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Evaluation of Interleukin-23 and Transforming Growth Factor-β in Iraqi Women with Rheumatoid Arthritis

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Abstract

This study emphasizes the significance of inflammatory and anti-inflammatory levels in Iraqi females with rheumatoid arthritis, the role of markers in disease treatment, and the need for balance in autoimmune conditions.

Objectives: The study investigates the impact of inflammatory markers like IL-23 and TGF-β as anti-inflammatory markers on the development of rheumatoid arthritis in Iraqi females.

Materials and Methods: A case-control study involving 118 female subjects aged 30-70years, who were divided into two groups: 71 RA patients and 47 healthy controls. All these patients were subjected to full history taking through clinical examination, and laboratory investigations in sera including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IL-23 and TGF-β levels were measured using an ELISA technique

Results: The distribution of age groups is fairly similar across both groups, with a slight trend towards older ages in the patient group. Results were demonstrated that the patient group appears (69.4%) of patients were in the age range between 60-70 years as compared with control group (30.6%), Results were higher prevalence of obesity compared to the control group. All patients (100%) in the obesity category belong to the patient group, with none in the control group. The whole patients (100%) reported as a family history of the condition, whereas none (0%) in the control group, the mean ± SD difference of inflammation biomarkers for patients with RA patients showed an increasing range of IL-23 and TGF-β as compared to the healthy control groups. The mean ± SD levels of (CRP), (ESR) in RA which were significantly higher than that in control group. Receiver operating curve (ROC) was analysis for IL-23 levels indicate: statistically significant.

Conclusion: IL-23, an inflammatory marker, may be elevated in RA patients, affecting RA pathophysiology. It can be used as a biomarker for RA pathogenesis evaluation, while TGF-β may contribute to RA development.

تقييم الانترلوكين 23 (IL-23) وعامل النمو التحويلي (TGF- β) لدى النساء العراقيات المصابات بالتهاب المفاصل الروماتويدي

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الخلاصة

المقدمه: التهاب المفاصل الروماتويدي هومرض التهابي مناعي ذاتي يتسبب نتيجة كلا العاملين الوراثي والسببي. هذه الدراسة سلطت الضوء على أهمية تقييم مستويات الالتهابات ومضادات الالتهاب لدى الإناث العراقيات المصابات بالتهاب المفاصل الروماتويدي، وتسليط الضوء على دور العلامات في علاج المرض، والتأكيد على ضرورة التوازن في حالات المناعة الذاتية.

الاهداف: يبحث البحث في تأثير العلامات الالتهابية مثل انترلاكن 23 والعلامة المضادة للالتهابات عامل النمو المتحول بيتا على تطور التهاب المفاصل الروماتويدي لدى الاناث العراقيات.

المواد والطرق: دراسة الحالات والشواهد التي شملت 118 أنثى تتراوح أعمارهن بين 30-70 سنة، تم تقسيمهن إلى مجموعتين: 71 مريضة بالتهاب المفاصل الروماتويدي و 47 فرداً سليماً كمجموعة سيطرة. تم إخضاع جميع هؤلاء المرضى لأخذ التاريخ الكامل من خلال الفحص السريري، و تم قياس الفحوصات المختبرية في المصل بما في ذلك معدل ترسيب كرات الدم الحمراء (ESR) وبروتين سي التفاعلي (CRP) وانتر لوكين (IL-23) وعامل النمو المتحول بيتا (TGF-β) باستخدام تقنية (ELISA)

