Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder, characterized by symmetrical polyarthritis effecting joints. RA main symptoms are persistent inflammation, joints swelling, and pain and may progress into gradual joint destruction, including both bone erosion and deformity. Although the etiology of the disease is still not clear. Risk factors are widely accepted, as they play a crucial role in disease development, including individual's genetic background, epigenetic markers, and environmental agents. This study aimed to investigate the potential roles of global DNA methylation changes (5mC and 5hmC) alongside interleukins-24 (IL-24) and interleukin-38 (IL-38) as biomarkers associated with the disease progression. Accordingly, Overall, 60 RA patients (15 males and 45 females) and 40 healthy controls (10 males and 30 females), with an age range of 30-70 years, were enrolled in this study. Blood samples were collected from all participants to assess the levels of the Complete Blood Count (CBC), Erythrocyte Sedimentation Rate (ESR), and global DNA methylation markers, specifically 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC).while the serum samples were utilized to assess Rheumatoid Factor (RF), C-reactive protein (CRP), anti-cyclic citrullinated peptides (anti-CCP), and interleukins 24, 38. The results revealed a significant increase in ESR levels of RA patients compared to controls. Serological markers, including anti-CCP and CRP, exhibited elevated levels in RA patients, and RF was detected in 73.33% of the cases. The analysis of global DNA methylation demonstrates a significant decrease in 5mC levels (P=0.0003), correlated with a corresponding increase in 5hmC levels. The patients with RA exhibited higher levels of IL-24 (75.056 ± 1.5) and IL-38 (19.8 ± 0.4) compared with the control group (33.44 ± 1.45 and 13.25 ± 0.46, respectively). In addition, no significant difference in the cytokines and global DNA methylation levels was observed across different age groups or between sexes. The findings of the study underscore the role of epigenetic changes and inflammatory cytokines in rheumatoid arthritis development. suggesting their potential utility as biomarkers for disease activity and progression.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune progressing disease characterized by symmetrical symptoms. It mainly affects small joints before progressing to larger ones [1]. The common symptoms of RA include joint pain, swelling, stiffness, and reduced mobility. Furthermore, diverse organs and systems throughout the body can also be affected by the disease, leading to systemic

manifestations such as fatigue, fever, and unintended weight loss[2] Rheumatoid arthritis effects an estimated 0.5-1% of the adult population worldwide, and its prevalence is 2–3 times higher in females than in males [3]. The pathogenesis of RA remains unknown , although evidence suggests that development of disease result from intricate interactions involving genetic predisposition, epigenetic regulation, and environmental factors [4]. In RA, the immune system mistakenly targets the joints, leading to chronic inflammation, synovial hyperplasia, and subsequent destruction of joints, cartilage, and bone [5]. The treatment of RA involves corticosteroids and nonsteroidal anti-inflammatory medications (NSAIDs), which aid in reducing inflammation and discomfort. Disease-modifying anti-rheumatic drugs (DMARDs) are commonly used to delay joint degradation and associated symptom mitigation. Recent treatment options include methotrexate, hydrochloroquine, interleukin inhibitors, tumor necrosis factor (TNF) inhibitors, physical and occupational therapy, nutritional supplements, and complementary therapies [6].

Epigenetics refers to inheritable chemical changes that regulate gene expression and DNA transcription without directly altering the DNA sequence. Key epigenetic mechanisms include DNA methylation and histone modifications [7]. DNA methylation (5-methylcytosine 5mC) involves the addition of a methyl group to the 5-carbon position of cytosine on CpG sites, which are genomic regions with a rich C and G base pair, which regulates gene expression. Methylation of these sites near gene promoters leads to gene silencing [8]. DNA hydroxymethylation (5-hydroxymethylcytosine, 5hmC) is a stable DNA methylation derivative generated via the oxidation of 5-methylcytosine by the ten-eleven translocation (TET) family of enzymes [9]. 5-Hydroxymethylcytosine plays a critical role as an intermediary in the DNA demethylation process, by which 5mC returns to normal cytosine [10].

