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MicroRNA-5196 as an Accurate Biomarker of Disease Activity and Response to Treatment in Rheumatoid Arthritis

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Abstract

Objective: To investigate the association between serum expression levels of miRNA-5196 and rheumatoid arthritis (RA), particularly its potential as a biomarker for disease progression and response to TNF-α inhibitor therapy.

Methods: One hundred patients divided into two groups' 50 responders and 50 non responders with 50 healthy individuals were studied. The serum expression ofmiRNA-5196 was detected by using real-time polymerase chain reaction.

Results: miRNA-5196 expression was significantly higher in rheumatoid arthritis patients versus control group P <0.05. Sub-group of patients into responders and non-responders showed that miRNA-5196 expression higher in non-responders than responders. miRNA-5196 expression related with response to TNF-α inhibitor and disease activity in patients with rheumatoid arthritis.

Conclusion: miRNA-5196 may consider as a valid target for monitoring progression of RA disease and response to TNF-α inhibitor therapy.

Keywords: miRNA-5196, rheumatoid arthritis, TNF inhibitor, RA

Introduction

Progress of the rheumatoid arthritis (RA) often starts years before the beginning of symptoms with the development of certain autoantibodies, such as anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF).[1]

The epigenetic components have been linked to RA. MiRNAs negatively regulate gene expression of target messenger RNA (mRNA).[2] The expression of miRNAs can be changed under conditions of pathophysiological stress, disease or treatment.[2] Thus, identification of miRNAs which will be operational as biomarkers determining early diagnosis and response to anti-TNF-α therapy might be of great notice in RA. Infliximab (IFX) is a chimeric monoclonal antibody against tumor necrosis factor (TNF) alpha that work by blocking its activity on cell surface receptors.[3] At some point IFX fails to produce a suitable clinical response in patients with RA, and this lead to the generation of anti-drug antibodies (ADAs) or to other side effect,[4] the infliximab immunogenicity, likely due to the chimeric nature of this molecule.[5] Treatment cost and the degree of effectiveness of anti-TNF-α treatment have major problems and fail to provide a solution to every patient.[6] So, before the start of therapy in patients suffering for RA, there is a strong need to identify the markers which allow predicting a successful therapy outcome.

Therefore this study shed light on potential using the expression of miRNA-5196 as a biomarker for predicting the response for anti-TNF-α therapy in Iraqi RA patients.

Materials and Methods

One hundred patients admitted to the unit of Rheumatology of Baghdad Teaching Hospital suffering from rheumatoid arthritis after infliximab infusion therapy) ≤ 6 months were included in the study and they were divided into two groups 50 responders and 50 non responders. This case-control study was conducted from November 2022 to August 2023. Inclusion criteria included Rheumatoid arthritis patient under the treatment of TNF alpha inhibitors ≤ 6 months duration with age ≥ 18 years while Exclusion criteria included early diagnosed RA patients, pregnant women and patients with other chronic diseases. Ethical approved was obtained from the Institutional Review Board of College of Medicine /Al-Nahrain University (I.R.B/ 25 in 28/12/2022). As well as all samples were obtained with informed consent from patients in accordance with Baghdad Teaching Hospital. 2 ml of the blood was collected from each patient (prior to the treatment infusion) and control group in a gel tube and left at room temperature for 30 minutes and then underwent centrifugation at 3000 rpm for 10 minutes for miRNA extraction.

Molecular Technique for Detection of miRNA-5196

miRNA was extracted from serum sample using a ready kit from Promega Company, USA, according to the manufacturer's instructions. Quantus Fluorometer was used to detect the concentration of extracted cDNA in order to detect the quality of samples for downstream applications. The cDNA sequences of (miRNA) gene were obtained from the NCBI GenBank database. As shown in Table 1.

