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Evaluation of circulating miR-16-5p and miR-223-5p in association with musculoskeletal ultrasonography seven-joint score in the assessment of rheumatoid arthritis activity

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Abstract

Rheumatoid arthritis (RA) is characterized by ongoing joint destruction. MicroRNAs (miRs) are blood-based biomarkers linked to RA pathogenesis. The musculoskeletal ultrasonography seven-joint score (US7) is an objective tool to assess RA activity. We aimed to evaluate miR-223 and miR-16 roles in monitoring RA activity and to investigate if there is a link between their plasma levels and US7 score. This study enrolled 76 RA patients classified according to Disease Activity Score 28-joint count with erythrocyte sediment rate (DAS28-ESR) to inactive cases (n = 38) and active cases (n = 38). Each patient's joint was scored for synovial proliferation (gray-scale ultrasound 'GSUS7') and vascularization (power Doppler ultrasound 'PDUS7'). Real-time quantitative PCR was used to measure the expression levels of miR-16 and miR-223 in plasma. When compared to inactive group, the active group revealed significant upregulation of miR-16 and miR-223, (P = 0.001 and P = 0.02, respectively). miR-16 and miR-223 levels were correlated with synovitis PDUS7 (r = 0.34, $p < 0.01$ and r= 0.25, P = 0.03, respectively). miR-16 was also positively correlated with synovitis GSUS7 (r= 0.42, $p < 0.001$). miR-223 upregulation discriminated active from inactive RA patients at AUC = 0.64, with 76% sensitivity and 50% specificity at cutoff > 2.8-fold change), whereas miR-16 distinguished the two groups at AUC = 0.78 with 87% sensitivity and 53% specificity at cutoff >38.27-fold change. In conclusion, upregulated miR-16 may have more potential to serve as activity biomarkers than miR-223 in RA. The miR-16 level was linked to synovitis GSUS7 and synovitis PDUS7 changes but miR-223 only linked to synovitis PDUS.

Keywords: miR-16, miR-223, Ultrasonography seven-joint score**,** RA activity discriminator**s.**

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Introduction

Rheumatoid arthritis (RA) is a common chronic inflammatory autoimmune illness that, if not recognized and treated promptly, can lead to disability. Patients with RA may develop depression as a result of their poor quality of life.[1]

Disease activity indicators such as the Disease Activity Score 28-joint count with erythrocyte sediment rate (DAS28-ESR) and Clinical Disease Activity Index (CDAI) include subjective metrics, such as patient-reported outcomes. Furthermore, different indices weight identical factors to varying degrees.[2] Another issue is diagnosing seronegative RA and distinguishing it from psoriatic arthritis, which only affects the tiny joints of the hands, highlighting the importance of applying objective, new, and accurate tools such as musculoskeletal ultrasonography (US) to identify specific patterns of joint and tendon involvement, which may help in RA evaluation and therapy.[3]

The exact number of joints that should be inspected is a source of debate. A reduced joint count examination is more practical than a comprehensive one.[4] Because examining several joints in the US can be time-consuming, many US score structures have been updated to analyze a smaller number of joints while maintaining the quality of the records acquired.[5]

The ultrasound 7 score (US7 score), a semiquantitative US scoring system, has been suggested to study the existing RA. It was created to standardize the US examination in everyday rheumatology practice as well as multicenter studies. Each joint score for synovial proliferation (gray-scale ultrasound 'GSUS7') and vascularization (power Doppler ultrasound 'PDUS7') is combined into the validated seven-joint score (US7).[6]

MicroRNAs (miRs) are short, non-coding RNAs that regulate gene expression after transcription. They have a length of roughly 22 nucleotides. MiRs bind to one or more messenger RNAs (mRNAs), controlling protein production by translation inhibition or increased mRNA turnover and destruction. Over 30% of human protein-coding genes are regulated by miRs.[7-8] As a result, miRs with aberrant expression are linked to the onset and progression of illnesses. Even under extreme conditions such as heat, extreme pH, long-term storage, and repeated freezing and thawing, miRs have been proven to be extremely stable. The exceptional stability of circulating miRs makes them ideal biomarkers for a variety of illnesses.[8-9]

