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Plasma levels of microRNA-24-3p as a potential diagnostic biomarker for rheumatoid arthritis in some Egyptian patients

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Abstract

Background Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that presents as a symmetrical polyarthritis characterized by joint pain, swelling, and stiffness. This study aimed to evaluate the expression of microRNA-24-3p (miRNA-24-3p), in the plasma of RA patients and compare it with that of healthy controls with the goal of establishing a specific expression profile for early diagnosis and improved patient care.

Methodology This case-control study was carried out on 100 RA patients attending Rehabilitation and Physical Medicine department and 50 age and sex-matched control individuals. Disease severity score (DAS-28) was used to assess the disease's level. Real-time polymerase chain reaction (qPCR) was used for detection of plasma level of miRNA-24-3p and other lab tests including blood picture, c-reactive protein (CRP), erythrocytes sedimentation rate (ESR) and anti-cyclic citrullinated peptide (anti-CCP) were also performed.

Results The miRNA-24-3p expression was statistically significant higher among RA cases than controls ($p < 0.001$) and was positively associated with higher ESR, white blood cells (WBCs) and along with the number of tendered/swallow joints ($p < 0.05$). Higher CRP, ESR, Anti-CCP, and miRNA-24-3p expression were found to be significant predictors of DAS28 scores in univariable analysis.
Higher CRP and miRNA-24-3p expression exhibit substantial impact in multivariate analysis, indicating a strong statistical significance in their association with higher DAS28 grades.

Conclusion The elevated expression of miR-24-3p suggests its probable contribution to both the onset of RA and the activity measured by DAS28 score. Consequently, assessing its expression levels may serve as a valuable tool for the diagnosis and management of RA.

Keywords Rheumatoid Arthritis, miRNA 24-3p, Gene expression, Disease activity

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Introduction

Rheumatoid arthritis is a multifactorial inflammatory polyarticular autoimmune disease that progressively progresses to arthralgia, bone or cartilage disintegration, and disability. Any synovial joint in the body may be impacted, though it usually begins in the tiny joints of the hands and feet. It may have an effect on all facets of daily life.

The prevalence of RA is approximately 240 per 100,000 people, and high levels of inflammation are linked to fatigue and a reduced ability to participate in social, recreational, and occupational responsibilities [1]. In addition, the prevalence of RA is rising as the world's population ages at an accelerated rate. There is still much to learn about the etiology and pathophysiology of RA, which includes genetic predisposition and environmental triggers [2].

The symptoms of the patient, the clinical examination, joint evaluation by ultrasound sonography, and lab tests like elevated levels of CRP and ESR and the identification of RA-specific autoantibodies like rheumatoid factor and anti-CCP Ab are typically used to diagnose RA [3].

MiRNAs are small single stranded, endogenous non-coding RNAs (ncRNAs) of ~ 22 nucleotides in size that are crucial for gene regulation [4, 5]. MiRNAs act at the post transcription level by binding to the 3' UTR of target mRNAs, preventing their translation into proteins, or enhancing degradation [6].

Numerous investigations have demonstrated that ncRNAs control nearly every facet of inflammatory reactions as well as the etiology of immune-related conditions like RA. Modification of miRNA expression, for instance, has been linked to mechanisms that maintain the auto-immune vicious cycle, such as increased release of pro-inflammatory cytokines and activated inflammatory signaling pathways [7].

Circulating miRNAs have emerged as promising biomarkers in RA, reflecting immune dysregulation and inflammatory activity. Among these, miRNA-24/miR-24-3p has been repeatedly reported as altered in RA. Murata et al. identified plasma miR-24 among RA-associated circulating miRNAs, supporting its utility as a potential blood-based marker (Murata et al., 2013). In addition, subsequent work reported elevated plasma miR-24-3p in RA and suggested diagnostic relevance in specific RA subsets [8].

More recent literature continues to support the broader relevance of circulating miRNA signatures (including miR-24-3p) to RA-related immune pathways and their potential clinical utility [9].

However, available evidence remains heterogeneous across cohorts, sample types, and laboratory workflows, and data from Middle Eastern/North African populations are limited. Therefore, the current study intended to evaluate the association of plasma miR-24-3p expression level with the disease and disease activity among Egyptian RA patients.

