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ORIGINAL ARTICLE

Association of Micro RNA-223 with Disease activity and Severity in Rheumatoid Arthritis Patients

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ABSTRACT

Background: *Rheumatoid arthritis (RA) is the most common autoimmune inflammatory arthritis, with a lifetime prevalence of up to 1% around the world. It is characterized by an inflammatory polyarthritis that preferentially affects the small joints leading to joint damage and eventual deformity and disability, and can also present with extra-articular manifestations. Micro RNA (miRNA) is a class of non-coding RNAs which negatively regulate messenger RNA (mRNA) expression.* ***Objective:*** *To evaluate the expression of miRNA-223 in rheumatoid arthritis (RA) patients and its potential association with disease activity and severity.* ***Methodology:*** *Blood samples from 35 patients with RA, according to the classification criteria of the American College of Rheumatology/ European League against Rheumatism (ACR/EULAR) for RA[1] and 35 apparently healthy volunteers as controls were allocated. Real-time PCR was used to determine the expression levels of miRNA 223.* ***Results:*** *miRNA 223 expression level was significantly higher in RA group compared with controls (RA group: 2.19± 1.28 vs 1.0± 0.02; P <0.001). There was significant association between serum miRNA 223 (RQ) level with Disease Activity Score 28 (p= 0.026). A significant positive correlation was found between miRNA 223 expression level and Rheumatoid Arthritis Severity Score activity (r=0.469, p =0.004).* ***Conclusions:*** *Plasma micro RNA223 level was significantly elevated in RA patients which clarifies its possible role in RA pathogenesis and correlates positively with disease severity and DAS-28. Targeting Micro RNA223 may provide a promising role in suppressing RA.*

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease in which the underlying mechanisms of autoimmunity and the nature of contributing antigens have not been understood [2]. (RA) is the most common autoimmune inflammatory disease with arthritis, its prevalence is up to 1% around world. It has a critical impact on occupational and daily activities, as well as mortality[3]. Measurement of autoantibodies has been important for physicians shared in the treatment of rheumatoid arthritis (RA) patients. Autoantibody analyses are increasingly being prioritized in guidelines nowadays, and because the field is so dynamic, it is important for doctors to be aware of cost-benefit of such testing.[4] For diagnosis and classification of rheumatoid arthritis (RA), measurement of two groups of autoantibodies, rheumatoid factor (RF) and Anti-cyclic citrullinated peptide antibodies (Anti-CCP) should be done and they are very substantial[4].

The pathological picture of rheumatoid arthritis (RA) is characterized by chronic inflammation and progressive joint damage because of lymphocyte infiltration of the synovial membrane. RA is developed as a result of impaired cell death (apoptosis). Because of impaired apoptosis, activated cells in joints as lymphocytes and fibroblast-like synoviocytes (FLS), survive for a long period as well as increased cell infiltration and cell proliferation may be a cause for developing these disease. Endogenous microRNAs (miRNAs) are single-stranded RNAs that are 19–24 nucleotides long and not important for coding [5]. Protein synthesis and translation are inhibited when these posttranscriptional regulators attach to the 3' untranslated region (UTR) of the target gene, destabilizing the mRNA and repressing translation[6].

Although the precise mechanisms leading to RA remain incompletely understood, extensive key suggests that B-cells play a role in its pathogenesis. MiR-223 is a hematopoietic miRNA that is important for B-cell differentiation and development[7]. Several studies have been conducted to comprehend the functions of miR-223 that dictate its cellular and tissue expression. These studies showed that miR-223 expression is altered during the inflammatory response of various cell types, including macrophages, dendritic cells (DCs), granulocytes, T cells, epithelial cells and endothelial cells. This change in miR-223 expression regulates

many roles of the cells and attenuates or exacerbates the tissue inflammation[8].

