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MicroRNA146a gene polymorphism in patients with rheumatoid arthritis and the relevant value with disease activity and extra-articular manifestations

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ABSTRACT

Aim of the work: To analyze the relationship between miRNA-146a rs2910164 and rheumatoid arthritis (RA) susceptibility and clarifying its association with disease activity and extra-articular involvement.
Patients and methods: The study enrolled 50 RA patients and 40 controls. DNA extraction from whole blood was done. Genotyping was performed by real-time polymerase chain reaction. The selected single nucleotide polymorphism was rs2910164 in the miRNA-146a gene. Genotyping was performed on genomic DNA samples by allelic discrimination assay. Disease activity score (DAS28) and health assessment questionnaire-disability index (HAQ-DI) were assessed.
Results: The mean age of patients was 44.8 ± 9.9 years while age at onset was 37.2 ± 9.8 years. Female:male was 5.3 *vs* 1. Females had significantly higher DAS28, visual analogue scale and rheumatoid factor (RF) than males (p = 0.04, p = 0.009, p = 0.03). The frequency of GC genotype was more in both males and females (62.5 *vs* 47.6%). GC genotype and G allele were the most frequent in patients. No significant difference in the frequency distribution of genotypes and alleles was observed between patients and controls. There was a significant difference in the number of swollen joints (p = 0.04) and sicca symptoms (p = 0.01) being higher in those with CC genotype with a tendency of increased DAS28, HAQ-DI, deformities and rheumatoid nodules with CC genotype and interstitial pulmonary fibrosis and RF with GG genotype.
Conclusions: CC genotype was associated with sicca symptoms and swollen joints, deformities, disease activity and functional disability. miRNA-146a may be a potential biomarker for extra-articular manifestations of RA that need more attention and warrant aggressive therapy.

1. Introduction

Rheumatoid arthritis (RA) is a systemic progressive autoimmune disease that results from a complex interaction between genes and environment leading to a breakdown of immune tolerance, and chronic synovial joint inflammation which leads to irreversible joint destruction [1].There is a strong influence of immunological markers on the clinical presentation of Sjögrens syndrome (SS) [2]. More than a quarter of patients with primary SS may have systemic manifestations not considered in the classification criteria [3]. It has been reported that metabolic disorders and chronic inflammation are significantly affected by RA rather than SS [4].

MicroRNAs (miRNAs) are small non-coding RNA molecules that participate in transcriptional and translational regulation [5]. miRNAs play a central role in cell proliferation, differentiation, apoptosis, and inflammation, and act as key regulators of innate and adaptive immune responses [6]. miRNA-146a (miR-146a) is induced in response to cytokine and/or pathogen products such as lipopolysaccharide and acts as a negative regulator of the Toll-like receptor (TLR))/ nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathway by targeting tumor

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necrosis factor receptor-associated factor 6 (TRAF6), and interleukin-1 receptor-associated kinase 1 (IRAK1) [7]. Single nucleotide polymorphisms (SNPs) of the miRNA gene region may affect the property of miRNA via altering the miRNA expression and/or maturation, resulting in aberrant miRNA regulation [8]. The elevated expression of miR-146a might be due to the polymorphism of miRNA-146a rs2910164 which affects the specificity of mature miR-146a in binding to its targets [9,10]. In previous studies on Egyptian systemic lupus erythematosus (SLE) [11] and osteoarthritis [12] patients, a potential role of miRNA-146a has been revealed.

The expression of miR-146a was believed to be increased in RA but it is unable to properly function, leading to prolonged TNF-α production [13] that permits persistent inflammation which is one of the characteristics of the pathogenesis of RA [14].

The current study aims to analyze the relationship between miR-146a rs2910164 and RA susceptibility and to clarify its association with disease activity and extra-articular involvement.

2. Patients and methods

The present study was conducted between January and December 2018 and included 50 RA patients recruited from the Rheumatology and Rehabilitation clinics, Assiut University Hospitals and fulfilled the 2010 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) revised criteria for RA [15] in addition to, 40 healthy, age and sex-matched controls from the blood bank donors were included in the current study. The study was approved by the Ethical Committee of our university and was conducted following the ethical principles of the Declaration of Helsinki. Written Informed consent was obtained from all participants.

Demographic and clinical information were collected. Clinical evaluation included tender joint count (TJC) and swollen joint count (SJC), visual analogue scale (VAS) of pain, detection of extra-articular manifestations (EAM), and disease activity score (DAS28) [16]. The laboratory assessment included rheumatoid factor (RF), anti-cyclic citrullinated peptides (anti-CCP) antibodies, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Functional disability level was assessed using the Health assessment questionnaire-disability index (HAQ-DI) [17].