Subjects and methods

Subjects

This case-control study was conducted between September 2023 to April 2024 and included 100 patients with recently diagnosed RA within the last 6 months prior to enrollment and 50 subjects of age and gender matched healthy controls. The patients had previously been diagnosed according to the American College of Rheumatology/ European League against Rheumatism (ACR/EULAR) 2010 criteria [10], attending at Outpatient and Inpatient clinics, Department of Rehabilitation and Physical Medicine, Faculty of Medicine, Benha University Hospitals, Benha, Egypt. Patients with any autoimmune disease other than RA at the time of study were excluded. Each patient underwent a thorough clinical assessment and documentation of their medical history. Assessment was conducted for ESR, CRP, RF and ACPA.

Global health was assessed using a 10-cm Visual Analogue Scale (VAS). Patients were asked to mark on a 10-cm horizontal line representing their overall health status (0 = best possible health, 10 = worst possible health). The global health score was calculated as the distance (in cm) from the left end to the patient's mark (range: 0–10). The DAS 28 was recorded and classified into four categories: remission (score ≤ 2.6), low (≥ 2.6 - ≤ 3.2), moderate (> 3.2 - ≤ 5.1), and high (> 5.1) activities [11].

Sampling and methods

Six milliliters of venous blood were withdrawn and separated into 3 tubes; EDTA tube for complete blood picture and real-time PCR to detection of plasma level of MiRNA-24-3p, plain tube for separation of serum RF, anti-CCP, and CRP and sodium citrate tube for ESR.

MiRNA extraction and expression by real-time PCR

MiR-24-3p expression was measured using a commercial SYBR Green–based qPCR kit according to the manufacturer's instructions. The reverse transcription and amplification steps, including miRNA-specific priming and cDNA synthesis suitable for short RNA molecules "poly A", were performed as part of the proprietary kit protocol. Data normalization was performed using U6 small nuclear RNA as an internal reference, and relative expression levels were calculated using the $2^{-}\Delta Ct$ method. Each PCR reaction consisted of: 10 µl Master Mix (2x), 1 µl of both forward (5′GCCTACTGAGCTGAAAC – 3′) and reverse primers (5′GAACATGTCTGCGTATCTC – 3′) (200nM), 250ng of DNA template and 20 µl nuclease free water.

Table 1 Demographic, clinical, and lab data of the study population

	RA ($n=100$)	Control ($n=50$)	*P*
Age (years), median (range)	36.5 (20.0–50.0)	32.0 (20.0–50.0)	0.223
Sex			
(female: male)	80:20	34:16	0.105
Family history			
Positive	45	5	< 0.001*
Negative	55	45	
Number of tender joints, median (range)	10 (0–28.0)	-	NA
Number of swollen joints, median (range)	0 (0–11.0)	-	NA
Hemoglobin (g/dL), median (range)	11.4 (7.60–17.20)	13.40 (12.0–15.3)	< 0.001 *
RBCS (10^6/μL), median (range)	4.46 (1.46–7.12)	4.85 (4.24–6.17)	< 0.001 *
WBCs (10^3/μL), median (range)	6.5 (2.90–21.0)	7.10 (5.0–12.0)	0.122
Platelets (10^3/μL), median (range)	260 (78–588)	285 (212.0–473.0)	0.078
CRP (mg/L), median (range)	23 (2.0–150.0)	4 (2.0–5.0)	< 0.001*
ESR (mm/h), median (range)	30.5 (7.0–140.0)	6 (1.0–9.0)	< 0.001 *
RF (IU/L), mean ± SD	90.35 ± 121.3	-	NA
Anti-CCP (IU/L), mean ± SD	71.97 ± 62.30	-	NA
DAS28 score, mean ± SD	4.72 ± 1.67	-	NA
MiRNA- 24-3p expression (relative expression "arbitrary units"), median (range)	2.110 (0.893–2.960)	0.998 (0.893–1.70)	< 0.001*

*significant ($p<0.05$), *RBCs* Red blood cells, *WBCs* White blood cells, *CRP* C-reactive protein, *ESR* Erythrocytes sedimentation rate, *RF* Rheumatoid factor, *anti-CCP* Anti cyclic citrullinated peptide DAS28: disease Activity Score-28

The thermal cycler conditions were: initial denaturation (1 cycle) 95 °C for 10 min, following by 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min.

Ethical consideration

Informed written consent from all patients; it included data about the study design and methods. Official permission from the administrators of ethical committee, Faculty of Medicine, Benha University to conduct this study was gained (approval No.: MS 28-11-2023).

Statistical analysis

Using Statistical program for Social Science (IBM Corporation Released 2017), the data that was acquired was cleaned up, coded, tabulated, and then uploaded to a personal computer.

