(Research Article)

Assessment of MicroRNA (96) and MicroRNA (298) as Biomarkers for Diagnosis and Prognosis of Rheumatoid Arthritis in Egyptian Patients

Gamil Mohamed Abdallah [1,2], Sara Mohamed Sayed [3], Hany Mohamed Aly [4], Mohamed Basiouny Yahia[5] and Nada Raafat Mohamed [2]*

[1] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Boys), Al Azhar University, Cairo, Egypt.

[2] Department of Biochemistry, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt.

[3] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Girls), Al Azhar University, Cairo, Egypt.

[4] Department of Rheumatology and Rehabilitation, Faculty of medicine (Boys), Al Azhar University, Cairo, Egypt.

[5] Department of Clinical Pathology, Faculty of medicine (Boys), Al Azhar University, Cairo, Egypt.

*Correspondence: nada_raafat@eru.edu.eg

Abstract: Rheumatoid arthritis (RA) is a chronic symmetric polyarticular arthritic disease characterized by symptoms of inflammation and pain in the joints .It was reported that some microRNAs (miRs, miRNAs) are markedly dysregulated in RA. So, this study aimed to identify the expression pattern of miR-96-5p and miR-298 as diagnostic biomarkers for RA and to see how patients with early RA respond to therapy based on that pattern. This study was conducted on 120 individuals enrolled in the outpatient clinic of El Hussein University Hospital. They were divided into three groups; early diagnosed (ERA) patients, methotrexate (MTX) -treated RA patients for 6 months, and healthy controls (HC). Total RNA was extracted from entire sera and the expression of miR-96-5p and miR-298 was analyzed by Real-Time Quantitative polymerase chain reaction (qRT-PCR). Receiver operating characteristic (ROC) curve analyses were conducted for miR-96-5p and miR-298 in the diagnosis of RA and the prediction of therapeutic efficacy in MTX -treated RA patients. In the current study, we reported that miR-96-5p and miR-298 levels were significantly higher in ERA patients in comparison with healthy participants. And interestingly, both of them were down-regulated significantly in MTX-treated RA patients when compared with ERA patients group. Moreover, the study illustrated that they could be used as a RA biomarker for the diagnosis and also for the prediction of therapeutic efficacy in MTX -treated RA patients.

Key Words: Rheumatoid arthritis, miR-96-5p, miR-298, methotrexate, miRNA analyses, RA diagnosis.

1.INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory illness that affects roughly 1% of the global population and 0.29% of Egyptians. It is associated with persistent inflammation and synovium hyperplasia, which can lead to severe joint deterioration and joint abnormalities if not treated correctly[1,2]. The etiology of this disease is still unclear[3]. A combination of genetic predisposition (shared epi-tope, Human Leucocyte Antigen-DR isotype (HLA-DR) genes, family genetic predisposition) and several environmental and lifestyle factors (viruses, mucosal inflammation, bacteria, smoking, periodontal as well as lung diseases) plays a crucial role in the development of RA[4,5]. Surprisingly, the treatment of RA has shifted significantly towards biological response modifiers in recent years, albeit the first therapeutic option remains the traditional disease-modifying anti-rheumatic drugs (DMARD)[6]. Methotrexate (MTX) is the most often used first-line DMARD for RA treatment because of its high efficacy, low cost, early onset of action, and convenience of administration[7,8]. However, in ~30–40% of patients treated with MTX, disease activity is not adequately controlled[9]. As a result, other RA treatments are still required for the huge number of patients who do not respond effectively to conventional or biological DMARDs[10]. A recent study has conclusively proven the relevance of epigenetic dysregulation, notably in the expression

profile of microRNA (miRNA), in immunological dysregulation that participates in the etiology of RA[11]. MicroRNAs (miRs, miRNAs) are a type of non-coding RNA with a length of about 21 nucleotides[12]. MiRNAs identify target genes' mRNA by complementary pairing with their 3'-UTR in mammalian cells and then contribute to mRNA degradation or translational inhibition[13]. Numerous studies have found that miRNAs have a crucial role in a variety of disorders, including cancer, virus infection, inflammation, cardiac and metabolic diseases[6,14]. Additionally, They have been shown to influence the development of all aspects of the immune system, and are presently being utilized to predict RA patients' responsiveness to biological therapy[7]. Previous studies have reported miR-96 and miR-298 to have a role in autoimmune response[15,16]. It was found that miR-96 is responsible for regulating T cell activation[17]. Besides, its role in Pro-inflammatory proteins such as interleukin 6 (IL-6), IL-10, and tumor necrosis factor alpha (TNF-α) elevation[18]. MicroRNA-298 also coupled with IL-1β, TNF-α and interferon gamma (IFN-γ) regulation [19]. To date, there are no studies about miR-96 and miR-298 expressions in RA patients and their predictive capabilities on MTX therapy response. Therefore, we aimed to determine whether miR-96-5p and miR-298 may be used as RA biomarkers for the diagnosis and the prediction of MTX efficacy in patients with RA.

