Effects of global epigenetic methylation changes and interleukins-15 and -29 on the progression of rheumatoid arthritis

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Received September 10, 2024; Accepted December 10, 2024

DOI: 10.3892/wasj.2025.310

Abstract. Rheumatoid arthritis (RA) is a chronic, systemic disease characterized by inflammation. However, the mechanisms underlying the pathogenesis of RA remain unclear, although multiple genetic and environmental agents have been implicated. The present study investigated the roles of interleukin (IL)-15 and IL-29) along with DNA methylation changes in the progression of RA, aiming to elucidate their potential as prognostic markers. For this purpose, 60 patients with RA (45 females and 15 males) and 40 healthy controls were selected for the study. Blood samples were collected from all participants to evaluate the sedimentation rate (ESR), and complete blood count, while the serum of participants was used to assess rheumatoid factor, C-reactive protein, anti-cyclic citrullinated peptides, IL-15 and IL-29. In addition, the levels of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) were determined as markers of global DNA methylation. The results indicated a significant elevation in ESR and a decrease in red blood cell count in patients with RA compared with the controls. The patients with RA exhibited higher levels of IL-15 (464.61±13.89) and IL-29 (349.45±7.87) compared with the control group (291.47±10.32 and 255.37±5.45 ng/ml, respectively). Age-related analysis revealed elevated levels of IL-15, but not IL-29 in the older age groups. Additionally, the patients with RA exhibited reduced levels of 5mC, indicative of global DNA hypomethylation, and increased levels of 5hmC, suggesting altered methylation dynamics. These changes are implicated in immune regulation and inflammatory responses, potentially contributing to the pathogenesis of RA. On the whole, the findings of the present study underscore the importance of cytokine regulation and epigenetic changes in the progression of RA, presenting avenues for personalized therapies aimed at mitigating joint inflammation and damage.

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Key words: rheumatoid arthritis, global DNA methylation, DNA hydroxymethylation, interleukin-15, interleukin-29

Introduction

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory disease worldwide, primarily affecting the joints. It is marked by a progressive course that can result in pannus formation and joint destruction if left untreated (1,2). Rheumatoid arthritis affects 0.5 to 1.0% of the adult population, with the incidence varying by region (3). The disease is more prevalent among females than males and the incidence tends to increase with age (4). The main symptoms of RA include pain, stiffness and swelling, primarily affecting the joints of the hands and feet, although other joints can also become inflamed. Additionally, RA may affect other organs (5). Research suggests that multiple genetic and environmental factors contribute to the development of RA (6,7). Despite advancements in more effective treatments over the past two decades, achieving disease remission in RA remains uncommon (8).

Previous studies have demonstrated that cytokine networks play a crucial role in the pathogenesis of RA (9). Interleukin (IL)-15, a cytokine produced by myeloid cells, plays a critical role in the proliferation and survival of natural killer cells, memory $CD8^+$ T-cells and invariant natural killer T-cells (10). It is also implicated in the pathogenesis of RA and may be considered a potential biomarker for the progression of RA. Furthermore, elevated levels of IL-15, rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) have been reported in patients with RA (11,12). IL-29 is a key member of the type III interferon family and the most active cytokine in this group. It plays a role in modulating autoimmune inflammation and protection against viral infections. Previous studies have indicated that IL-29 is expressed in the lining layers of RA synovium by $CD68^+$ macrophages and FGF-2^+ fibroblasts. Moreover, the production of IL-29 is significantly elevated in the serum, peripheral blood mononuclear cells (PBMCs) and synovial tissue of patients with RA compared to healthy controls. However, the precise molecular mechanisms underlying the role of IL-29 in RA are not yet fully understood (13,14).

Epigenetics is the alteration in gene expression and function without changes in the DNA sequences, leading to heritable phenotypes. Epigenetic modifications involve DNA methylation, the post-translational modifications of proteins and the post-transcriptional regulation of genes. Epigenetics plays a key role in autoimmune diseases, including RA (15,16).

DNA methylation is a process in which a CH_3 group is added to cytosine at cytosine-guanine (CG) dinucleotide sites, known as CpG islands. Under normal conditions, the DNA methylation of CpG islands prevents transcription factors from binding to the DNA through various mechanisms, leading to the inhibition of gene expression (17,18). Hydroxymethylcytosine (5hmC) is an epigenetic modifier formed through the active demethylation of DNA via oxidation by ten-eleven-translocation (TET) enzymes. Previous studies have reported that patients with RA exhibit differential methylation patterns in peripheral blood mononuclear cells, fibroblast-like synoviocytes and synovial T-cells. As a result, DNA methylation in peripheral blood, or the methylome, has been proposed as a biomarker for predicting the response of patients with RA to treatment (19,20). Furthermore, DNA methylation can be considered as a biomarker for RA susceptibility. Detectable DNA methylation signatures at the earliest stages of the disease provide the optimal opportunity to initiate early treatment and potentially achieve remission (21).