The media (min-max.) for numerically non-parametric data and non-numerical data broken down by frequency and proportion. The Mann Whitney Test, the Chi-Square test, The Kruskal Wallis test and Fisher's exact test were all used in the process of doing analytical statistics.

The adjustment for multiple comparisons was performed using Dunn's post hoc test with Bonferroni-adjusted p-values. In order to evaluate how strongly two quantitative factors are associated with one another, a Spearman correlation study was carried out. The receiver operating characteristic (ROC) Curve, is used to assess the sensitivity and specificity of quantitative diagnostic tests.

The area under the curve (AUC) suggests that a test has high accuracy if it has a value more than 0.9, that the range 0.7–0.9 indicates moderate accuracy, that the range 0.5–0.7indicates poor accuracy, and that 0.5 implies a chance result. In order to make accurate predictions about risk variables, logistic and ordinal regression analyses were employed. If a p value is less than 0.05 at a confidence interval of 95%, then it is deemed significant.

Table 2 Correlation between miRNA- 24-3p expression and other variables among RA cases

	miRNA-24-3p expression	
	rs	*P*
Age	0.030	0.769
No. of tender joints	0.363	< 0.001 *
No. of swollen joints	0.541	< 0.001*
Hemoglobin	-0.083	0.414
RBCS	-0.005	0.962
WBCS	0.461	< 0.001 *
Platelets	0.328	0.001 *
Urea	-0.060	0.554
Creatinine	-0.057	0.574
CRP	0.495	< 0.001 *
ESR	0.542	< 0.001 *
RF	0.206	0.040 *
Anti-CCP	0.185	0.066
Global health Score	0.516	< 0.001*
DAS28 score	0.894	< 0.001*

*Significant ($p<0.05$), *rs* Spearman coefficient, *RBCs* Red blood cells, *WBCs* White blood cells, *CRP* C-reactive protein, *ESR* Erythrocytes sedimentation rate, *RF* Rheumatoid factor, *anti-CCP* Anti cyclic citrullinated peptide DAS28: disease Activity Score-28

Results

This study showed that the median age of RA cases was 36.5, ranged from 20 to 50 years. Females had a larger proportion of both groups, with 80% in the RA group and 68% in the control group. Higher percentages of patients with RA have a positive family history (45%) than the control group (10%). The majority (75.0%) tested positive for the RF. Similarly, anti-CCP antibodies were detected in 92.0% of patients, Remission was identified in 14%, low activity in 10%, moderate activity in 28%, high activity in 48% (Table 1).

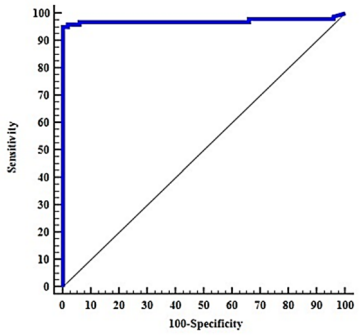

Fig. 1 ROC Curve of miRNA- 24-3p expression to discriminate between RA cases and the controls

Table 2 The miRNA-24-3p gene expression showed significant positive correlations with no. of tender joints, no. of swollen joints, WBCs, platelet count, CRP, ESR, RF, global health score and DAS28 score. Otherwise, no significant correlations were found with other studied parameters.

Table 3 evaluates the association between miRNA-24-3p expression and DAS28 grades among RA cases. The median gene expression levels for different DAS28 grades had a descending manner from high to remission grades, with values of 2.279 for high, 2.007 for moderate, 1.988 for low, and 1.730 for remission. The statistical test showed a $p < 0.001$, indicating a significant difference in gene expression levels across the various DAS28 grades. Pairwise comparisons also revealed significant differences between different grade categories, with $p < 0.001$ for each comparison.

Table 4 examined the validity of miRNA-24-3p expression to distinguish between RA and the controls. The AUC was 0.973 (p<0.001), suggesting a potential discriminatory power of miRNA-24-3p gene expression between RA patients and controls. With a cutoff value >1.7, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were 95.0%, 100.0%, 100.0%, 90.91%, and 96.67%, respectively (Fig. 1). In addition, Table 5 evaluates the validity of miRNA-24-3p expression for discriminating between cases with active and inactive RA. The AUC was 0.999 (p<0.001). At cutoff value >1.972, the sensitivity, specificity, PPV, NPV, and accuracy were 100%, 98.8%, 93.1%, 100%, and 99%, respectively. These results suggest that miRNA-24-3p gene expression can effectively differentiate between active and inactive RA patients (Fig. 2).