2. METHODS

2.1 Clinical Data Collection

Forty early diagnosed patients with RA and sixty MTX-treated RA patients for 6 months without taking any other DMARD with ages ranging from (30-70) years fulfilling (American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 classification criteria)[20] were included in the study. The control group comprised of 20 healthy people who were age and sex-matched. Rheumatoid arthritis patients with any other inflammatory autoimmune disease were excluded from the study. Clinical information about the condition, physician and patient assessments, the health assessment questionnaire (HAQ), and the 28-joint disease activity score (DAS28) were all determined at baseline (BL). Serum samples were collected for miRNA analysis. RA patients were enrolled in the outpatient clinic of El Hussein University Hospital, from October 2020 to September 2021.

2.2 Serum Samples Collection

The Direct venous puncture was used to draw whole blood from the participants, the serum supernatant was isolated and then kept at -80°C. Erythrocyte sedimentation rate (ESR) was determined using Westergren method[21]. A rheumatoid factor of IgG, IgM and IgA isotypes was quantitatively measured using ELISA kit (provided by ORGENTEC Diagnostica GmbH, Mainz, Germany) (catalog no. ORG522G, ORG522M and ORG522A, respectively) according to ***Ernst et al., (1988)***[22]

Anti-cyclic citrullinated peptide antibodies (Anti-CCP) were quantitatively measured using third-generation assays (Quanta Lite® CCP3 IgG ELISA; Inova Diagnostics, San Diego, CA, USA)) (catalog no.704535) according to ***Bizzaro et al., (2001)***[23]. Positive anti-CCP titers were considered at a concentration of >20 U/mL. Quantitative Serum high sensitive C reactive protein CRP (hs-CRP) levels were measured by ELISA using (Accu-Bind™ ELISA Microplate Test System, Monobind Inc., USA) (catalog no.3125-300) according to ***Kindmark (1972)***[24].

2.3 miRNA Analyses

2.3.1 Serum RNA Isolation

QIAzol lysis reagent (Qiagen®) and a set of miRNeasy Mini Kits were used to extract total serum RNA from 5ml volumes of serum (217004; Qiagen®, Hilden, Germany) according to the manufacturer's instructions. Residual The RNase-Free DNase Set (Qiagen®) was used to eliminate any remaining DNA contamination, as directed by the manufacturer. The NanoDrop® spectrophotometer (Thermo Scientific®) was used to determine the concentration and purity of RNA.

2.3.2 miRNA quantification using RT-qPCR

Using a miRCURY LNA reverse transcription kit, whole RNA was reverse transcribed into cDNA (Qiagen®, Germany). Amplification of miR-96-5p and miR-298 was conducted using a miRCURY SYBR® Green PCR Kit with miRCURY LNA miRNA Primer assays (Qiagen®, Germany). We used Readymade primers from (Qiagen®). The primer sequences were listed as follows:
hsa-miR-96-5p (assay ID: YP00204417)
Mature miRNA sequence: UUUGGCACUAGCACAUUUUUGCU
miRNA sequence (pre-miR): UGGCCGAUUUUGGCACUAGCACAUUUUUG CUUGUGUCUCUCCGCUCUGAGCAAUCAUG UGCAGUGCCAAUAUGGGAAA

hsa-miR-298 (assay ID: YP00204115)
Mature miRNA sequence: AGCAGAAGCAGGGAGGUUCUCCCA

miRNA sequence (pre-miR):
UCAGGUCUUCAGCAGAAGCAGGGAGGUUC
UCCCAGUGGUUUUCCUUGACUGUGAGGAA
CUAGCCUGCUGCUGCUUUGCUCAGGAGUG
AGCU
Normalized by U6 snRNA (assay ID: YP00203907)
Mature miRNA sequence:
GUGCUCGCUUCGGCAGCACAUAUACUAAA
AUUGGAACGAUACAGAGAAGAUUAGCAUG
GCCCCUGCGCAAGGAUGACACGCAAAUUC
GUGAAGCGUUCCAUAUUUUU; The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression.

