GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gscbps.gsconlinepress.com/

(RESEARCH ARTICLE)

Check for updates

ASSESSMENT OF SERUM INTERLEUKIN-6, FAMILIAL, AND INFECTIOUS FACTORS ASSOCIATED WITH RHEUMATOID ARTHRITIS: A CASE–CONTROL STUDY

Mustafa Jawad Al-Imari *

Department of Medical Microbiology, Hammurabi College of Medicine, University of Babylon, Al-Hillah, Babylon, Iraq
*Corresponding Author; Email: mustafa.jawad@uobabylon.edu.iq

ORCID Details
Mustafa Jawad Al-Imari: https://orcid.org/0000-0002-7313-7580

GSC Biological and Pharmaceutical Sciences, 2026, 36(02), 088–097

Publication history: Received on 12 July 2026; revised on 18 August 2026; accepted on 20 August 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.36.2.0294

ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease involving interactions between genetic susceptibility, infectious exposures, and immune dysregulation. This study assessed familial and infectious factors associated with RA and evaluated serum interleukin-6 as a potential biomarker.

Methods: A case–control study included 47 participants: 28 patients with RA and 19 healthy controls. Demographic characteristics, family history of RA and autoimmune disease, severe viral infection, environmental and reproductive factors, and COVID-19 vaccination status were assessed. Serum IL-6 concentrations were compared between RA patients and controls.

Results: Mean age was comparable between RA patients and controls (49.89 ± 15.38 vs 49.63 ± 4.52 years, P = 0.935), whereas females were significantly more frequent among RA patients (96.4% vs 57.9%, P = 0.0016). Family history of RA was significantly associated with RA (50.0% vs 5.3%; OR = 18.00, P = 0.0013), while family history of autoimmune disease showed a stronger association (71.4% vs 5.3%; OR = 45.00, P < 0.0001). Severe viral infection was also significantly associated with RA (39.3% vs 5.3%; OR = 11.65, P = 0.0149). COVID-19 vaccination was lower among RA patients than controls (35.7% vs 72.2%; OR = 0.21, P = 0.0331). Serum IL-6 concentrations did not differ significantly between RA patients and controls (87.61 ± 9.64 vs 86.88 ± 13.03 pg/mL), with identical median values (90.34 pg/mL). The estimated ROC AUC of approximately 0.50–0.60 indicated weak discriminatory performance.

Conclusion: Familial autoimmune susceptibility and severe viral infection were significantly associated with RA. Despite its biological importance in RA inflammation, serum IL-6 demonstrated limited standalone diagnostic discrimination in this cohort. Larger studies are needed to validate these associations and develop integrated clinical and immunological models for RA assessment.

Keywords: Rheumatoid Arthritis; Interleukin-6; Family History; Autoimmune Disease; Viral Infection; COVID-19 Vaccination; Case–Control Study

1 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by persistent synovial inflammation, progressive cartilage and bone destruction, functional impairment, and a range of extra-articular manifestations. Its pathogenesis is complex and results from interactions among genetic susceptibility, environmental exposures, hormonal influences, infectious or microbial stimuli, and dysregulated innate and adaptive immune responses [1]. Recent advances indicate that RA should not be considered the consequence of a single pathogenic factor; rather, disease development occurs through a multistage process in which inherited susceptibility interacts with environmental and immunological triggers to produce loss of immune tolerance and sustained inflammation [2].

Genetic susceptibility represents one of the most important determinants of RA development. The heritability of RA is estimated to be substantial, particularly in seropositive disease, with human leukocyte antigen (HLA) variants accounting for an important proportion of genetic risk [3]. Familial aggregation therefore provides an important epidemiological indicator of inherited susceptibility [1], [4]. Even stronger association was observed for a family history of autoimmune disease supporting the concept that genetic susceptibility may extend beyond disease-specific inheritance toward broader defects in immune tolerance [3].

Sex is another important epidemiological characteristic of RA [5]. The disease occurs considerably more frequently in women, with proposed explanations involving sex hormones, X-chromosome-related mechanisms, genetic factors, and differences in immune regulation [6]. Estrogen can influence B-cell activity, cytokine production, antigen presentation, and other components of adaptive and innate immunity. Recent evidence also suggests that reproductive and menopausal factors may modify susceptibility and disease expression [6], [7]. Environmental exposures may interact with genetic susceptibility to initiate or amplify autoimmune responses [8]. Smoking remains one of the most established environmental risk factors, particularly in genetically susceptible individuals, and microbial dysbiosis has increasingly been implicated in RA pathogenesis [9]. Alterations in host–microbiome interactions may promote mucosal inflammation, impaired barrier integrity, antigen presentation, and activation of autoreactive immune responses [10]. Infectious exposures may similarly function as environmental "second hits," although a single microorganism has not been established as a universal cause of RA [11]. Inflammatory cytokines are central to the transition from immune activation to persistent synovitis [12]. Interleukin-6 (IL-6) is particularly important because it contributes to acute-phase responses, T-cell differentiation, B-cell activity, and chronic inflammatory signaling [13]. Nevertheless, biological importance does not necessarily imply that a biomarker has sufficient diagnostic discrimination when measured in peripheral blood. Contemporary biomarker research similarly emphasizes the need to integrate inflammatory biomarkers with clinical, serological, and imaging findings rather than relying on a single marker [14].