Regression analysis revealed that positive family history, higher ESR, CRP, and miRNA-24-3p expression exhibit significant associations with RA susceptibility in uni- and multivariate analyses Table 6. Moreover, Table 7 highlights the influence of different factors on grades of DAS28 scores, showing that higher CRP, ESR, RF, Anti-CCP, and miRNA-24-3p expression were found to be significant predictors of DAS28 scores in univariable analysis.

Table 3 Association between miRNA- 24-3p expression and DAS28 grades among patients with RA

	miRNA- 24-3p expression		Test	p1	p2	Pairwise
	Median	Min. – Max.				
DAS28 grades						
Remission, $n=14$	1.730	0.893–1.972	H= 83.577*	< 0.001*		
Low, $n=10$	1.988	1.962–1.995			< 0.001*	p3 < 0.001* p4 < 0.001* p5 < 0.001*
Moderate, $n=28$	2.007	1.995–2.137			< 0.001*	
High, $n=48$	2.279	2.102–2.960			< 0.001*	

p1: Comparing different grades, p2: Comparing remission vs. each other grades, p3: Comparing low vs. moderate, p4: Comparing low vs. high, p5: Comparing moderate vs. high, *: significant ($p < 0.05$). Overall comparison was performed using the Kruskal–Wallis test. Pairwise comparisons were performed using Dunn's post hoc test with Bonferroni-adjusted p-values

Table 4 Validity of miRNA- 24-3p expression for discrimination between RA cases and the controls

miRNA- 24-3p gene expression								
AUC	95% CI	*p*	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
0.973	0.944-1.0	< 0.001*	> 1.7	95.0	100.0	100.0	90.91	96.67

*Significant ($p < 0.05$), *AUC* Area under the curve

Table 5 Validity of miRNA- 24-3p expression for discrimination between active and inactive groups

miRNA- 24-3p gene expression								
AUC	95% CI	*p*	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
0.999	0. 962-1.0	< 0.001*	> 1.972	100	98.8	93.1	100.0	99.0

*Significant ($p < 0.05$), *AUC* Area under the curve

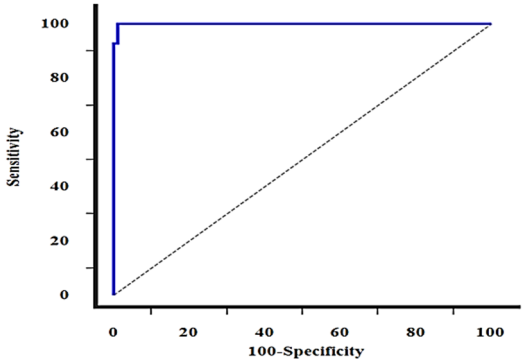

Fig. 2 ROC curve of miRNA- 24-3p expression for discrimination between active and inactive RA cases

Among these factors, higher CRP and miRNA-24-3p expression exhibit substantial impact in multivariate analysis, indicating a strong statistical significance in their association with higher DAS28 grades.

Discussion

Prolonged articular damage and extra-articular symptoms are hallmarks of rheumatoid arthritis (RA), a chronic rheumatic condition that has a higher death rate than the general population and can cause lifelong disability. Among the rheumatic inflammatory musculoskeletal disorders, RA is the most common systemic autoimmune illness. RA often requires lifetime therapy and can be challenging to cure [12].

From a mechanistic perspective, miRNA-24-3p may plausibly contribute to RA pathogenesis through regulation of genes involved in inflammatory and immune signaling. MicroRNAs are known to fine-tune cytokine networks and intracellular pathways such as NF-κB and MAPK, which are central to synovial inflammation. Dysregulated miRNA-24-3p could therefore influence the expression of downstream inflammatory mediators (e.g., IL-6, TNF-related signaling) and promote persistence of inflammation. In addition, miRNAs can affect the activation, proliferation, and apoptosis of immune cells as well as fibroblast-like synoviocytes, the key effector cells responsible for pannus formation and joint destruction in RA. Thus, the observed elevation of circulating miRNA-24-3p may reflect ongoing immune activation and synovial inflammatory processes. Nonetheless, functional studies are needed to confirm causal targets and define the exact role of miRNA-24-3p in RA [13, 14].