The present study aimed to assess the potential roles of global DNA methylation levels, along with certain physiological and immunological markers, in the development of RA.

Materials and methods

Subjects and blood collection. A total of 60 patients with RA (45 females and 15 males) visiting the Department of Rheumatology in Private Nursing Home Hospital in Medical City, Baghdad, Iraq, from October, 2023 to January, 2024 were recruited for the study. All patients were diagnosed according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria. The patients with RA were aged between 30 and 70 years, and were newly diagnosed. Patients with prolonged disease duration, those on extended treatment and other autoimmune diseases, active infections, or who were pregnant or breastfeeding, were excluded from the study. Additionally, 40 healthy control samples (10 males and 30 females; aged 30 to 66 years) with no history of autoimmune or chronic diseases and who appeared to be in good health were enrolled in the study. The present study was approved by the Ethics Committee of the Department of Biology, College of Science, University of Baghdad, under reference no. CSEC/0923/0105, on September 25, 2023. Written informed consent was obtained from all patients in the study. The research adhered to the standards set by the latest revision of the Declaration of Helsinki.

Sample collection. A total of 5 ml venous blood was drawn from the radial vein of the participants using disposable syringes. In total, 3 ml were slowly transferred into disposable serum tubes containing separating gel, allowed to clot at room temperature for 10 to 15 min, and then centrifuged at 1,508.3 x g for 10 to 15 min at room temperature. The serum was then distributed into Eppendorf tubes (EPPENDORF) in equal amounts and stored at -20˚C for later use in serological tests. The remaining 2 ml blood were transferred to ethylenediaminetetraacetic acid (EDTA)-containing tubes (Hangzhou Ciping Medical Devices Co., Ltd.) for complete blood count (CBC), erythrocyte sedimentation rate (ESR), and the measurements of 5mC and 5hmC.

Measurement of immunological markers using enzyme-linked immunosorbent assay (ELISA). The quantification of C-reactive protein (CRP) and anti-cyclic citrullinated peptides (anti-CCPs) was performed using the human CRP ELISA kit (cat. no. MBS564038, MyBioSource) and the human anti-CCP antibody ELISA kit (cat. no. MBS7235871, MyBioSource) all materials mentioned hereafter are components of these kits. These kits are based on ELISA, a diagnostic method widely used in medicine. Additionally, ELISA is used in biomedical research as an analytical tool for the detection and measurement of specific antigens or antibodies in selected samples. The principle of ELISA relies on the fundamental immunological concept of antigens binding to specific antibodies, allowing for the detection of small quantities of antigens, such as proteins, peptides, hormones, or antibodies in fluid samples. This process was conducted according to the manufacturer's instructions. All reagents and samples were allowed to reach room temperature prior to use. A total of 100 μl of standards and samples were added to their respective wells, followed by incubation for 30 min at 37˚C and washing with 300 μl of wash buffer. Subsequently, 100 μl HRP conjugate were added to each well followed by incubation for 30 min at 37˚C, then washed again. Subsequently, 50 μl Substrates A and B were added followed by incubation for 10 min at 37˚C. Finally, 50 μl stop solution were added before measuring the absorbance at 450 nm using an ELISA reader (BioTek Instruments, Inc.).

Detection of IL-15 and IL-29 levels using ELISA. The levels of IL-15 and IL-29 were evaluated using human IL ELISA kits (cat. nos. E0097Hu and E0040Hu, respectively, BT LAB) all materials mentioned hereafter are components of these kits. Following the manufacturer's instructions, all reagents and samples were brought to room temperature prior to use. A total of 50 μl of standard and 40 μl of sample were added to their respective wells. Subsequently, 10 μl anti-IL-15 antibody and human IL-29 were added to the sample wells, followed by the addition of 50 μl streptavidin-HRP to both the sample and standard wells (but not the blank well). The wells were then incubated for 60 min at 37˚C and washed with 300 μl wash buffer. Following this, 50 μl substrate solutions A and B were added to each well followed by incubation for 10 min at 37˚C in the dark. Finally, 50 μl stop solution were added to each well, and the absorbance was read at 450 nm using an ELISA reader (BioTek Instruments, Inc.).

Measurement of RF levels. The RF-latex slide agglutination test (cat. no. SL003, Bioresearch) was performed to determine the RF levels all materials mentioned hereafter are components of this kit. First, 40 μl of the sample and one drop each of the negative (usually contains animal serum, which not contain RF) and positive controls (Human serum with a RF concentration >30 IU/ml) were placed into separate circles on the test slide. Subsequently, 40 μl RF latex reagent was added adjacent to the sample and the negative and positive controls for testing. Thirdly, the RF reagent was mixed with the sample, positive and negative controls, and spread over the entire surface of each circle using a stirrer. Finally, the slide was placed on a mechanical rotator at 80 to 100 rpm for 2 min. The presence or absence of visible agglutination was assessed immediately after removing the slide from the rotator.