Cytokines are proteins responsible for intercellular signaling that play a crucial role in nearly every aspect of human immunology, ranging from immune responses against pathogen to inflammation that causes tissue damage [11]. complex network of diverse cytokines involves in the disease pathogenesis inducing proliferation of synovial cells and cause destruction of bone and cartilage [12]. Interleukin-24 is a cytokine that belongs to IL-10 family. IL-24 is produced by various immune cells involves T and B cells, natural killer cell, lymphoid cells, dendritic cells, monocytes, and macrophages. Additionally, it is produced by non-immune cells such as melanocytes, dermal keratinocytes, and epithelial stem cells at wound edges. it play

several critical roles ranging from antitumor activity , wound healing , host defense, immune regulation, and inflammation.

[13] Later studies revealed that elevated levels of IL-24 in the plasma of patients with RA, highlighting the role of this cytokine in the etiology of RA, this cytokine appears to exerts a suppressive influence on immune cells such as T cells, B cells , NK cells, and macrophages . furthermore IL-24 is a multifunctional cytokine with both pathogenic and tolerogenic properties, exhibiting complex regulatory functions in immune cells and related diseases. This understanding may pave the way for novel therapeutic strategies for this disease[14], [15]. Interleukin-38 is a recently identified cytokine within the IL-1 family. This cytokine is produced by epithelial cells, monocytes, macrophages, and immune cells mainly in immune related tissues and less prevalent in immune-inactive tissues. IL-38 plays a crucial role in a variety of diseases [16], [17]. previous studies have demonstrated that IL-38 exhibits both pro-inflammatory and anti-inflammatory properties which are determined by factors like the concentration of protein, existing form, posttranslational modification, or the context of cellular environmental. Recent studies has indicated that IL-38 is involved in the development of RA . In RA patients IL-38 levels was significantly increased in the serum, synovial tissue, and synovial fluid. The abnormal expression of IL-38 suggests it has role in immune response modulating. IL-38 is associated with inflammatory cytokines production like IL-1β, IL-6, or IL-1Ra, and it inhibits the secretion of IL-6 and IL-8 . Additionally, by binding to receptors such as IL-36R and IL-1RAPL1, IL-38 exhibit an anti-inflammatory function by blocking pro-inflammatory cytokines. Therefore IL-38 may be regarded as promising therapeutic target for RA management [18], [19], [20]. The present study aimed to illustrate the roles of the cytokines IL-24 and IL-38, alongside global DNA methylation alterations, in the progression of RA.

Method and material

Study Population and Ethical Approval

In total 100 participant including 60 RA patients (45 females and 15 male; aged 30 to 70 years) and 40 healthy controls (10 males and 30 females; aged 30 to 66 years) were enrolled in present study at Department of Rheumatology in Private Nursing Home Hospital in Medical City, Baghdad, Iraq, from October, 2023 to January, 2024. All patients diagnosed according to the revised diagnostic criteria that were established by the 2010 American College of Rheumatology/European League Against Rheumatology (ACR/EULAR) classification criteria. The RA patients were

newly diagnosed. Individuals with an extended disease duration, those who were undergoing prolonged treatment, patients with other autoimmune diseases or with active infections, and pregnant or breastfeeding females were excluded from the study. The 40 healthy control samples were from people with no autoimmune history or chronic diseases and who appeared to be in good health and were recruited for the study. The present study was approved by (ethical approval). 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

Five millimeters of venous blood were drawn from the radial vein of each participant using disposable syringes. Subsequently, three milliliters were gradually loaded into serum tubes (containing separating gel), allowed to clot at room temperature for 10 to 15 minutes, and subsequently centrifuged at 3000 rpm for 10 to 15 minutes at room temperature. Then the serum was aliquoted into Eppendorf tubes in equal volumes and stored at -20°C for later use in serological tests. The remaining volume of blood was transferred to EDTA tubes (containing ethylenediaminetetraacetic acid) for complete blood count (CBC), erythrocyte sedimentation rate (ESR), and the assessment of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC).

Measurement of serological markers

C-reactive protein (CRP) and anti-cyclic citrullinated peptides (anti-CCP) levels were analyzed utilizing enzyme-linked immunosorbent assay (ELISA) kits, specifically the CRP kit (cat. no. MBS564038, MyBioSource) and the human anti-CCP antibody kit (cat. no. MBS7235871, MyBioSource) All materials referenced herein were components of these kits. ELISA is a widely used diagnostic technique in biomedical research for the detection and quantification of selected antigens or antibodies in biological samples. In brief, the ELISA method is based on the basic immunological concept of antigen-antibody specific binding, which enables the detection of minute antigen quantities, including hormones, antibodies, proteins, and peptides in fluid samples. The analysis was performed following the manufacturer's instructions. All reagents and samples were brought to room temperature before use. Standards and samples (100 µl each) were introduced to their respective wells and incubated for 30 min at 37°C. Subsequently, the plate was washed with 300 µl of wash buffer. A 100 µl volume of HRP conjugate was then added to each well, followed by a 30-minute incubation at 37°C, after which another wash step was performed. This step was followed by the addition of 50 µl of