Finally, vaccination represents an important consideration in patients with autoimmune rheumatic disease. Differences in healthcare access, treatment-related concerns, vaccine hesitancy, and perceptions regarding disease exacerbation may contribute to the observed difference. Current evidence supports COVID-19 vaccination in patients with rheumatic diseases, while recognizing that immunomodulatory treatment can influence vaccine responses [15].

This study aimed to investigate the association of familial and infectious factors with rheumatoid arthritis and to assess the serum interleukin-6 (IL-6) level as a potential biomarker in RA patients compared with healthy controls.

2 MATERIAL AND METHODS

2.1 Study Design and Setting

A case–control study was conducted to investigate familial, infectious, reproductive, environmental, lifestyle, and vaccination-related factors associated with rheumatoid arthritis (RA), as well as to evaluate serum interleukin-6 (IL-6) concentrations among patients with RA compared with healthy controls. Participants were recruited from Al-Hillah, Babylon Province, Iraq, through a private rheumatology center under the supervision of a specialist physician. The study was conducted during the period from January to April 2026.

2.2 Study Participants

A total of 47 participants were enrolled in the study, comprising 28 patients with confirmed RA and 19 healthy controls. Patients with RA constituted the case group, whereas individuals without a known history of RA or autoimmune disease were included as the control group, according to the study recruitment procedure.

2.3 Data Collection

Data were collected using a structured questionnaire administered to all participants. The questionnaire was designed to obtain demographic, familial, infectious, reproductive, environmental, lifestyle, and vaccination-related information.

The collected variables included age, sex, residence, family history of RA, family history of autoimmune diseases, previous severe viral infection, recurrent childhood infections, pregnancy before symptom onset, previous hormonal therapy, smoking status, chemical or environmental exposure, prolonged sun exposure, and COVID-19 vaccination status. Participants provided information based on their personal and medical history. Questionnaire responses were recorded using a standardized data collection form and subsequently entered into the statistical database.

2.4 Blood Sample Collection and Serum Preparation

Three milliliters of venous blood were collected from each participant into a gel-containing tube without anticoagulant for serum preparation. Blood samples were processed to obtain serum for subsequent IL-6 determination.

2.5 Measurement of Serum IL-6

Serum IL-6 concentrations were determined using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Elabscience®, USA), according to the manufacturer's instructions. The assay was performed using the serum samples collected from participants in the RA and control groups. Optical density was measured at 450 nm using a BioTek ELx800 microplate reader (BioTek, USA). IL-6 concentrations were calculated from the standard calibration curve and expressed as picograms per milliliter (pg/mL). All samples were analyzed in duplicate in accordance with the manufacturer's protocol to improve analytical reliability and reproducibility.

2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables were summarized as mean ± standard deviation (SD). Odds ratios (ORs) were calculated to estimate the strength of association between selected categorical exposures and RA. Receiver operating characteristic (ROC) analysis was used to explore the ability of serum IL-6 concentrations to discriminate participants with RA from healthy controls. All statistical tests were two-sided, and a P-value <0.05 was considered statistically significant.

2.7 Ethical Considerations

The study protocol was reviewed and approved by the Research Ethics Committee of Hammurabi College of Medicine, University of Babylon, under approval number 28, dated 31 December 2025.

3 RESULTS

3.1 Demographic Characteristics of the Study Participants

A total of 47 participants were included in the study, comprising 28 patients with rheumatoid arthritis (RA) and 19 healthy controls. The mean age was 49.89 ± 15.38 years among RA patients and 49.63 ± 4.52 years among healthy controls. No statistically significant difference in mean age was observed between the two groups (P = 0.935). A significant difference was observed in sex distribution. Among patients with RA, 27 of 28 participants (96.4%) were female and 1 (3.6%) was male, compared with 11 of 19 controls (57.9%) who were female and 8 (42.1%) who were male (P = 0.0016). The distribution of urban residence was reported as comparable between the two groups and did not demonstrate a statistically significant difference. As shown in Table 1.