In our study, the RA patients presented with a median age of 36.5 (20.0–50.0), where approximately 80% of them were females. This was in line with a large Egyptian study of RA in which the mean age of the patients was 38.4 ± 11.6 years (range 28 to 50) [15].

In contrast, Saccon et al. found that the age of RA patients was much higher with a mean of 51 ± 13.3 (28 to 73 years) [16]. Regarding gender distribution, our results were in accordance with Cunningham et al. who found sex bias to female, with the same ratio. The main factors affecting the differences between female and male

Table 6 Logistic regression analysis for prediction of RA susceptibility

	Univariate			Multivariate		
	P	OR	95% CI	*P*	OR	95% C. I
Sex	0.109	1.477	0.917–2.379			
Age	0.206	1.015	0.992–1.037			
Family history	< 0.001*	3.177	1.862–5.419	0.008*	8.346	1.732–40.218
ESR	< 0.001*	1.687	1.283–2.217	0.011*	1.145	1.032–1.271
CRP	< 0.001*	1.390	1.222–1.580	0.003*	1.267	1.086–1.479
miRNA- 24-3p expression	< 0.001*	1.469	1.306–1.654	< 0.001*	1.128	2.695–22.727

*Significant ($p < 0.05$), *CRP* C-reactive protein, *ESR* Erythrocytes sedimentation rate

Table 7 Ordinal regression analysis for prediction of grades of the DAS28 score among patients with RA

	Univariate			Multivariate		
	P	OR	95% CI	*P*	OR	95% CI
Sex	0.083	0.587	0.321–1.072			
Age	0.385	1.011	0.987–1.036			
Family history	0.401	0.827	0.531–1.288			
CRP	< 0.001*	1.024	1.013–1.035	0.003*	1.002	1.001–1.003
ESR	< 0.001*	1.026	1.015–1.036	0.072	1.297	0.895-1.300
RF	0.001*	1.004	1.002–1.006	0.292	1.001	0.999–1.004
Anti-CCP	0.002*	1.010	1.003–1.016	0.185	1.001	0.997–1.002
ANA	0.097	1.294	0.954–1.754			
miRNA-24-3p expression	< 0.001*	5.578	2.157–14.430	< 0.001*	2.345	1.715–3.205

*Significant ($p < 0.05$), *CRP* C-reactive protein, *ESR* Erythrocytes sedimentation rate

immune systems are the sex hormones and the different response to environmental factors [9].

The present study revealed that there was positive family history among 45% of the patients. Family history of RA is known to be an important predictor, even after accounting for genetic and environmental factors. Supporting these findings, studies have shown that the heritability of RA is estimated to be 50–60%. Further, first-degree relatives of RA patients have an increased likelihood of having inflammatory joint signs [17].

Regarding the laboratory parameters in the present study, the hemoglobin concentration and RBCs count of RA patients were lowered than the control group with a significant p-value of (< 0.001 for both). Iron deficiency anemia and anemia of chronic disease (ACD) are characteristic disorder that affects the health of rheumatoid patients. The ACD is the most common, developing soon after the onset of the disease and often reflecting its severity. Iron deficiency anemia mainly caused by blood loss due to non-steroidal anti-inflammatory drugs-induced gastric erosions [18].

In addition, our results showed no significant variations in platelet count, which were not matched with the study conducted by Tekeoglu et al. as they reported high platelet count which directly proportional to RA activity [19].

Also, the lab results revealed no significant variations in urea, or creatinine levels and urine examination between the two groups which were not in line with Tang et al. who found an impaired renal function which manifested by an increased blood urea and creatinine and a decrease in glomerular filtration rate and renal functional reserve. They explained these dysfunctions as probably associated with the long-term drug treatment for RA [20].

The CRP is a sensitive acute-phase protein marker of systemic inflammation synthesized by the liver in response to pro-inflammatory cytokines, and its elevated levels are commonly observed in active RA [21]. In the present study 90% of RA patients had a positive CRP test and their ESR values were strongly higher than controls. This was matched with the results of the studies carried out by Bobirca et al. [22] and Ormseth et al. [8] as both studies found significant percentage of positive CRP test (94.3%) and also agreed with Smyrnova, who recorded an increased ESR value in all patients, but normal in all the controls [23].

Rheumatoid factor and anti-CCP are well-known serological markers for RA diagnosis according to the 2010 American College of Rheumatology/European League against Rheumatism classification criteria. The first autoantibody detected in rheumatoid patients was RF which are antibodies directed against the human IgG Fc region [24]. In our study, the majority (75.0% and 92%) tested positive for the RF and anti-CCP, respectively.