Substrates A and B then incubated for 10 min at 37°C. Finally, 50 µl of stop solution was added to terminate the reaction, and absorbance was measured at 450 nm using an ELISA reader (BioTek Instruments, Inc.).

Measurement of RF levels

Rheumatoid factor detection was performed using a latex agglutination test kit (cat. no. SL003, Bioresearch). According to the manufacturer's protocol, 40 µl of the test sample was placed on a slide alongside negative control (usually contains animal serum), and lacking RF and positive control (contains human serum with an RF concentration >30 IU/ml). Then, 40 µl of the supplied RF latex reagent was added to each. Each reaction circle was mixed and uniformly spread using a stirrer. The slide was subsequently placed on a mechanical rotator and agitated at 80-100 revolutions per minute for a duration of 2 minutes. The results were interpreted by immediately inspecting for visible agglutination.

DNA extraction and global DNA methylation evaluation.

The extraction of Genomic DNA was performed using the gSYNC™ DNA extraction kit (cat. no. GS100, Geneaid Biotech Ltd.). NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc.) were used to measure the purity and concentration of the genomic DNA by assessing the A260/A280 absorbance ratio. A ratio ranging from 1.8 to 2.0 was considered indicative of high-quality genomic DNA. The emulation of global methylation was conducted using MethylFlash™ Global DNA Methylation (5mC) and MethylFlash™ Hydroxymethylated DNA (5hmC) Quantification kits (cat. nos. P-1034 and P-1036, EpigenTek). A 100 ng of extracted genomic DNA from each blood sample was diluted in the supplied binding solution included with the well assay strip kit. in brief, the DNA methylation fraction that bound to the monoclonal antibodies on the well-assay strip was captured for identification in the subsequent assay stages. This process involved the addition of wash solution, detection antibody, enhancer solution, developer, and stop reaction solution, each step followed by incubation and a washing process. Ultimately, the quantification of 5mC and 5hmC was determined as proportional to the optical density (OD) intensity measured at 450 nm using a microplate reader (BioTek Instruments, Inc.). The kit is highly sensitive, with detection limits of 0.02 ng for 5mC and 0.04 ng for 5hmC DNA. Furthermore, it demonstrates high specificity, as the antibody exclusively detects 5mC and 5hmC.

Measurement of Serum IL-24 and IL-38 Levels

Interleukin-24 (IL-24) and Interleukin-38 (IL-1θ) quantification were performed utilizing enzyme-linked immunosorbent assay (ELISA) kits (Human IL-24 ELISA Kit, Cat. No. ELK2454; Human IL-1θ ELISA Kit, Cat. No. ELK7424; ELK Biotechnology, China) according to the manufacturer's instructions. In brief, all reagents, standards, and samples were brought to room temperature prior to use. The biotinylated detection antibody and streptavidin-horseradish peroxidase (HRP) conjugate were diluted 1:100 with their respective diluents to create working solutions. A total of 100 µL of each standard and serum sample were added to the appropriate wells of the antibody pre-coated microplate. Then the plate was covered and incubated for 80 minutes at 37°C. After incubation, the liquid was disposed, and each well washed three times with 200 µL of 1× wash buffer. subsequent, 100 µL of the biotinylated antibody working solution was added to each well, followed by the plate incubation for 50 minutes at 37°C and another three washes. 100 µL of the streptavidin-HRP working solution was added, then the plate was incubated for a further 50 minutes at 37°C and washed five times. subsequently, 90 µL of tetramethylbenzidine (TMB) substrate solution was added to each well, and the plate was incubated for 20 minutes at 37°C in the dark. Finally, 50 µL of stop solution was added, and the absorbance was read at 450 nm using an ELISA reader (BioTek Instruments, Inc.).

