, they have been researched as potential therapeutic targets in the management of several autoimmune diseases [5]. IL-35 is secreted primarily by regulatory T cells (Treg) and has anti-inflammatory and immunosuppressive properties. It can enhance Treg proliferation and inhibit T helper-17 (Th17) cell differentiation. Therefore, by maintaining the balance between Th17 and Tregs cells, IL-35 is crucial to the progression of RA. IL-35 is closely related to the incidence of inflammation in infections and autoimmune diseases [6]. IL-39 is a new member of the IL-12 cytokine family, which was recently identified. It has been shown that IL-39 plays a role in SLE pathogenesis *in vivo* and induces differentiation and activation of neutrophils. B cells express IL-39 more frequently by secreting the B-cell stimulating factor [7]. IL-35 is strictly anti-inflammatory, while IL-39 is relatively less well-characterized, but accumulating evidence points to its pro-inflammatory actions. Therefore, the aim of current study was to evaluate the levels of interleukins 35 and 39 and their ratio in RA patients in Iraq.

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Patients and methods:

Patients

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 used as a control sample and were matched with patients based on their gender, age, and ethnicity.

Diagnosis of RA: After a clinical examination of the patients by the hospital medical staff, we performed an erythrocyte sedimentation rate (ESR) test (using the standard Westergren method), an anti-cyclic citrullinated peptide (Anti-CCP) test (Hotgen, China), a C-reactive protein (CRP) and rheumatoid factors (RF) tests (SPINREACT, Spain), on the blood samples. According to the laboratory tests and information sheet for each subject, the samples were divided into RA and other rheumatic diseases (excluded).

Measurement of IL-35 and IL-39 Serum Levels

The levels of IL-35 and IL-39 were measured in the blood of RA patients and healthy controls using sandwich ELISA kits from Bioassay Technology Laboratory in China. These kits were designed for quantitative measurement of human cytokines based on the same principles.

Statistical analysis

Statistical analysis was performed using SPSS (version 14), and all data was reported as 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.

Result

The results showed a high prevalence of RA in females compared to males (87.5% vs. 12.5%, respectively), and the age (Mean ± Standard Deviation) of RA patients was 50.107 ± 12.422 years. When distributing RA patients according to age groups, the largest proportion of RA patients (88%) were older than 40 years. In addition, the results of this study showed that the serum level of IL-39 in RA patients compared with the control group was (196.392 ± 26.532 vs. 132.790 ± 9.227 ng/L), while IL-35 was (16.018 ± 2.074 vs. 11.059 ± 0.833 ng/ml), as shown in table (1). A significantly increased in IL-39 concentration was observed in RA patients compared to controls, but the increment in IL-35 didn't show a statistically significant difference (*P* = 0.055). The cytokine ratios (IL-39/IL-35) were the same for the groups, and there were no significant differences when comparing patients to controls, (14.30 ± 1.47 vs. 13.18 ± 0.71).

Table 1: Serum levels of IL-35 and IL-39 in RA patients and healthy controls

Cytokines	Mean ± Stander Error (S.E.)		t-test	*P*-values	95% C.I.
	Controls n=44	Patients N= 56			
IL-35 (ng/ml)	11.059 ± 0.833	16.018 ± 2.074	1.940	0.055	0.116 - 10.034
IL-39 (ng/L)	132.790 ± 9.227	196.392 ± 26.532	2.046	0.043	1.835 - 125.368

Regarding therapy status, the results of the present study showed that 46 (82.1%) of RA patients received therapy, while 10 (17.9%) of RA patients did not receive any therapy (diagnosis onset). When studying the value of cytokine levels in these two types of therapy in RA patients, the results showed elevated serum levels of cytokines in patients under therapy when compared to those not under therapy, with significant differences in IL-39 (211.594 ± 32.210 vs. 134.064 ± 22.426 ng/L), while there are no significant differences in IL-35 (17.044 ± 2.484 vs. 11.299 ± 1.504 ng/ml), figure (1).

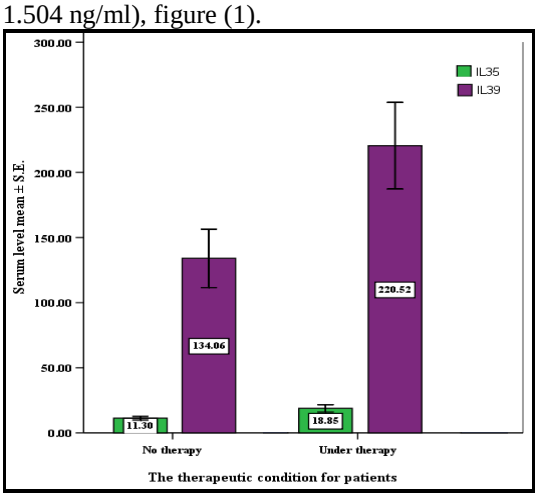

Figure 1: Distribution of cytokine serum levels in RA patients according to the therapeutic state.