Table I. Comparison of age, BMI, ESR and CBC parameters between patients with and the control group.

	Mean ± SE				
Parameter	Patients with RA	Controls	t-test	P-value	Cohen's d
Sex	Females (n=45) (75%)	Females (n=30) (75%)	-	0.0087[a]	-
	Males (n=15) (25%)	Males (n=10) (25%)	-	0.0087[a]	-
Age (years)	48.72±1.32	45.60±1.63	-	0.141 NS	0.303
BMI (kg/m^2)	28.52±0.46	27.81±0.44	-	0.294 NS	0.215
ESR (mm/h)	39.67±1.91	8.69±0.59	4.752[b]	0.0001	2.641
RBC (x10^6/μl)	4.49±0.07	4.97±0.10	0.239[b]	0.0002	-0.808
WBC (10^3/μl)	6.97±0.27	6.35±0.21	0.745 NS	0.102	0.339
PLT (10^3/μl)	265.20±9.82	261.00±9.73	28.462 NS	0.769	0.060
MCV (fl)	86.19±0.79	83.98±0.60	2.132[a]	0.0424	0.423
MCHC (g/dl)	33.49±0.37	33.04±0.15	0.926 NS	0.333	0.200
MCH (pg)	29.03±0.53	28.95±0.39	1.425 NS	0.913	0.022
HCT (%)	39.35±0.92	41.79±0.50	2.308[a]	0.0386	-0.513
HGB (g/dl)	13.06±0.23	13.57±0.20	0.647 NS	0.117	-0.325
Neutrophils (%)	57.96±1.38	51.06±0.93	3.652[b]	0.0003	0.771
Lymphocytes (%)	32.02±1.24	26.09±1.38	3.745[b]	0.0022	0.646
Monocytes (%)	8.17±0.34	6.13±0.28	0.941[b]	0.0001	0.886
Eosinophils (%)	5.37±0.31	3.21±0.18	0.824[b]	0.0001	1.066

Data are presented as the mean ± standard error (SE); [a]P<0.05 and [b]P<0.01 between the two groups; NS, not significant; Cohen's d indicates the t-test effect size (small size, 0.2; medium size, 0.5; large size, 0.8). RA, rheumatoid arthritis; BMI, body mass index; ESR, erythrocyte sedimentation rate; RBC, red blood cell; WBC, white blood cell; PLT, platelets; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit; HGB, hemoglobin.

DNA extraction and assessment of global DNA methylation. Whole DNA was extracted from the blood samples of all the studied cases and healthy controls using the gSYNC™ DNA extraction kit (cat. no. GS100, Geneaid Biotech Ltd.). The purity and concentration of the genomic DNA were assessed by measuring the A_{260}/A_{280} absorbance using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc.). A ratio between 1.8 and 2.0 indicates that the genomic DNA is of high quality. The evaluation of global methylation was performed using the MethylFlash™ Global DNA Methylation (5mC) and MethylFlash™ Hydroxymethylated DNA (5hmC) Quantification kits (cat. nos. P‑1034 and P‑1036, EpigenTek). A total of 100 ng of the extracted genomic DNA from each test sample were diluted in the supplied binding solution provided with the 8‑well assay strip kit. In brief, the DNA methylation fraction that bound to the monoclonal antibodies of the well‑assay strip was captured for detection in the subsequent assay steps. This process included the addition of wash solution, detection antibody, enhancer solution, developer and stop reaction solution. Ultimately, the quantification of 5mC and 5hmC was calculated as proportional to the optical density (OD) intensity read at 450 nm using a microplate reader (BioTek Instruments, Inc.). The kit demonstrates excellent sensitivity, with a detection limit of 0.02 ng for 5mC and 0.04 ng for 5hmC DNA. Additionally, it has high specificity, as the antibody selectively detects only 5mC and 5hmC.

Statistical analysis. Graph Pad Prism (Version 8) was employed to create various graphical representations of the data. The Statistical Analysis System (SAS) program (2018) was used to assess the effects of different groups (patients and controls) on the study parameters. The mean ± standard error (SE) were calculated for each group. An unpaired t‑test was employed to evaluate the significance of differences between the means. One‑way ANOVA was used to assess the differences among the age groups. Tukey's Honest Significant Difference (HSD) post hoc test was performed for pairwise comparisons. Cohen's d was calculated to assess the effect size of the difference between the two group means. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

A total of 45 females (75%) and 15 males (25%) with RA participated in the present study, while the control group comprised 30 females (75%) and 10 males (25%), as presented in Table I. The mean age of the patients with RA was 48.72±1.32 years, compared to 45.60±1.63 years for the control group. The body mass index (BMI) results for both the patients with RA and the control group are presented in Table I. The mean BMI of the patients with RA was 28.52±0.46, while the control group had a mean BMI of 27.81±0.44, indicating that both groups were classified as overweight. However, no significant differences were observed in age or sex based on the obtained P‑values. Additionally, the present study demonstrated a significant increase in the ESR among patients with RA compared with the control group, with values of 39.67±1.91 and 8.69±0.59 mm/h, respectively. The results

Table II. Comparison of CRP, anti-CCP and RF between patients with RA and the controls.