2.4 Statistical Analyses

IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2015) was utilized to perform statistical analysis on the data. GraphPad Prism 7 (Softhead, Inc.) was utilized to plot figures. The data were represented as the mean ± standard error (SE). The following tests were done: Independent-samples t test for comparison between two independent groups for parametric data. One-way ANOVA test to compare more than two patient groups. Pearson correlation test to study the possible correlation between each two variables among each group for parametric data.

P <0.05 was used to determine whether differences were significant. Receiver operating characteristic (ROC) curve analyses, plotting the true positive rate (sensitivity) vs. the false positive rate (1-specificity) at various threshold settings were performed for serum miRNAs for the diagnosis of RA and also for the prediction of therapeutic efficacy in in MTX -treated RA patients. SPSS was used to calculate the areas under the curve (AUCs). We regarded an AUC-ROC of 0.7 to be only marginally indicative of therapy success, 0.7-0.8 to be moderately predictive, and > 0.8 to be highly predictive.

3. RESULTS

3.1 Patient's characteristics

Table 1 shows the clinical, demographic, and laboratory data for all of the participants in this study. All groups were age- and sex matched.

3.2 Expression of miR-96 and miR-298 in patients with early-diagnosed RA

Real-time PCR study of miR-96 and miR-298 expression in the sera of patients with early diagnosed- RA (ERA) was undertaken to assess their role in RA. Interestingly, the level of them was observed to be significantly higher in the patients

Table 1. Parameters for early diagnosed RA, MTX-treated patients and healthy controls.

Variable	Control (n=20)	Early Diagnosis (n=40)	Treated (n=60)	P-value
Gender (female/male)	18/2	36/4	54/6	0.22
Age (years)	50.45±2.5	47.9±1.7	43.3±1.3	0.27
ESR (mm/hr)	8.9±0.8	70.8±5 [a]	33.9±2.3 [a,b]	< 0.001
hs-CRP (mg/L)	6.8±0.3	55.33±5 [a]	18.25±1.9 [a,b]	< 0.001
RF (U/mL)	8.9±1	84.3±7 [a]	54.9±5.8 [a,b]	< 0.001
RF-IgG (U/mL)	15.25±0.75	75.14±1.7 [a]	34.84±2.6 [a,b]	< 0.001
RF-IgM (U/mL)	7.33±0.36	71.44±0.87 [a]	39.63±2.09 [a,b]	< 0.001
RF-IgA (U/mL)	7.1±0.5	63.9±2.4 [a]	29.6±1.8 [a,b]	< 0.001
Anti-CCP (U/mL)	4.1±0.1	77.8±6.3 [a]	44.6±4.2 [a,b]	< 0.001
TJC	0	11.1±0.3 [a]	3.6±0.4 [a,b]	< 0.001
SJC	0	8.5±0.3 [a]	2.8 ±0.4 [a,b]	< 0.001
DAS28-ESR	0	5.7±0.08 [a]	3.7±0.18 [a,b]	<0.001

The data are represented as the mean±SE. a: Significant difference compared to controls. b: Significant difference compared to early diagnosed RA patients' group.
ESR: erythrocyte sedimentation rate; hs-CRP: high-sensitivity C-reactive protein; RF: rheumatoid factor; Anti-CCP: anti-cyclic citrullinated peptide antibodies; TJC: tender joint count; SJC: swollen joint count; DAS28: The 28-joint disease activity score.

sera with ERA (2.02 ± 0.1) vs (4.1 ± 0.47) respectively compared with healthy controls, (P < 0.05) vs (P<0.001) respectively as in fig (2). ROC analysis was conducted for further investigation to see if the expression of miR-96 and miR-298 might be used as a potential RA diagnostic biomarker. We found that the AUC for miRNA-96-5p was 0.82, with a 100% sensitivity and 75% specificity at the best cutoff, as well as AUC was 0.94 for miR-298, with a 90% sensitivity and 95% specificity at the best cutoff as shown in table (2) and Fig (3).

This means that the miR-96-5p & miR-298 levels were significant diagnostic biomarkers for RA. Furthermore, miR-298 expression was correlated with DAS28 (P< 0.01) (Table 4), demonstrating that its expression was correlated with disease activity.