Statistical Analysis

GraphPad Prism 8.4.3 software using ordinary one-way ANOVA and Newman-Keuls multiple comparisons post hoc test, and unpaired t-test were used in statistical analysis. The lower level of accepted statistical significant difference is equal

Table 1. **Primers sequence of miRNA-5196**

Primer name	Sequence 5`-3`	Annealing temp. (°C)
miR5196-RT	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCCCAAC	57
miR5196-F1	TTGAGGGAAGGGGACGAG	
RNU43_RT	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATCAG	
RNU43_F	GTGAACTTATTGACGGGCG	55
Universal Reverse	GTGCAGGGTCCGAGGT	

Table 2. **Distribution of age (years) in different groups**

		Patients group	Healthy control group
Age (years)	≤ 35	21	10
		42%	20%
	36–45	28	13
		56%	26%
	46–55	28	15
		56%	30%
	≥ 56	23	12
		46%	24%

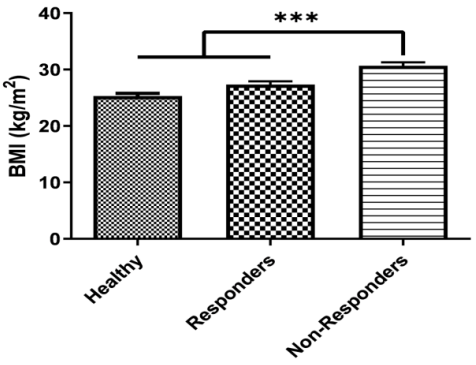

Fig. 1 **Body mass index in studied groups.**

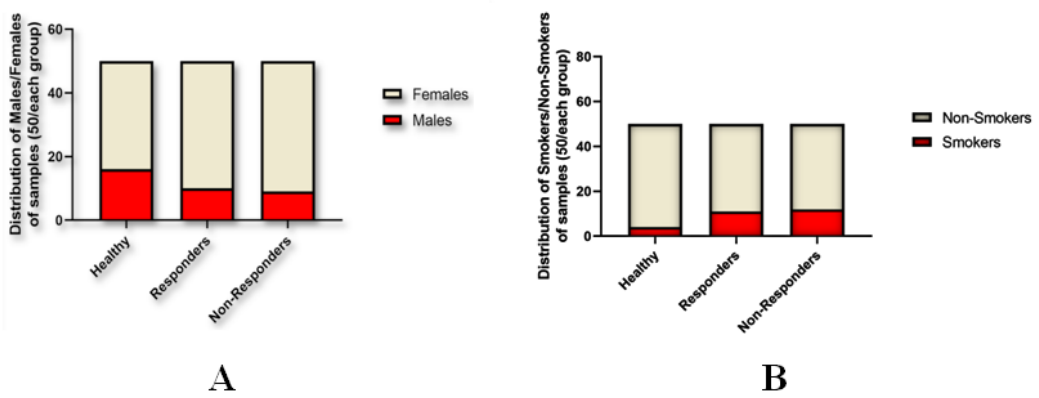

Fig. 2 **(A) Distribution of males and females in responders, non-responders and healthy groups; (B) Distribution of smokers and nonsmokers in the three different groups.**

or bellows to 0.005. Spearman correlation was used to estimate the correlation between studied variables.

Results

Mean age of Healthy was (46.82 ± 12.01) while in RA was (45.99 ± 11.08). Mean age of non-Responders was (46.40 ± 11.32), while Responders was (45.58 ± 10.94). The occurrence of RA was higher in age group (36–55) (Table 2). There was a significant association between RA and BMI (P<0.001) (Figure 1). Females were more than males to RA develops, with a rate was 3.05:1 (Figure 2A). The current results showed that the smokers were 28 (10 responders, 12 non-responders and 6 control) while non-smokers individuals 122 (38 responders, 37 non-responders and 47 control) (Figure 2B). Related to medication of RA, Patients taken Methotrexate were 70 (37 Responder and 33 non-Responder) but there were 30 patients (13 Responder and 17 non-Responder) without MTX (Figure 3A and 3B).

Clinical Disease Activity Index (CDAI) showed significant difference between patients (responders and non-responders) P<0.0001(4A). The result of study found 68% of responder patients with RF positive while non-responders were 88% seropositive (Figure 4B). In addition the results showed that non-responders have significantly higher ACPA serum positivity is suggesting they may have a more evident autoimmune response against citrullinated proteins (Figure 5A). According to current study RA patients who have ACPA positive have a reduced response anti-TNF-α therapy. Non-responders RA patients in comparison to the control group, miRNA-5196 expression was greater (P value = 0.072) (Figure 5B), (Table 3).