	Mean ± SE			
Parameter	Patients with RA	Controls	P-value	Cohen's d
CRP	11.50±1.12	4.83±0.38	0.0003	0.985
Anti-CCP	0.629±0.03	0.307±0.01	0.0001	1.962
RF %	73.33%	0%	0.0001	-

RA, rheumatoid arthritis; CRP, C-reactive protein; anti-CCP, anti-cyclic citrullinated peptides; RF, rheumatoid factor.

Table III. Comparison of IL-15 and IL-29 levels between patients with RA and the controls.

	Mean ± SE	
Group	IL-15 (ng/l)	IL-29 (ng/l)
Patients with RA	464.61±13.89	349.45±7.87
Control	291.47±10.32	255.37±5.45
P-value	0.001	0.001
Cohen's d	1.859	1.807

RA, rheumatoid arthritis; IL, interleukin.

of the CBC indicated that the mean red blood cell (RBC) count of patients with RA was 4.49±0.07x10^6/μl, compared to 4.97±0.10x10^6/μl in the control group, exhibiting a significant decrease (P-value=0.0002). However, the white blood cell (WBC) and platelet (PLT) counts did not exhibit significant differences between the patients with RA and the control group. Additionally, there was a significant increase in the mean corpuscular volume in patients with RA compared with the control group (Table I). A significant increase in hematocrit (HCT) levels was observed in the control group compared with the patients with RA. The mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and hemoglobin levels did not exhibit any significant differences between the patients with RA and the control group. The results of the WBC count indicated that the levels of neutrophils (57.96%), lymphocytes (32.02%), monocytes (8.17%) and eosinophils (5.37%) in the patients with RA were higher compared to those in the control group, which had levels of 51.06, 26.09, 6.13 and 3.21%, respectively. These results indicated a significant increase in the levels of neutrophils (P-value=0.0003), lymphocytes (P-value=0.0022), monocytes (P-value=0.0001) and eosinophils (P-value=0.0001) in the patients with RA compared to the levels in the control group (Table I).

The results presented in Table II indicated that the anti-CCP levels in the patients with RA were significantly higher (P-value=0.0001) than those in the control group. A significant difference (P-value=0.0003) was also observed in the CRP levels between the patients with RA and the controls. The RF levels in the patients with RA were 73.33%, which differed significantly (P-value=0.0001) from those in the control group. Furthermore, the results from the analysis of the levels of IL-15 and IL-29 indicated that the patients with RA exhibited higher levels of both ILs compared with the control group, with a P-value of 0.001, demonstrating a highly significant difference between the two groups (Table III and Fig. 1). Additionally, the results presented in Table IV and Fig. 2 indicate a significant difference in IL-15 and IL-29 levels among the patients based on sex, with the levels being higher in females compared to males (Fig. 2). The results regarding the age groups indicated that the P-value for the IL-15 levels was 0.037, suggesting a significant difference across the age groups. By contrast, the P-value for IL-29 was 0.772, indicating no significant difference in its levels among the age groups (Table IV and Fig. 3). The results of the analysis of global DNA methylation (5mC and 5hmC; Table V) revealed a highly significant decrease in 5mC levels in the patients with RA compared with the control group, with a P-value of 0.0001. Conversely, the 5hmC levels were elevated in the patients with RA compared with the control group, exhibiting a significant difference (P-value=0.0004), as illustrated in Fig. 4. Additionally, the results indicated no significant differences in the 5hmC levels based on age groups or sex among the patients with RA, as presented in Table VI.

Discussion

The results of the present study indicated no significant differences between the patients with RA and the control group as regards age and sex. However, clinical and laboratory data from previous research suggest that demographic factors, such as age, sex, disease duration and clinical parameters, including ESR and anti-CCP levels, do not differ significantly among patients with RA with mild, moderate and severe disease (22). The serum marker findings from the present study align with those observed in previous research (23), particularly the increased levels of the inflammatory marker, CRP, in the patients with RA compared with the controls. Furthermore, the levels of autoantibodies, specifically anti-CCP, were significantly elevated in the patient group compared with the controls. RF autoantibodies were detected in 73.33% of the patients with RA. These findings are consistent with those in the studies by Bagdi *et al* (24) and Hashiam and Aldahhan (25). Elevated CRP levels are associated with higher disease activity in RA and play a role in bone damage and disease progression. Anti-CCP and RF autoantibodies have been utilized as diagnostic markers for RA for several years. However, ~25% of patients with RA do not produce RF (26), which is consistent

Table IV. Distribution of IL-15 and IL-29 in patients with RA according to age groups and sex.