Specific circulating miR levels were found to be related to RA activity, suggesting that they could be used as blood-based biomarkers for activity.[10] MiR-223 has previously been shown to play a role in cell apoptosis, immunological activation, and bone metabolism. By inhibiting the synthesis of vascular endothelial growth factor, miR-223 has been linked to the inhibition of chondrogenesis and angiogenesis.[11] MiR-16 is thought to play a role in the development of autoimmune diseases by regulating the expression of cytokines such as tumor necrosis factor-alpha (TNF-), interleukin (IL)-8, IL-6, and IL-4, affecting the proliferation and differentiation of T helper-17 (Th17) cells and regulatory T (Treg) cells. In addition, miR-16 is involved in triggering TNF-induced apoptosis.[12] We aimed to assess the clinical potential of miR-223 and miR-16 as non-invasive biomarkers in monitoring RA activity and to investigate if there is a link between their level of expression and US7 findings.

Subjects and Methods

Study participants

In this prospective cross-sectional comparative study, 76 RA patients were classified into two groups: active disease patients (n = 38) and remission patients (n = 38). In addition, 10 sex and age-matched control individuals were included for detection of miR-16 and miR-223. All patients were recruited from the Internal Medicine Department's Outpatient Rheumatology Clinic and Inpatient Rheumatology Unit at AL-Zahraa Hospital, Al-Azhar University, Cairo, Egypt.

The American College of Rheumatology/European League Against Rheumatism 2010 updated criteria for RA were

used to diagnose patients with RA.[13] To measure activity, the DAS28-ESR Score was generated for all patients. Exclusion criteria included autoimmune illnesses other than RA, ongoing inflammatory joint diseases other than RA, signs of cancer or bacterial and/or viral infection.

Ethical statement

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls, Al Azhar University, Cairo, Egypt (Approval no. 202106863). An informed written consent was obtained from each participant.

Patient's assessment & ultrasonographic examination

All patients were subjected to a full history taking and complete clinical examination, calculation of DAS score 28, and musculoskeletal US7 score on the wrist, hand, and forefoot, performed with the US device with a 14 MHz probe (Siemens high-end machine S2000, Siemens Medical Solutions, USA). All patients were assessed and examined by two experienced rheumatology sonographers. Furthermore, power Doppler (PD) was employed with the following parameters: pulse repetition frequency (PRF) 0.7 KHz, gain 18–30 dB, and low filter. This score included US evaluation of the following joints of the clinically most affected hand and forefoot, wrist, metacarpophalangeal (MCP II, III), proximal interphalangeal (PIP-II, III), and metatarsophalangeal (MTP II, and V), which were assessed for synovitis, tenosynovitis/peritonitis, and erosions. GSUS and PDUS assessed synovitis and synovial tenosynovial vascularity semi-quantitatively (grade 0–3), as well as tenosynovitis and erosions for their existence (0/1). [14] The Grey Scale (GS) synovitis score ranged from 0–27, the Power Doppler (PD) synovitis score ranged from 0–39, the GS tenosynovitis score ranged from 0–7, the PD tenosynovitis score ranged from 0–21, and the erosions score ranged from 0–14 excluding wrist examination and 0–17 together with wrist examination.[6] An example is illustrated in Figure 1

Sampling and Biochemical analysis

Blood samples were collected from study subjects. Each blood sample was divided into four aliquots. The first, aliquot of 2 mL of blood was put into an EDTA tube for a complete blood count and differential count using an automated blood counter (Sysmex, Kobe, Japan). The second aliquot of 1.6 ml of blood was transferred into a citrate Westergren tube for erythrocyte sedimentation rate (ESR) measurement. The third aliquot of 2 ml of blood was transferred to a yellow capped vacutainer for serum separation for biochemical tests including C-reactive protein (CRP). The fourth aliquot (2 mL of blood) was placed into an EDTA tube and spun for 10 minutes at 1900xg, after which the plasma was carefully withdrawn and centrifuged again for 10 minutes at 1600xg to remove additional cellular nucleic acids attached to cell debris. The supernatant was then moved to microcentrifuge vials and kept at -80°C for extraction and real-time quantitative PCR (RT qPCR) detection of miR-16 and miR-223.