3.3 MiR-96 & MiR-298 levels in methotrexate treated group.
Next, we investigated to see if both miR-96 and miR-298 could predict MTX therapy response in RA patients.
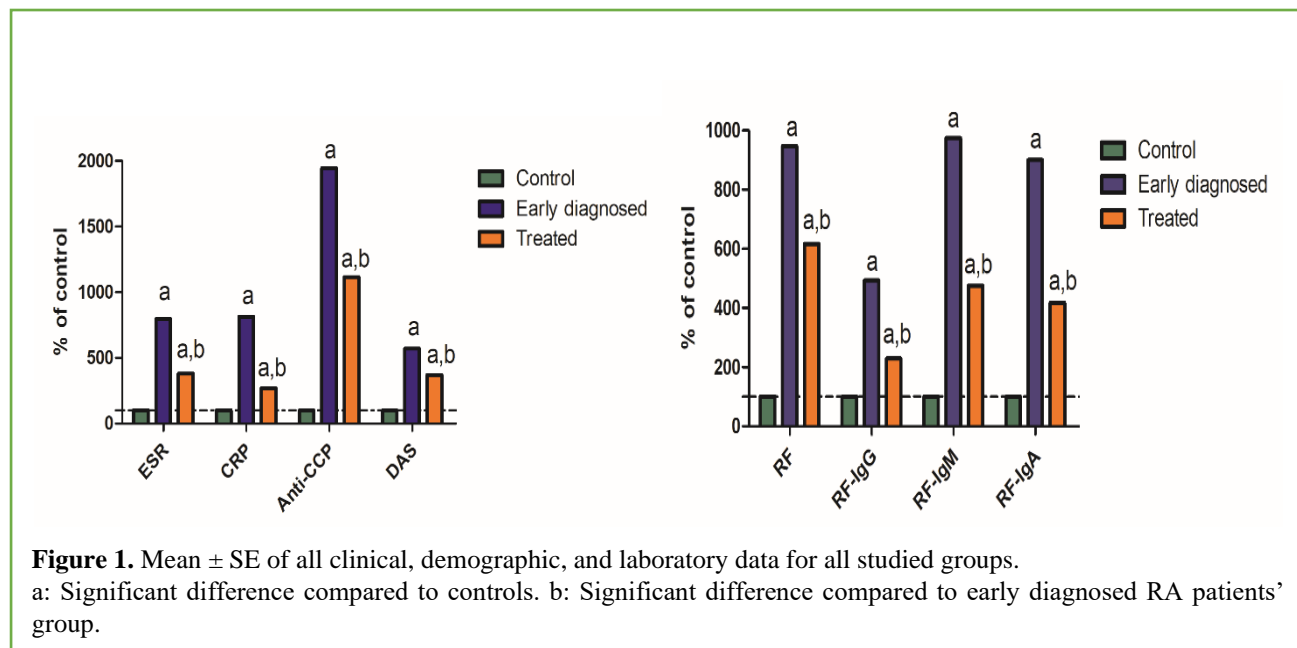

Figure 1. Mean ± SE of all clinical, demographic, and laboratory data for all studied groups.
a: Significant difference compared to controls. b: Significant difference compared to early diagnosed RA patients' group.

Table 2. ROC curve of serum miR-96-5p and miR-298 gene expression for discrimination between ERA patients and controls.

Markers	AUC	Sensitivity	Specificity	Best cutoff value
miR-96-5p	0.82	100%	75%	0.4
miR-298	0.94	90%	95%	1.8

Table 3. ROC curve of serum miR-96-5p and miR-298 gene expression for discrimination between ERA patients and treated group.

Markers	AUC	Sensitivity	Specificity	Best cutoff value
MiR-96-5p	0.78	90%	75%	1.17
MiR-298	0.76	90%	61.7%	1.9

As demonstrated in Table 1, MTX-treated RA patients had a better prognosis (RF, Anti-CCP, CRP, and ESR were all lower) than RA patients who did not get treatment. Particularly, levels of miR-96 & miR-298 were significantly down-regulated in MTX-treated RA patients as a value of (1.2 ± 0.23) vs (2.3 ± 0.38) respectively, $(P<0.05)$ vs $(P<0.01)$ respectively (Fig. **2**).