These observations were quit similar to a study done by the Egyptian College of Rheumatology who reported RF positivity in 73.7% and anti-CCP positivity in 66.7% of RA patients [15]. Antinuclear antibodies refer to a diverse group of autoantibodies targeting various nuclear and cytoplasmic components. Regarding the disease activity, in the present study, DAS28 score with a mean of 4.72 ± 1.67 suggested that 48.0% of patients had high disease activity, while 28.0% had moderate activity [25].

Only 14.0% of patients were in remission, while 10.0% had minimal disease activity. This is in line with Murata et al. [26] who found 11% of patients were in remission, 32% of patients had high disease activity and 13% of patients had low disease activity, with an average score of 3.42 ± 1.07 but unlike Bobirca et al. who found 43.2% of the patients had moderate activity of the disease, 21.6%were in remission, 20.5% high activity and 14.8% low activity [22].

Importantly, in our investigation, there was significantly higher plasma miRNA-24-3p expression in RA group than the control group ($p < 0.001$) which was consistent with Murata et al. who reported that the expression of plasma miR-24-3p in RA patients was shown to be three-fold higher in comparison to healthy individuals [26]. Ormseth et al. found that miR-24-3p concentrations was elevated in the plasma of patients with RA and had the strongest diagnostic accuracy for seronegative RA [8]. Cunningham et al. also stated that miR-24-3p elevated in the plasma of RA patients compared to healthy control, so circulating miRNAs may be utilized as potential biomarkers for RA [9].

In our study we found no statistically significant difference in miRNA24-3P gene expression levels based on sex. This was in conformity with Mooney et al. who found that miRNA 24-3p levels were very consistent between males and females. By comparison of miRNA – 24-3P expression in our results according to lab measurements and disease scores, it was showed that there was statistically significant correlation in gene expression in relation to RF, CRP, Anti- CCP and DAS28 score [27].

This was in keeping with Murata et al. [26] who found that plasma miR-24 had a positive correlation with CRP, RF, ESR, anti-CPP, global health and DAS28 scores and not agreed with Ormseth et al. who found no correlation with DAS28 score, tender joint count, global health score, ESR, or CRP ($p > 0.08$) [8].

In this research, miRNA- 24-3p gene expression showed significant positive correlations with no. of tender joints and no. of swollen joints ($p < 0.001$ for both), suggesting that higher miR-24-3p expression is associated with increased joint tenderness and inflammation severity. Aligned with this finding, Murata et al. [26] found that miR-24-3p was positively correlated with

tender and swollen joint count and was in disagreement with Ormseth et al. who found that miR-24-3p not correlated with tender or swollen joint and not associated with disease activity [8].

The positive correlation found between miRNA- 24-3p and WBCs ($p < 0.001$) in this work suggests that miR-24-3p is upregulated in response to systemic inflammation and also the significant positive correlation with platelets ($p = 0.001$), indicating a link between miR-24-3p and inflammation-associated thrombocytosis in RA.

ROC curve displayed that miRNA-24-3P gene expression can significantly diagnose RA with high accuracy at cutoff value greater than 1.7. A study conducted by Ormseth et al. found that miR-24-3p having the highest (AUC = 0.772) for RA diagnosis using individual miRNA [8].

The very high diagnostic performance observed in our ROC analysis should be interpreted with caution, i.e. differences from previous reports (e.g., lower AUC values) may relate to variations in patient selection, disease stage, sample type, and laboratory workflows. Therefore, external validation in larger, independent cohorts is warranted.

The logistic regression analysis in our study suggested that the increased CRP, ESR, RF, Anti-CCP, and miRNA-24-3p gene expression were significant predictors of higher grades of DAS28 score.

Finally, miR-24 has a diagnostic potential even in RA patients. Importantly, plasma miR-24-3p can be used as a marker of disease activity of RA since the expression level was shown to correlate with CRP, the scale of general health and DAS28. MiR-24-3p may enhance the inflammation by targeting the expression of the Furin enzyme which is important for the processing of latent transforming growth factor beta 1 (TGF-β1) into its active form and required for the maintenance of peripheral tolerance.