PATIENTS AND METHODS

Sample size calculation:

MedCalc® version 12.3.0.0 program "Ostend, Belgium" was used for calculations of sample size, statistical calculator based on 95% confidence interval and power of the study 80% with α error 5%**,** According to a previous study [9]**,** showed that the mean of miRNA223 in RA 0.44±0.93 and control group 1.20±1.00, with p-value <0.05 significant. Sample size was calculated according to these values produced a minimal samples size of 64 cases were enough to find such a difference. Assuming a drop-out ratio of 5%, so sample size will be 35 individuals in each group.

Participants:

In this cross-sectional study, 35 consecutive patients who were diagnosed with disease according to the 2010 American College of Rheumatology/European League against Rheumatism Classification Criteria [1] and 35 healthy were enrolled in the study. The patients were recruited during the period from Jan 2023 to Jan 2024 from the Outpatient Clinic of the Rheumatology, Rehabilitation and Physical Medicine Department, Benha University Hospital.

Subjects with history of autoimmune diseases, cardiac diseases, liver diseases, renal diseases, malignancy, and any other chronic diseases were excluded from this study. Written consents were obtained from all subjects and the study was approved by The University Ethics Committee (Ms.19.1.2023) and in accordance with the Declaration of Helsinki 2013.

All subjects underwent thorough medical history taking and thorough physical examination. Clinical assessment of the RA patients included recording of the swollen and tender joint count (SJC and TJC respectively). The disease activity was measured by the Disease Activity Score 28 (DAS28).The disease severity was assessed by Rheumatoid Arthritis Severity Scale (RASS). All patients were subjected to the following investigations: Anticyclic citrullinated antibodies (anti-CCP), (RF), ESR and (CRP).

Measurement of plasma miRNA-223 expression

Total RNA from plasma samples was extracted using RNeasy Mini Kit according to the instructions of the manufacturer instructions. (Thermo Fisher Scientific. cat. No. 217004). The total RNA was reversely transcribed into cDNA using TaqMan reverse transcription kit (Applied Biosystems;Thermo Fisher Scientific cat. No. 217004) according to manufacturer's instructions. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to measure miRNA-223 expression level with HERA SYBR Green qPCR on an ABI Step-one PCR system. We used the 2–ΔΔCt method to measure miRNA-223 expression levels using U6 as the internal reference.

Statistical analysis

IBM-SPSS software version 26.0 was used for statistical analysis. Continuous variables were tested for normality of distribution prior to statistical analysis. Student t-test was used for normally distributed quantitative variables, to compare between two studied groups. Mann Whitney test was used for abnormally distributed quantitative variables, to compare between two studied groups. Analysis of variance (ANOVA or F test): was used for continuous data to test for significant difference between more than two normally distributed groups. Receiver operating characteristic (ROC) curve was used to evaluate the values of miRNA-223 and its validity in predicting RA and activity. For all statistical procedures, significance threshold was set if $P \leq 0.05$.

RESULTS

A non-significant difference was found between the two groups regarding age and gender (p>0.05). According to DASS-28, 3 (8.6%) of RA patients showed low disease-activity, 12 (34.3%) of them had (moderate disease activity), and 20 (57.1%) of patients had (high disease activity). Regarding RASS, the disease activity and functional impairment had median of 4, the median of physical damage was 5 and the median of total score was 40. The most common drug used was steroid in all cases followed by methotrexate in 21 patients (60%) then Hydroxy-chloroquine in 51.4% and Leflunomide in 22.9%. The least drug used was biological drugs in 20% of cases. There was significant increase of TLC, ESR, CRP, RF and anti-CCP in RA patients when compared to control group, as shown in table (1).

Table 1: Demographic, clinical & laboratory data among RA patients and controls.