ROC studies backed up those findings which exhibited that miR-96 in MTX-treated RA patients,

with 1.17 cutoff value and 0.78 AUC value, with 90% sensitivity, and 75% specificity, and for miR-298, with 1.9 cutoff value and 0.75 AUC, with 90% sensitivity and 61.7% specificity implying a moderate prediction of both of them in response to MTX therapy as shown in table (3) and Fig (**4**). Additionally, miR-298 expression was correlated with DAS28 $(P< 0.01)$ (Table 5), indicating that its expression was correlated with disease activity.
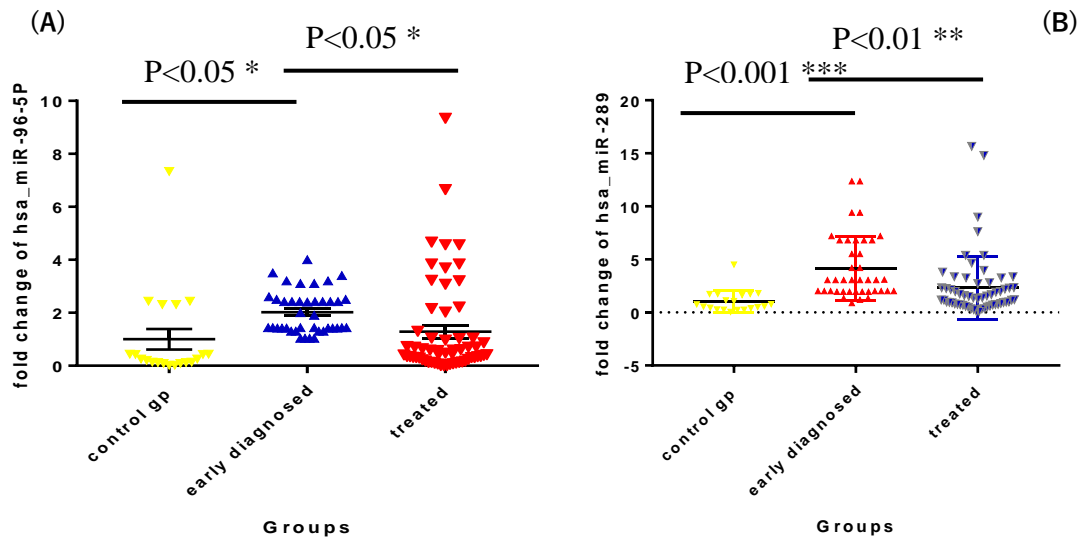

Figure 2. The expression of miR-96 and miR-298 was upregulated in early diagnosed-RA patients when compared to healthy control. After MTX treatment their expression showed downregulation.
Statistical significance at *: *P<0.05*; **: *P<0.01*; ***: *P<0.001*.
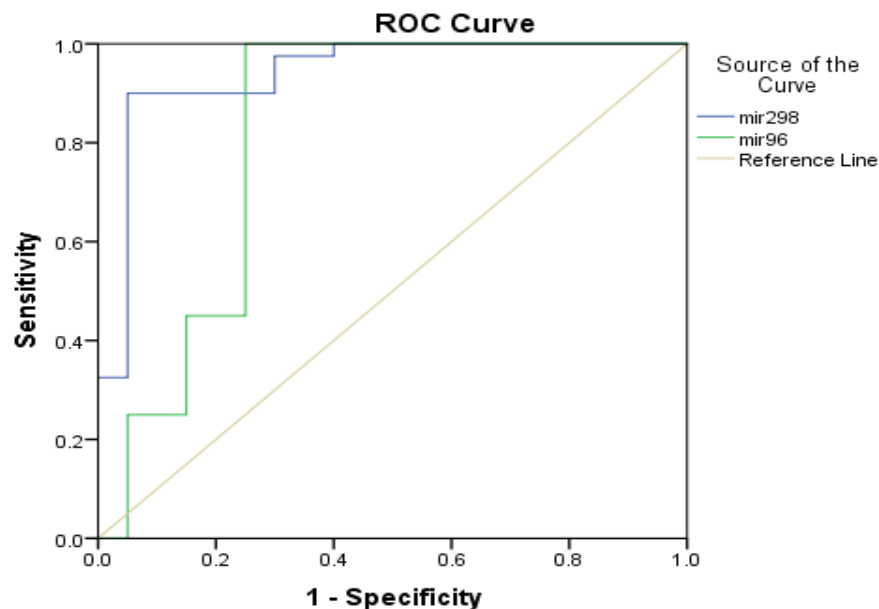