RNA extraction and real-time PCR

Using 200 µl of plasma and a miRNeasy commercial kit (Cat. NO. 217004, Qiagen, Germany), we extracted plasma mature miRNAs, miR-223, and miR-16 according to the manufacturer's instructions. A commercial miScript II RT Kit (Cat. No. 218161, Qiagen, Germany) was used, according to the manufacturer's instructions, to reverse-transcribe the extracted RNA to complementary DNA. miRNA expression was determined using the miScript SYBR Green PCR kit (Cat. No. 204145, Qiagen, Germany), manufacturer's instructions, on a real-time PCR quaint studio 5 system (Applied Biosystem, USA). An internal housekeeping endogenous control, miRNA SNORD68, was employed.

The RT qPCR cycling conditions were : 95°C for thirty min, then 40 cycles, each of 15 minutes at 94°C, 30 seconds at 55°C, and 30 seconds at 70°C. Δ cycle threshold (Ct) was calculated by subtracting the Ct values of SNORD68 from the Ct values of the target miRNAs in all patient samples together with

samples from the 10 control individuals. Fold change was calculated using $2^{-\Delta\Delta Ct}$ for relative quantification.

Statistical analysis

The Statistical Package for the Social Science (SPSS, Inc, Chicago, Illinois, USA), version 18, was used to code, assess, and statistically analyze the data collected. In the case of quantitative data, the median (interquartile range) was used. Whereas, in the case of qualitative data, the frequency and percentage were used. For qualitative data comparison, the X^2-test was utilized. When comparing quantitative data between two groups, the Mann-Whitney test was used. When comparing nonparametric data between more than two groups, the Kruskal Wallis test was employed. To investigate correlations between different biomarkers, Spearman correlation tests were used. A $p < 0.05$ was used as the significance level. The medians of fold change were used to represent miR data. The p-values were calculated based on the Mann-Whitney -test of the replicate $2^{-\Delta\Delta CT}$ values for each miR in the studied groups. The discriminative accuracy of miRs was evaluated by rhea receiver-operating-characteristic (ROC) analysis.