		RA patients (N=35)	Controls (N=35)	*p*-value
Age (years)	**Median**	45.0	47.0	0.223(NS)
Gender	**Male**	7, 20.0%	10, 28.6%	0.403
	Female	28, 80.0%	25, 71.4%	
Duration of RA (years)	**Median**	5.0		
Duration of morning stiffness (hours)	**Mean± SD**	1.67±0.74		
TJC	**Median**	6.0		
SJC	**Mean± SD**	8.11±3.89		
VAS	**Mean± SD**	5.77±2.03		
Larsen score	**Median**	28.0		
DAS-28	**Low activity (< 3.2)**	3, 8.6%		
	Moderate (3.2-5.1)	12, 34.3%		
	High (> 5.1)	20, 57.1%		
RASS	**Mean± SD**	50.76±17.4		
	Steroids	35, 100.0%		
Drug intake	**Methotrexate**	21, 60.0%		
	Hydroxy-chloroquine	18, 51.4%		
	Leflunomide	8, 22.9%		
	Biological	7, 20.0%		
Hb (g/dL)	**Median (IQR)**	11.4 (11.0- 12.6)	12.2 (11.4- 12.7)	0.176
TLC ($\times 10^9$/L)		8.55 (6.54- 13.56)	6.8 (5.18- 8.30)	0.012
Platelets count ($\times 10^9$/L)		275.0 (232.0- 390.0)	273.0 (230.0- 302.0)	0.434
ESR titer		65.0 (50.0- 92.0)	18.0 (15.0- 21.0)	<0.001
CRP titer		40.0 (26.0- 45.0)	3.5 (2.25 – 9.0)	<0.001
RF titer		64.0 (54.0- 110.0)	9.0 (0.0- 30.0)	<0.001
Anti-CCP titer		65.0 (65.0- 200.0)	20.5 (18.0- 35.0)	0.001

RA: Rheumatoid arthritis, CCP: cyclic citrullinated peptide, CRP C reactive protein, DAS: disease activity score, ESR erythrocyte sedimentation rate, RF: rheumatoid factor, SJC: swollen joint count, TJC: tender joint count, TLC: Leucocytic count, VAS: Visual analogue scale, RASS: Rheumatoid Arthritis Severity Scale, NS: non significant

The mean miRNA 223 expression level was 2.19± 1.28 in RA group and 1.0± 0.02 in control group. There was a statistically significant increase in miRNA 223 expression level in RA group when compared to control group (*P* <0.001), the results is shown in table 2 & Fig. 1.

Table (2): Comparison of miRNA 223 expression level between RA patients and control groups

		RA patients (N=35)	Controls (N=35)	*p*-value
miRNA 223 (RQ)	Mean± SD	2.19± 1.28	1.0± 0.02	<0.001
	Range	0.03- 4.51	1.0- 1.07	

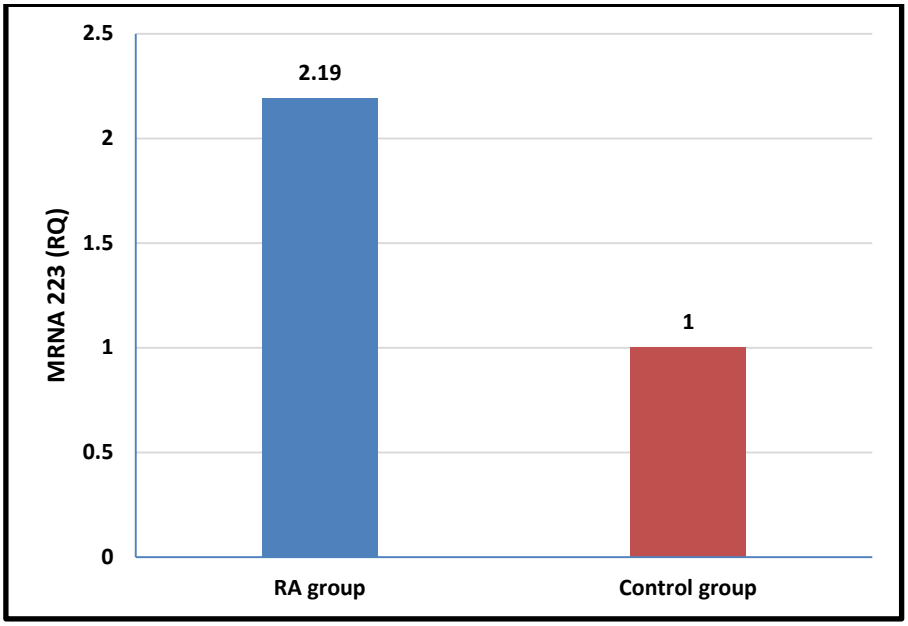

Fig.1: Bar-chart showing comparison between RA group and control group groups regarding expression level.