Figure 3. ROC curve analysis showing the diagnostic performance of has- miR-298 and has- miR-96-5p for discriminating early diagnosed from those control.
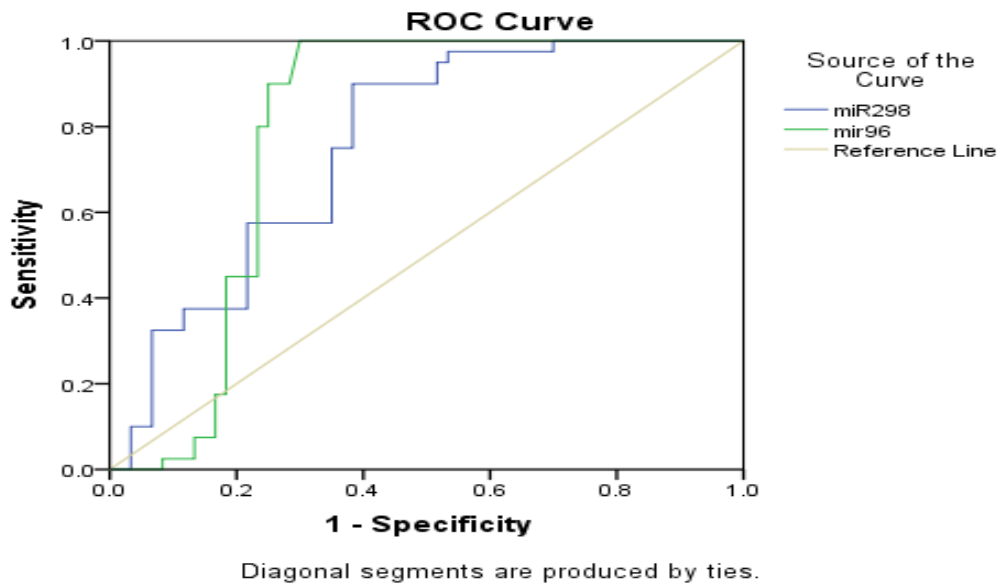
ROC Curve

Figure 4. ROC curve analysis showing the prognostic performance of has- miR-298 and has- miR-96-5p for discriminating early diagnosed from those treated.

4.4 Correlation between serum miR-96, miR-298 and other laboratory data

Table 4. Pearson correlations (r) between serum miRNAs and clinical and laboratory data in early diagnosed RA patients.

	hsa-miR.96-5p		hsa-miR-298	
	R	P	R	P
ESR	0.25	0.12	0.7	0.000***
CRP	0.03	0.84	0.22	0.17
RF	0.56	0.000***	0.03	0.83
RF-IgG	0.46	0.002**	0.14	0.36
RF-IgM	0.38	0.015*	0.11	0.47
RF-IgA	0.25	0.11	0.3	0.05
Anti-CCP	0.15	0.35	0.8	0.000***
TJC	0.38	0.01*	0.5	0.001**
SJC	0.14	0.38	0.54	0.000***
DAS	0.28	0.08	0.7	0.000***

Statistical significance at *: *P<0.05*; **: *P<0.01*; ***: *P<0.001*.

This table is showing a correlation between miR-96 and miR-298 biomarkers and other parameters, in early diagnosed RA patients, miR-96 was positively correlated to RF and its isotypes (RF-IgG & RF-IgM) and TJC, with no correlation to other parameters, but there was a strong positive correlation between miR-298 and ESR, Anti-CCP, TJC, SJC and DAS.

Table 5. Pearson correlations (r) between serum miRNAs and clinical and laboratory data in RA patients treated with MTX.

clinical and laboratory data in RA	hsa-miR.96-5p		hsa-miR-298	
	R	P	R	P
ESR	0.2	0.09	0.69	0.000***
CRP	0.07	0.57	0.27	0.04*
RF	0.84	0.000***	0.09	0.49
RF-IgG	0.77	0.000***	0.17	0.19
RF-IgM	0.71	0.000***	0.2	0.11
RF-IgA	0.67	0.000***	0.21	0.1
Anti-CCP	0.1	0.4	0.77	0.000***
TJC	0.06	0.64	0.4	0.001**
SJC	0.07	0.59	0.38	0.002**
DAS	0.15	0.26	0.48	0.004**

Statistical significance at *: *P<0.05*; **: *P<0.01*; ***: *P<0.001*.