Table 1: Demographic characteristics of rheumatoid arthritis patients and healthy controls

Demographic characteristic	RA patients (n=28)	Healthy controls (n=19)	p-value
Age, years, mean ± SD	49.89 ± 15.38	49.63 ± 4.52	0.935
Female sex, n (%)	27 (96.4%)	11 (57.9%)	0.0016
Male sex, n (%)	1 (3.6%)	8 (42.1%)	0.0016
Urban residence	Comparable	Comparable	NS

RA: rheumatoid arthritis; **SD:** standard deviation; **NS:** not significant.

3.2 Familial and Infectious Factors Associated with RA

A significantly greater proportion of RA patients reported a family history of RA compared with healthy controls. A family history of RA was reported by 14 of 28 RA patients (50.0%) compared with 1 of 19 controls (5.3%), with a reported odds ratio (OR) of 18.00 (P = 0.0013). Similarly, a family history of autoimmune disease was reported in 20 of 28 RA patients (71.4%) compared with 1 of 19 controls (5.3%). This association was statistically significant, with a

reported OR of 45.00 (P < 0.0001). Previous severe viral infection was also more frequently reported among RA patients than controls. Eleven of 28 RA patients (39.3%) reported a history of severe viral infection compared with 1 of 19 controls (5.3%), corresponding to a reported OR of 11.65 (P = 0.0149). These findings indicate that family history of RA, family history of autoimmune disease, and previous severe viral infection were significantly associated with RA in the study population, as shown in Table 2.

Table 2: Major risk factors associated with RA

Variable	RA n/N (%)	Control n/N (%)	Crude OR	95% CI	Fisher's exact P
Family history of RA	14/28 (50.0)	1/19 (5.3)	18.00	2.11–153.86	0.0013
Family history of autoimmune disease	20/28 (71.4)	1/19 (5.3)	45.00	5.12–395.80	<0.0001
Severe viral infection	11/28 (39.3)	1/19 (5.3)	11.65	1.35–100.17	0.0149

3.3 Hormonal, Reproductive, Environmental, and Lifestyle Factors for RA patients

Several reproductive, hormonal, environmental, and lifestyle variables were evaluated. The number of participants available for individual analyses varied because of missing responses. Pregnancy before symptom onset was reported among 15 of 26 RA patients (57.7%) compared with 3 of 16 controls (18.2%), with a reported P-value of 0.0236. Previous hormonal therapy was reported by 13 of 27 RA patients (48.1%) compared with 8 of 11 controls (72.7%), and the difference was not statistically significant (P = 0.2817). Current smoking was reported by 8 of 27 RA patients (29.6%) and 8 of 18 controls (44.4%), with no statistically significant difference between groups (P = 0.3544). Chemical exposure was reported by 10 of 28 RA patients (35.7%) compared with 10 of 19 controls (52.6%), also without a statistically significant difference (P = 0.3680). Prolonged sun exposure was reported by 17 of 28 RA patients (60.7%) compared with 19 of 19 controls (100.0%). The difference was statistically significant (P = 0.0014), as shown in Figure 1.

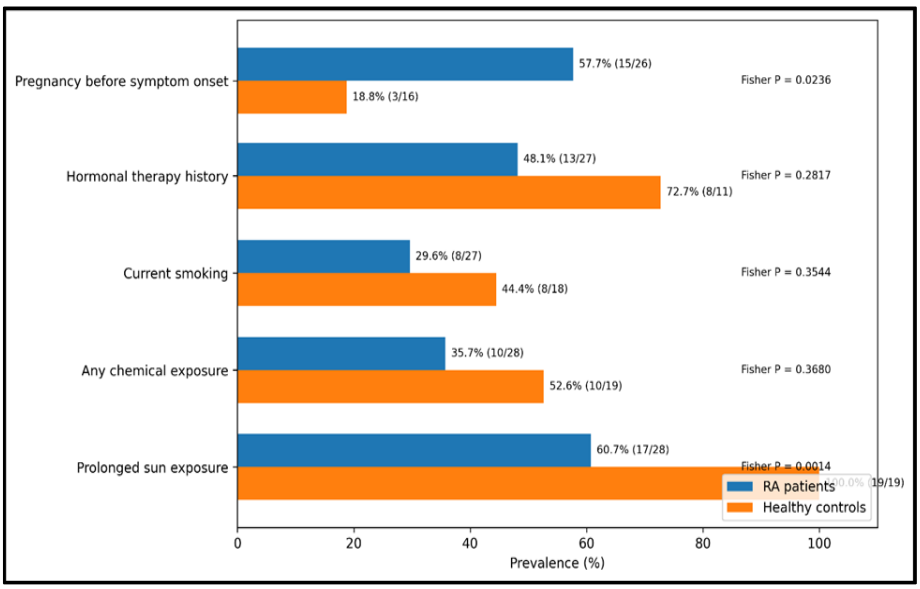

Figure 1: Comparison of reproductive, hormonal, environmental, and lifestyle variables between RA patients and healthy controls. Percentages are accompanied by the number of participants analyzed for each variable. Fisher's exact P-values were calculated for each variable in study groups.