Results

As presented in Table 1, the study comprised 45 females (75%) and 15 males (25%) diagnosed with RA. While the control group included 30 females (75%) and 10 males (25%). The mean age of RA patients was 48.72±1.32 years, compared to 45.60±1.63 years in the control group. As shown in table 1 the BMI for RA patients was 28.52±0.46, while it was 27.81±0.44 in the control group. According to BMI classification both groups considered as overweight. No statisticaly significant results in age and sex distribution were observed between study group, as indicated by the corresponding P-values. Furthermore, erythrocyte sedimentation rate (ESR) was markedly elevated in RA patients with value of 39.67±1.91 compared to 8.69±0.59 mm/h in the control group. The complete blood count (CBC) showed that the mean red blood cell (RBC) count was 4.49±0.07x10^6/µl in RA patients, and 4.97±0.10x10^6/µl in the control group, indicating a significant reduction in RA

patients. although the white blood cell (WBC) count did not show a significant difference between the study groups, the differential WBC analysis revealed increased levels in RA patients of neutrophils (57.96%), lymphocytes (32.02%), monocytes (8.17%), and eosinophils (5.37%) compared to 51.06%, 26.09%, 6.13%, and 3.21%, respectively in the control group. Statistical analysis indicated a significant difference among study group with P-value of 0.0003 for neutrophil, 0.0022 for lymphocytes, 0.0001 for monocytes, and 0.0001 for eosinophils (Table I). In addition, mean corpuscular volume results exhibit a significant increase in RA patients compared to the control group (Table I). hematocrit (HCT) levels were significantly increased in the control group compared to patients with RA. However, significant differences were observed in mean corpuscular hemoglobin concentration (MCV**)**, mean corpuscular hemoglobin (MCH), hemoglobin levels (Hb), and platelet (PLT) counts between study group.

Table 1 Comparison between RA patients and control group according to age, BMI, ESR and CBC parameters.

	Groups (Mean± SE)		
Parameters	**Control**	**RA**	**P-value**
Sex	**Females (n=45) (75%)**	**Females (n=30) (75%)**	**0.0087a**
	Males (n=15) (25%)	**Males (n=10) (25%)**	**0.0087a**
Age (years)	**48.72±1.32**	**45.60±1.63**	**0.141 NS**
BMI (kg/m2)	**28.52±0.46**	**27.81±0.44**	**0.294 NS**
ESR mm/h	**8.69 ± 0.6**	**39.67 ± 1.9**	**<0.0001****
(RBC) x106/µl)	**4.97 ± 0.1**	**4.5 ± 0.072**	**<0.0001****
WBC (103/µl)	**6.35 ± 0.21**	**6.97 ± 0.27**	**0.102**
Lymphocytes%	**26.095**	**32.024**	**0.002****
Neutrophils	**51.058**	**57.962**	**<0.0001****
Monocyte (%)	**6.1300**	**8.1734**	**<0.0001****
Eosinophil (%)	**3.215**	**5.376**	**<0.0001****
Hb (g/dl)	**13.573**	**13.057**	**0.117**
HCT (%)	**41.79 ± 0.5**	**37.34 ± 1.43**	**0.014***
MCV (fl)	**83.98 ± 0.6**	**86.195 ± 0.8**	**0.042***
MCH pg	**28.953 ± 0.39**	**29.031 ± 0.53**	**0.913 NS**
MCHC (g/dl)	**33.043 ± 0.15**	**33.5 ± 0.4**	**0.333 NS**
Platelet (10x/µl)	**261 ± 9.74**	**265.21 ± 9.83**	**0.770**

***Data are presented as mean ± standard error (SE). *P<0.05; **P<0.01; NS: Non-Significant.**

AS shown in Table 2 the anti-cyclic citrullinated peptide (anti-CCP) levels were significantly higher (P-value=0.0001) in RA patients than those in the control group.

The C-reactive protein (CRP) levels also exhibited a significant difference (P-value=0.0003) between the RA patients and the controls. The rheumatoid factor (RF) was detected in 73.33% of RA patients, showing significant difference (p-value=0.0001) compared to the control group.

Table 2: Comparison of CRP, anti-CCP and RF between RA patients and controls.

	Groups (Mean± SE)			
Parameters	**Control**	**RA**	***P-value***	***Cohen's d***
ANTI-CCP	0.31 ± 0.12	0.63 ± 0.03	<0.0001**	1.962
CRP	4.83 ± 0.39	35.58 ± 24.11	<0.0001**	0.214
RF %	0%	73.33%	<0.0001**	-

Data are presented as mean ± standard error (SE). *P<0.05; **P<0.01; NS: Non-Significant.