النتائج: تم تضمين الخصائص الديموغرافية لإجمالي 118 مشاركًا في هذه الدراسة، 71 مريضًا بالتهاب المفاصل الروماتويدي و47 ضابطًا، مقسمة إلى مجموعات فرعية على أساس العمر والتاريخ العائلي ومجموعات مؤشر كتلة الجسم. الفئات العمرية متشابه إلى حد ما بين المجموعتين، مع وجود اتجاه طفيف نحو الأعمار الأكبر في مجموعة المرضى. كانت أعلى نسبةً حوالي (69.4%) من المرضى في الفئة العمرية بين 60-70 سنة مقارنة مع المجموعة الضابطة (30.6%)، أظهرت النتائج أن مجموعة المرضى يبدو أن لديهم معدل انتشار أعلى للوزن الزائد والسمنة مقارنة بالمجموعة الضابطة. ينتمي جميع المرضى (100%) في فئة السمنة إلى مجموعة المرضى، ولا يوجد أي منهم في المجموعة الضابطة. وفيما يتعلق بتاريخ العائلة، فقد أظهرت هذه الخاصية وجود اختلاف بين المجموعات. أبلغ جميع المرضى (100٪) عن تاريخ عائلي للحالة، في حين لم يكن هناك أي (0٪) في المجموعة الضابطة. أظهر الفرق المتوسط ± SD للمؤشرات الحيوية للالتهاب للمرضى الذين يعانون من مرضى التهاب المفاصل الروماتويدي نطاقًا متزايدًا من IL-23 وTGF بالمقارنة مع المرضى الذين يعانون من التهاب المفاصل الروماتويدي. إلى مجموعات المراقبة الصحية. أشارت النتائج إلى وجود فرق ذو دلالة إحصائية عالية في مستويات IL-23 وTGF بين المجموعات، كان متوسط مستويات ± SD لـ (CRP) و (ESR) في مرضى التهاب المفاصل الروماتويدي والتي كانت أعلى بكثير من تلك الموجودة في المجموعة الضابطة. تظهر دراسة الارتباط العديد من الارتباطات الهامة بين المعلمة المقاسة. يشير تحليل منحنى تشغيل المستقبِل (ROC) لمستويات IL-23 ، وكانت القيم P للمساحة تحت المنحنى <0.05 وذات دلالة إحصائية.

الاستنتاج: قد تكون علامات الالتهاب (IL-23) مرتفعة إلى حد أكبر في مرضى التهاب المفاصل الروماتويدي وتلعب دورا هاما في الفيزيولوجيا المرضية لمرض RA، لذلك يمكن اعتبار IL-23 مؤشرات حيوية لتقييم التهاب المفاصل الروماتويدي. تأثير علامة الالتهاب (TGF-β) على تطور التهاب المفاصل الروماتويدي عن طريق زيادتها بالتوازن مع علامة الالتهاب.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease resulting from both genetic and ecological factors. It has been hypothesized that genetic predilection, in combination with environmental factors, leads to cascade of events causing synovitis and eventually destructive arthritis (Jahid, Khan and Ahmed, 2023)(Babaahmadi et al., 2023)**.**Early diagnosis could prevent joint damage; a large body of evidence indicates that statistically significant difference permanent joint damage can occur within the first two years of disease onset (Chen et al., 2021)**.** Rheumatoid arthritis is typically diagnosed according to the 2010 ACR-EULAR (American college of rheumatology-European league against rheumatism (Aletaha et al., 2010)The overproduction of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and some of interleukins, results in the proliferation of synovial cells in joints and the consequent formation of pannus, cartilage destruction, and bone erosions (Nattagh-Eshtivani et al., 2021). The CD4+ T cells and their cytokines are believed to play major parts in the induction and propagation of the pathogenic inflammatory conditions. The T cell-associated cytokines interleukin (IL-23) have recently been proposed as important regulators of this process (Engelen et al., 2022)**..** This is supported by the recent discovery that IL-23, produced by CD4+ T cells and dendritic cells, respectively, promote the development of the IL-17A-producing pro-inflammatory CD4+ T cell subset T helper (Th) 17. Angiogenesis is a remarkably active process in RA, especially in early stages and is regulated by various pro-angiogenic mediators such as TGF– β, angiopoietin, placenta growth factor, FGF, and vascular growth factor (VEGF) (Ba et al., 2021).The Erythrocyte Sedimentation Rate (ESR) increases as a result of any cause or focus of inflammation. When an inflammatory process is present, fibrinogen enters the blood in high amounts and causes red cells to stick to each other, which raises the ESR (Lapić et al., 2020). ESR is the elevation is typically 3 to 4 times above normal .ESR also can be very helpful in diagnosing and monitoring chronic pain patients (Tennant, 2014)**.**C-Reactive Protein(CRP) is synthesized in the liver. Its physiologic role is to bind to phosphocholine expressed on the surface of dead or dying (apoptosis) cells in order to activate the complement/immune system, which enhances phagocytosis by macrophages (Bajic et al., 2015) . Levels of(CRP) begin to rise within 2 hours of an insult, and have a half-life of about 18 hours(Malin and Witkowska-Piłaszewicz, 2022)**.**

