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Evaluating tumor necrosis factor-alpha and interleukin 17 as plasma biomarkers for rheumatoid arthritis diagnosis

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ABSTRACT

Introduction and aim. Rheumatoid arthritis (RA) is a complex chronic inflammatory autoimmune disease with significant global health implications. In this study, our aim was to evaluate the clinical utility of plasma tumor necrosis factor-alpha (TNF-α) and interleukin-17 (IL-17) along with immunological parameters as diagnostic biomarkers for patients with RA.

Material and methods. A case-control study was conducted involving 75 RA cases and 75 age- and sex-matched controls. Plasma levels of TNF-α and IL-17 were measured using ELISA kits. Statistical analyses included receiver operating characteristic (ROC) curve analysis and correlation tests to evaluate the diagnostic accuracy, predictive value, and associations with disease activity parameters.

Results. RA patients exhibited significantly elevated TNF-α (275.5±99.9 vs. 46.2±8.4 pg/mL, $p<0.001$) and IL-17 (313.8±95.4 vs. 42.2±10.96 pg/mL, $p<0.001$) compared to controls. Patients with a family history of RA had higher TNF-α ($p=0.019$) and IL-17 ($p=0.03$) levels. ROC analysis revealed perfect diagnostic accuracy for both biomarkers (100% sensitivity and specificity) at cut-offs >65.2 pg/mL (TNF-α) and >83 pg/mL (IL-17).

Conclusion. There was a positive correlation between serum levels of IL-17 and TNF-α. Therefore, these biomarkers distinguish rheumatoid arthritis patients from healthy controls.

Keywords. anti-cyclic citrullinated peptide, biomarkers, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by immune-mediated inflammation of synovial joints, which leads to progressive cartilage and bone destruction, functional impairment, and potential multi-organ involvement.[1] This condition represents a complex interplay of genetic predisposition, environmental triggers, and dysregulated immune responses, resulting in the production of autoantibodies that target citrullinated peptides and immunoglobulins.[2] RA is characterized by symmetrical inflammatory polyarthritis, predominantly affecting the small joints of the hands and feet before progressing to larger joints.[3,4] This inflammatory cascade results in a hallmark clinical triad of pain, swelling, and morning stiffness lasting more than 30 minutes.[5]

RA affects a substantial proportion of the global population, with epidemiological studies generally indicating a population prevalence ranging from 0.5% to 1%.[6] Multiple studies have documented an increasing trend in the prevalence of RA in the recent decades. The age-standardized global prevalence rate increased by 14.1% between 1990 and 2020, with other studies reporting increases of 0.37%, 14.1%, and 6.4% from 1990 to 2019, 2020, and 2017, respectively.[5] According to systematic reviews, the worldwide average point prevalence is estimated to be 0.51%, whereas the period prevalence is slightly higher, at 0.56%. However, these figures mask the significant variations across the different populations and methodologies used for case identification.[7] Thus, the global burden of RA is considerable. In 2020, an estimated 17.6 million people were living with RA worldwide, with an age-standardized global prevalence rate of 208.8 cases per 100,000. This represents a 14.1% increase since 1990, indicating a rising disease burden, despite advances in treatment.[8] Furthermore, it has been demonstrated that the inflammatory marker C-reactive protein (CRP) increases the synthesis of proinflammatory cytokines, but its specificity is poor.[9] Among serological markers, rheumatoid factor (RF) and anti-citrullinated protein antibodies are particularly prominent. RF, a classical autoantibody discovered over eight decades ago, has been the cornerstone of RA diagnostics and research. Although its utility has been debated due to its presence in other autoimmune and inflammatory conditions, recent advancements have shed new light on its role, mechanisms, and potential implications in the pathophysiology and treatment of RA.[10] Cytokines are also thought to play an important role in causing inflammation, joint destruction, and extra-articular manifestations associated with RA. Therefore, the role of biomarkers in clinical diagnosis and decision-making is crucial. However, traditional biomarkers have low specificity and sensitivity in diagnosing infections in patients with RA, and specific infection markers are urgently needed.[9] In this study, we discuss tumor necrosis factor-alpha (TNF-α) and interleukin-17 (IL-17) as biomarkers and their potential clinical applications in RA.