The mean miRNA 223 level with low activity was 0.47, in moderate activity it was 2.05 and it was 2.53 in patients with high grade. There was a statistically significant relation between serum miRNA 223 (RQ) level with DAS-28 among RA group (***p***= 0.026) as it was significantly higher in patients with high grade compared to patients with low activity (**Fig. 2).**

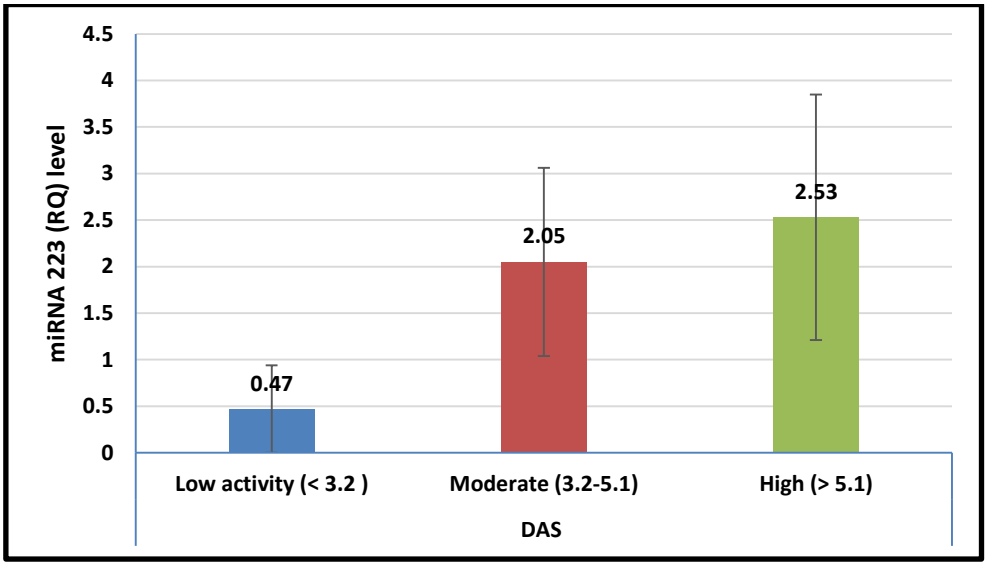

Fig.2: Relation between serum miRNA 223 (RQ) level with DAS among RA group**.**

There was a significant positive correlation between serum miRNA 223 (RQ) level and morning stiffness (r=0.389, *P* =0.021), TJC (r=0.399, *P*=0.018), SJC (r=0.506, *P*=0.002). In addition, there was significant positive (+) correlation between serum miRNA 223 (RQ) level and DAS-28 (r=0.434, *P*=0.009), functional impairment (r=0.036, *P* =0.021), physical damage (r=0.545, *P* =0.001), total RASS (r=0.469, *P* =0.004), ESR (r=0.442, *P* =0.008) and Larsen score (r=0.429, *P* =0.01) as shown in **Fig. 3.**