Epidemiologic variations in the incidence and prevalence of rheumatoid arthritis have been observed based on ethnic and geographic dispersion (Finckh et al., 2022). The estimated global prevalence of RA ranges from 0.24 to 1%, although rates differ by region and country. The epidemiology of RA is poorly understood in the Middle East and North Africa (MENA) region, data on its prevalence and disease activity among Arab populations are rare(Bedaiwi et al., 2019) . The incidence of RA in Iraq was 1.1% in 2014 and 2.2% in 2019, compared to 1.6% and 2.1% in 2001 and 2011, respectively. Although this variation is not statistically significant difference, it may be attributable to disruptions in the healthcare system and immigration during this time period (Al_Badran et al., 2022)**.** The etiology of RA is complex and can occur at any age, especially in middle-aged people, with a high disability rate, and there is currently no complete cure(Guo et al., 2020) **.** Currently, there is an increasing interest in treating patients at risk of rheumatoid arthritis (RA) to prevent the development of this chronic disease. In this sense, research has focused attention on the early identification of predictive factors of this disease (Novella-Navarro et al., 2021)**.** IL-23 is an inflammatory marker and a member of the IL-12 cytokine family composed of the IL-23 p19 subunit and the IL-12/23 p40 subunit. It is secreted by activated macrophages and dendritic cells in peripheral tissues such as skin, intestinal

mucosa, joints, and lungs (Schinocca et al., 2021)**.** IL-23 was associated with disease activity in rheumatoid arthritis (RA)(Rasmussen et al., 2010) **.** IL-23 can induce chronic inflammation through two independent pathways. The first pathway is via enhancing the secretion of IL-17 by non-T cells, and the second pathway is by the activation of Th17 cells. Once activated, Th17 cells produce IL17, IL-6, IL-22, and TNFα and other factors that are associated with immune-mediated inflammation (Guo et al., 2020)**.** IL-23 is not only a sensitive biomarker but also a key player in the pathogenesis of RA, making it a valuable target for diagnosis and potential therapeutic interventions in rheumatoid arthritis and other autoimmune diseases (Tsukazaki and Kaito, 2020) (Wajda et al., 2023) **.** Anti-inflammatory cytokines might effectively inhibit arthritis, either by affecting innate immune cells or by interfering with the activation of B cells or T cells (Chen et al., 2019)**.** (TGF-β) is anti-Inflammatory a member of a family of secreted cytokines with vital biological functions in cells. The abnormal expression of TGF-β signaling is a common finding in pathological conditions are considered to be an important immune regulatory cytokine, which is predominantly expressed in the immune system (Huang et al., 2022)**.** Increasing evidence has demonstrated the role of TGF-β signaling in the regulation of many physiological processes, such as wound healing, inflammation, apoptosis, differentiation, and embryogenesis. Furthermore, TGF-β signaling participates in pathological processes including, diabetes, and cancer (Kimawaha et al., 2020) **.** In RA synovial tissues, TGF-β is expressed at high levels and has been linked to synovial hyperplasia, inflammation, and angiogenesis. Inhibition of TGF-β signaling has shown promise in down-regulating rheumatoid synoviocytes and preventing arthritis induction, suggesting a potential therapeutic target for RA treatment (Sakuma et al., 2007)(Su et al., 2024)**.** Increased expression of TGF-β and its receptor I in rheumatoid synovial fibroblasts was found to correlate positively with clinical markers of disease activity, pointing to a correlation between TGF-β and inflammation (• Wajda, A., Stypińska, B., Czarnecka, M., Hasan, D., Jarończyk, M., Hładyj, E.& Paradowska-Gorycka, 2023)**.** The presented work aimed to investigates the effect of inflammatory marker such as (IL-23) and (TGF-β) as anti-inflammatory marker in sera of RA patients to study their effects on the development of and pathogenesis of the diseases in Iraqi women.