TNF-α is a central mediator of RA pathogenesis, driving synovial inflammation, cartilage degradation, and systemic complications.[11] As a pleiotropic cytokine, TNF-α orchestrates a cascade of immunological and structural changes that define the progression.[12] TNF-α activates synovial fibroblasts, inducing the overproduction of matrix metalloproteinases (MMPs) and cathepsins, enzymes responsible for degrading collagen and proteoglycans in cartilage and bone.[13] This enzymatic activity leads to progressive joint erosion, a hallmark of RA. Concurrently, TNF-α promotes synovial hyperplasia and angiogenesis, facilitating the formation of invasive pannus tissue that exacerbates joint damage.[14] IL-17 is a proinflammatory cytokine family comprising six members (IL-17A–F), with IL-17A and IL-17F being the most studied.[15] Produced by Th17 cells, γδ T cells, and innate lymphoid cells, IL-17 coordinates host defense against pathogens but also drives immunopathology in autoimmune diseases such as RA.[16] Its dual role in balancing protective immunity and inflammatory damage makes it a critical focus in understanding RA pathogenesis.[17] IL-17 is a linchpin of RA pathogenesis, orchestrating synovial inflammation, bone erosion, and systemic complications.[18]

Aim

This study evaluated plasma TNF-α and IL-17 levels as diagnostic biomarkers of RA. We examined their correlation with RF/anti-CCP levels, discriminatory capacity against healthy controls, and predictive validity for adverse joint outcomes.

Material and methods

This case-control study was conducted between November 2024 and January 2025 at the Marjan Teaching Hospital in Babylon Province, Iraq, and included 75 RA patients and 75 age- and sex-matched healthy controls. The RA diagnosis was confirmed by a joint consultant physician through clinical examination and diagnostic criteria, including RF assessment. RA diagnosis was confirmed by a consultant rheumatologist using the 2010 ACR/EULAR classification criteria, incorporating clinical joint examination, seropositivity for RF, and symptom duration. All patients fulfilled ≥6 points on this validated scoring system. The patient groups were recruited from rheumatology clinics, and demographic and clinical data (e.g., age, sex, family history, comorbidities, treatment regimens, residential status, and smoking habits) were collected using structured questionnaires. The 75 healthy individuals in the control group, systematically selected to mirror the age and sex distributions of the patient group for comparability, were recruited through both hospital staff volunteers and community advertising with age/sex matching criteria. All potential controls underwent screening via a self-reported questionnaire to verify the absence of a personal or familial history of RA or other autoimmune disorders, excluding individuals with current inflammatory symptoms, and eliminating those using immunomodulatory drugs, ensuring that they had no autoimmune conditions. The exclusion criteria for both groups were incomplete medical histories or refusal to participate. Ethical approval

(document number 1887, date: November 10, 2024) and informed consent were obtained prior to data collection and adherence to institutional guidelines.

A priori power analysis (G*Power 3.1) determined that 68 participants per group would provide 90% power (α=0.05, two-tailed) to detect clinically relevant effect sizes (d=0.7) in cytokine levels between RA and controls[19]. To account for subgroup analyses and potential exclusions, we enrolled 75 participants per group (n=150).

Blood collection

A venous blood sample (5 mL) was collected from both the patients and control group in a gel tube under sterile conditions. After centrifugation at 15000 rpm for 10 min, the serum was separated and 0.4 mL was placed in Eppendorf tubes. All samples were labeled with a serial number and the person's name, immediately frozen by deep freezing in the hospital for further processing, and refreezing was avoided.

Biochemical measurement

The analytical tools employed in this study include a range of specialized diagnostic kits sourced from reputable international manufacturers. A set of immunoassay kits produced by BT LAB (China) was used, including the Human IL-17 ELISA kit (Lot No: 202412021), TNF-α kit (Lot No: 202501007), and ANTI-CCP kit (Lot No: 202412021). These kits are based on ELISA and are designed to detect various immunological markers. Additionally, CRP (Lot No: AAUGH02EX) and RF (Lot No: CPUHC05Z) kits from Chroma (Korea), which rely on rapid immunodiagnostic technology, were used. Complete blood count (CBC) analysis was conducted using equipment from Horiba (France) (Lot No: _241028I1), which is known for its high accuracy in routine hematological assessments. Together, these tools enhance the precision of the laboratory results and support the reliability of the data collected throughout the study.

Statistical analysis

GraphPad Prism® software (version 9.3.1, Boston, AM, USA) was used for statistical analyses to compare demographic and clinical parameters between RA patients and healthy controls. The chi-square test and independent samples t-test were employed, and Pearson's correlation coefficient was used for statistical analysis. In addition, normality of continuous variables was assessed using Shapiro-Wilk tests: all biomarkers (TNF-α, IL-17, RF, etc.) normality assumptions ($W<0.90$, $p<0.05$) and biomarkers were normally distributed ($W>0.95$, $p>0.1$).