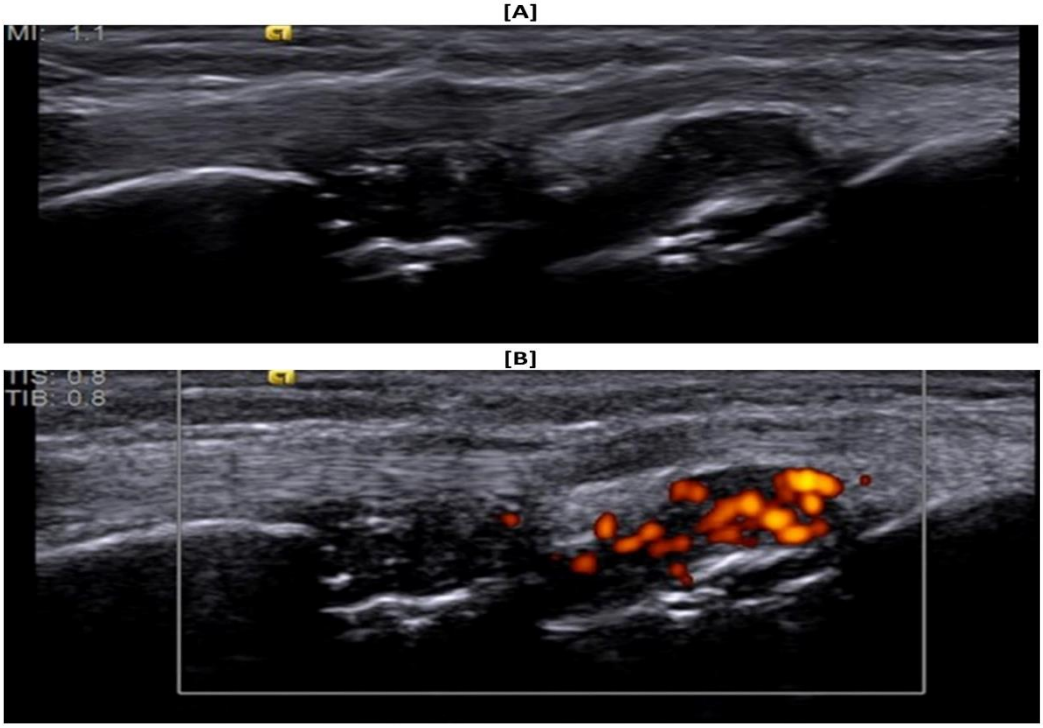

Figure 1. [A] Dorsal longitudinal scan of wrist joint showing grade 2 synovitis with grayscale [B] Dorsal longitudinal scan of wrist joint showing grade 2 synovitis with positive doppler signals.

Results

This cross-sectional study participants consisted of 76 patients with RA divided into two groups, the inactive group (n=38) and the active group (n=38). Comparisons of demographic data and laboratory findings between the two groups are shown in Table1.

The active group showed significant up-regulation of plasma miR-16 and miR-223 levels when compared to the inactive cases (p <0.001 and P=0.02, respectively) (Figure. 2). The relevant data are presented in Table 1.

Table 1. Comparison of demographic and clinical data, and laboratory findings among studied individuals.

Parameter	RA active group	RA inactive group	*p*-value
	Median (interquartile range)		
Age in years (range)	44 (37-54)	44 (35-52)	NS
Disease duration in year (range)	5 (3-7.3)	5(2.8-9.3)	NS
Sex			
Females no (%)	37 (97.4%)	36 (94.7 %)	NS
Males no (%)	1 (2.6 %)	2 (5.3 %)	
TLC [10^3/μl]	5.9(4.4-8.3)	5.2(4.1-7.8)	NS
N/L Ratio	8.6(5.3-14.6)	1.2(0.95-1.7)	<0.001
ESR mm/hour	45(30-68)	28 (17 -51)	0.02
CRP (mg/l)	13.3 (11-22)	4.7 (3-6)	<0.001
Anti CCP (u/ml)	123 (112-155)	115(111-133)	NS
MiR-16 /fold change	40.2 (111.6-138)	3.5(0.3-31.1)	<0.001
MiR- 223 /fold change	2.8 (0.2-12.6)	0.4 (0.03-2.6)	0.02

Anti-CCP, Anticyclic citrullinated peptide; CRP, C reactive protein; N/L; Neutrophil lymphocyte ratio; TLC, total leukocyte count; DAS28-ESR, Disease Activity Score in 28 joints-erythrocyte sedimentation rates; ESR, Erythrocytic sedimentation rate; MiR; micro-RNA; GSUS; Grey scale ultrasound, PDUS; Power Doppler ultrasound. *P* > 0.05 is not significant (NS).

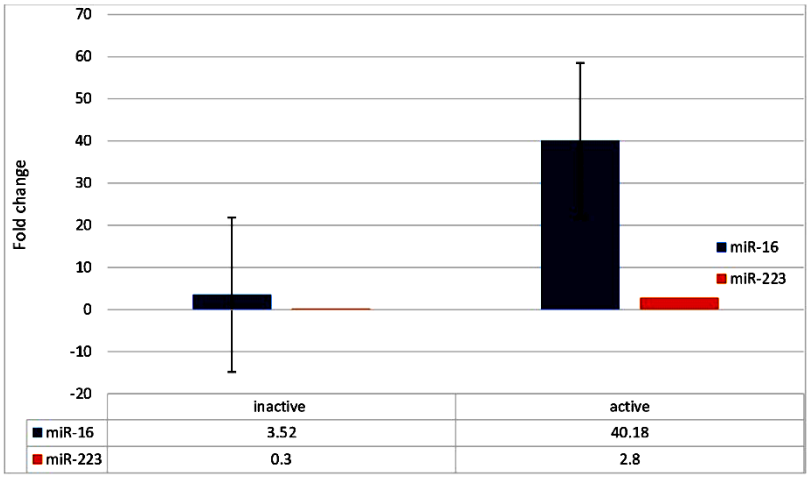

Figure 2. Comparison between miR-16 and miR-223 fold changes in active RA patients compared to inactive RA patients. The active group showed significant up-regulation of plasma miR-16 and miR-223 levels when compared to inactive cases (p <0.001 and p =0.02, respectively).