2. Materials and Methods

A case-control research approach was utilized together data from 118 females within the period from Oct., 2023 to May, 2024. Females matched ages ranged between 30-70 years and were obtained from the Rheumatology consultant unit, Al-Hassan Teaching Hospital, Kerbela Health Directorates / Kerbela – Iraq. They were divided into two groups: the first group contained 71 patients with rheumatoid arthritis (diagnosed by a rheumatologist according to clinical examination and laboratory testing to ensure inclusion in the American College of Rheumatology (ACR). European League Against Rheumatism (EULAR) 2010 (ACR/EULAR-2010**)** and another participant 47 sample were obtained from an apparently healthy control group, divided all participants to four subgroups according to age group. The exclusion criteria included people with any excluded with malignant diseases, pregnant and breastfeeding. A questionnaire was developed for the study based on the literature review and discussions between the researcher and the supervisory team. The questionnaire included age, duration, and family history of rheumatoid arthritis.

3. Results

Table1: The distribution of age groups is fairly similar across both groups, with a slight trend towards older ages in the patient group. More than half of patients (57.1%) fall within this age range (**30-39) years** compared to controls (42.9%). Similar distribution for the (40-49) years range, with a slightly higher percentage in the patient group (67.7%) compared to controls (32.3%). A slightly higher percentage of controls (57.5%) are in the age range (50-59) years compared to patients (42.5%), while about of patients (69.4%) were in the age range (60-70) years compared to controls (30.6%).

Results were demonstrated that the patient group appears to have a higher prevalence of overweight and obesity compared to the control group about (42.9%) patients were classified as normal weight compared to controls (57.1%). A higher percentage of patients (74.6%) were overweight compared to controls (25.4%). All patients (100%) in the obesity category belong to the patient group, with none in the control group, Regarding the Family History, this characteristic shown a difference between the groups. All patients (100%) reported a family history of the condition, whereas none (0%) in the control group, the percentage of controls was (47.5%) do not have a family history, compared to only half (52.5%) of patients.

Table 1: Demographics Characteristic of The Study Groups

Variable	Groups	Patient N=71	Control N=47	Total N=118
Age. Groups Years	30-39 Years	18(57.1%)	13(42.9%)	31(100%)
	40-49 Years	30(67.7%)	15(32.3%)	45(100%)
	50-59 Years	10(42.5%)	13(57.5%)	23(100%)
	60-70 Years	13(69.4%)	6(30.6%)	19(100%)
BMI. Groups	Normal weight	29(42.9%)	37(57.1%)	66(100%)
	Over weight	31(74.6%)	10(25.4%)	41(100%)
	Obesity	11(100%)	0(0.0%)	11(100%)
Family History	Yes	21(100%)	0(0.0%)	21(100%)
	No	50(52.5%)	47(47.5%)	97(100%)

Table 2 indicated the mean ± SD levels of inflammatory and anti-inflammatory biomarkers in sera of rheumatoid arthritis patients. A significantly higher levels of each of IL-23, TGF-β, CRP and ESR were observed in sera of RA (41.32±14.15 pg/ml) (40.56 ± 16.35 pg/ml), (5.98 ± 1.89 mg/l) and (50.11 ± 14.18 mm/hr) as compared with their levels in apparently healthy control group respectively (9.16 ± 1.01 pg/ml), (4.42 ± 2.79 pg/ml), (0.43 ± 0.22 mg/l) and (14.17 ± 4.84 mm/hr) respectively ($P < 0.001$).

Table 2: The Mean ± SD Levels of Inflammatory and Anti-Inflammatory Biomarkers In Sera of RA Patients Compared With Haelthy Control.