Results

Table 1 presents the demographic characteristics and biomarker profiles of the 75 RA patients and 75 healthy controls. The RA cohort exhibited a marginally higher mean age (42±13.2 years) compared to

controls (38.9±5.3 years; p=0.08), though this difference was not statistically significant. Sex distribution was comparable between the groups, with 35% of males in the RA group versus 38% in controls (p=0.2). A family history of RA has been reported in 32% of RA patients. BMI was significantly elevated in RA patients (28.7±2.9 kg/m²) relative to controls (24.6±2.1 kg/m²; p=0.005). Biomarker analysis revealed distinct profiles: RA patients demonstrated lower mean WBC counts (7.3±2.3 vs. 8.3±2.3 $\times 10^9$/L; p=0.06) and higher hemoglobin levels (12.9±1.9 vs. 12.0±1.4 g/dL; p=0.04) compared to controls. Markers of inflammation and autoimmunity, including RF: 16.4±7.4 vs. 9.8±2.9 IU/mL CRP: 10.2±6.8 vs. 5.2±1.7 mg/dL), anti-cyclic citrullinated peptide (anti-CCP: 2.3±0.68 vs. 0.2±0.05 pg/mL), TNF-α: 275.5±99.9 vs. 46.2±8.4 pg/mL), and IL-17: 313.8±95.4 vs. 42.2±10.96 pg/mL), were all significantly elevated in RA patients (p<0.001).

Table 1. Demographic and clinical characteristics of study participants

Demographic and biomarker		**Healthy group n=75**	**RA n=75**	**p**
Age (years), mean±SD		38.9±5.3	42±13.2	0.08[a]
Gender	Male, n (%)	28 (38%)	26 (35%)	0.2[b]
	Female, n (%)	47 (62%)	49 (65%)	
Family history	Yes, n (%)	-	24 (32%)	
	No, n (%)	-	51 (70%)	
BMI (kg/m^2), mean±SD		24.6±2.1	28.7±2.9	0.005[a]
WBC(x10^9/L), mean±SD		8.3±2.4	7.3±2.3	0.06[a]
HB (g/dL), mean±SD		12.0±1.4	12.9±1.9	0.04[a] *
RF (IU/mL), mean±SD		9.8±2.9	16.4±7.4	<0.001[a]
CRP (mg/dL), mean±SD		5.2±1.7	10.2±6.8	<0.001[a]
Anti-CCP (pg/mL), mean±SD		0.2±0.05	2.3±0.68	<0.001[a]
TNF-α (pg/mL), mean±SD		46.2±8.4	275.5±99.9	<0.001[a]
IL-17 (pg/mL), mean±SD		42.2±10.96	313.8±95.4	<0.001[a]

n – number of cases, SD – standard deviation, [a] – independent sample t-test. [b] – Chi-square

Table 2 presents a comparative analysis of serum inflammatory biomarker levels between patients with RA with (n=18) and without (n=42) family history of the disease. All biomarkers exhibited significantly higher mean concentrations in the family history group than those in the non-family history group. TNF-α levels were elevated in the family history cohort (309.6±122 pg/mL vs. 253.3±64.4 pg/mL; p=0.019), and IL-17 levels were significantly higher in this group (345.7±91 pg/mL vs. 298.4±83.8 pg/mL; p=0.03). Statistical significance ($p<0.05$).

Table 2. Inflammatory biomarker levels in RA patients stratified by family history (independent sample t-test)

Biomarkers		Family history n=24	Without family history n=51	p
TNF-α (pg/mL)	Mean±SD	309.6±122	253.3±64.4	0.019
IL 17 (pg/mL)	Mean±SD	345.7±91	298.4±83.8	0.03

Receiver operating characteristic (ROC) curve analysis assessed the diagnostic accuracy of TNF-α and IL-17 for RA (Fig. 1), revealing that both biomarkers achieved perfect discrimination with 100% sensitivity and 100% specificity at their optimal cut-off values (TNF-α>65.2 pg/mL, IL-17>83 pg/mL), resulting in perfect area under the curve (AUC) values of 100% ($p<0.001$ for each), where the 95% confidence interval for TNF-α sensitivity was (95.0–100) and for specificity was (89.4-100), while for IL-17 the sensitivity 95% CI was (94.3–100) and the specificity 95% CI was (89.57–100).