		Parameters Mean ± SE	
Descriptive data		IL-15 (ng/l)	IL-29 (ng/l)
Sex	Female	479.35±15.86	359.68±7.38
	Male	420.40±26.43	318.73±20.98
	P-value	0.0498	0.0212
	Cohen's d	0.6	0.7
Age categories (years)	30-40	440.29±25.524	351.21±14.267
	41-50	485.67±21.174	338.43±13.530
	51-60	498.71±25.813	357.53±16.520
	61-70	379.50±42.338	358.13±21.915
	P-value	0.037[a]	0.772
	Eta-squared	0.139	0.020

Data are presented as the mean ± standard error (SE). An independent t-test was performed to compare males and females, and one-way ANOVA was performed to compare the different age groups. Tukey's Honest Significant Difference (HSD) post hoc test was used for pairwise comparisons. For IL-15, statistically significant differences were observed between the following age categories: 51-60 vs. 61-70, P=0.028; 41-50 vs. 61-70, P=0.041; t-test effect size (small size, 0.2; medium size, 0.5; large size, 0.8). ANOVA effect size (small size, 0.01; medium size, 0.06; large size, 0.14). [a]P<0.05. RA, rheumatoid arthritis; IL, interleukin.

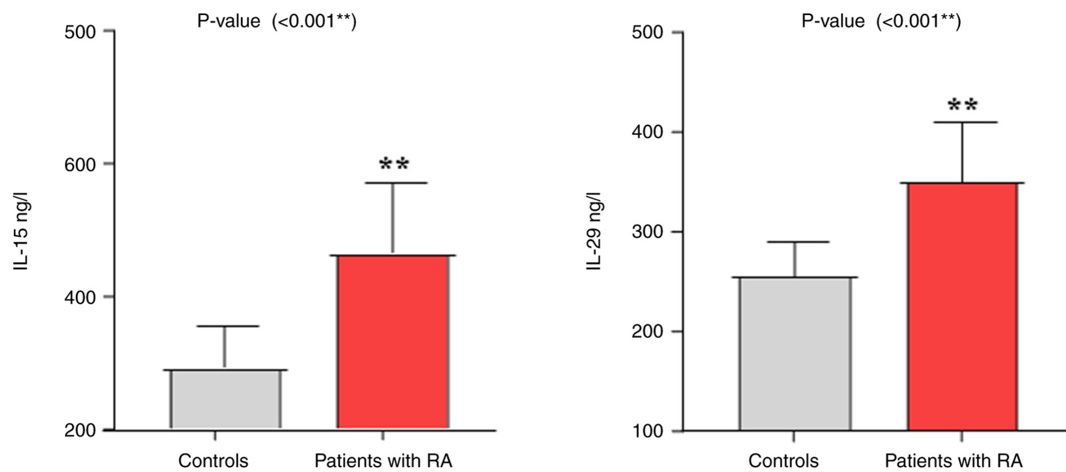

Figure 1. Serum levels of IL-15 and IL-29 (ng/l) in patients with RA and the control group. Bar graphs depict the mean levels of IL-15 and IL-29 in the control group and patients with RA. **P<0.001, statistically significant vs. the control group. IL, interleukin; RA, rheumatoid arthritis.

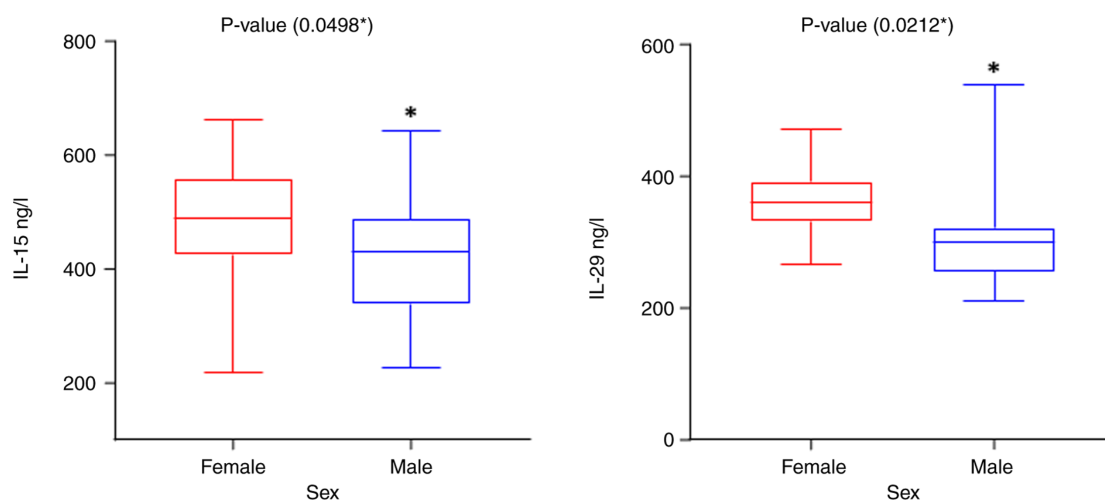

Figure 2. Distribution of IL-15 and IL-29 (ng/l) in patients with rheumatoid arthritis according to sex. Box plots illustrates the distribution of IL-15 and IL-29 levels in female and male in patients with RA. *P<0.05, statistically significant vs. females. IL, interleukin.