Biomarkers	RA Mean ± SD N=71	Health Control Mean ± SD N=47	P value
TGF, pg/ml	40.56±16.35	4.42±2.79	**<0.001[S]**
IL-23, pg/ml	41.32±14.15	9.16±1.01	**<0.001[S]**
CRP, mg/ml	5.98±1.89	0.43±0.22	**<0.001[S]**
ESR, mm/hr	50.11±14.18	14.17±4.84	**<0.001[S]**
T -test was: significant at $p \leq 0.05$; SD: standard deviation; [S]: significant; NS= Non-significant.			

Table 3 and Fig.1 indicated the results obtained after applying the receiver operating curve (ROC) analysis for only IL-23 biomarker which was (sensitivity 63.2%, specificity 68.1%) at a level = 21.37, the *P*-values of the AUC were <0.05 and statistically significant**.**

Table 3: The area under the curve (AUC), sensitivity and specificity of IL-23 determined by applying the ROC analysis in RA disease

Test Result Variable(s)	IL-23
AUC	66.2%
Sensitivity %	63.2%
Specificity %	68.1%
Youden index	0.312
Cut-off points	21.37
CI (95%)	0.542-0.781
PPV	84%
NPV	77%
Accuracy	91%
P value	0.011[S]
S= Significant, PPV= Positive protective value, NPV= Negative predictive value, AUC= Area under curve, CI= confidence interval	

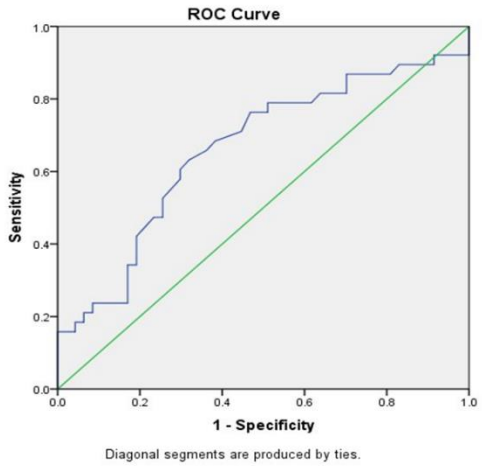

Figure 1: Receiver Operating Characteristics (ROC) Curve Analysis of IL-23 in Sera of RA Patients.

4. Discussion

Inflammatory processes play a pivotal role in the pathogenesis of rheumatoid arthritis. (RA) markers of inflammation such as interleukin (IL-23), play an important role in the pathophysiology of RA (Huang et al., 2022); (Shrivastava and Pandey, 2013)**.** IL-23 is playing important role and considering a key cytokine controlling inflammation in peripheral tissues leading to the development of autoimmune diseases (Al Fadl et al., 2013) . TGF-β activation leads to functional immune-modulatory effects according to environmental conditions. The function of TGF-β in the development of arthritis in murine models has been extensively studied with controversial results (Al Fadl et al., 2013)**.** The observed results indicated a descriptive data between RA patients and control with the age in which the age (60-70) years old were highly percentage (69.4%) and more susceptibility to RA, these results were agreed with those study which reported of age between RA patients and control that recorded by (Gadallah et al., 2015) . BMI groups show in higher percentage (100%) in obese patients than other BMI groups, these results were agreement with that recorded by (Shrivastava et al., 2015) . While not agreement with study recorded by (George et al., 2017). Highly percentage of family history (100%) of RA disease from other group of patients, family history is similar with that obtained by other study (Jang et al., 2022)**.**