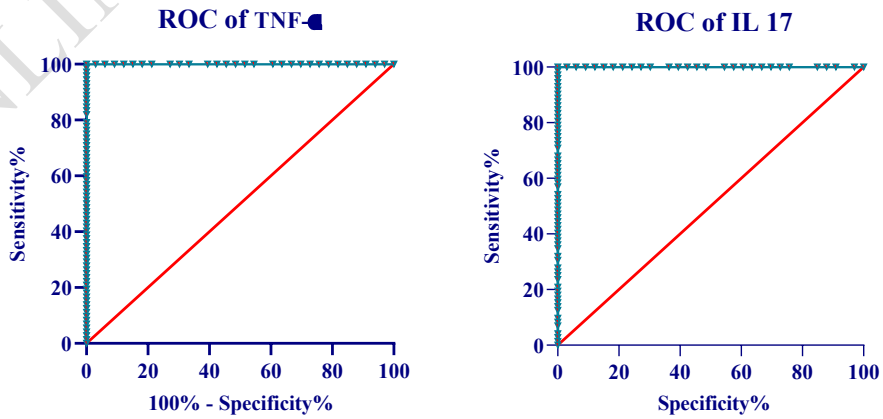

Fig. 1. The ROC curve for (TNF-α, IL-17)

A correlation matrix revealed a moderately positive association between TNF-α and RF (r=0.396, p=0.018, Fig. 2). Anti-CCP exhibited a weak positive correlation with TNF-α level (r=0.290, p=0.013). No significant correlations were observed between CRP or RF levels and the other variables (p>0.05).

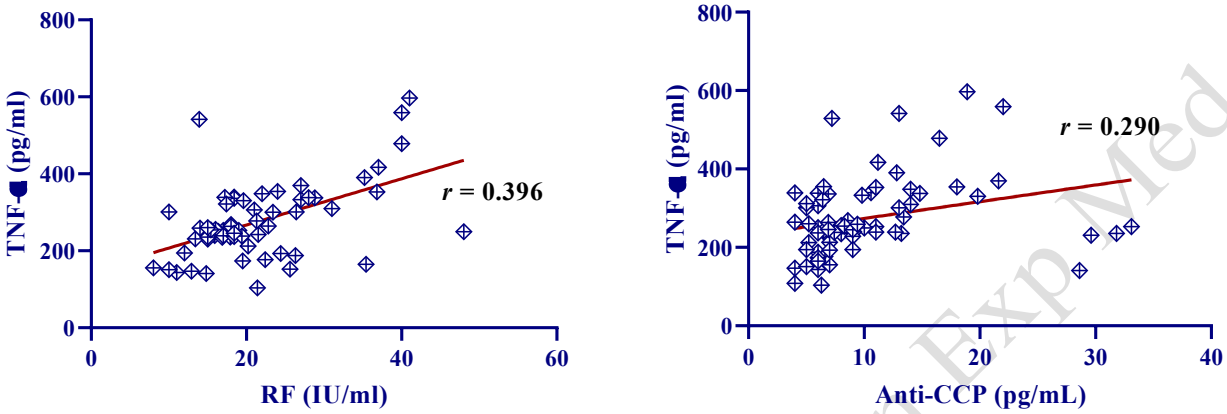

Fig. 2. Positive correlation between the TNF-α and different biomarkers RF and Anti-CCP

In addition, although no statistically significant relationship was found between IL-17 and RF/anti-CCP, where IL-17 demonstrated exceptional diagnostic accuracy and was elevated in RA patients (particularly those with a family history), our correlation analysis revealed no significant association with RF or anti-CCP levels.

Discussion

RA exhibits pronounced sex disparity, with women being disproportionately affected across all populations studied. This condition predominantly impacts women, with a female-to-male ratio of around 3:1 being commonly seen.[20] More precise estimates from global burden of disease studies indicate a 2.45 female-to-male ratio of recurrence, confirming this substantial sex difference.[21] The sex disparity in the RA incidence was similarly pronounced, with a female-to-male incidence rate ratio of 2.3. Interestingly, this disparity appeared most pronounced among young adults, with the peak differential observed among individuals aged 20-29 years.[22] This age-specific pattern suggests potential hormonal influences on disease onset, and supports the hypothesis that reproductive factors may modulate RA risk in women. Although RA can manifest at any age, it demonstrates a characteristic age-dependent pattern of onset and prevalence. The peak in both prevalence and incidence typically occurs around the eighth decade of life,[22] although global data suggest a peak around the age of 60.[21] This age-related pattern has important implications for healthcare planning, particularly given the global demographic shift toward aging populations.