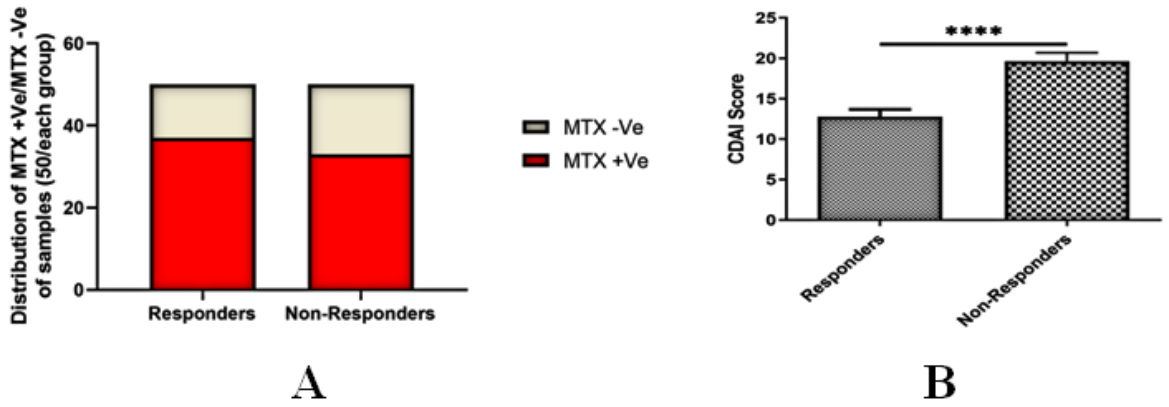

Fig. 3 **(A) Distribution of MTX positive\negative status in three different groups; (B) CDAI score in responders and non-responders patients.**

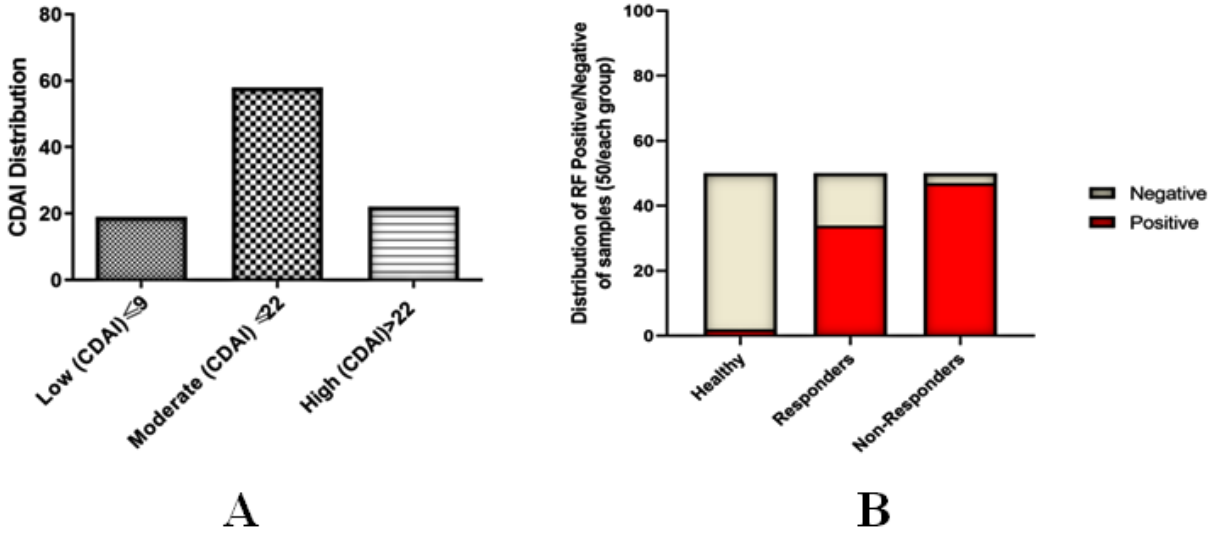

Fig. 4 **(A) CDAI distribution score in patients; (B) Distribution of RF positive\negative status in three studied groups.**