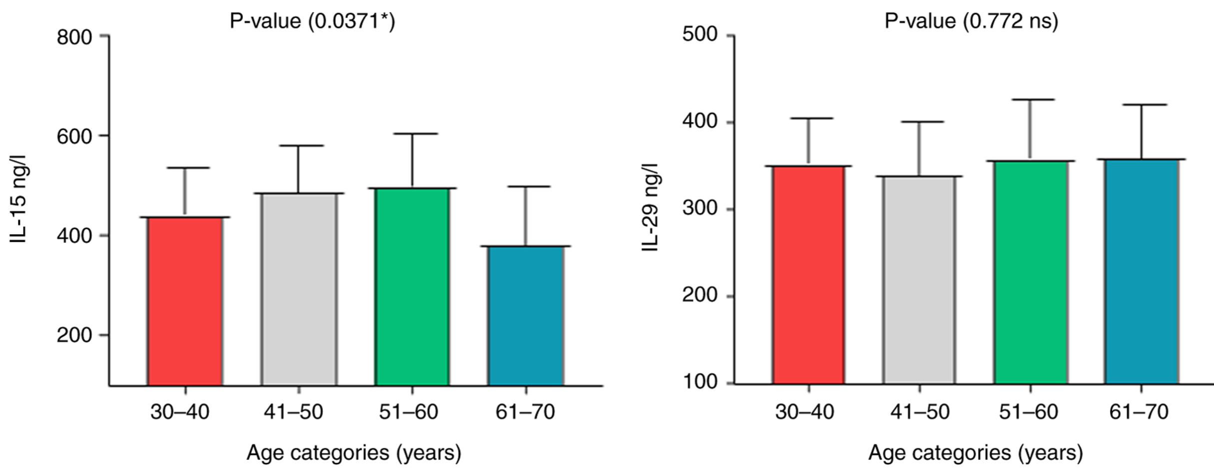

Figure 3. Distribution of IL-15 and IL-29 (ng/l) in patients with rheumatoid arthritis according to age group. Bar graphs demonstrates the mean ± SM of IL-15 and IL-29 levels in different age groups of patients with RA. [*]P<0.05, indicates a statistically significant difference between the age group for IL-15. No significant differences were observed for IL-29 levels across age groups. IL, interleukin. Tukey's Honest Significant Difference (HSD) post hoc test for pairwise comparisons. For IL-15, statistically significant differences were observed between the following age categories: 51-60 vs. 61-70, P=0.028; 41-50 vs. 61-70, P=0.041.

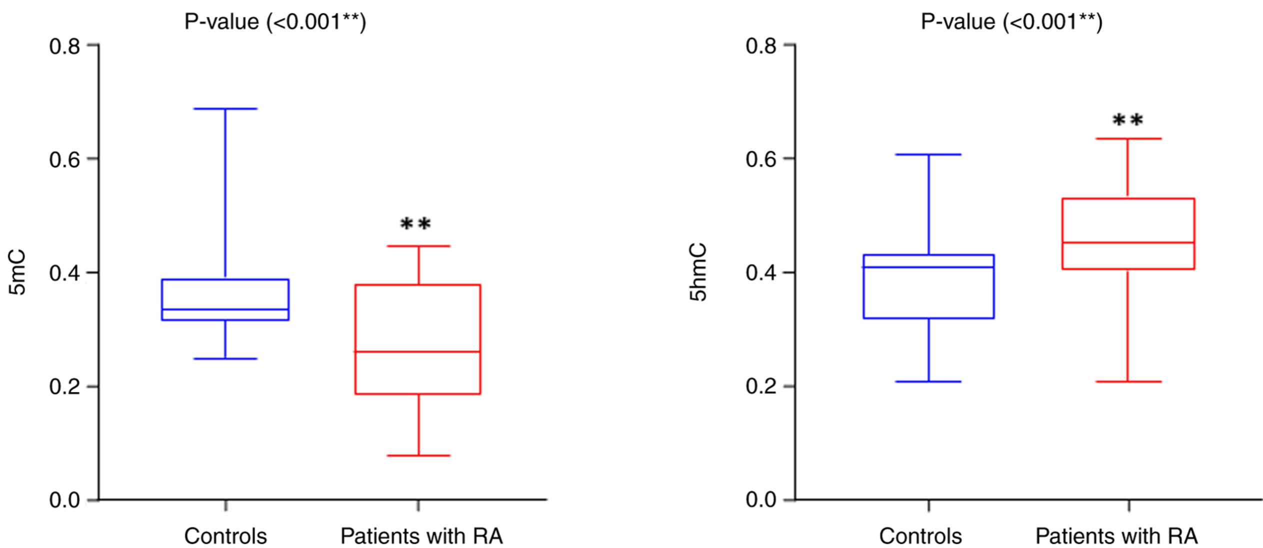

Figure 4. Comparison of 5mC and 5hmC levels between patients with RA and the control groups. Box plots represent the mean of 5mC and 5hmC levels in patients with RA and the control group. [**]P<0.001, statistically significant vs. the control group. 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; RA, rheumatoid arthritis.

with the findings of the current study. Anti-CCP exhibited a specificity of 95-99% and a sensitivity of 60-75%, rendering them a valuable tool for the early diagnosis of RA (27).

