Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 2, 199-205

Assessment of MicroRNA (miR-146a) as Potential Biomarkers and Disease Activity in Iraqi Patients with Rheumatoid Arthritis after Biological Treatment

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Received: September 24, 2023 / Accepted: November 21, 2023 / Published: October 30, 2024

Abstract: Rheumatoid arthritis (RA) is a debilitating form of persistent autoimmune inflammation that affects the joints. Rheumatoid arthritis's primary treatment is disease-modifying anti-rheumatic agents (DMARDs), such as methotrexate (MTX) and Enbrel. The current study aimed to measure the gene expression level of MiR146a as well as demographic data and clinical presentations for patients with RA compare with control models after biological treatment. The study includes 70 patients with rheumatoid arthritis and 30 healthy individuals as a control group, with an age range between 20 to 60 years. Blood was drawn from everyone who participated in this study. In this study, ESR, HB, and WBC were evaluated in rheumatoid arthritis patients. The findings showed that the majority of age groups were in the third group (40-49 years), where there was a 26.1% percentage, and that the lowest number was in the first group, which included ages 29, where it was 8.7 percent. Erythrocytes Sedimentation Rate (ESR), C-reactive Protein (CRP), CDAI and smoking were examined in this study with concentrations (8.0±2.7 × 10^9/L, 10.56±1.40, 13.95± 9.2, 16(23.2%)) respectively. It was concluded that MiR-146a transcript levels were higher in RA patient whole blood samples than in healthy control samples.

Keywords: autoimmune disease, rheumatoid arthritis,MiR146a, gene expression.

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Introduction

Rheumatoid arthritis (RA) is a debilitating autoimmune disorder that is characterized largely by joint damage, erosive arthritis, and persistent joint inflammation(1). RA affects between 0.3% and 1.0% of people worldwide, and it is more common in industrialized nations and among women. The exact origin of RA is not fully understood, however it has been shown that a variety of genetic, environmental, and immunological variables are linked to RA susceptibility (2).

Patients with RA in Iraq are treated with methotrexate as a first-line option and are then qualified to start a biologic, such as etanercept, provided their symptoms have been established, severe, and active for at least three month (25 mg/week; or alternative (3) conventional synthetic DMARD in case of methotrexate intolerance) and ≥2 of the following prognostic factors:

smoking, very high erythrocyte sedimentation rate (ESR) and C-reactive. protein .results, very high rheumatoid factor or anti-citrullinated protein antibody results, radiologic evidence of joint damage, or the presence of nodules or other extra-articular features (4). MicroRNAs are small, non-coding RNA molecules that limit protein translation and promote mRNA cleavage to reduce gene expression (5). However, recent advances in our understanding of the immune system suggest that miRNA also play a significant role in immune regulation and can therefore act as both agents and targets of immunotherapy. Studies of miRNA biology have, for the most part, concentrated on their role as either oncogenes or tumor suppressors (6). MiR-146a It seems to be an intriguing illustration of a master regulator in a number of immunity-related features. By building endotoxin tolerance and cross-tolerance, it specifically helps to regulate the overproduction of cytokines, such as TNF-, and it serves as a negative feedback regulator of innate immunity in toll-like receptor (TLR) signaling during repeated bacterial infection(7). Few research, however, have looked at the connection between the severity of the illness and the amount of miR-146a present in the whole blood of RA patients. In order to assess the possible roles of miR-146a as potential indicators in the onset, severity, and activity of RA patients with established illness, the expression of miR-146 in RA blood patients was examined in this study.

Subjects and sample collection

The study was conducted in Al Yarmouk teaching hospital in Baghdad. A Cross sectional study was conducted in the period between beginning of July 2022 to the end of November 2022. Blood drawn from everyone participate in this study. 70 Patients were under chemotherapy and take MTX with folic acid or biologic therapy and take Etanercept which is sold under the brand name Enbrel. Patients with other diseases besides rheumatoid arthritis such as diabetes, hypertension, hyperthyroidism, systemic lupus erthromytous and psoriasis were excluded. Using a disposable syringe, seven milliliters (ml) of venipuncture blood were drawn. Both EDTA tubes for DNA isolation and hematological test while the gel tubes have been used to separate the collected blood. EDTA tubes containing the obtained blood are kept at -20°C. Centrifuging the blood in the gel tube for 15 minutes at 10,000 rpm yielded the serum, which was then separated into five aliquots (each containing 50 µl) and kept at -20°C until analysis. In order to prevent repeated freezing and thawing, each serum aliquot is only utilized once.