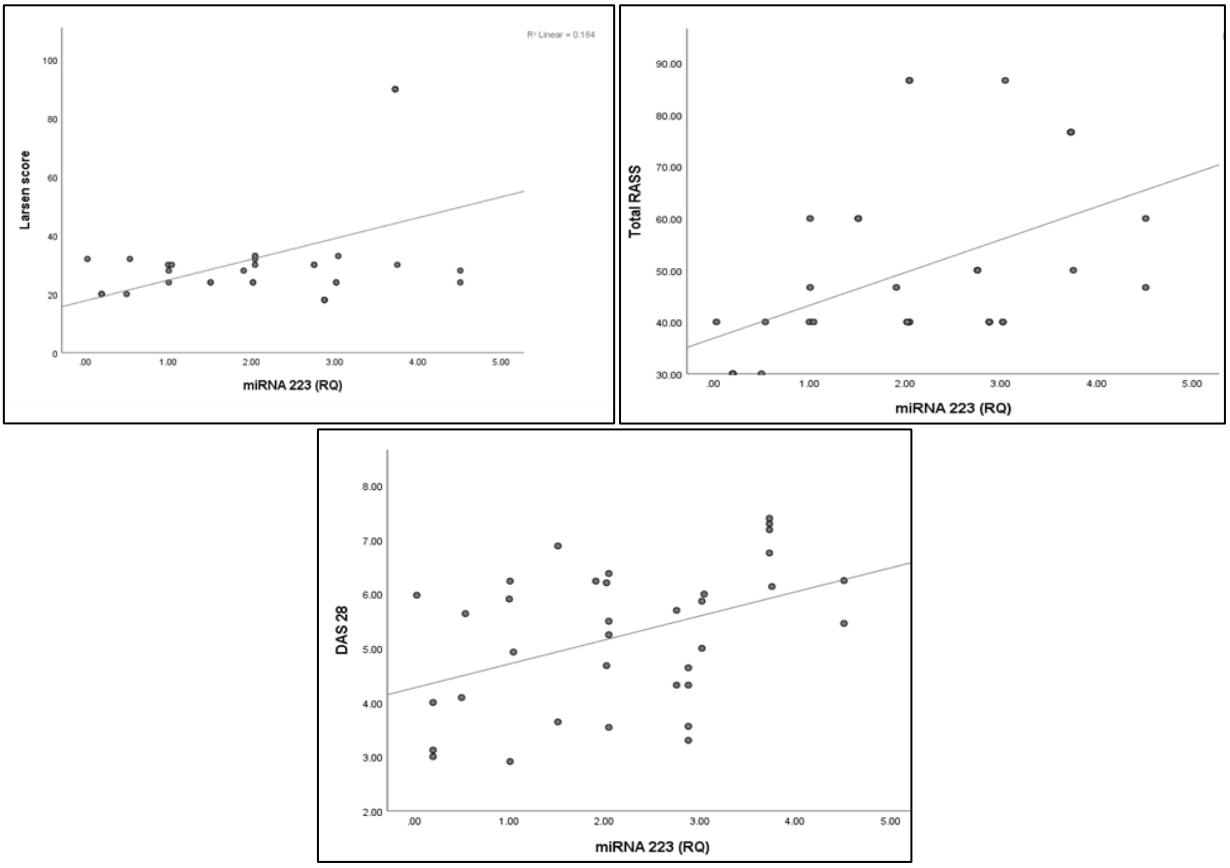

Fig.3: Scatter plot showing significant positive correlation between serum miRNA 223 (RQ) level and different parameters. (DAS 28, Total RASS and Larsen score.).

There was no statistically significant relation between serum miRNA 223 (RQ) level with gender among RA group (P >0.05). Serum miRNA 223 (RQ) level was significantly higher in diabetic patients compared to patients without (*P*=0.004). While no significant relation was found between serum miRNA 223 (RQ) level with hypertension, obesity and cardiac diseases among RA group (*P*>0.05).

By using ROC-curve analysis, Serum miRNA 223 (RQ) level can significantly (p<0.001) differentiates RA patients from controls with 80% sensitivity, 91.4% specificity, 90.3% positive (+) predictive value and 82% negative (-) predictive value when the cutoff point was >1 with AUC was 0.807 and 95% CI of 0.695 - 0.892 as shown in **Fig.4**.

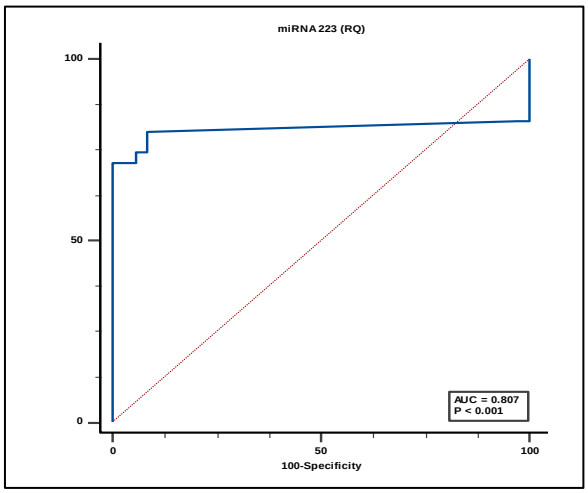

Fig.4: ROC curve for the performance of Serum miRNA 223 (RQ) level in detection of RA.