3.4 Infectious History and COVID-19 Vaccination

A history of severe viral infection was significantly more frequent among RA patients than healthy controls, being reported by 11 of 28 patients (39.3%) compared with 1 of 19 controls (5.3%) (OR = 11.65, P = 0.0149). Recurrent childhood infections were reported by 12 of 28 RA patients (42.9%) and 9 of 18 controls (50.0%). No statistically significant difference was observed between the groups (P = 0.7640; reported OR = 0.75). COVID-19 vaccination was

reported by 10 of 28 RA patients (35.7%) compared with 13 of 18 controls (72.2%). The difference was statistically significant (reported OR = 0.21, P = 0.0331). The denominator of 18 controls indicates that vaccination information was available for 18 of the 19 controls for this analysis, as shown in Figure 2.

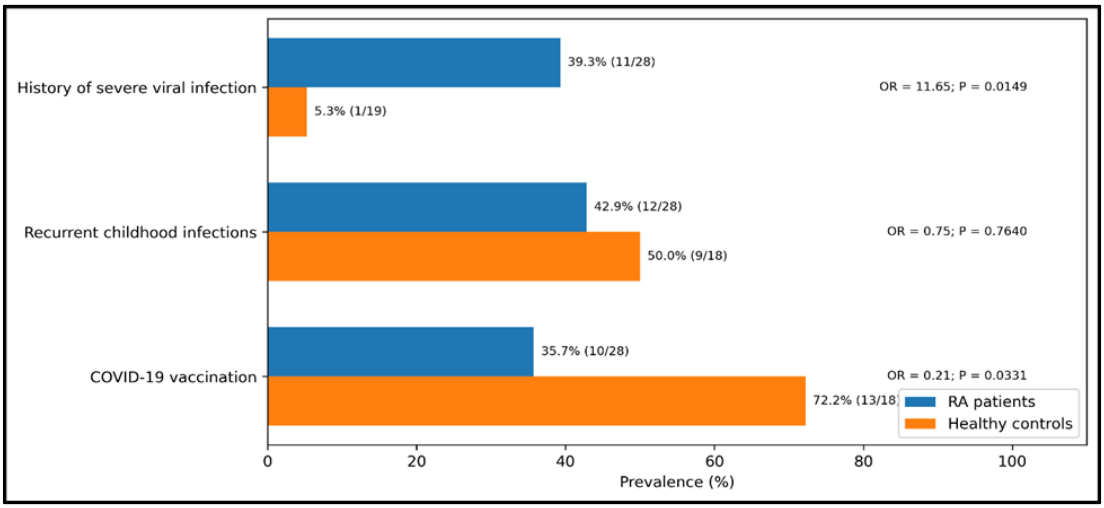

Figure 2: Comparison of infectious history and COVID-19 vaccination status between RA patients and healthy controls. Percentages are accompanied by the number of participants analyzed for each variable. Fisher's exact P-values and crude odds ratios are shown where estimable.

3.5 Serum IL-6 Concentrations in RA Patients versus Healthy Controls

The IL-6 analysis included RA patients and healthy controls. The mean serum IL-6 concentration was 87.61 ± 9.64 pg/mL in RA patients and 86.88 ± 13.03 pg/mL in controls. The median concentration was 90.34 pg/mL in both groups. No statistically significant difference was observed between the groups using either the Mann–Whitney U test or Welch's t-test (P > 0.05; Mann–Whitney U test, P = 0.418). The observed distribution showed substantial overlap between the two groups. The estimated ROC AUC was approximately 0.50–0.60, indicating weak discriminatory performance. Because IL-6 concentrations were not normally distributed, the Mann–Whitney U test was used for between-group comparison. Welch's t-test was additionally performed as a sensitivity analysis, as shown in Figure 3.