Gene expression

Using the Magna Pure LC RNA Isolation Kit III (Roche) and the Magna Pure LC 2.0 Instrument, messenger RNA (mRNA) was extracted from the leukocyte lysate. Total RNA content (ng/uL) was determined by measuring absorbance at 260 nm using a NanoPhotometer (Implen GmbH, Germany), and the A260-280-nm absorbance ratio was >1.8 (quality). The complementary DNA (cDNA) was then created using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Applied Science); each reaction required 100 ng of RNA in a final volume of 15 ul. The TaqMan Universal

PCR Master Mix and the particular probes were used with the 7500 Fast Real-Time PCR System (Applied Biosystems, Applera UK, Cheshire, UK) to perform the quantitative polymerase chain reaction (qPCR).

Statistical analysis

Version 26 of SPSS statistical software was used to examine the data. Independent-Samples The results of the Student t-test between the patient and control groups were presented as mean and standard deviation (SD). With a 95% confidence range, the statistical tests were significant at p0.05 and highly significant at p0.01. AUC (area under the curve) and 95% confidence interval (CI) ROC (receirver operating characterstic curve) curves were created for the event "clinical activity.

Results and discussion

The demographic data and clinical presentations of the RA patients and healthy subjects are shown in Table1. Among the 70 RA patients sex distribution was 49(69.6%) females and 21(30.4%) males. Therefore, the ratio of female to male was 4:1, the prevelage of womens number were agreed with results of Kavanaugh(9) study who revealed that women were substantially more likely to have RA than men (female to male ratio: roughly 3:1). 84% of female RA patients had RA, compared to 16% of male patients; this incidence is rather greater (26).

Table (1): Characteristics of rheumatoid arthritis patients

Characteristics	RA patients (n= 70-)	Controls (n= 30)
Gender, Male /Female	21(30.4%)/49(69.6%)	15(50%)/15(50%)
Smoker/Non-smoker	16(23.2%)/54(76.8%)	30 (100%)
Age, year (Mean±SD)	47.6±12.0	34.1±4.0
CDAI	13.95± 9.2	-
ESR (mm/h)	28.2 ±12.2	10.5 ±4.5
CRP (mg/L)	10.56±1.40	6.97±2.2

Dividing the RA patients into two groups-smokers as 16 people in a 23.2%proportion and non-smokers as 54 76.8% people in a percentage-while the controls were all non-smokers in a 100% percentage. According to the Swedish BARFOT research, passive smoking and tobacco usage in the past had no effect on RA activity (10). The situation with active smoking is different. Active smoking does appear to be linked to more significant joint inflammation in both established forms (American VARA registry) and early stages of RA, as seen by a greater number of swollen joints and higher CRP readings (11).

The result of CDAI (Clinical Disease Activity Index) in patients show significant difference (P-value 0.001) patients and control in represented by mean and S.D (13.95± 9.2 vs 0.0± 0.0). Our result agreed with Van Nies,(12) who observed that after one year of therapy, patients with a mean duration of RA symptoms previous to starting etanercept of less than a year exhibited a substantial improvement in CDAI. Overall, our findings show the significance of starting pharmacotherapeutic therapy

for RA as soon as symptoms appear (12). The average ESR value was 28.2±12.2 in patients with 70 cases, compared to 10.5 4.5 in controls with 30 cases. With a large significant difference between them, the P-Value was (0.001). ESR readings are often greater in female patients and those who have chronic illnesses (13). It has been discovered that the patient's age and ESR are connected. ESR values increase with patient age (14).

Table (2): Mean of *Erythrocyte sedimentation rate* to Patients and Controls.

Group	N	ESR Mean ± S.D. (mm/hr.)	P-Value
Patients	70	28.2 ±12.2	**0.001**
Controls	30	10.5 ±4.5	
Normal Value		**Men** = 0 to 15 mm/hr. **Women**= 0 to 20 mm/hr	

ESR= *Erythrocyte sedimentation rate,* S.D. = standard deviation, N= Frequency, p= Probability.

The mean value of C-Reactive protein in 70 patients and control were (10.56±1.40 vs 6.97±2.2) respectively. There were high significant differences between them P-value=0.001. This result indicate elevated in the CRP after biological treatment in bone destruction in RA.

Table (3): Mean of C-Reactive protein serum level to Patients and Controls.

Group	N	Mean ± S.D. (pg/mL)	P-value
Patients	**70**	10.56±1.40	0.001
Controls	**30**	6.97±2.2	

S.D. = Standard Deviation, N= Frequency, p= Probability.