Another notable finding of the present study is the elevated concentrations of IL-15 and IL-29 in patients with RA, indicating a highly significant difference compared with the control group. These findings align with those of previous studies (14,28). Age groups influence IL-15 levels in RA patients, while IL-29 levels remain unaffected by age. Additionally, the levels of both ILs are not affected by sex. Elevated IL-15 and IL-29 levels could be implicated in the pathogenesis of RA by several mechanisms. IL-15 plays a critical role in the pathogenesis and progression of RA. It activates and promotes the proliferation of T-cells and natural killer cells, which produce pro-inflammatory cytokines, leading to increased inflammation and joint damage. Additionally, IL-15

Table V. Comparison of 5mC and 5hmC levels between patients with and the controls.

	Mean ± SE	
Group	5mC	5hmC
Patients with RA	0.269±0.01	0.227±0.01
Control	0.376±0.02	0.193±0.01
t-test	0.0438[a]	0.0187[a]
P-value	0.0001	0.0001
Cohen's d	0.984	0.754

Data are presented as the mean ± standard error (SE); [a]P<0.01. RA, rheumatoid arthritis; 5mC, DNA methylation; 5hmC, DNA hydroxymethylation.

Table VI. Distribution of 5mC and 5hmC in patients with RA according to age groups and sex.

		Parameter (mean ± SE)	
Descriptive data		5mC	5hmC
Sex	Female	0.270±0.02	0.231±0.01
	Male	0.268±0.03	0.217±0.01
	P-value	0.939 NS	0.328 NS
	Cohen's d	0.023	0.294
Age categories (years)	30-40	0.296±0.03	0.231±0.011
	41-50	0.255±0.023	0.222±0.01
	51-60	0.260±0.024	0.228±0.012
	61-70	0.283±0.04	0.237±0.019
	P-value	0.699 NS	0.877 NS
	Eta-squared	0.025	0.012

Data are presented as the mean ± standard error (SE). An independent t-test was performed to compare males and females, and one-way ANOVA was performed to compare the different age groups. Tukey's Honest Significant Difference (HSD) post hoc test was used for pairwise comparisons. For, 5mC, 5hmC statistically significant differences were not observed between the sex and the following age categories: 41-50 vs. 61-70, P=0.723; 51-60 vs. 61-70, P=0.640. t-test effect size (small size, 0.2; medium size, 0.5; large size, 0.8). ANOVA effect size (small size, 0.01; medium size, 0.06; large size, 0.14). NS, not significant; RA, rheumatoid arthritis; 5mC, DNA methylation; 5hmC, DNA hydroxymethylation.

is involved in the activation of neutrophils and macrophages, which play a crucial role in modulating inflammation, as well as in the survival and proliferation of synovial fibroblasts, leading to pro-inflammatory cytokine production, contributing to synovial hyperplasia. Furthermore, this cytokine stimulates the production of TNF-α and IL-17, exacerbating joint inflammation and osteoclastogenesis, which is associated with the progression of joint destruction (10,29). Furthermore, IL-15 plays a critical role in the early stages of RA development (11), and has been identified as a potential therapeutic target (30). In patients with undifferentiated arthritis (UA), IL-15 levels were more pronounced in those who later developed RA compared to those with RF or anti-CCP antibodies. Consequently, IL-15 has been proposed as a serum marker for RA, demonstrating greater sensitivity and specificity than the aforementioned autoantibodies (31). Furthermore, the role of IL-29 in the development of RA has recently been discovered. IL-29 contributes to neutrophil chemotaxis, triggering inflammatory responses and tissue damage, inhibiting the differentiation of T-follicular helper cells and stimulating fibroblast-like synoviocytes, which leads to synovial hyperplasia and contributes to joint bone destruction; it also induces the production of pro-inflammatory cytokines involved in the pathogenesis of RA, such as IL-6, IL-8 and IL-10. This cascade promotes inflammation, exacerbating joint inflammation and damage. Additionally, IL-29 stimulates the activation and differentiation of B-cells, which are responsible for producing RF and anti-CCP antibodies; these autoantibodies play a crucial role in the development and progression of RA by triggering inflammation, the formation of the immune complex and tissue damage (32,33). The use of IL-29 biologics in patients with RA blocks the production of T-follicular helper cells, which may help suppress disease progression and provide novel targets for clinical treatment (14,34). Overall, the findings of the present study suggest that the levels of IL-15 and IL-29 in patients with RA could serve as prognostic risk factors and may be utilized in the diagnosis of the disease.