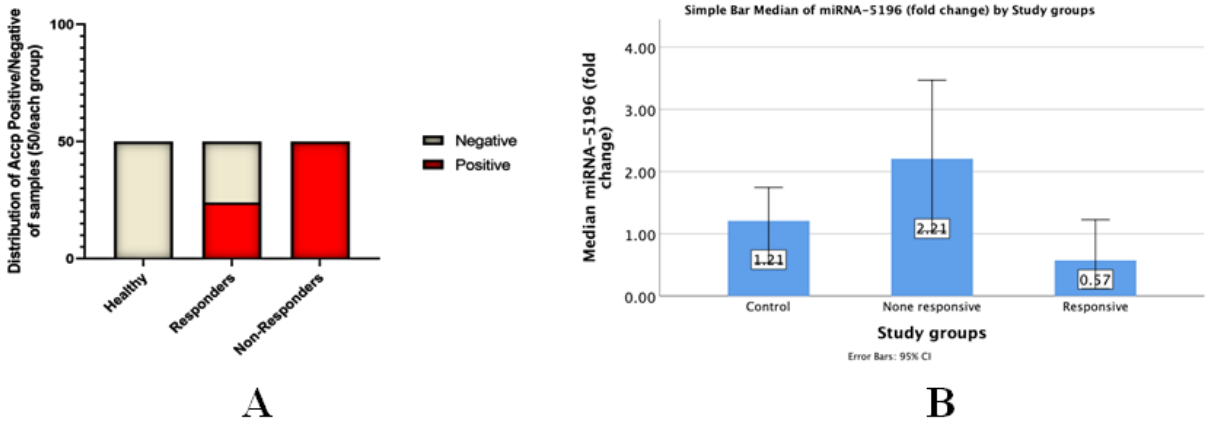

Fig. 5 **(A) Distribution of ACPA positive\negative status in the three different groups; (B) Expression of miRNA-5196 in patients and control group (ns: non-significant (*P*>0.05), *(*P*<0.05)).**

In this study there was a significant correlation between miRNA-5196 with CDAI and ACPA, as well as there was a correlation between ACPA and CDAI, as shown in Table 4.

Discussion

Present study shown that the most occurrence of RA in age group of 36-55 years (Table 2), RA can develop at any age, typical onset occurs between 30 and 50 years.[7]

Table 3. **Demographic characteristics, CDAI, RF, ACPA and miRN-5196 in studied groups**

		Study groups			*P* value		
		Control	None responsive	Responsive	Control vs None responsive	Control vs Responsive	None responsive vs Responsive
Sex	Female	34 68.00%	41 82.00%	38 76.00%	0.165	0.505	0.624
	Male	16 32.00%	9 18.00%	12 24.00%			
Body mass index		24.6 (23.9–25.5)	30 (29.4–31.1)	27.95 (25.9–29.4)	0.018	0.442	0.108
Rheumatoid factor	Negative	48 96.00%	3 6.00%	17 34.00%	<0.001	<0.001	<0.001
	Positive	2 4.00%	47 94.00%	33 66.00%			
Anti-cyclicitrullinated protein	Negative	50 100.00%	0 0.00%	26 52.00%	<0.001	<0.001	<0.001
	Positive	0 0.00%	50 100.00%	24 48.00%			
Smoking habit	Yes	4 8.00%	12 24.00%	12 24.00%	0.056	0.056	0.999
	No	46 92.00%	38 76.00%	38 76.00%			
Methotroxate treatment	Yes		33 66.00%	38 76.00%			0.378
	No		17 34.00%	12 24.00%			
Clinical disease activity index		.	20 (18–23)	12 (11–15)			<0.001
miRNA-5196 (fold change)		1.21 (0.61–1.71)	2.21 (1.05–3.28)	0.57 (0.14–1.22)	0.317	0.186	0.072

Table 4. **Correlation among BMI, CDAI, RF, ACCP and miRNA**

		Body mass index	Clinical disease activity index	Rheumatoid factor	Anti-cyclic citrulinated protein	miRNA-5196 (fold change)
Body mass index	r	1.000	0.088	0.088	0.074	0.038
	p		0.385	0.384	0.465	0.706
Clinical disease activity index	r	0.088	1.000	0.172	.260	.211
	p	0.385		0.087	0.009	0.035
Rheumatoid factor	r	0.088	0.172	1.000	0.046	0.087
	p	0.384	0.087		0.652	0.392
Anti-cyclic citrulinated protein	r	0.074	.260	0.046	1.000	.244
	p	0.465	0.009	0.652		0.014
miRNA-5196 (fold change)	r	0.038	.211	0.087	.244	1.000
	p	0.706	0.035	0.392	0.014	