Research indicates that TNF-α, a proinflammatory cytokine, is markedly greater in RA patients than in healthy controls, showing a strong diagnostic potential. Additionally, research has shown that individuals

with a relative history of RA exhibit higher TNF-α concentrations than those without a genetic predisposition, suggesting a hereditary component in disease-related inflammation. These findings align with a 2024 Iraqi study involving 63 newly diagnosed RA patients and 20 healthy controls, which reported significantly increased TNF-α levels in RA cohorts, particularly among severe cases compared to mild to moderate cases.[23] Notably, genetic predisposition may influence TNF-α expression, as patients with a family history of RA exhibit higher levels of TNF-α than those without, implicating hereditary factors in inflammatory dysregulation. Diagnostic evaluations further support the clinical relevance of TNF-α. ROC curve analysis identified a cut-off of 4.25 pg/mL, yielding 76% sensitivity and 70% specificity for distinguishing patients with RA from controls. These findings collectively emphasize the role of TNF-α as a biomarker for RA diagnosis and progression monitoring.[24] Additional case-control studies examining TNF-α in RA, including a study involving 150 participants (119 RA and 31 healthy controls) conducted by Farrugia, demonstrate that RA patients' serum TNF-α levels were considerably greater than those of healthy people. TNF-α levels were substantially linked with age, BMI, and biological therapy.[25] Nonetheless, our results suggest that TNF-α may be a potential biomarker for the pathogenesis of RA.

Previous investigations have has indicated that older persons had higher plasma levels of TNF.[26–29] These findings suggest that TNF-α modulates joint degradation. These observations indicated that elevated systemic and local TNF-α levels are associated with RA.[30] Therefore, in our RA study, the relationship with serum TNF-α levels and RF anti-CCP was statistically significant. Collectively, these results suggest that TNF-α is not only a biomarker for RA, but also a key factor in disease progression and therapy response.[31]

IL-17, another key inflammatory mediator, was considerably increased in patients with RA as opposed to controls. Furthermore, TNF, IL-17 has demonstrated ideal diagnostic accuracy, distinguishing RA with complete sensitivity and specificity. Furthermore, patients with relatives with a history of RA also exhibited higher IL-17 levels, suggesting a role in genetic susceptibility. A study by Samaan et. al. in Egypt compared 45 cases with RA and 45 controls. RA patients' serum IL-17 levels were significantly higher than those of the control group, with a cut-off of >175 pg/mL predicting illness activity ranging from mild to high (DAS28 ≥3.2) with 100% sensitivity and specificity. This highlights IL-17 utility in arranging patients according to the severity of their conditions and monitoring therapeutic responses.[32] This study focused on the function of IL-17 in RA, revealing that serum IL-17 levels were elevated in patients with RA, suggesting a potential role of IL-17 in the pathogenesis of CarP-positive RA. These findings highlight IL-17 possible involvement in specific RA subgroups, as reported by Selimov et al.[33] Although these mechanisms bolster host defense, IL-17 dysregulation in RA exacerbates pathology by driving synovial inflammation (synovitis), bone destruction via osteoclastogenesis, and systemic complications, highlighting its dual role in protective immunity and inflammatory diseases.[34,35] Therefore, in this work, we looked at how IL-17, a

significant Th1 cytokine generated by activated T cells, contributes to the activation of synovial fibroblasts in RA.

Based on these findings, we propose that the rise in these indicators may be due to interactions between the immune system and cells within the inflamed joints that stimulate the release of inflammatory cytokines, contributing to enhanced inflammation and tissue destruction.[36] Farag et al. found that serum IL-17 levels were significantly elevated in patients with RA compared to those in patients with osteoarthritis and controls. These elevated levels correlated positively with disease activity parameters, such as DAS-28, power Doppler ultrasound findings, and radiographic severity (Larsen score), suggesting IL-17's role in RA pathogenesis. The authors concluded that IL-17 may serve as a biomarker for disease activity and a potential therapeutic target.[37] Similarly, Nasef et al. demonstrated markedly increased IL-17 and Th17 cells in patients with RA relative to healthy controls, showing a strong correlation with DAS28. Following anti-rheumatic therapy, both IL-17 levels and Th17 cell frequencies decreased along with clinical improvement, further underscoring their pathogenic role.[38] A study by Atwa et al. on RA patients and healthy controls observed that serum IL-17 levels were 4.7-fold higher in patients with RA. IL-17 was positively relates with DAS28, and IL-17 predicted disease activity with 81.2% sensitivity and 75% specificity.[39] The positive relationship between TNF-α and both anti-CCP and RF stems from TNF-α-activating B cells producing these autoantibodies, which in turn contributes to a continuous inflammatory environment.