In MTX-treated RA patients also miR-96 was positively correlated to RF and its isotypes (RF-IgG, RF-IgM &RF-IgA) while miR-298 was positively correlated to ESR, CRP, Anti-CCP, TJC, SJC and DAS.

4. DISCUSSION

Rheumatoid arthritis (RA) is a chronic inflammatory illness of the joint[25]. So current study on RA epigenetics has mostly emphasized on miRNA abnormalities in RA, elucidates many aspects of RA pathogenesis, introduces novel biomarkers for RA diagnosis and therapy response prediction, and opens the door to the development of new, customized drugs for RA patients[26]. The miRNA network's significance in the pathogenesis of autoimmune diseases like RA[27], Sjogren's syndrome, systemic lupus erythematosus, and ulcerative colitis is being studied more and more[28]. To date, there has never been any evidence of a direct link between RA and miR-96 and miR-298; hence, our work has demonstrated both miR-96 & miR-298 as a prospective option for further investigation for RA diagnosis and prediction of MTX efficacy in RA patients.

When compared to a healthy control group in our study, miR-96-5p was found to be markedly increased in the serum of patients with early-stage RA. This result is in line with *Ma et al.* who reported that miR-96 overexpression stimulates the differentiation of osteoblast and bone production in Ankylosing spondylitis through activation of the wingless-int (wnt) signaling pathway[18]. Furthermore, miR-96 has been shown to promote osteogenic differentiation in osteoblastic cells[29]. Also, it has been shown to be upregulated in serum samples from individuals with the autoimmune thyroid disorders Hashimoto thyroiditis and Graves' disease[15]. The

main question is how serum miR-96-5p relates to RA. The Forkhead Box O (FoxO1) pathway was revealed to be the major pathway implicated. As reported from previous studies FOXO1 is a target gene of miR-96-5p in many diseases like Alzheimer's disease[30], papillary thyroid carcinoma[31], gastric cancer[32] and in aging humans[33]. Furthermore, increasing expression of miR-183-96-182 cluster repressed Foxo1 expression and promoted pathogenic cytokine expression of T helper 17 (Th17) cells in autoimmune diseases[34,35]. And it was proven that Th17 cells and their effector molecules such as IL-17A, IL-17F, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor have been implicated in the pathogenesis of RA[36,37]. Also, during the early stages of the illness, interactions between Th17 cells and other immune cells or stromal cells located in synovial tissue may result in persistent inflammation, irreversible cartilage degeneration, and bone erosion[38]. Furthermore, as part of a complex network of cytokines, the pro-inflammatory cytokine IL-17 plays a significant role in the pathogenesis of RA by acting on osteoclast and osteoblast-related pathways[39,40]. As a consequence, we expected that the increase of miR-96-5p expression in ERA patients in our study may have a negative effect on FOXO1 expression, leading to disease progression via increased Th17 cell expression.

To the best of our knowledge, this study is the first to reveal the influence of MTX treatment on serum miR-96 levels. When analyzing miR-96

expression in MTX treated RA patients, it was found to be considerably decreased. Interestingly, one of MTX's main mechanisms is to increase adenosine synthesis[41]. Moreover, adenosine signaling affects different types of inflammatory cells through the A2A receptor[42]. Also, *Friedman et al.* discovered that adenosine A2AR stimulation promotes FoxO1 and FoxO3 activation and nuclear localization[43]. Finally, we conclude that MTX treatment in RA might lead to miR-96 downregulation that led to FOXO1 stimulation. This data is also supported by *Garg et al.* who discovered that MTX-loaded NLCs decreased inflammation and caused apoptosis via the NF-kB and FOXO1 pathways in rheumatoid arthritis cells[44].