Also, the correlation studies in all study cases (n = 76) revealed a positive correlation of miR-16 with neutrophil-lymphocyte (N/L) ratio, and with DAS28-ESR (r=0.38, *p*<0.001) (r = 0.23, *p*=0.046, and r=0.38, *p*<0.001, respectively). Both miR-16 and miR-223 levels were positively correlated with CRP levels (r = 0.44, *p*<0.001 and r = 0.31, *p*<0.01, respectively).

Also, correlation studies revealed that miR-16 and miR-223 levels were positively correlated with synovitis PDUS7 (r=0.34, *p*<0.01, and r=0.25, *p*=0.03, respectively), Furthermore, miR-16 was positively correlated with synovitis GSUS7 (r=0.42, *p*<0.001) as shown in Table 2.

Table 2. Correlation between fold changes of miR-16, and miR-223 and the clinical, biochemical, and ultrasound findings in the study 76 patients.

	RA patients	MiR- 223	MiR- 16
Age in years	Correlation Coefficient	0.01	0.06
	p value	NS	NS
Disease duration in years	Correlation Coefficient	0.08	0.04
	p value	NS	NS
ESR mm/hour	Correlation coefficient	0.15	0.19
	p value	NS	NS
N/L	Correlation Coefficient	0.16	0.38
	p value	NS	<0.001
CRP (mg/l)	Correlation Coefficient	0.31	0.44
	p value	<0.01	<0.001
Anti CCP (u/ml)	Correlation Coefficient	0.08	0.03
	p value	NS	NS
DAS28-ESR Score	Correlation Coefficient	0.14	0.38
	p value	NS	<0.001
MiR-223	Correlation Coefficient	…….	0.18
	p-value	……..	NS
Synovitis GSUS	Correlation coefficient	0.19	0.42
	p value	NS	<0.001
Synovitis PDUS	Correlation coefficient	0.25	0.34
	p value	0.03	<0.01
Tenosynovitis GSUS	Correlation coefficient	0.18	0.17
	p value	NS	NS
Tenosynovitis PDUS	Correlation coefficient	0.06	0.19
	P value	NS	NS
Erosion GSUS	Correlation coefficient	0.15	0.01
	p value	NS	NS

Anti-CCP, Anticyclic citrullinated peptide; CRP, C reactive protein; N/L; Neutrophil lymphocyte ratio; DAS28-ESR, Disease Activity Score in 28 joints-erythrocyte sedimentation rates; ESR, Erythrocytic sedimentation rate; MiR; micro-RNA; GSUS; Grey scale ultrasound, PDUS; Power Doppler ultrasound. . $p > 0.05$ is not significant (NS).

In addition, DAS28-ESR score was positively correlated with synovitis GSUS (r=0.5, *p*<0.001), synovitis PDUS (r=0.7, *p*<0.001), tenosynovitis GSUS (r=0.5, *p*<0.001), tenosynovitis PDUS (r=0.52, *p*<0.001) and erosion GSUS (r=0.33, *p*<0.01), as shown in Table 3.

Table 3. Correlation of components of ultrasound 7 scores with other clinical, biochemical findings in the 78 RA patients.

