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ORIGINAL ARTICLE

# MicroRNA-146a expression as a potential biomarker for rheumatoid arthritis in Egypt

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**Abstract** *Background:* MicroRNAs (miRNAs) are small non-coding RNAs, whose role in regulating diverse immune functions, suggests they might play a role as biomarkers for immune mediated disorders. Studies showed that miRNA-146a (miR-146a) expression is increased by proinflammatory cytokines and is an important modulator of differentiation and function of cells of innate and adaptive immunity.

*Aim of the work:* The current study aimed to evaluate the expression of miR-146a as a potential biomarker for diagnosis of rheumatoid arthritis (RA) and to explore its association with disease activity.

*Subjects and methods:* The study enrolled 50 Egyptian subjects divided into a patient group, which comprised 25 RA patients, and a control group which comprised 25 healthy individuals. The disease activity for the patients' group was determined by simplified disease activity index. Relative quantification of miR-146a expression in whole blood was determined using reverse transcriptase quantitative real time polymerase chain reaction.

*Results:* There were highly significant statistical differences between patients and healthy controls as regards miR-146a relative expression, erythrocyte sedimentation rate (ESR) and anti-cyclic citrullinated peptide (anti-CCP) ($p < 0.001$). Highly significant statistical differences ($p < 0.001$) were also found between different patients' subgroups as regards miR-146a relative expression and ESR. miR-146a levels correlated positively with those of ESR, C-reactive protein and anti-CCP ($p < 0.001$).

miR-146a illustrated best performance in diagnosing RA, showing the highest sensitivity and specificity (96% and 100%, respectively) (AUC: 0.992 at a cut off value of $\geqslant 2.16$) compared to anti-CCP (sensitivity: 68%, specificity: 100% and AUC: 0.87 at a cut off value of $\geqslant 22$ U/ml) and RF (sensitivity: 56%, specificity: 80% and AUC: 0.992 at a cut off value of $\geqslant 13$ U/ml).

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*Conclusion:* This study demonstrated that miR-146a expression was highly significantly elevated in whole blood of patients with RA. Its diagnostic performance was better than anti-CCP and RF and its level of expression correlates with disease activity.



## 1. Introduction

Rheumatoid arthritis (RA) is a systemic progressive autoimmune disease characterized by chronic inflammation of the synovial tissue, which leads to irreversible joint destruction and subsequent disability [1]. Considering the variable and heterogeneous clinical presentation of the disease, its diagnosis is primarily based on the 2010 revised American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria which includes the evaluation of several clinical and serum parameters as anti-citrullinated protein/peptide antibodies, rheumatoid factor (RF) and acute phase reactants [2]. Nevertheless, there is still no specific pathognomonic test for the diagnosis and identification of at risk patients, which necessitates defining new biomarkers for RA [3].

MicroRNAs (miRNAs) are small non-coding RNAs, whose role in regulating diverse immune functions including B and T cell selection and maturation, suggests that they might play a central regulatory role in immunological tolerance and can play a potential role as biomarkers for various immune mediated disorders [4–7]. miRNA-146a (miR-146a) was first described by Taganov et al. who demonstrated its increased expression in response to stimulation with lipopolysaccharides, and its role as a regulator of the Toll-like receptor signaling by targeting tumor necrosis factor (TNF) receptor-associated factor 6 and interleukin-1 receptor-associated kinase 1 [8]. Other studies showed that its expression is increased by proinflammatory cytokines and is considered an important modulator of differentiation and function of cells of innate and adaptive immunity [9,10]. It has been implicated in the pathogenesis of RA via regulation of multiple target genes linked to inflammation and apoptosis [11]. Levels of miR-146a expression have been investigated in synovial tissues, fibroblasts [12–14], T-cells, B-cells, IL-17 producing CD4 cells [15], peripheral blood-derived mononuclear cells (PBMC) [14] and plasma [13] of RA patients. However, There is conflicting evidence regarding the association between the level of miR-146a and the disease course [11].

The current study aimed to evaluate the expression of miR-146a as a potential biomarker for diagnosis of RA using whole blood owing to the feasibility and the minimally required manipulation of the sampling that won't alter the molecules level in samples, and to explore its association with disease activity.

## 2. Subjects and methods

### 2.1. Study design

This observational case–control study was conducted in the period from March to August, 2015.

The work was approved by Ain Shams University (ASU) Ethics Committee. It was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. Informed consent was obtained from all participants.

### 2.2. Patients and controls

The study involved 50 Egyptian subjects, who were divided into two groups; patients and controls.

Patient Group: Included 25 patients recruited from the rheumatology outpatient clinic, ASU Hospitals. Patients were included in this group after being diagnosed with RA according to the ACR/EULAR classification criteria [2]. Patients suffering from any other autoimmune disorders, chronic illnesses or comorbidities were excluded from the study.

Control Group: Included 25 apparently healthy individuals whose age and sex were matched with those in the patients group.

### 2.3. Data collection

Relevant sociodemographic and clinical data were collected in a standardized collection form. Results of clinical rheumatology examination including disease duration, number of tender swollen joints, morning stiffness, extra articular manifestations, in addition to the simplified disease activity index (SDAI) score [16] were recorded for each patient.

### 2.4. Laboratory investigations

#### 2.4.1. Sample collection

Adequate blood samples were collected from each subject under complete aseptic condition. The samples were divided into two parts; one part was used for analysis of the erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP, mg/L), complete blood picture, RF and anti-cyclic citrullinated peptide (anti-CCP). The other part was preserved in tubes containing EDTA at −80 °C for the relative quantification (RQ) of miR-146a expression.

#### 2.4.2. RQ of the miR-146a expression

(a) *Purification of RNA from blood:* RNA was isolated from blood using the blood MicroRNA extraction kit "miRNeasy Mini Kit" (Qiagen, Hilden, Germany) according to manufacturer's instruction. Briefly, after thawing the samples, QIAzol Lysis Reagent was added. Chloroform was then added to the lysate to separate the aqueous phase. Samples were then vortexed, incubated at room temperature for 2–3 min, and centrifuged for 15 min at