A recent study by Okutan et al. found that three novel inflammatory markers, pan-immune-inflammation value, systemic immune-inflammation index, and systemic inflammation response index, were significantly elevated in rheumatoid arthritis patients compared to healthy controls and showed positive correlations with disease severity is evaluated by DAS28 values.[40] ROC analysis revealed a perfect diagnostic accuracy (AUC=1.0). While statistically robust in our study, such perfect discrimination is uncommon in biomarker studies and may reflect stringent control selection, homogenous RA groups, and technical precision. Nevertheless, these results require validation in larger, heterogeneous populations, including patients with non-RA inflammatory conditions, to confirm their real-world applicability.

Study limitations and strengths

While TNF-α and IL-17 demonstrated perfect diagnostic accuracy (100% sensitivity/specificity) in distinguishing RA patients from healthy controls in this study, this finding is context-specific, and several limitations must be acknowledged. The cohort was relatively small (75 RA cases and 75 age/sex-matched controls) and sourced from a single Iraqi center, necessitating validation in larger multicenter studies across diverse populations to confirm generalizability. Crucially, the control group lacked patients with other inflammatory rheumatic diseases (e.g., SLE, psoriatic arthritis, osteoarthritis, and spondyloarthropathies); therefore, the diagnostic performance and specificity of these biomarkers for differentiating RA from conditions with potentially elevated cytokine levels remain unknown and require assessment in mixed

cohorts reflective of clinical practice. Furthermore, the RA patients were not stratified by disease activity (e.g., DAS28), seropositivity status, treatment history, factors that may influence cytokine levels, and diagnostic utility. Finally, longitudinal data are required to evaluate biomarker dynamics during development of the disease or in response to therapy.

Conclusion

This research showed the significance of TNF-α and IL-17 in patients with RA, highlighting their diagnostic utility. Elevated TNF-α and IL-17 levels effectively distinguished patients with RA from healthy controls, achieving exceptional diagnostic accuracy. It has emerged as an independent predictor of adverse joint outcomes, underscoring its unique role in RA pathogenesis. In addition, patients with a family history of RA exhibit heightened inflammatory biomarker levels, suggesting genetic and epigenetic influences on disease susceptibility. These findings highlight the pronounced dysregulation in inflammatory and autoimmune biomarkers in RA, underscoring their potential roles in disease pathogenesis and clinical differentiation from healthy individuals.

Declarations

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Author contributions

Conceptualization, A.H.A.A. and F.M.K.; Methodology, F.M.K.; Software, F.M.K.; Validation, A.H.A.A. and F.M.K.; Formal Analysis, F.M.K.; Investigation, A.H.A.A.; Resources, F.M.K.; Data Curation, F.M.K.; Writing – Original Draft Preparation, F.M.K.; Writing – Review & Editing, A.H.A.A.; Supervision, A.H.A.A.; Project Administration, A.H.A.A. and F.M.K.

Conflicts of interest:

No conflicts of interest.

Data availability

The datasets utilized and analyzed in the present research are available from the author upon reasonable request.

Ethics approval

Before collecting the samples, a local ethics committee reviewed and authorized the research protocol, subject information, and consent form. This approval was granted by document number 1887, date: November 10, 2024.