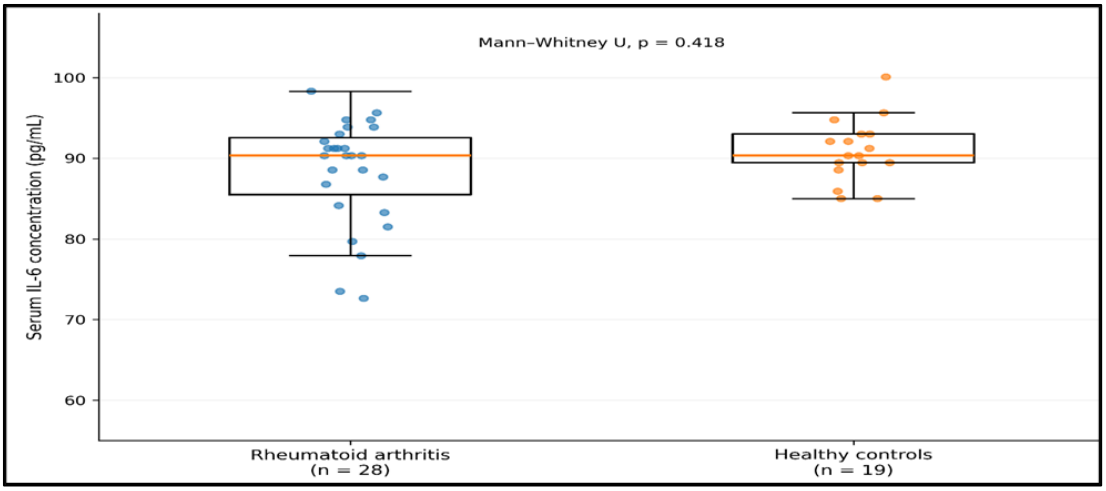

Figure 3: Comparative distribution of serum interleukin-6 concentrations between patients with rheumatoid arthritis and healthy controls. Boxes represent the interquartile range, horizontal lines indicate the median, whiskers represent the distribution of observations, and individual points represent individual participants. Statistical comparison was performed using the Mann–Whitney U test.

4 DISCUSSION

Rheumatoid arthritis (RA) is a complex systemic autoimmune disease in which genetic susceptibility, environmental exposures, infectious events, hormonal influences, and dysregulated immune responses interact to initiate and sustain chronic inflammation. The present case–control study identified several important associations with RA, particularly a

strong familial aggregation of both RA and autoimmune diseases, a marked female predominance, and a significant association with previous severe viral infection. In contrast, environmental and reproductive factors demonstrated less consistent associations, while COVID-19 vaccination was significantly less frequent among patients with RA [15], [16]. Despite the recognized biological importance of interleukin-6 (IL-6) in RA pathogenesis, serum IL-6 concentrations did not significantly discriminate patients with RA from healthy controls in the present cohort. These findings collectively support a multifactorial model in which inherited susceptibility and environmental or infectious triggers interact rather than acting independently.

The absence of a significant difference in age between patients with RA and healthy controls indicates that the two groups were appropriately comparable with respect to age. This is important because age can influence both the prevalence and clinical expression of RA as well as the frequency of inflammatory and autoimmune abnormalities. The close age distribution in the present study therefore reduces the possibility that age-related differences substantially influenced the observed associations [1].

In contrast, a pronounced female predominance was observed among patients with RA. This finding is consistent with the established epidemiological pattern of RA, which occurs substantially more frequently in women than in men. Contemporary evidence suggests that this sex difference is unlikely to be explained by a single mechanism and instead reflects interactions between hormonal regulation, genetic susceptibility, immune-cell function, and sex-specific inflammatory pathways [5].

Estrogen is particularly relevant because of its capacity to influence both innate and adaptive immunity. Hormonal signaling can affect B-cell maturation, antibody production, cytokine secretion, and T-cell responses, potentially contributing to enhanced autoimmune reactivity in susceptible women [6]. In addition, sex-linked genetic mechanisms may influence immune regulation and contribute to the higher prevalence of autoimmune diseases among females [5], [6].

However, the substantial sex imbalance in the present cohort requires caution when interpreting female-specific risk factors. Because almost all RA patients were female, it is difficult to determine whether pregnancy or hormonal therapy independently contributed to disease susceptibility. Therefore, these variables should be regarded as exploratory findings rather than definitive independent risk factors.

One of the most important findings of the present study was the strong association between family history and RA. A family history of RA was substantially more frequent among patients than controls, indicating marked familial aggregation. An even stronger association was observed for a family history of autoimmune disease, suggesting that susceptibility in this cohort may extend beyond disease-specific inheritance [3].

This observation is consistent with the established contribution of genetic factors to RA development. RA has a substantial heritable component, with HLA-associated genetic variation representing one of the major determinants of susceptibility. In particular, HLA-DRB1 alleles containing the so-called shared epitope have been strongly associated with RA, while multiple non-HLA genetic variants contribute to immune dysregulation and disease susceptibility [4].

The particularly strong association between RA and a broader family history of autoimmune disease is also biologically plausible [2]. Autoimmune disorders share several pathogenic mechanisms, including impaired central and peripheral tolerance, abnormal antigen presentation, dysregulated T- and B-cell activation, and altered cytokine signaling. Consequently, individuals may inherit a general predisposition toward immune dysregulation rather than susceptibility to one specific autoimmune disease [2], [3].