Cohen's d t-test effect size (Small size=0.2, medium size= 0.5, large size= 0.8)

Global DNA methylation (5mC and 5mhC) results as illustrated in figure 2, were previously published in our earlier study [21] and are not detailed here.

Moreover, IL-24 and IL-38 levels were significantly elevated in patient with RA compared to control group, indicating a significant difference among the study groups with a P-value of 0.001 (Table 3 and Fig. 1). While results presented in table 4 demonstrated no significant difference in interleukins levels between RA patients across various age groups or sex.

Table 3: Comparison of IL-24 and IL-38 between RA patients and controls.

	Mean ± SE	
Group	**IL-24 (ng/l)**	**IL-38 (ng/l)**
Patients with RA	75.056 ± 1.5	19.8 ± 0.4
Control	33.44 ± 1.45	13.25 ± 0.46
P-value	<0.0001**	<0.0001**
Cohen's d	3.958	2.256

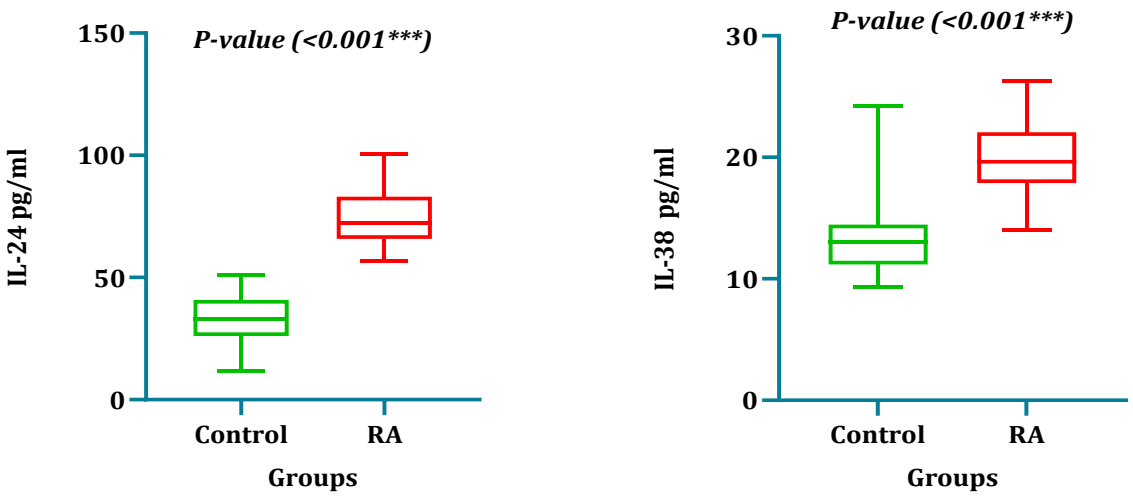

Figure 1. The serum levels of IL-24 and IL-38 in RA patients and control group.

Table 4. Distribution of IL-24 and IL-38 in RA patients according to age groups and sex.

	Mean ± SE	
Age categories (Years)	**IL-24 pg/ml**	**IL-38 pg/mL**
30-40 (14)	73.45 ± 3.13	20.58 ± 0.89
41-50 (21)	74.52 ± 2.34	19.1 ± 0.68
51-60 (17)	78.76 ± 2.42	19.85 ± 0.62
61-70 (8)	71.73 ± 5.4	19.95 ± 1.09
P-value	0.447 NS	0.556NS
Gender		
Male (15)	75.2 ± 2.43	20.9 ± 0.86
Female (45)	75.01 ± 1.8	19.4 ± 0.42
P-value	0.096 NS	0.958 NS

Data are presented as mean ± standard error (SE). *P<0.05; **P<0.01; NS: Non-Significant. . Independent t-test was used to compare sex, and one-way ANOVA was used to compare the different age groups. Tukey's Honest Significant Difference (HSD) post hoc test was performed for pairwise comparisons.