Regarding miR-298, we also found that miR-298 was overexpressed in ERA patients when compared to healthy control. And that is compatible with *Dudics et al.* who demonstrated that expression of miR-298 has been observed to be enhanced during TNF-α-stimulated macrophage activation[16,45]. Additionally, it has been demonstrated that its expression is higher in lupus patients than in controls[16]. In terms of miR-298 explanation, earlier research has shown that Wnt signaling is important in maintaining the equilibrium of the osteoblast-osteoclast axis in RA pathogenesis[46]. In addition, *Miao et al.* proved that Wnt signaling is inhibited in osteoblasts over RA progression[47]. This backs with the findings of *Shi et al.*, who found that inhibiting the wnt signaling pathway promotes bone erosion and increases the catabolic model in the RA patients' bone loss process[48]. In our study, we hypothesize that miR-298 upregulation might inhibit the differentiation and function of osteoblast and this contributes to the pathogenesis of arthritic bone loss in RA through wnt signaling inhibition. As it was proven from previous studies that miR-298 correlates negatively with its target gene polycomb protein enhancer of zeste2 *(EZH2)* in ovarian cancer[12,49]. As well, the authors identified secreted frizzled-related protein 1 (SFRP1) which is a wnt signaling antagonist as a target gene of *EZH2* in rheumatoid arthritis synovial fibroblasts and found that *EZH2* silencing results in a significant increase in SFRP1 mRNA[50,51]. Besides wnt signaling inhibition, SFRP overexpression may also cause a marked increase of Th17 cells in RA synovial fluid[40,52]. So, we conclude that miR-298 might lead to wnt signaling inhibition and increasing Th17 cells expression through negatively targeting *(EZH2)* gene that leads to SFRP (Wnt signaling inhibitor) overexpression.

There is no previous study has looked at the changes in serum miR-298 profiles in RA patients after treatment with MTX. The expression of miR-298 was found to be considerably decreased in RA

patients getting MTX according to our findings. it was discovered that adenosine A2AR stimulation that results from MTX treatment activates the wnt signaling pathway in dermal fibrosis[53]. Also, *Kim et al.*[54] suggested that the Wnt/β-catenin signaling pathway was stimulated by adenosine via adenosine receptor activation. So MTX treatment might help in wnt signaling pathway regulation through adenosine signaling pathway. Based on our findings, this study is the first to assess miR-96 and miR298 as potential biomarkers for RA diagnosis. As well, they appear to be moderate predictors for MTX therapy response.

However, the current study has certain limitations: (1) The sample size in this study is modest, and a bigger sample size investigation is required to corroborate the current experimental results; (2) The object of our study is only Egyptian, and there are no foreigners. (3) The mechanism of miR-96-5p & miR-298 in rheumatoid arthritis remains poorly understood and needs further investigation. Therefore, we suggest that further studies are needed to support our findings.

5. CONCLUSIONS

In summary, our data show for the first time that miR-96 and miR-298 might be used as novel powerful biomarkers for RA diagnosis. furthermore, they act as moderate predictors of response to MTX therapy. Our findings also shed light on the role of FOXO1 and wnt signaling pathways on miR-96 and miR-298 respectively but this needs further studies to confirm.

Conflicts of Interest: The authors report no conflict of interest.

Ethical Statement: The present work was approved by the Research Ethical Committee of the Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt (REC number: 241).

Author Contribution: This work was carried out in collaboration between all authors.

Nada R. Mohamed: methodology, formal analysis and writing the manuscript; **Gamil.M. Abdallah:** Supervision, editing the manuscript; **Sara. M. Sayed:** data analysis, visualization, editing manuscript, and gaining ethical approval. **Hany M. Aly:** clinical diagnosis of RA patients; **Mohamed. B. Yahia:** the practical part and data curation. All authors have read and approved the final manuscript.

List of Abbreviations: RA: rheumatoid arthritis; miRNA-96: microRNA-96; miRNA-298: microRNA-298; ERA: early diagnosed rheumatoid arthritis; MTX: methotrexate; HC: healthy controls; qRT-PCR: Real-Time Quantitative polymerase chain reaction; ROC: Receiver operating characteristics; HLA-DR: Human Leucocyte Antigen-DR isotype; DMARD: disease-modifying anti-rheumatic drugs; 3'-UTR: the three prime untranslated region; miRNAs, miRs : MicroRNAs; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon gamma; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; HAQ: health assessment questionnaire; DAS28: The 28-joint disease activity score; BL: baseline; ESR: erythrocyte sedimentation rate; Anti-CCP: anti-cyclic citrullinated peptide antibodies; hs-CRP: high-sensitivity C-reactive protein; SPSS: Statistical package for social science; SE: Standard error; AUC: Area under the curve; RF: rheumatoid factor; TJC: tender joint count; SJC: swollen joint count; mRNA: Messenger RNA; WNT: wingless-int; FOXO: The Forkhead Box O; Th17: T helper 17 cells; IL: interleukin; EZH2: polycomb protein enhancer of zeste2; SFRP1: secreted frizzled-related protein 1; NLC: nanostructured lipid carrier; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells.

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