Peripheral blood samples were collected from all patients at the time of enrollment and were then stored at −18 °C for later genetic analysis. DNA extraction from whole blood was done using (Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit) then genotyping was performed by real-time polymerase chain reaction (PCR). The selected SNP was rs2910164 in the miR-146a gene. Genotyping was performed on genomic DNA samples by allelic discrimination assay using (Applied BiosystemsTaqMan SNP Genotyping assays and 7500 fast real-time PCR system, Thermo Fisher Scientific, USA).

2.1. Statistical analysis

Data was collected and analyzed using MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015). Descriptive statistics were expressed using counts and their corresponding percentages for categorical variables, and as means, medians, interquartile ranges, and ranges for quantitative variables. Bivariate associations of interest were evaluated using Independent-samples Mann-Whitney *U* test for one quantitative variable and one binary variable, Kruskal-Wallis test for one quantitative variable and one multinomial variable, and Fisher's exact test for two nominal variables. P-value was considered significant if <0.05.

3. Results

Clinical and demographic features of RA patients are reported in Table 1. Table 2 shows the characteristics of the RA patients according to their gender. There are significant differences between both sexes as regards DAS28, VAS, and the level of RF ($p = 0.04$, $p = 0.009$, $p = 0.03$). No significant difference of various genotypes or alleles is found between patients and controls (Table 3). In Table 4 there was a significant difference in the number of swollen joints ($p = 0.04$) and sicca symptoms ($p = 0.01$) being higher in those with CC genotype. Associations of miR-146a alleles with clinical and laboratory parameters of patients are reported in Table 5.

4. Discussion

The miRNAs are small molecules with well-known sequence, preserved among species, stable in plasma or serum, easy to assess, and measurable at disease onset. They may be used as biomarkers that have several advantages over the use of conventional biomarkers to differentiate the RA from other rheumatic diseases and detection the disease activity [18]. The majority of miRNAs are found intracellular while the extracellular miRNAs are secreted into exosomes or microvesicles [19,20]. The current study evaluated the relations between miR-146a rs2910164 and RA. The VAS and DAS28 were significantly higher in females. Earlier studies reported the same associations in addition to a significant association between the sex and each TJC, SJC, and HAQ-DI [21,22]. There was a significant difference in RF positivity among males and females, on the contrary, no difference was found in a previous study [21]. On analyzing genotypes distribution between patients with RA based on gender, it was found that the frequency of miR-146a rs2910164 genotype GC was more in both males and females with no significant difference with the distribution of various genotypes. Similarly, a study observed no significant differences in miR-146a rs2910164 genotype distribution in Chinese female RA patients compared to males [23] while, *Zhou et al.* found a significant association between miR-146a rs2910164 genotype GG and the increased risk of RA in Chinese female patients [24].

It was demonstrated that there was a direct functional effect of the rs2910164 polymorphism on the miR-146a capacity to inhibit its target genes (TRAF6 and IRAK1) [25] taking into consideration that TRAF6 and IRAK1 have been implicated in RA pathogenesis, this polymorphism may contribute to RA development [26]. The miR-146a rs2910164 polymorphism has been studied concerning the susceptibility, activity, and severity of RA but the results have been controversial. In the current study as regards the RA susceptibility, although the GC genotype and G allele were more frequently seen in RA patients than controls there was no significant difference between the disease and control groups regarding neither the genotypic distribution nor the allelic frequencies. This result matches most of the published data before which all revealed no association between this SNP and RA susceptibility [27–30]. However, *Ayeldeen et al.* reported a significant difference in the distribution of miR-146a rs2910164 genotypes between patients and controls, as the GG genotype and the G allele were more found in patients than in controls [31]. *Ahmadi et al.* showed that there is a risk of developing RA in individuals carrying GC versus GG genotype among the Iranian population and in GC + CC versus GG genotype in females only rather than males [32].

A significant association between miR-146a rs2910164 CC genotype and the SJC was detected. The TJC, CRP, DAS28 tended to be higher in miR-146a CC genotype. This matches the findings of *Zhou et al.* who reported in their meta-analysis that disease

Table 1
Clinical and demographic features of the rheumatoid arthritis patients.