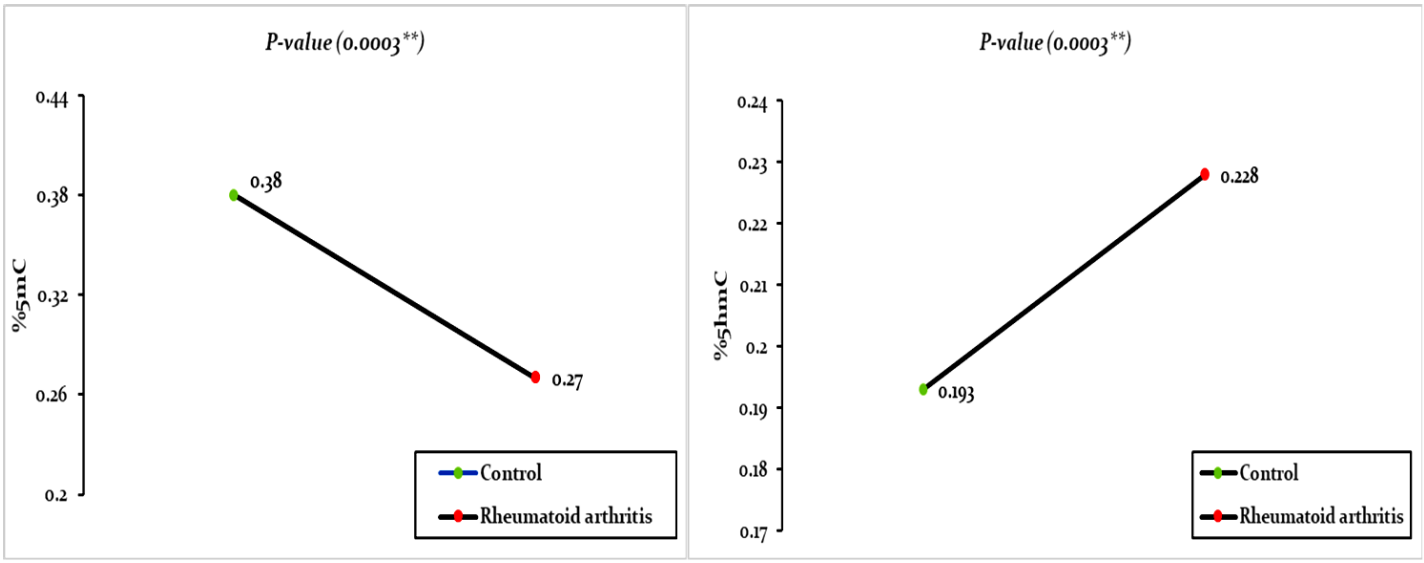

Figure 2. the levels of of 5mC and 5hmC in the patients with RA and the control groups.

Discussion

the study's demographical and clinical results align with the well-established profile of RA. results of the serological markers in present study align with those observed in previous research [22], [23], [24]. Increased ESR levels reflect changes in plasma protein composition during inflammation, which promote red blood cell aggregation leading to accelerate sedimentation. Elevated ESR positively correlate with the degree of synovial tissue inflammation, supporting their utility as a clinical marker for assessing disease activity. CRP levels were also significantly increased in RA patients [25]. CRP is an acute-phase protein produced by the liver in response to synovial membrane inflammation (synovitis) in RA. CRP elevated levels in RA patients associated with severe inflammation and with disease activity [26]. Together ESR and CRP represent a complementary inflammatory markers in RA, reflecting both chronic and acute phases of synovial inflammation and offers valuable insight into treatment response and disease monitoring. Anti-CCP are antibodies produced during the citrullination process in RA, this process triggered by citrullinated proteins synthesis. Higher Anti-CCP levels are indicative of a more severe disease, erosive progression, and joint damage [27]. Anti-CCP is considered a highly specific and valuable biomarker for diagnosis that can be detected in the early stages of RA, generally before clinical symptom onset[28]. Rheumatoid factor (RF) is an autoantibody with ability to activate inflammatory cells via formation of immune complexes, which deposit in joint tissues, and stimulate pro-inflammatory cytokine release, consequently contributing in joint destruction [29]. RF was

detected in the majority of patients with RA in this study. despite RF not being specific to RA, it still widely used as diagnostic marker in clinical practice [29]. Anti-CCP represents a highly specific marker for early diagnosis of the disease. while RF provides a prognostic tool indicating the disease severity. The utilization of both markers improve diagnostic accuracy and aids in treatment decision - making in patients with RA [30].