	RA patients	Synovitis GSUS	Synovitis PDUS	Tenosynovitis GSUS	Tenosynovitis PDUS	Erosion GSUS
Age in years	Correlation Coefficient	-0.05	-0.008	-0.002	-0.07	0.03
	P value	NS	NS	NS	NS	NS
Disease Duration in years	Correlation Coefficient	-0.07	0.05	-0.08	-0.02	-0.19
	P value	NS	NS	NS	NS	NS
ESR Mm/hour	Correlation coefficient	0.22	0.19	0.28	0.08	0.04
	P value	NS	NS	0.02	NS	NS
TLC [$10^3/\mu l$]	Correlation Coefficient	0.06	-0.08	0.07	0.02	0.12
	P value	NS	NS	NS	NS	NS
N/L	Correlation Coefficient	0.44	0.59	0.33	0.49	0.24
	p value	<0.001	<0.001	<0.001	<0.001	<0.05
CRP (mg/l)	Correlation Coefficient	0.51	0.61	0.39	0.49	0.25
	p value	<0.001	<0.001	<0.001	<0.001	<0.05
Anti CCP(u/ml)	Correlation Coefficient	0.14	0.04	-0.01	-.008	0.06
	p value	NS	NS	NS	NS	NS
DAS28-ESR Score	Correlation Coefficient	0.5	0.7	0.5	0.52	0.33
	p value	<0.001	<0.001	<0.001	<0.001	<0.01

Anti-CCP, Anti cyclic citrullinated peptide; CRP, C reactive protein; N/L; Neutrophil lymphocyte ratio; TLC, total leukocyte count; DAS28-ESR, Disease Activity Score in 28 joints-erythrocyte sedimentation rates; ESR, Erythrocytic sedimentation rate; MiR; micro-RNA; GSUS; Grey scale ultrasound, PDUS; Power Doppler ultrasound. $p > 0.05$ is not significant (NS).

By receiver-operating-characteristic analysis, miR-223 upregulation distinguished active from inactive RA patients at AUC = 0.64 (with 76 % sensitivity and 50 % specificity at cutoff > 2.8-fold change), whereas miR-16 superiorly distinguished the two groups at AUC=0.78 (87 % sensitivity and 53 % specificity at cutoff >38.27-fold change), as shown in Table 4 and Figure 3.

Table 4. Data of the ROC curve for miR-223 and miR-16 to differentiate between active and inactive RA patients.

Micro RNA	AUC	*p value	Cut off	Sensitivity	Specificity
MiR-223 fold change	0.64	<0.05	2.8	76%	50%
MiR-16 fold change	0.78	<0.001	38.27	87%	53%

MiR; micro RNA. *$p \leq 0.05$ is significant.

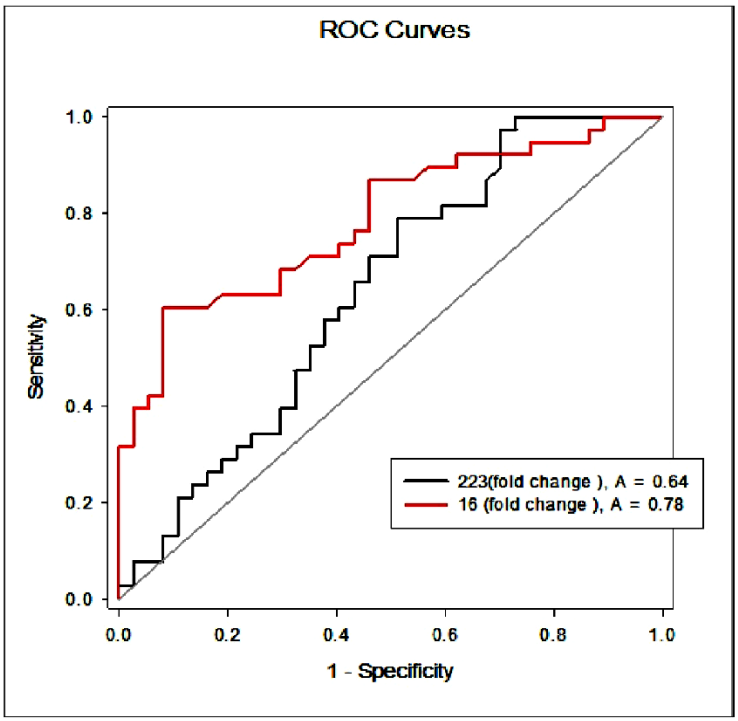

Figure 3. Output data of ROC curves regarding fold changes of miR-16 and miR-223 to differentiate between active from inactive RA states. miR-223 upregulation distinguished active from inactive RA patients with 76 % sensitivity and 50 % specificity. miR-16 superiorly distinguished the two groups with 87 % sensitivity and 53 %, specificity.