Nevertheless, the magnitude of the observed odds ratios should be interpreted cautiously. The relatively small sample size and case-control design may result in wide uncertainty around the estimated effects, while family-history information may be affected by recall or reporting bias. Larger studies involving first-degree relatives and molecular genetic analysis would be valuable to determine whether the strong familial associations observed here are attributable to specific genetic variants or broader familial environmental factors.

A significant association was observed between previous severe viral infection and RA. This finding is of particular interest because it supports the concept that environmental exposures may act in conjunction with inherited susceptibility to initiate autoimmune disease [8].

Current models of RA pathogenesis increasingly describe disease development as a multistep process. Genetic predisposition alone may not be sufficient to produce clinical disease; instead, environmental stimuli may provide an additional inflammatory stimulus that promotes loss of immune tolerance [11]. Severe infections may represent such a stimulus by inducing intense activation of innate immunity, inflammatory cytokine production, antigen presentation, and tissue damage.

The findings of the present study are therefore compatible with a potential "second-hit" model, whereby inherited susceptibility establishes a permissive immunological background and a major infectious event subsequently promotes persistent immune activation [9]. This interpretation is consistent with evidence suggesting that infections can influence the development or acceleration of autoimmune responses through sustained inflammatory signaling and interactions between host immunity and microbial antigens [11].

However, the association observed in this study should not be interpreted as evidence that viral infection directly causes RA. The retrospective case-control design cannot establish temporal or causal relationships with certainty. In addition, the study did not identify a specific viral pathogen or characterize the severity, duration, or timing of each infection in sufficient detail to establish a pathogen-specific mechanism. Future prospective studies incorporating virological testing and longitudinal immune profiling would therefore be required to clarify this relationship [17], [18].

Interestingly, recurrent childhood infections did not demonstrate a similarly strong association. This may indicate that the severity and immunological consequences of an infectious event may be more relevant than the simple frequency of exposure. Nevertheless, this interpretation remains hypothesis-generating and should be examined in larger populations [17].

Hormonal and reproductive variables showed less pronounced associations than familial and infectious factors in the present cohort. This finding should be interpreted in the context of the marked female predominance. Reproductive events may influence immune function and the timing or expression of autoimmune disease, but they are unlikely to represent sufficient independent causes of RA in most individuals [6].

Hormonal transitions can influence cytokine profiles, B-cell activity, and immune tolerance, suggesting that pregnancy and other reproductive events may modify disease expression rather than independently determine disease onset [7]. Therefore, the absence of a strong association for individual reproductive variables in this cohort does not necessarily exclude their biological relevance.

Environmental and lifestyle factors, including smoking and chemical exposure, also demonstrated less consistent associations. This differs somewhat from the substantial body of evidence identifying smoking as an important environmental risk factor for RA, particularly in genetically susceptible individuals [8]. The discrepancy may reflect differences in sample size, exposure intensity, smoking history, disease phenotype, or genetic background.

The present findings therefore support an interpretation in which environmental exposures may function as modifiers within a genetically susceptible host, rather than acting as universally independent determinants of RA. This multifactorial interpretation is consistent with current models of RA pathogenesis [1], [2].

The present study identified a significantly lower prevalence of COVID-19 vaccination among patients with RA compared with healthy controls. Although the association was statistically significant, it should be interpreted carefully.

Importantly, the observed odds ratio should not be interpreted as evidence that COVID-19 vaccination protects against RA or that vaccination is biologically associated with reduced disease development. Because vaccination status was assessed after disease classification, several sources of confounding are possible. Patients with RA may differ from healthy individuals in healthcare behavior, access to vaccination, treatment-related concerns, perception of vaccine safety, or willingness to receive vaccination [16].

This interpretation is particularly relevant because patients with autoimmune rheumatic diseases may have concerns regarding vaccination while receiving immunomodulatory or immunosuppressive therapies. Evidence indicates that COVID-19 vaccines can generate meaningful immune responses in patients with rheumatic diseases, although the magnitude of the response may vary according to the immunomodulatory regimen [15], [16].

In particular, B-cell-depleting therapies and some targeted immunotherapies may reduce vaccine immunogenicity, which can influence clinical decisions regarding the timing of vaccination [15]. Therefore, the lower vaccination prevalence observed in the present cohort may reflect treatment-related or healthcare-related factors rather than a biological relationship between vaccination and RA development.

Future investigations should collect detailed information regarding vaccination timing, number of doses, vaccine type, immunosuppressive treatment, disease activity, education, socioeconomic status, healthcare accessibility, and reasons for vaccine acceptance or refusal [12].

IL-6 is an important cytokine in RA pathogenesis and contributes to several processes involved in chronic inflammation, including acute-phase responses, T-cell differentiation, B-cell activation, and maintenance of inflammatory signaling

[13]. The biological importance of IL-6 has led to considerable interest in its potential use as a biomarker and therapeutic target in RA.