The analysis of epigenetic markers revealed a remarkable decrease in 5-methylcytosine (5-mC) and a significant corresponding increase in 5-hydroxymethylcytosine (5-hmC) in the peripheral blood mononuclear cells (PBMCs) of RA patients compared to controls. These findings are in line with previous studies highlighting epigenetic dysregulation in autoimmune diseases, including RA [21], [31]. DNA methylation is responsible for gene expression regulation in immune cells, affecting their development, differentiation, and function. Therefore, it plays a crucial role in the maintenance of immune homeostasis and ensures the immune response is appropriate. DNA hypomethylation observed in the disease leading to alterations in gene expression of immune cells, which result in loss of immune tolerance and chronic inflammation[32], [33].Various mechanisms may participate in this hypomethylation, encompassing pro-inflammatory cytokines, such as TNF-α and IL-1. These cytokines can decrease the expression of DNA methyltransferases (DNMTs), which are the enzymes maintaining the DNA methylation process. furthermore, environmental factors, specifically smoking and oxidative stress, play an important role in stimulating DNA methylation modifications [34], [35], [36]. Conversely the 5-hmC increased levels in patients with RA suggest persistent epigenetic remodeling as triggered by inflammatory stimuli [37]. Kawabe et al. (2022) [38]study indicated that pro-inflammatory cytokines, particularly TNFα, upregulated the TET3 enzyme and accordingly 5-hmC levels in cultured fibroblast-like synoviocytes (FLS) derived from RA patients. This suggests that inflammatory cytokines during RA development stimulate the expression of TET3, resulting in the hydroxylation of methylated DNA that reveals active epigenetic changes.

Study results are consistent with previous findings [39], [40], [41], [42] that demonstrate increased IL-24 and IL-38 levels in patients with RA. These results reflect the sophisticated pro- and anti-inflammatory cytokine interaction in the disease progression. This upregulation observed in both interleukins suggests their pivotal utility as biomarkers indicating disease activity and as promising targets in immunological therapies. IL-24 a member of the IL-10 cytokine family, has both

pro- and anti-inflammatory effects, depending on the cellular context and nature of the disease. [43]. In this study, elevated IL-24 levels may indeed show an anti-inflammatory effect in response to persistent synovial inflammation, thus reflecting its crucial role in the regulation of immune responses. as they contribute to processes that play an important in inflammation and autoimmunity management, including modulating activation of macrophage, regulate T-cell proliferation, and impacts secretion of cytokines [44]. Additionally, its ability to stimulate inflammatory cascades in particular settings highlights its complex role in RA etiology. IL-38 a relatively new member in the IL-1 family, has growing importance for its anti-inflammatory properties specifically through the antagonizing of IL-36 receptor signaling, which lead to the inhibition of pro-inflammatory cytokine production, such as IL-6 and IL-8 [45]. The elevated IL-38 levels indicated in the present study may reflect an endogenous mechanism involved in reducing joint damage and chronic synovial inflammation. It still to be specifies whether these elevation effects are protective or an insufficient against persistent immune activation.

Moreover, no significant results were observed in either cytokine or methylation marker levels across different age groups or between sexes, demonstrating that both biomarkers could have broad applicability in the monitoring of the disease across different patient populations. This enhances their potential utility in early diagnosis, prognosis, and as targets in therapeutic strategies.

The present study has limitations that should be acknowledged. In the first place, its cross-sectional design restricts the ability to establish causal correlation between progression of disease, cytokine levels, and epigenetic changes. In the second place, the relatively small sample size may limit the generalizability of the findings to wider RA populations. Despite these limitations, they do not distract from the results, which offer valuable insights into the immunological and epigenetic landscape of RA.

Future studies should focus on longitudinal studies to indicate the changes in IL-24 and IL-38 levels, besides DNA methylation and hydroxymethylation alterations over time. In addition, mechanistic studies are essential to explain the interaction between these cytokines and epigenetic regulation in immune cells. Such studies may help in specifying whether targeted modulation of IL-24 and IL-38 activity influences the disease development, severity, or treatment response. Finally, this line of studies can aid in the identification of new biomarkers and therapeutic strategies for RA.

In conclusion, the combined elevation of IL-24 and IL-38, alongside global epigenetic modifications, provides new insights into the immunopathogenesis of RA. These biomarkers may serve as important biomarkers and potential therapeutic targets in RA, paving the way for innovative interventions aimed at modulating immune responses and restoring epigenetic balance in RA patients.

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