Variable	RA patients (n = 50)
Age (years)	44.8 ± 9.9
Female:male	42:8 (5.3:1)
Disease duration (years)	6 (0.17–23)
Age at onset (years)	37.2 ± 9.8
Morning stiffness (min.)	15 (0–90)
Deformities	23 (46)
Sicca symptoms	10 (20)
IPF	6 (12)
RN	10 (20)
Vasculitis	1 (2)
TJC	19.5 (0–28)
SJC	15.5 (0–26)
VAS	5 (0–10)
DAS28	6.3 ± 1.5
HAQ-DI	1.2 (0–2.75)

RA: rheumatoid arthritis, IPF: interstitial pulmonary fibrosis, RN: rheumatoid nodule, TJC: tender joint count, SJC: swollen joint count, VAS: visual analogue scale, DAS28: disease activity index, HAQ-DI: health assessment questionnaire-disability index. Results are presented as mean ± SD, median (range) or n(%).

Table 2
Characteristics of the rheumatoid arthritis patients according to their gender.

Variable median (range) or n(%)	RA patients (n = 50)		
	Male (n = 8)	Female (n = 42)	p
Deformities	5 (62.5)	18 (42.9)	0.44
Sicca symptoms	2 (25)	8 (19)	0.65
IPF	0 (0)	6 (14.3)	–
RN	1 (12.5)	9 (21.4)	–
Vasculitis	1 (12.5)	0 (0)	–
DAS28	5.7 (4.4–6.4)	6.9 (5.6–7.5)	**0.04**
VAS	3.5 (1–5)	5.0 (5–10)	**0.009**
HAQ-DI	0.5 (0.1–1.5)	1.3 (0.4–2)	0.1
RF	3 (37.5)	33 (78.6)	**0.03**
Anti-CCP	5 (62.5)	32 (76.2)	0.41
miRNA-146a genotypes			
GC	5 (62.5)	20 (47.6)	0.58
GG	1 (12.5)	14 (33.3)	
CC	2 (25)	8 (19.1)	

RA: rheumatoid arthritis, IPF: interstitial pulmonary fibrosis, RN: rheumatoid nodule, DAS28: disease activity score, VAS: visual analogue scale, HAQ-DI: health assessment questionnaire-disability index, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide. Bold values are significant at $p < 0.05$.

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Table 3
Genotypes and alleles frequency distribution ofmiRNA-146a (rs2910164) polymorphism in rheumatoid arthritis patients and controls.

Variable n(%)	RA patients (n = 50)	Control (n = 40)	p
miR-146a genotypes			
GC	25 (50)	14 (35)	
GG	15 (30)	14 (35)	0.26
CC	10 (20)	12 (30)	
miR-146a alleles			
G	55 (56.1)	42 (52.5)	0.65
C	43 (43.9)	38 (47.5)	

RA: rheumatoid arthritis, miR-146a: miRNA-146a, n: Number.

activity may be significantly influenced by CC genotype [24]. *Pauley et al.* found an association between high miR-146a expression levels and disease activity [13]. On the other hand, others reported no association between miR-146a gene polymorphism and disease activity scores [31,33].

In the present study EAMs were found in 46% of the patients of them the most common were both RN and sicca symptoms (20% of the patients each), interstitial pulmonary fibrosis (IPF) was seen in 12% of the patients. Rheumatoid nodules are the most commonly reported EAM followed by ocular symptoms according to *Vela* [34]. *Turesson et al.* found a 28.2% frequency of RN, 9.5% for sicca symptoms, and 5.6% for IPF [35]. *Calguneri et al.* detected 18.1% frequency of RN, 11.4 for sicca symptoms, and 4.8% for IPF [36]. This variance in the reported values is probably attributed to different ethnic groups. It was observed that the sicca symptoms were more associated with the CC genotype with the significant difference among different genotypes with more frequent C allele. Also, CC genotype and C allele were higher in RN while GG genotype and G allele were higher in IPF. *Hu et al.* reported an association between miR-146a gene polymorphism and EAM; they found it to be associated with GG genotype [37]. Others found no association with EAM [26]. The present study demonstrates that miR-146a rs2910164 polymorphisms may be used as a diagnostic biomarker for EAM of RA in the future therefore these results need to be confirmed in a larger cohort. Furthermore, the relationship between the miR-146a rs2910164 genotypes and the severity parameters of RA (deformities, HAQ-DI, RF and anti-CCP) has been determined. The current results showed that the CC genotype and C allele were higher in presence of deformities. This agrees with *El-Shal et al.* [33], and *Ben Hassine et al.* [38] who did not find an association between this SNP and joint erosions in Egyptian and Tunisian RA patients. On the contrary, a cohort of Egyptian RA patients revealed that patients without deformities carried more likely the CC genotype of miR-146a rs2910164 and had a higher expression level of miR-146a than patients with deformities, thus suggesting a protective effect [31]. There was also no significant association between miR-146a rs2910164 different genotypes and the HAQ-DI, this is consistent with the results of *El-Shal et al.* who reported the same finding [33]. Moreover, current results revealed that GG genotype and G allele were more frequent in the positive RF and anti-CCP autoantibodies. Similarly, earlier studies reported that there was no significant difference between the SNP rs2910164 and RF, anti-CCP antibodies [24,33,39]. In the study of *Ciccacci et al.* [30] the variant allele of the miR-146a was associated with negative RF status, consistent with the evidence that this polymorphism leads to a reduced expression of mature miR-146a [26,39]. Considering all of the above, it is evident that miR-146a rs2910164 polymorphism affects the development of various symptoms of RA. Studies on the association between the genetic variants and disease can improve insights about the pathogenic mechanisms, and they also have the potential for direct clinical application by providing markers of risk, diagnosis, recognize the RA in an earlier stage, prognosis and possible therapeutic targets, so much more work is warranted in this field. The small sample size and the lack of confirmed functional characterization of the currently studied SNP are limitations of this study. Further studies of the association in larger cohorts and the functions of this SNP in the expression of miR-146a are required to clarify its role in the pathogenic mechanisms of the RA. More extensive wide range studies on different ethnicities are needed to prove or reject our findings.