Despite this recognized pathogenic role, the present study found no statistically significant difference in serum IL-6 concentrations between RA patients and controls. The similarity in mean and median concentrations resulted in substantial overlap between the two groups. Consequently, serum IL-6 demonstrated limited discriminatory capacity in this cohort [12].

This finding illustrates an important distinction between biological relevance and diagnostic utility. A cytokine can be central to disease pathogenesis without necessarily being an effective standalone diagnostic biomarker. Circulating IL-6 concentrations may reflect the intensity of systemic inflammation rather than the mere presence or absence of RA [14].

Several factors could explain the absence of a significant difference. First, IL-6 concentrations may vary according to disease activity and inflammatory burden. Patients with clinically controlled or low-activity disease may have lower systemic cytokine concentrations than patients with active synovitis. Second, pharmacological treatment can alter cytokine production and circulating concentrations. Third, biological and analytical variability may influence serum measurements. Finally, the control group may include individuals with subclinical or nonspecific inflammatory activity, thereby increasing overlap between groups [11].

The weak estimated ROC discrimination in the present study further supports the conclusion that serum IL-6 should not be used as an isolated diagnostic marker for RA. Rather, IL-6 may have greater value when integrated with established clinical and laboratory parameters. Contemporary approaches increasingly favor multimarker models that combine inflammatory mediators with autoantibodies, clinical disease-activity measures, and other biomarkers to improve disease characterization and prognostic assessment [14].

The findings of this study have several important implications. First, the strong association with family history emphasizes the importance of genetic and familial susceptibility in RA. Second, the association with severe viral infection supports further investigation of infectious triggers as potential environmental modifiers in genetically predisposed individuals. Third, the marked female predominance reinforces the importance of sex-related immunological mechanisms while also highlighting the need for appropriately balanced study populations when evaluating hormonal risk factors [11], [9], [6].

The IL-6 findings are equally important because they demonstrate that a biologically important inflammatory mediator may have limited diagnostic value when measured in isolation. This distinction is particularly relevant when developing biomarker-based diagnostic approaches for autoimmune diseases [13], [14].

Finally, the lower COVID-19 vaccination rate among RA patients highlights the importance of examining behavioral and healthcare determinants rather than interpreting vaccination status solely from a biological perspective. Current evidence continues to support vaccination for patients with rheumatic diseases, with individualized consideration of treatment and immune response [15].

The present study has several strengths, including the case-control design, age comparability between groups, assessment of familial, infectious, reproductive, environmental, and vaccination-related factors, and evaluation of serum IL-6 as a potential biomarker. The integration of epidemiological risk factors with an immunological biomarker provides a broader perspective on RA susceptibility [6], [14].

However, several limitations should be considered. The relatively small sample size limits statistical power and may produce unstable estimates of effect size, particularly for variables with low frequencies. The marked female predominance among RA patients also limits assessment of sex-specific and reproductive factors. Furthermore, several exposures were based on historical or self-reported information, which may introduce recall bias. The cross-sectional assessment of IL-6 prevents determination of whether cytokine concentrations vary with disease activity or treatment. Finally, the case–control design identifies associations but cannot establish causality, particularly for the relationship between severe viral infection, vaccination status, and RA [9], [14].

5 CONCLUSION

Overall, the present findings reinforce the concept that RA develops through a complex interaction between inherited susceptibility and environmental or infectious exposures. The strongest associations in this cohort were observed for family history of RA and family history of autoimmune disease, highlighting the importance of inherited immune susceptibility. The significant association with severe viral infection provides additional support for a potential

environmental "second-hit" mechanism. The marked female predominance is consistent with established epidemiological patterns but requires cautious interpretation of hormonal and reproductive variables because of the substantial sex imbalance.

The significantly lower COVID-19 vaccination rate among RA patients appears more likely to reflect healthcare, behavioral, or treatment-related factors than a direct biological relationship with RA. Finally, although IL-6 remains an important mediator of RA inflammation, its serum concentration showed poor standalone discriminatory performance in this cohort. These findings emphasize that RA diagnosis and risk assessment should rely on an integrated clinical, genetic, immunological, and environmental framework rather than on any single biomarker or risk factor.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The author would like to thank all participants who contributed to this study and the clinical staff who assisted with participant recruitment and sample collection.

Funding Source

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

The author declares that they have no competing interests.

Author Contributions

The author was responsible for conceptualization, methodology, investigation, data collection, formal analysis, laboratory investigation, data curation, writing the original draft, and writing, review, and editing. The author reviewed and approved the final version of the manuscript.

REFERENCES

[1] D'Orazio A, Cirillo AL, Greco G, Di Ruscio E, Latorre M, Pisani F, et al. Pathogenesis of rheumatoid arthritis: one year in review 2024. Clin Exp Rheumatol. 2024;42(9):1707-13. doi:10.55563/clinexprheumatol/0307ed.