DISCUSSION

In the present study, there was a high statistically significant increase in miRNA 223 in RA group when compared to control group (P <0.001) This was in agreement with results of previous studies [7] that revealed that miR-223 was differentially expressed in sera of RA patients and was a positive predictor of RA diagnosis, implicating miR-223 as potential biomarker of RA as the tested miRNA223 was positively correlated in RA, suggesting their concomitant dysregulation in RA, and this concurs with Khalifa et al[12] ,who found a tendency to overexpression of miR-223 in RA patients. And Filková et al[9], who reported that miR-223 was increased in synovial tissue of RA patients. However, Villigas et al[13] found that plasma miR-223 expression in RA patients did not differ from controls.

There was a high statistically significant association between serum miRNA 223 (RQ) level with DAS-28 among RA group (p= 0.026). This was in aggrement with results of previous studies of Evangelatos et al[12] who found that serum miR-223 levels have been related to disease relapse and RA activity. On contrary this result did not agree with the result of Andonian et al[11] who revealed that plasma expression of miR-223 in RA patients did not associate with RA disease activity or inflammation. And also was not in agreement with result of Khalifa et al[10] who found no change between healthy subjects and patients with non-active RA.

In the present study, There was a significant positive (+) correlation between serum miRNA 223 (RQ) level and DAS-28 (r=0.434, P=0.009), however Khalifa et al[10] revealed that no correlation was found between miR-223 expression and biological parameters of RA patients such as DAS28. Similarly, Andonian et al[11] reported that miR-223 in plasma was not correlated to inflammatory markers or disease activity in RA patients, and this concurs with Filková et al[9] who found that level of miR-223 that circulating in plasma of RA patients was correlated with DAS28, and was used as predictor for disease prognosis (outcome) and also as a marker of disease activity**.** This also was in agreement with Castro-Villegas et al[13] who found that the changes observed in miRNA223 significantly correlated with the changes observed in clinical parameters (that is, DAS28), and with changes in inflammatory parameters such as CRP or ESR.

In the present study, there was a significant positive correlation between serum miRNA 223 (RQ) level and total RASS (r=0.469, P =0.004) and this was in agreement with Taha et al[7] who illustrated an increasing of serum miRNA-223 in RA patients that was characterized by the presence of extra articular manifestations as subcutaneous nodules, linking this miRNA to RA severity and progresion. Also Filková et al[9] who reported that silencing of miR-223 reduced disease severity in experimental arthritis. Zhang et al[14] demonstrated that suppression of miR-223 could decrease disease severity so for management of disease we should inhibit miR-223.

In the present study, no correlation was found between miR-223 expression and some of parameters of RA patients like CRP, anti-CCP and RF. This was in line with results of previous studies that revealed no correlation between miR-223 expression and biological parameters of RA patients such as CRP, anti-CCP and RF[10].

In the present study, By using ROC-curve analysis, Serum miRNA 223 (RQ) level can significantly determine RA patients from controls with high sensitivity and specificity was 80% and 91.4% when the cutoff point was >1 with AUC was 0.807. This concurs with results of previous studies that illustrated that serum miR-223 differentiate RA patients from controls with AUC = 0.85[7].

CONCLUSION

Serum Micro RNA223 level was significantly elevated in RA patients which clarifies its possible role in RA pathogenesis and correlates positively with disease severity and DAS-28. Targeting Micro RNA223 may give us a promising role in suppressing RA.

The article had not been published or under consideration by another journal or any other reviewed media. No financial or non-financial conflict of interest have been declared by authors. All authors participated equally to the manuscript and approved the version submitted.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

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

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