Different biomarkers in sera of RA patients and control were studied and the observed results indicated a highly significant association for more than one parameter as (ESR, IL-23, CRP, TGF-β). The current study showed that the mean ± SD of TGF-β was highly significantly difference between patients and control group ($P<0.001$) in which its levels was increased and reached to (40.56 ± 16.35 pg/ml) in RA patients, compared with healthy control group (4.42 ± 2.79 pg/ml), therefore, inhibition TGF-β play role in treatment of RA because of it's a multifunctional cytokine that regulates cell growth, inflammation and angiogenesis by acting on various cell types, and these data agree with result (Gonzalo-Gil and Galindo-Izquierdo, 2014)**.** These results were agreed with other investigations who reported the elevation of TGF-β in RA cases. The reason behind that is activated macrophages which are the major producers of inflammatory cytokines, including tumor necrosis factor-alpha (TNF–α). This, in turn, induce the release of other inflammatory cytokines such as interleukin (IL)-1, IL-6 or IL-8, involved in angiogenesis and the proliferation of the synovial fibroblasts and the production of factor platelet-derived growth (PDGF), fibroblast growth factor (FGF) and TGF–βC (Huang et al., 2015).The sustained activation of these cell types leads to RA's structural alterations (Gonzalo-Gil and Galindo-Izquierdo, 2014)**.** The mean ± SD of ESR level observed was (50.11 ± 14.18 mm/hr) in RA patients, while its level in control was (14.17 ± 4.84 mm/hr) which highly significant association in between ($p<0.001$), this study was agreed with that recorded by others (Khadim and Al-Fartusie, 2021). CRP, the typical inflammatory biomarker produced in the liver, is regulated by adipocyte-derived pro-inflammatory cytokines(den Hartigh et al., 2023) **.** The current study showed that the mean ± SD of CRP was highly significant ($p<0.001$) in RA patients as compared with healthy control (5.98±1.89 mg/l) in RA patients and control (0.431 ± 0.22 mg/l) which agreement with other data reported previously (Khadim and Al-Fartusie, 2021)**.**

IL-23 plays a role in local inflammation., there is an increased production of IL-23 by peripheral blood mononuclear cells (PBMCs) surrounding the islets (Luo et al., 2024). Furthermore, an up-regulation of the IL-23 subunit was observed in pancreatic islets from individuals (Krupa and Kowalska, 2021)**.** The current study indicates the mean ± SD of IL-23 was highly significant ($P<0.001$) in RA patients (41.32 ± 14.15 pg/ml) as compared with control (9.16 ± 1.01 pg/ml) and these data were agrees with other results performed by other(Lucaciu et al., 2021) **.** Elevated IL-23

might be reflected by their central role in the development and maintenance of autoimmune diseases like RA. Increased IL-23 levels might contribute to enhanced activation and proliferation of immune cells that damage joint tissues in RA patients (Xiong et al., 2022)**.**

The study findings highlight a potential link between interleukin-23 (IL-23), inflammation (CRP), in patients with Rheumatoid Arthritis (RA). This indicated that higher IL-23 levels coincide with increased inflammation (CRP). IL-23 is known to play a crucial role in promoting inflammatory responses in autoimmune diseases like RA (Tsukazaki and Kaito, 2020). The IL-23 levels may be considered as a biomarker used for the diagnosis of RA, and this data is consistent with other studies(Xiong et al., 2022);(Loures et al., 2019).Results of the receiver operating (ROC) curve and AUC of IL-23 showed good diagnostic performance for predication of the RA compared to the control groups. For IL-23 levels (sensitivity 63.2%, specificity 68.1%) at a level = 21.37, the p-values of the AUC were <0.05 and statistically significant accuracy 91%. while there is non-significant relationship of rock operative curve for TGF-β and each of cut-off, sensitivity and specificity were un-impotence, because it is not statistically or clinical importance

5. Conclusion

IL-23 was significantly compared with control; therefore, these are more importance beneficial in monitors and early detection of disease**.** Increased transforming growth factor is an important cytokine in rheumatoid arthritis and has both pro- and anti-inflammatory effects. Its increase is due to a balancing act when inflammatory markers high. The complex role of TGF-β has anti-inflammatory properties. Increase of TGF-β combined with increased inflammation (CRP) has been the body's attempt to counteract the ongoing inflammatory process IL-23, CRP, and ESR were significantly compared with control; therefore, these are more important, IL-23 important in monitoring, early detection and diagnosis of RA, while CRP and ESR there are classification criteria of RA.

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7. Ethical approval:

The ethical approvals were obtained from the ethical committee team, College of Medicine, University of Kerbela and the Kerbela Health Directorates / Kerbela – Iraq, (Ethic board, 32 on 02/11/2023).

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