References

1. O'Neil LJ, Alpízar-Rodríguez D, Deane KD. Rheumatoid Arthritis: The Continuum of Disease and Strategies for Prediction, Early Intervention, and Prevention. *J Rheumatol*. 2024;51(4):337-349. doi: 10.3899/jrheum.2023-0334
2. Smith MH, Berman JR. What Is Rheumatoid Arthritis? *JAMA*. 2022;327(12):1194. doi: 10.1001/jama.2022.0786
3. Shi W, Liang X, Zhang H, et al. Burden of rheumatoid arthritis in India from 1990 to 2021: insights from the Global Burden of Disease Database. *Front Med.* 2025;12:1526218. doi: 10.3389/fmed.2025.1526218
4. Gabal MMA, Eldin AB, Elserwy MA, Mohammed MA. Simplified vs modified (reduced) ultrasound 6 joint score in assessing disease activity in rheumatoid arthritis patients. *Eur J Clin Exp Med.* 2024;22(1):36-44. doi: 10.15584/ejcem.2024.1.4
5. Uke P, Maharaj A, Adebajo A. A review on the epidemiology of rheumatoid arthritis: An update and trends from current literature. *Best Pract Res Clin Rheumatol*. 2025;39(1):102036. doi: 10.1016/j.berh.2025.102036
6. Finckh A, Gilbert B, Hodkinson B, et al. Global epidemiology of rheumatoid arthritis. *Nat Rev Rheumatol*. 2022;18(10):591-602. doi: 10.1038/s41584-022-00827-y
7. Almutairi KB, Nossent JC, Preen DB, Keen HI, Inderjeeth CA. The prevalence of rheumatoid arthritis: a systematic review of population-based studies. *J Rheumatol.* 2021;48(5):669-676. doi: 10.3899/jrheum.200367
8. Black RJ, Cross M, Haile LM, et al. Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. *Lancet Rheumatol.* 2023;5(10):e594-610. doi: 10.1016/S2665-9913(23)00211-4
9. Hussein OA, El-Toukhy MA, El-Rahman HS. Neutrophil CD64 expression in inflammatory autoimmune diseases: its value in distinguishing infection from disease flare. *Immunol Invest.* 2010;39(7):699-712. doi: 10.3109/08820139.2010.491520
10. Togashi T, Ishihara R, Watanabe R, et al. Rheumatoid Factor: Diagnostic and Prognostic Performance and Therapeutic Implications in Rheumatoid Arthritis. *J Clin Med.* 2025;14(5):1529. doi: 10.3390/jcm14051529
11. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. *Int J Mol Sci.* 2019;20(23):6008.

doi: 10.3390/ijms20236008

12. Kondo N, Kuroda T, Kobayashi D. Cytokine networks in the pathogenesis of rheumatoid arthritis. *Int J Mol Sci.* 2021;22(20):10922. doi: 10.3390/ijms222010922
13. Jang DI, Lee AH, Shin HY, et al. The Role of Tumor Necrosis Factor Alpha (TNF-α) in Autoimmune Disease and Current TNF-α Inhibitors in Therapeutics. *Int J Mol Sci.* 2021;22(5):2719. doi: 10.3390/ijms22052719
14. Orsini F, Crotti C, Cincinelli G, et al. Bone involvement in rheumatoid arthritis and spondyloartritis: an updated review. *Biology*. 2023;12(10):1320. doi: 10.3390/biology12101320
15. Mills KHG. IL-17 and IL-17-producing cells in protection versus pathology. *Nat Rev Immunol.* 2023;23(1):38-54. doi: 10.1038/s41577-022-00746-9
16. Sun L, Wang L, Moore BB, et al. IL-17: Balancing Protective Immunity and Pathogenesis. *J Immunol Res.* 2023;2023:3360310. doi: 10.1155/2023/3360310
17. Taams LS. Interleukin-17 in rheumatoid arthritis: Trials and tribulations. *J Exp Med.* 2020;217(3):e20192048. doi: 10.1084/jem.20192048
18. Gremese E, Tolusso B, Bruno D, Perniola S, Ferraccioli G, Alivernini S. The forgotten key players in rheumatoid arthritis: IL-8 and IL-17 – Unmet needs and therapeutic perspectives. *Front Med.* 2023;10:956127. doi: 10.3389/fmed.2023.956127
19. Gail MH, Haneuse S. *Power and sample size for case-control studies.* In: *Handbook of statistical methods for case-control studies*. New York: Chapman and Hall/CRC; 2018: 163–188. doi: 10.1201/9781315154084
20. Venetsanopoulou AI, Alamanos Y, Voulgari P V, Drosos AA. Epidemiology and Risk Factors for Rheumatoid Arthritis Development. *Mediterr J Rheumatol.* 2023;34(4):404-413. doi: 10.31138/mjr.301223.eaf
21. Zhang Z, Gao X, Liu S, et al. Global, regional, and national epidemiology of rheumatoid arthritis among people aged 20–54 years from 1990 to 2021. *Sci Rep.* 2025;15(1):10736. doi: 10.1038/s41598-025-92150-1
22. Zen M, Salmaso L, Amidei CB, et al. AB1628 the incidence and prevalence of rheumatoid arthritis in Italy in the last decade. *Ann Rheum Dis.* 2023;82(1):2048. doi: 10.1136/annrheumdis-2023-eular.1432
23. Hashim ZM, Abdulameer KA. The Effects of -806 T/C and -857 T/C Single Nucleotide Polymorphisms in the TNF-α Gene on Rheumatoid Arthritis Severity and Inflammatory Markers. *Al-Rafidain J Med Sci*. 2024;6:188–194. doi: 10.54133/ajms.v6i1.517
24. Mosavi E, Bandehpour M, Mostafazadeh A, et al. Plasma TNF-α Elevation in Biologic Naive Rheumatoid Arthritis Patients Belonging to a Population with New Mutations in TLR4 and CYP51A1 genes without Association with Disease-Related Antibodies Levels. *Int J Mol Cell Med.*