This study has several limitations. First, the sample size was relatively limited and the study was conducted in a single study where no independent external validation set was available, which may limit generalizability. Second, being a case–control study, it provides evidence of association but cannot establish a causal relationship between plasma miRNA-24-3p expression and the development or progression of RA. Third, circulating miRNA levels may be influenced by potential confounders, including comorbidities, inflammatory or infectious conditions, and medications (e.g., corticosteroids, conventional DMARDs, and biologic therapies), which were not fully controlled.

Additionally, pre-analytical variables such as sample handling may affect circulating miRNA measurements. Therefore, larger prospective studies with external validation and standardized protocols are needed to confirm reproducibility and clinical utility.

Future studies with larger, multicentric cohorts and longitudinal designs are recommended to confirm these findings and better elucidate the temporal and mechanistic relationship between miRNA-24-3p and RA activity. From a clinical perspective, circulating miRNA assays may offer an additional tool to complement existing biomarkers in RA. Measurement of plasma miRNA-24-3p by qRT-PCR is technically feasible in laboratories with molecular facilities and can be performed on small volumes of peripheral blood. However, translation into routine practice requires standardized pre-analytical handling, robust normalization strategies, and inter-laboratory reproducibility. In terms of cost, qRT-PCR–based miRNA testing is generally more expensive and resource-intensive than conventional inflammatory markers (ESR/CRP), which may limit widespread use as a first-line test. Therefore, miRNA-24-3p may be more realistically positioned as an adjunct biomarker to support RA assessment—particularly for aiding disease activity stratification or risk profiling—rather than replacing current diagnostic criteria based on clinical evaluation and established serological markers (RF and anti-CCP).

In conclusion, there was significantly higher miRNA-24-3p gene expression in plasma sample of RA group than the control group. MiRNA-24-3p gene expression was found to be significant predictor of DAS28 scores and is associated with higher DAS28 grades.

Abbreviations

ACD	Anemia of chronic disease
Anti-CCP	Anti-Cyclic Citrullinated Peptide
CRP	C-reactive protein
DAS	Disease severity score
ESR	Erythrocytes sedimentation rate
miRNA	Micro RNA
ncRNA	Non-coding RNA
RA	Rheumatoid arthritis
RBCs	Red blood cells
RF	Rheumatoid factor
ROC	Receiver operating characteristic
TGF-β1	Transforming growth factor beta 1
WBCs	White blood cells

Authors' contributions

Ibrahim Mohammed Rageh contributed to project administration, investigation, and the performance of the statistical analysis. Ahmed Mamdouh Awadallah contributed to methodology, formal analysis and supervision. Noha Hosni Ibrahim and Rana Gamal Abd-Elnasser contributed in validation, writing, and editing, Seham Gouda Ameen contributed to software development, data curation and formal analysis.

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nil.

Data availability

The dataset utilized in the preparation of this study will be available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All procedures accomplished in this work, including human subjects, were in accordance with the ethical guidelines of the institutional research committee and with the 1964 Helsinki declaration and its adjustments.
Informed consent was obtained from all participants in this work.

Competing interests
One of the authors (Noha Hosni Ibrahim) is a member of the editorial team in the Egyptian journal of Rheumatology and Rehabilitation.