[2] Shakeel L, Shaukat A, Khaliq N, Kashif A, Mujeeb A, Adnan Z, et al. Rheumatoid arthritis: a comprehensive overview of genetic markers, emerging therapies, and personalized medicine. Ann Med Surg (Lond). 2025;87(2):696-710. doi:10.1097/MS9.0000000000002890.

[3] Sharma SD, Leung SH, Viatte S. Genetics of rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2024;38(4):101968. doi:10.1016/j.berh.2024.101968.

[4] Kronzer VL, Sparks JA, Raychaudhuri S, Cerhan JR. Low-frequency and rare genetic variants associated with rheumatoid arthritis risk. Nat Rev Rheumatol. 2024;20(5):290-300. doi:10.1038/s41584-024-01096-7.

[5] Venetsanopoulou AI, Alamanos Y, Voulgari PV, Drosos AA. Epidemiology and risk factors for rheumatoid arthritis development. Mediterr J Rheumatol. 2023;34(4):404-13. doi:10.31138/mjr.301223.eaf.

[6] Malik S, Chakraborty D, Agnihotri P, Sharma A, Biswas S. Mitochondrial functioning in rheumatoid arthritis modulated by estrogen: evidence-based insight into the sex-based influence on mitochondria and disease. Mitochondrion. 2024;76:101854. doi:10.1016/j.mito.2024.101854.

[7] Namavari N, Jokar M, Ghodsian A, Kargar Jahromi H, Rahmanian V. Menopausal state and rheumatoid arthritis: a systematic review and meta-analysis. BMC Rheumatol. 2024;8(1):48. doi:10.1186/s41927-024-00418-2.

[8] Klareskog L, Padyukov L, Lorentzen J, Alfredsson L. Mechanisms of disease: genetic susceptibility and environmental triggers in the development of rheumatoid arthritis. Nat Clin Pract Rheumatol. 2006;2(8):425-33. doi:10.1038/ncprheum0249.

[9] Seymour BJ, Allen BE, Kuhn KA. Microbial mechanisms of rheumatoid arthritis pathogenesis. Curr Rheumatol Rep. 2024;26(4):124-32. doi:10.1007/s11926-024-01135-y.

[10] Mahroum N, Seida R, Shoenfeld Y. Triggers and regulation: the gut microbiome in rheumatoid arthritis. Expert Rev Clin Immunol. 2023;19(12):1449-56. doi:10.1080/1744666X.2023.2260103.

[11] Arleevskaya MI, Novikov AA, Valeeva AR, Korovina MO, Serdiuk IL, Popov VA, et al. At early rheumatoid arthritis stage, the infectious spectrum is driven by non-familial factors and anti-CCP immunization. J Clin Med. 2024;13(10):2796. doi:10.3390/jcm13102796.

[12] Nishimoto N, Kishimoto T. Interleukin-6: from bench to bedside. Nat Clin Pract Rheumatol. 2006;2(11):619-26. doi:10.1038/ncprheum0338.

[13] Avci AB, Feist E, Burmester GR. Targeting IL-6 or IL-6 receptor in rheumatoid arthritis: what have we learned? BioDrugs. 2024;38(1):61-71. doi:10.1007/s40259-023-00634-1.

[14] Sahin D, Di Matteo A, Emery P. Biomarkers in the diagnosis, prognosis and management of rheumatoid arthritis: a comprehensive review. Ann Clin Biochem. 2025;62(1):3-21. doi:10.1177/00045632241285843.

[15] Curtis JR, Johnson SR, Anthony DD, Arasaratnam RJ, Baden LR, Bass AR, et al. American College of Rheumatology guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases: version 4. Arthritis Rheumatol. 2022;74(5):e21-e36. doi:10.1002/art.42109.

[16] Al-Imari MJ, Baqer ZM, Karbul NA and Sabbar HM. Impact of COVID-19 Vaccination and Estrogen on the Severity of Rheumatoid Arthritis in Postmenopausal Iraqi Females. International Journal of Science and Research Archive 16(2):993-996 https://doi.org/10.30574/ijsra.2025.16.2.2423

[17] Tuompo R, Hannu T, Paimela L, Kautiainen H, Leirisalo-Repo M, Koivuniemi R. Do infections play a role in the development of chronic inflammatory arthritis? A 14–year follow-up study of patients with early arthritis. BMC Rheumatology. 2025 Apr 11;9(1):41.

[18] Kudaeva FM, Speechley MR, Pope JE. A systematic review of viral exposures as a risk for rheumatoid arthritis. Semin Arthritis Rheum. 2019;48(4):587-596.