In conclusion, this work revealed that the distribution of miR-146a rs2910164 genotypes in RA patients did not differ from that in control subjects, however, CC genotype was more frequently associated with sicca symptoms, RN, presence of deformities, TJC, SJC, CRP, DAS28, and HAQ-DI but GG genotype was higher in IPF, positive RF and anti-CCP antibodies. The number of swollen joints and frequency of sicca symptoms were higher with the CC genotype. miR-146a rs2910164 may be considered as a potential biomarker for extra-articular manifestations of RA that need more attention and warrant aggressive therapy.

Table 4
Associations of clinical, and laboratory parameters of rheumatoid arthritis patients with different genotypes of miR-146a rs2910164.

Variable mean ± SD or n(%)	miR-146a genotypes in RA patients (n = 50)			P
	GC	GG	CC	
Age at onset (years)	39.2 ± 9.2	36.5 ± 10.5	32.2 ± 10.3	0.17
MS (min)	17.1 ± 7.8	25.0 ± 6.6	34.4 ± 13.1	0.31
TJC	15.4 ± 10.7	17.5 ± 10.4	18.2 ± 10.9	0.62
SJC	12.1 ± 6.8	15.9 ± 6.5	18.2 ± 6.3	**0.04**
VAS	5.5 ± 3	6.5 ± 3.4	6.3 ± 3.2	0.63
RF	45.8 ± 18.8	89.7 ± 25.6	74.4 ± 27.0	0.89
Anti-CCP	89.2 ± 29.1	118.1 ± 31	118.6 ± 35.9	0.28
ESR	46.1 ± 22.7	29.1 ± 18.5	46.3 ± 30.3	0.12
CRP	20.2 ± 10.6	14.6 ± 10.1	23.9 ± 16.2	0.14
DAS 28	6.2 ± 1.6	6.4 ± 1.3	6.8 ± 1.7	0.58
HAQ-DI	1 ± 0.9	1.3 ± 0.6	1.5 ± 1	0.23
Deformities	10 (40)	7 (46.7)	6 (60)	0.75
Sicca symptoms	2 (8)	3 (20)	5 (50)	**0.01**
IPF	3 (12)	2 (13.3)	1 (10)	0.69
RN	5 (20)	2 13.3)	3 (33.3)	0.51
Vasculitis	1 (4)	0 (0)	0 (0)	–

miR-146a: miRNA-146a, RA: rheumatoid arthritis, MS: Morning stiffness, TJC: tender joint count, SJC: swollen joint count, VAS: Visual analogue scale, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, DAS28: disease activity score, HAQ-DI: health assessment questionnaire-disability index, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, IPF: interstitial pulmonary fibrosis, RN: rheumatoid nodule. Bold values are significant at $p < 0.05$.

Table 5
Associations of miR-146a (rs2910164) alleles with clinical, and laboratory parameters of rheumatoid arthritis patients.

Variable n (%)	miR-146a alleles		p
	G	C	
Deformities	24 (43.6)	20 (46.5)	0.84
RN	9 (16.4)	11 (25.6)	0.32
Sicca symptoms	8 (14.5)	12 (27.9)	0.13
IPF	7 (12.7)	3 (7)	0.5
Vasculitis	1 (1.8)	1 (2.3)	–
RF	40 (72.7)	30 (69.8)	0.82
Anti-CCP	44 (80)	30 (69.8)	0.34

miR-146a: miRNA-146a, RN: rheumatoid nodule, IPF: Interstitial pulmonary fibrosis, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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