Discussion

A precise evaluation of RA activity can be obtained with the use of objective biological markers to monitor disease activity and drug efficacy.[15] Circulating nucleic acids have been identified as potential biomarkers for many autoimmune disorders.[16] Still, until now, the whole map of miRs as blood-based biomarkers for RA activity and associated joint injury remains a mystery.

The present study aimed to assess the clinical potential of miR-223 and miR-16 as non-invasive biomarkers in monitoring RA activity and to investigate whether there is a link between their level of expression and US7 findings.

In the present study, when the laboratory data from both RA groups were compared, ESR, CRP levels, and DAS28-ESR score levels were significantly elevated in the active group (Table 1). Our data regarding ESR and DAS28-ESR agreed with those of a study by Eldosoky et al., 2018.[16]

In the current study, we also observed that the patients with active RA had a higher median N/L. This matched the findings of a research study by Watanabe et al., 2018 who found that N/L was significantly increased in the RA active group and was positively linked with DAS28-ESR.[17] Similarly, Hu et al., 2018 found that N/L values were higher among individuals with primary Sjogren's syndrome and were linked to disease activity.[18]

In the current study, the RA active group showed a significant up-regulation of plasma miR-16 levels with a median level = 40.2-fold change increase when compared to inactive cases with a 3.5 median level of fold change increase (p<0.001). Dunaeva et al., 2018 documented that the serum miR-16 was higher in established RA patients when compared to controls.[15] In the present study, miR-223 was only upregulated in the active state of the disease with (2.8) fold change while no considerable upregulation was noted in the inactive state. Comparison between median levels of expression of miR-223 between active

and inactive states revealed a significant increase in the active RA patients (P = 0.02) (Table 1, Figure 2), suggesting that miRNA 223 may be considered a marker of disease activity. Filková et al., 2014 identified miR-223 as an indicator of disease activity which is similar to our findings and added that both miR-16 and miR-223 are probable predictors of RA outcome.[19]

According to Li et al., 2019 miR-223 overexpression in autoimmune disorders is a result of its role in monocyte/macrophage differentiation, osteoclast formation, and bone remodeling, as well as a regulator of autophagy. Autophagy is a mechanism in the cell that uses lysosomal digestion to destroy damaged organelles. Autophagy dysfunction has been linked to a variety of diseases, including autoimmune diseases. miR-223 deficiency increases autophagy and reduces inflammation and the development of autoimmune disorders.[20]

On the cellular level, Wu et al., 2016 stated that active RA patients showed increased miR-16 expression in Th17 and decreased miR-16 expression in Treg cells of peripheral blood mononuclear cells (PBMCs) when compared to the inactive group. [21]

In contrast to our findings, Huang and Kuang, 2022, reported that down-regulation of miR-16 is the cause behind the development of RA pathology via the focal adhesion kinase (FAK) signaling pathway in RA. This can increase Vascular Endothelial Growth Factor (VEGF) production in osteoblasts and facilitate angiogenesis and hyperplasia, the cause behind joint deformity taking place in RA.[22]

In the present study, there was no correlation between the level of expression of both plasma miR-16 and miR-223 with the disease duration (Table 2). This contradicts data observed by Dunaeva et al., 2018 who found that the levels of serum miR-223-3p and miR-16-5p were significantly lower in early RA patients when compared to those with established RA, indicating that the expression of both miRs differed depending on the duration of the disease.[15] Part of the controversy can be explained as that miR-223-duplex is formed of (miR-223-5p and miR-223-3p) In the current study we examined miR-223-5p with different primer sequence than that of miR-223-3p which was studied by Dunaeva et al., 2018. Also, Filková et al., 2014 reported that the downregulation of circulating miR-16-5p characterizes the early stage of the disease.[19]