Discussion

Pro-inflammatory cytokines are crucial in the processes that lead to inflammation, joint destruction, comorbidities associated with RA, and many other disorders brought on by an uncontrolled self-directed immune response [8] [9]. IL-39 is the modern member of the interleukin-12 cytokine family, which is important in autoimmune diseases, including SLE [10]. A significantly elevated IL-39 level was observed in RA patients in the current study, with a significant difference when compared to control. In studies conducted by [11] and [12], serum levels of IL39 were found to be significantly higher in patients with myocardial infarction disease and neuromyelitis disorders. Furthermore, it was observed that IL-39 in lupus-like mice produces an immune-pathogenic impact by promoting the inflammatory response. IL-39 increased the levels of IFN-γ, TNF-α, and IL-17, thus eliciting a pro-inflammatory state as well as demonstrating the potential to regulate the immune system. As a result, targeting IL-39 may provide a successful treatment for autoimmune diseases [13, 14]. Anti-inflammatory cytokines (for example Transforming growth factor beta (TGF-β), IL-4, IL-10, and IL-13) effectively prevent autoimmune disease, either by affecting innate cellular immunity, such as deviating macrophage polarization, or by interfering with the T cells' or B cells' activation [15]. IL-35 also belongs to the IL12 family, and IL-35 has been reported as an immuno-suppressive cytokine and is essentially produced by Tregs, which regulate immune functions by inhibiting the inflammatory response [16]. In this study, IL-35 in RA patients was

slightly higher than the controls with no significant difference, and that was almost in agreement with another study [17] in China that confirmed the raised serum IL-35 levels in RA related to disease activity. It has been hypothesized that IL-35 could possibly have an effect on RA by suppressing the Th-17/IL-17 related pathway. In addition, IL-35 can attenuate the pathogenesis and progression of RA by influencing the immune and pathological processes. Therefore, it is suggested that IL-35 could be used as a potential target for the future therapy of RA. Additionally, a previous study indicated that IL-35 facilitates the differentiation of human B cells into B regulatory cells that secrete IL-10. All of this evidence indicates that IL-35 significantly regulates immune responses [18]. The goals of RA treatment are to reduce inflammation and pain in the joints, improve joint efficiency, and stop joint damage and deformity. Traditional therapies for RA patients include immunosuppressive drugs such as corticosteroids, disease-modifying anti-rheumatic drugs/DMARDs and non-steroidal anti-inflammatory drugs/NSAIDs [19]. To our knowledge, there is no study regarding the impact of treatment on IL-35 and IL-39 levels in RA patients. These findings corroborate previous research and support the link between pro- and anti-inflammatory cytokines and disease progression.

Conclusion

Based on the findings of the present study, we can conclude that the levels of inflammatory cytokines IL-35 and IL-39 are augmented during RA disease, and the level of cytokine concentration is influenced according to treatment levels in RA patients. Therefore, elevated concentrations of cytokines represent a characteristic feature of RA and have potential value in the diagnosis of RA.

Author's Contributions

Raghda H. Omran: Carried out the experiment and verified the analytical methods.

Zahra'a A.Ahmed: Conceived the original idea, and supervised the findings of this work and the whole project.

Aymen A.O. Alrawi: Contributed to the preparation and diagnosis of the sample as well as contributed to the interpretation of the results.

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تقييم IL-35 و IL-39 في التهاب المفاصل الروماتويدي

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الخلاصة

الخلفية: الحركيات الخلوية لها مساهمة أساسية في الاستجابة الالتهابية وتطور الالتهاب المزمن. لذلك لها دور محوري في التسبب في التهاب المفاصل الروماتويدي. ترتبط البين ابيضاضي ارتباطًا بالتهاب المفاصل الروماتويدي ، والدور الدقيق لبعض البين ابيضاضيات في التسبب في التهاب المفاصل الروماتويدي غير معروف حتى الآن مثل IL-35 الذي له نشاط قمعي ، خاصة في الاورام الخبيثة وأمراض المناعة الذاتية، وكذلك IL-39 الذي يعزز الاستجابات الالتهابية وتنشيط الخلايا المناعية. لذلك كان الهدف من الدراسة الحالية هو تقييم مستويات البين ابيضاضيات ونسبتها في مرضى التهاب المفاصل الروماتويدي في العراق.

المرضى والطرق: تم استخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لقياس مستويات البين ابيضاضيات في دم 56 مريضًا مصابًا بالتهاب المفاصل الروماتويدي و 44 متطوعًا سليمًا تم تسجيلهم في الدراسة من تشرين الثاني 2021 إلى اذار 2022. تم تحليل مستوى البين ابيضاضي إحصائيًا باستخدام برنامج SPSS (الإصدار 14).

النتائج: كانت مستويات IL-39 في مصل الدم في مجموعات مرضى التهاب المفاصل الروماتويدي أعلى بكثير مما كانت عليه في مجموعات التحكم *(P = 0.043)*. في المقابل ، كان مستوى IL-35 أعلى قليلاً في مرضى التهاب المفاصل الروماتويدي ولكن ليس بقيم مختلفة بشكل كبير *(P = 0.055)*. كانت نسبة الحركيات الخلوية IL-35 / IL-39 هي نفس القيمة لكلا المجموعتين، ولم تكن هناك فروق ذات دلالة إحصائية عند مقارنة المرضى بالضوابط (14.30 ± 1.47 مقابل 13.18 ± 0.71). بالإضافة إلى ذلك ، كانت مستويات تركيز IL-39 أعلى بشكل ملحوظ في مرضى التهاب المفاصل الروماتويدي الخاضعين للعلاج مقارنة بمرضى التهاب المفاصل الروماتويدي مع التشخيص الأول وبدون علاج.

الإستنتاج: ارتبط التهاب المفاصل الروماتويدي بزيادة مستوى IL-35 و IL-39 ، لذلك قد يكون تقييم مستويات هذه الحركيات الخلوية مفيدًا في تأكيد نشاط التهاب المفاصل الروماتويدي.

الكلمات المفتاحية: المناعة الذاتية ، الحركيات الخلوية، IL-35، IL-39 ، التهاب المفاصل الروماتويدي.