In the current research the BMI of RA patients was significantly higher than healthy control group (Figure 1), obesity has been found to be associated with greater arthritis activity and a reduced probability of response to (TNF) agents. On the other hand, weight loss increases the chances of treatment success. In this study women get RA in greater numbers than do men for several reasons like sex hormones such as estrogens and their stronger response to infections, environmental triggers including stress and how they respond to external toxins and genetic factors.[8] In addition the current results showed that the smokers were 28 (12 responders, 12 non-responders and 4 control) while 122 (38 responders, 38 non-responders and 46 control) nonsmokers individuals (Figure 2A and B). A study conducted by van Wesemael et al., 2016[9] showed that Cigarette Smoking was associated with multiple autoantibody positivity (RF and ACPA), cigarette smoking may break tolerance to autoantigens in RA, which might be one of the triggers of RA onset in subsets of patients.[10]

Regarding the methotrexate treatment there were 70 patients on MTX therapy (37 Responder and 33 non-Responder) while there were 30 patients (13 Responder and 17 non-Responder) without MTX (Figure 3A). Methotrexate

(MTX) has been used in the treatment of rheumatoid arthritis, RA patients without MTX medication which increased CDAI score over than patients whom received treatments. Several studies have evaluated changes in disease activity after discontinuation of MTX in RA patients who achieved a good response to MTX plus bDMARDs.[11] MTX exerts anti-inflammatory effects by directly and indirectly regulating the function of most cell types involved in inflammation including neutrophils, monocytes, T cells, B cells, endothelial cells and fibroblast like synoviocytes.[12] On the other hand there were 68% of responder patients with RF positive while non-responders were 88% seropositive. Rheumatoid factors (RF) are found not only in RA but in a wide range of pathologies including other autoimmune and non-autoimmune diseases.[13] They have been found in up to 4% of young, healthy individuals and the elderly as well. RFs are probably the result of the immune response to inflammation (depending on genetic background) and may have regulatory effects on Ig production by controlling B cell activation.[14]

Also present study showed allnon-Responders and only 24 (48%) of Responders, were seropositive for ACPA suggesting they may have a more evident autoimmune response against citrullinated proteins (Figure 5A). RA patients who have received an ACPA positive have a reduced response anti-TNF-α therapy.[15] Previous results conclude the increase of ACPA in serum of RA patients was associated with development and progression of rheumatoid arthritis. High titer ACPA and RF antibodies are both associated with an increased risk of erosive joint damage; ACPA antibodies may confer a higher risk than RF.[16]

The expression of miRNA-5196 was significantly lower in responder as compared with non-responder and control group. Monocytes of RA patients has been shown abnormalities in microRNA (miRNA) expression are related to inflammatory cytokines production by T and B cells in several rheumatic diseases, however miRNAs have not been fully analyzed in monocytes population. Monocytes play an important role in autoimmune disease including rheumatoid arthritis (RA) or systemic sclerosis (SSc). Monocytes are the first immune cells which migrate from blood to the site of inflammation leading to tissue destruction due to enhanced proinflammatory cytokines secretion. miRNA-5196 binds to five seed regions within 3′UTR of Fra2, therefore, can negatively regulate gene expression of target mRNA.[17]

In the current study sera circulating miRNA-5196 is elevated in RA patients compared to healthy control. Following anti-TNF-α therapy, the level of miRNA-5196 (Figure 5B) was reduced in RA patients. Reduced expression of miRNA-5196 was seen in RA patients responding to anti-TNF-α therapy. In addition, this study demonstrated that a change in miRNA-5196 expression correlates with changes in CDAI in biologic therapy treatment of RA, these results similar to other studies.[18]

Conclusions

miRNA-5196 expression related with response to TNF-α inhibitor and disease activity in patients with RA.

List of Abbreviations

RA	Rheumatoid Arthritis
ACPA	Anti-Citrullinated Protein Antibodies
RF	Rheumatoid Factor
TNF	Tumor Necrosis Factor
ADAs	Anti-Drug Antibodies
CDAI	Clinical Disease Activity Index
BMI	Body Mass Index
SSc	Systemic Sclerosis

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Conflict of Interest

None.

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