Receptor activator of nuclear factor-kB ligand (RANKL) expression is increased, which drives osteoclastogenesis and causes bone resorption, which is the first step in the breakdown of bone. In the absence of RANKL, CRP enhances osteoclast development by inducing RANKL expression in peripheral blood monocytes (15). While the control group's values were within the normal range, RA patients showed higher levels of the biomarkers ESR, CRP, RF, and Anti-CCP (16). research by The CRP isoform may, however, affect how osteoclast differentiation is affected. By neutralizing RANKL, mCRP has been demonstrated to prevent RANKL-induced osteoclast development *in vitro*, perhaps providing a protective effect. (17).

MicroRNA 146a (mir-146a)

The current study find out that expression in mir-146a were elevated in treated patients with biological treatment in median as (1.40) compare to control

Differences between them (p-value= 0.06)figure (1) as (1.15). There is no significant The results of current According to the study, anti-TNF therapy had an impact on miR-146a expression, which showed an increase in serum expression compared to the control.

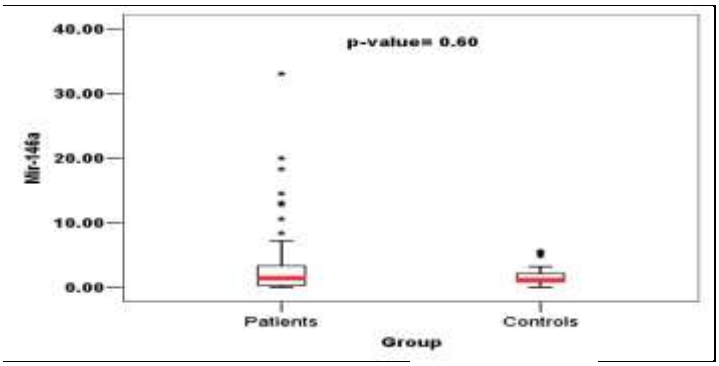

Figure (1): Median of Folding of gene expression to MicroRNA 146a (mir-146a) level to Patients and Controls.

Our research contradicted the findings of (18), who claimed that serum expression levels of miR-146a-5p were considerably lower in patients than in healthy people. Moreover, After three months of anti-TNF medication, patients' expression levels of miR-146a-5p increased. This finding suggests that anti-TNF- medication delivery gradually raises the miR-146-5p level in patients' sera, possibly reaching the level seen in healthy controls.

The lower miR-146a serum expression levels in patients with early RA as compared to healthy controls have previously been reported by Filková *et al*. (19), which is consistent with the findings of our current investigation. Additionally, miRNA-146a serum levels were noticeably lower in those with developed RA (20).

Additionally, the study by castro-Villegas, (21) looked at the variations in miR-146a levels in sera of RA patients before and after anti-TNF treatment. According to Murata *et al.* findings, patients receiving anti-TNF medication had considerably higher serum expression levels of the miR-146a. On the other hand, RA patients' synovial tissues, synovial fluid monocytes, peripheral blood-derived mononuclear cells, and serum all showed increased expression of the miR-146a (21).

Table (4): Correlations between mir-146a and clinicopathological characteristics of RA patients.

		Smoking	Age Group	Gender	CDAI	ESR	CRP
mir-146a	**r**	.263(*)	.202	.282(*)	.026	.017	-0.066

*** Correlation is significant at the 0.05 level (2-tailed)**

The results in Table(4) showed a significant positive correlation between smoking and gender with serum gene expression if Mir-146a, our result agreed with Murata, (22) Smoking was one among the first things found to have an impact on the world's miR machinery. Numerous research on humans that are backed by *in vitro* and *in vivo* experimental data establish a connection between smoking and changes in miR expression and present the profile of smoking-sensitive miRs, In the airway epithelium, smokers' and nonsmokers' miRNA expression patterns have mostly been compared (23).

The negative and positive correlation of (CRP, ESR) respectively with no significant difference between them and Mir-146a gene expression. The result disagreed with De Smet, (24) who found that this patient's miRNA levels remained increased relative to those of healthy control persons across the 2-month period, generally remaining

unaltered. This suggests that the patient's heightened miRNA expression may signify a lack of improvement, as shown by the patient's elevated CRP and ESR readings.

Our findings showed a positive association between age and disease activity in RA patients who received biological therapy, although not a significant one. This outcome contrasted with (25) who mentioned that miR-146 expression levels with patient clinical and demographic showed that there is no significant trends or correlations between high expression levels with age and medications. Patients receiving no medications at the time of miRNA analysis exhibited the same trend toward elevated miRNA expression, indicating that treatment with medications is not responsible for the increased miRNA expression in RA patients

Conclusion

It was discovered that MiR-146a transcript levels were higher in RA patient whole blood samples than in healthy control samples. Additionally, the degree of miRNA expression was correlated with the patients' clinicopathological traits, such as CDAI, ESR, CRP, RF, smoking, Age, and Gender. Despite this, more analyses could be useful in identifying a reliable biomarker for the RA condition.

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