The results of the present study also revealed a decreased level of 5mC in patients with RA, indicating DNA hypomethylation in PBMCs, which aligns with findings from previous research (35). In addition, there was no significant effect of age or sex on 5mC levels. The dysregulation of DNA methylation affects gene expression in immune cells by coordinating the control of immune cell differentiation and function. This dysregulation affects the activation of immune responses and inflammatory pathways, thereby contributing to the pathogenesis and development of RA (36,37). There are several potential explanations for the decrease in DNA methylation, leading to DNA hypomethylation in patients with RA. These include the action of pro-inflammatory cytokines, such as TNF-α, which can stimulate alterations in DNA methylation. Additionally, the inflammatory environment plays a critical role in influencing DNA methylation. Elevated levels of oxidative stress and environmental factors, such as smoking, can also induce changes in DNA methylation (38,39).

DNA hypomethylation has been observed in the PBMCs of patients with RA (40). Differential methylation patterns have also been reported in RA synovial fibroblasts, which correspond to the aggressive phenotype acquired by these fibroblasts and contribute to the development of RA. Abnormal gene expression resulting from DNA hypomethylation has been associated with increased levels of various genes, including growth factors and receptors, extracellular matrix proteins, adhesion molecules and matrix-degrading enzymes (41,42). By contrast, the present study found that levels of 5hmC were increased in patients with RA compared with the control group.

These findings align with those of previous research (43), indicating that 5hmC levels are higher in patients with both RA and osteoarthritis. It has been suggested that the TET3 enzyme functions as an epigenetic gatekeeper at the point of no return in the progression and chronicity of RA. It has been established that patients with RA exhibit distinct methylation patterns in their genomes compared to healthy individuals, as well as different DNA methylation profiles between treatment responders and non-responders (43). These findings suggest the potential for using DNA methylation as a predictive biomarker (44). The analysis of CpG sites within the promoters of genes in patients with RA indicates that changes in DNA methylation occur at a very early stage of the disease, with numerous genes displaying significant hypermethylation at their promoter sites. Thus, the identified epigenetic modifications in genes may serve as valuable prognostic biomarkers for the progression of RA (45). The findings of the present study also indicated no association between age and sex as regards DNA methylation and hydroxymethylation in patients with RA. However, previous studies (46,47) have revealed a positive association between age and the decline in global DNA methylation (5mC). One possible explanation is the age-related changes in the expression of enzymes involved in DNA methylation and demethylation. The findings of the present study have notable clinical implications. The elevated levels of IL-15 and IL-29, and the alteration of DNA methylation levels in the patient with RA indicates that these factors contribute to the pathogenesis of RA. Furthermore, this indicates that DNA methylation may serve as early diagnostic tool for the disease, and may also function as a target in novel treatment strategies. Increased DNA hydroxymethylation is associated with a decrease in DNA methylation levels (hypomethylation) in patients. This connection reflects a dynamic epigenetic backdrop in RA.

The present study is an extension of previous research in the field. examining the levels of IL-15, IL-29 and global DNA methylation modifications on the pathogenesis of RA. While previous studies (11,35,48) have examined the roles of cytokines and epigenetic changes separately, the present study provides novel insight by combining these cytokine levels with epigenetic changes. This integrated analysis provides a more comprehensive understanding of the interplay of these biomarkers in RA pathogenesis and may aid in the development of novel therapeutic strategies targeting both inflammatory and epigenetic pathways.

However, future research with a larger sample size could enable a more robust analysis. Further studies are required in the future to compare the results obtained in RA with those of other autoimmune diseases. Moreover, longitudinal follow-up studies that evaluate the association between epigenetic and immunological variations and disease progression, as well as assess changes in these markers with treatment, could provide valuable insight into the potential applications of these findings.

In conclusion, the present study indicated statistically significant differences in the levels of 5mC and 5hmC between patients with RA and healthy controls. However, further research is required to extensively explore the association between DNA methylation and demethylation patterns in genes associated with RA. Additionally, focusing on IL-15 levels in seronegative patients and targeting IL-29 in new therapies may provide insight into novel aspects of the pathogenesis of the disease and may lead to the identification of novel therapeutic targets.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Both authors (NAH and RMKAJ) contributed to the conception and design of the study, as well as in the preparation of materials, including the ELISA kits. Sample and data collection, analysis, and manuscript writing were carried out by NAH. RMKAJ was involved in the reviewing and editing of the manuscript, and supervised the study. Both authors have read and approved the final manuscript. Additionally, NAH and RMKAJ confirm the authenticity of all the raw data

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Department of Biology, College of Science, University of Baghdad (Baghdad, Iraq), under reference no. CSEC/0923/0105, on September 25, 2023. Written informed consent was obtained from all patients for their participation in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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