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References

1. Black RJ, Cross M, Haile LM, Culbreth GT, Steinmetz JD, Hagins H et al (2023) Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: A systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 5(10):1–12
2. Peng X, Wang Q, Li W, Ge G, Peng J, Xu Y et al (2023) Comprehensive overview of microRNA function in rheumatoid arthritis. Front Immunol 11(1):1–46
3. Burmester GR, Pope JE (2017) Novel treatment strategies in rheumatoid arthritis. Lancet 389(10086):2338–2348
4. Yang J, Li Z, Wang L, Yun X, Zeng Y, Ng JPL et al (2022) The role of noncoding RNAs (miRNA and lncRNA) in the clinical management of rheumatoid arthritis. Pharmacol Res 186:106549
5. He B, Zhao Z, Cai Q, Zhang Y, Zhang P, Shi S et al (2020) miRNA-based biomarkers, therapies, and resistance in cancer. Int J Biol Sci 16(14):2628–2641
6. O'Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol 9:402
7. Safari F, Damavand E, Rostamian AR, Movassaghi S, Imani-Saber Z, Saffari M et al (2021) Plasma levels of microRNA-146a-5p, microRNA-24-3p, and microRNA-125a-5p as potential diagnostic biomarkers for rheumatoid arthritis. Iran J Allergy Asthma Immunol 20(3):1–9
8. Ormseth MJ, Solus JF, Vickers KC, Oeser AM, Raggi P, Stein CM (2015) Utility of select plasma microRNA for disease and cardiovascular risk assessment in RA patients. J Rheumatol 42(10):1746–1751
9. Cunningham CC, Wade S, Floudas A, Orr C, McGarry T, Wade S et al (2021) Serum miRNA signature in rheumatoid arthritis and at-risk individuals. Front Immunol 12:633201
10. Ponchel F, Toomes C, Bransfield K, Leong FT, Douglas SH, Field SL et al (2003) Real-time PCR based on SYBR-Green I fluorescence: An alternative to the TaqMan assay. BMC Biotechnol 3:18
11. Prevoo MLL, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LBA, van Riel PLCM (1995) Modified disease activity scores including twenty-eight-joint counts: Development and validation in a longitudinal study of RA patients. Arthritis Rheum 38(1):44–48
12. Machaj F, Chmielewska-Jeznach M, Koryszewska-Bagińska A, Malinowski D, Pawlik A, Olędzka G (2025) MicroRNAs as biomarkers and therapeutic targets in rheumatoid arthritis. Int J Mol Sci 26(20):9950
13. Raslan HM, Atia HAE, Elshaer SS, Sabry SM, Mohammed YM, Amr KS (2024) Plasma expression and bioinformatic analysis of Mir-16, Mir-132, Mir-146 and Mir-223 in patients with rheumatoid arthritis. Hum Gene 42:201352
14. Pang X, Xu F, Fan C, Jiang H (2025) Global research trends of MicroRNAs in rheumatoid arthritis: bibliometrics and visualization analysis. Clin Rheumatol 44(1):53–66
15. Gheita TA, Raafat HA, El-Bakry SA, Elsaman A, El-Saadany HM, Hammam N et al (2023) Rheumatoid arthritis study of the Egyptian College of Rheumatology (ECR): Nationwide presentation and worldwide stance. Rheumatol Int 43(4):667–676
16. Saccon TD, Dhahbi JM, Schneider A, Nunez Lopez YO, Qasem A, Cavalcante MB et al (2022) Plasma miRNA profile of Crohn's disease and rheumatoid arthritis patients. Biology (Basel) 11(4):508
17. Sparks JA, Chang S, Deane KD, Gan RW, Demoruelle MK, Feser ML et al (2016) Associations of smoking and age with inflammatory joint signs in first-degree relatives of RA patients. Arthritis Rheumatol 68(8):1828–1838
18. Madu AJ, Ughasoro MD (2016) Anaemia of chronic disease: An in-depth review. Med Princ Pract 26(1):1–9
19. Tekeoğlu İ, Gürol G, Harman H, Karakeçe E, Çiftçi İH (2016) Overlooked hematological markers of disease activity in rheumatoid arthritis. Int J Rheum Dis 19(11):1078–1082
20. Tang Y, Varavko Y, Aringazina R, Menshikova I (2024) Changes in renal function and morphological variations of kidney diseases in rheumatoid arthritis patients. Asian J Urol 11(2):304–310
21. Sproston NR, Ashworth JJ (2018) Role of C-reactive protein at sites of inflammation and infection. Front Immunol 9:754
22. Bobirca A, Florescu AT, Alexandru C, Bobirca FT, Marinescu D, Musetescu A et al (2022) Characteristics of anemia in rheumatoid arthritis. Med Mod 29(3):1–6
23. Smyrnova VM (2015) Hematological abnormalities in Ukrainian patients with rheumatoid arthritis. J Arthritis 4(1):1000146
24. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd et al (2010) 2010 rheumatoid arthritis classification criteria: An ACR/EULAR collaborative initiative. Arthritis Rheum 62(9):2569–2581
25. Liu F, Wang XQ, Zou JW, Li M, Pan CC, Si YQ (2024) Association between serum antinuclear antibody and rheumatoid arthritis. Front Immunol 15:1358114
26. Murata K, Furu M, Yoshitomi H, Ishikawa M, Shibuya H, Hashimoto M et al (2013) Comprehensive microRNA analysis identifies miR-24 and miR-125a-5p as plasma biomarkers for rheumatoid arthritis. PLoS ONE 8(7):e69118
27. Mooney C, Raoof R, El-Naggar H, Sanz-Rodriguez A, Jimenez-Mateos EM, Henshall DC (2015) High throughput qPCR expression profiling of circulating microRNAs reveals minimal sex- and sample timing-related variation in plasma of healthy volunteers. PLoS ONE 10(12):e0145316

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