In the present study, there was a positive correlation of miR-16 with N/L, and with DAS28-ESR (p< 0.001 and p< 0.001, respectively). Also, miR-16 and miR-223 levels were positively correlated with CRP levels (p<0.001 and p<0.01, respectively) (Table 2). Filková et al., 2014 found a positive correlation between circulating miR-223 levels and CRP levels in early RA.[19]

Correlation studies revealed that miR-16 and miR-223 levels were positively correlated with synovitis PDUS7 p<0.01 and p=0.03, respectively. Whereas miR-16 was positively correlated with synovitis GSUS7 (p<0.001) (Table 2). These findings agreed with those of Chang et al., 2022 which documented that miRNA affects the synovial joints' micro-environment by targeting specific genes.[8]

MiR-16 may play a role in RA patients' Th17/Treg imbalance by affecting the expression of retinoic acid-related orphan receptor-γt (RORγt) and forkhead box P3 (FoxP3), according to Wu et al., 2016.[21] This information may indicate that miR-16 has a greater significance than miR-223 as biomarkers for identifying persistent synovial inflammation in patients who meet the set of apparent clinical remission criteria but still have residual synovial inflammation discovered by only ultrasonography.

In contrast to our findings, Wei et al., 2020 found that in RA, miR-15a/16 cluster is dramatically downregulated inside fibroblast-like synoviocytes (FLS). They added that a gene which encodes a member of the SOX (SRY-related HMG-box) family of transcription factors have aggressive nature and enhance the cell invasion in RA, which is suppressed in normal persons by miR-15a/16, but in RA-FLSs the miR-15a/16 is dramatically downregulated which leads to upregulation of this aggressive gene expression.[23] This controversy could be explained by the that former study measured mir15a/16 intracellular inside the FLSs while we

measured the free circulating miR-16 in the plasma where it was upregulated.

In the present study, DAS28-ESR score was positively correlated with synovitis GSUS (p<0.001), synovitis PDUS (p<0.001), tenosynovitis GSUS (p<0.001), tenosynovitis PDUS (p< 0.001) and erosion GSUS (p<0.01), respectively (Table 3). These findings agreed with those of Zhou et al., 2017 who correlated US7 with DAS28 as a reference standard of RA disease activity.[24] These data give strength to the validity of US7 score to effectively reflect RA disease activity with extra advantage being totally objective. Furthermore, according to the Kim et al., 2021 study, USGS and PD detected synovitis and joint effusion are important in assessing RA activity in response to treatment.[25]

Regarding US7 data, N/L was positively correlated with synovitis GSUS, Synovitis PDUS, Tenosynovitis GSUS, Tenosynovitis PDUS and Erosion GSUS (Table 3). These data contradict those reported by Lijuan et al., 2021[26], claimed that N/L is not a good independent supplementary measure for disease activity in RA patients. Even regarding the initial diagnosis of RA, Jin et al., 2021 found that N/L was less important than CRP and RF but superior to ESR.[27]

According to ROC curves analysis, miR-223 upregulation distinguished active from inactive RA patients. Whereas miR-16 was more superior in distinguishing the two study groups, as shown in Table 4 and Figure 3.

In conclusion, our study findings indicated that miR-16 may have more potential to serve as activity biomarkers than miR-223 in RA. The miR-16 level was linked to synovitis GSUS7 and synovitis PDUS7 changes while the miR-223 level was linked to PDUS7 only.

Author Contributions

RH, conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing original draft; writing review and editing. MAE, conceptualization; investigation; methodology; statistical analysis; writing original draft; writing review and editing. AAE, DAH, EFM, WEA, SE, WS, SME, MAS, MIA, SSA, conceptualization; investigation; methodology, writing review and editing. AK, conceptualization; investigation; methodology; supervision.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. (Approval No.202106863).

Informed consent

An informed written consent was obtained from every participant before being enrolled in the study.

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