2024;13(2): 171-185. doi: 10.22088/IJMCM.BUMS.13.2.171

25. Farrugia M, Baron B. The role of TNF-α in rheumatoid arthritis: a focus on regulatory T cells. *J Clin Transl Res*. 2016;2(3):84-90. doi: 10.18053/jctres.02.201603.005
26. Forsey RJ, Thompson JM, Ernerudh J, et al. Plasma cytokine profiles in elderly humans. *Mech Ageing Dev.* 2003;124(4):487-493. doi: 10.1016/S0047-6374(03)00025-3
27. Gerli R, Monti D, Bistoni O, et al. Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians. *Mech Ageing Dev.* 2001;121(1–3):37-46. doi: 10.1016/S0047-6374(00)00195-0
28. Anaya JM, Lozada-Martinez ID, Torres I, Shoenfeld Y. Autoimmunity in centenarians. A paradox. *J Transl Autoimmun*. 2024;8:100237. doi: 10.1016/j.jtauto.2024.100237
29. Pinti M, Gibellini L, Lo Tartaro D, et al. A comprehensive analysis of cytokine network in centenarians. *Int J Mol Sci.* 2023;24(3):2719. doi: 10.3390/ijms24032719
30. Samimi Z, Kardideh B, Chalabi M, Zafari P, Taghadosi M. The plasma Tumor Necrosis Factor-α (TNF-α) does not have any correlation with disease activity in rheumatoid arthritis patients treated with disease modifying anti-rheumatic drugs (DMARDs). *Braz J Pharm Sci*. 2020;56:e18551. doi: 10.1590/s2175-97902019000418551
31. Julià A, López-Lasanta M, Blanco F, et al. Interactions between rheumatoid arthritis antibodies are associated with the response to anti-tumor necrosis factor therapy. *BMC Musculoskelet Disord.* 2021;22(1):372. doi: 10.1186/s12891-021-04248-y
32. Samaan SF, Taha SI, Mahmoud FA, Elsaadawy Y, Khalil SA, Gamal DM. Role of Interleukin-17 in Predicting Activity of Rheumatoid Arthritis and Systemic Lupus Erythematosus. *Clin Med Insights Arthritis Musculoskelet Disord.* 2024;17. doi: 10.1177/11795441241276880
33. Selimov P, Karalilova R, Damjanovska L, et al. Rheumatoid arthritis and the proinflammatory cytokine IL-17. *Folia Med (Plovdiv)*. 2023;65(1):53-59. doi: 10.3897/folmed.65.e72448
34. Wang J, He L, Li W, Lv S. A Role of IL-17 in Rheumatoid Arthritis Patients Complicated With Atherosclerosis. *Front Pharmacol*. 2022;13:828933. doi: 10.3389/fphar.2022.828933
35. Robert M, Miossec P. IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels. *Front Med*. 2018;5:364. doi: 10.3389/fmed.2018.00364
36. Hwang SY, Kim JY, Kim KW, et al. IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways. *Arthritis Res Ther*. 2004;6(2):R120-R128. doi: 10.1186/ar1038
37. Farag MA, El Debaky FE, Abd El-Rahman SM, Abd el-khalek SM, Fawzy RM. Serum and synovial fluid interleukin-17 concentrations in rheumatoid arthritis patients: Relation to disease activity, radiographic severity and power Doppler ultrasound. *Egypt Rheumatol.* 2020;42(3):171-175. doi:

10.1016/j.ejr.2020.02.009

38. Nasef N, Elnagdy M, Younis W, et al. Helper 17 cells and interleukin-17 in patients with rheumatoid arthritis. *Int J Clin Rheumatol*. 2019;14(3):113-119.

39. Atwa SE, Azab MM, Mohamed MS. Serum Interleukin-17 level in patients with rheumatoid arthritis and its relation to disease activity. *Zagazig Univ Med J.* 2020;26(1):87-93.

40. Okutan İ, Aci R, Keskin Â, Bilgin M, Kızılet H. New inflammatory markers associated with disease activity in rheumatoid arthritis: pan-immune-inflammation value, systemic immune-inflammation index, and systemic inflammation response index. *Reumatologia.* 2024;62(6):439-446. doi: 10.5114/reum/196066