ABSTRACT

MicroRNA-124 (miR-124) is known as an important regulator of the immune system and inflammatory response. Studies have reported that this miRNA is dysregulated in autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). A functional analysis demonstrated that rs531564 (C>G) affects the biogenesis of primary microRNA transcript-124 (pri-miR-124) and changes the expression of mature miR-124. In the present study, for the first time, we intended to evaluate the possible association between rs531564 polymorphism with SLE and RA risk.

In this case-control study, 110 patients with SLE, 115 patients with RA, and 120 healthy subjects were enrolled to evaluate rs531564 genotypes with real-time polymerase chain reaction (PCR) high resolution melting method.

Our findings demonstrated that frequency of GC genotype and G allele were considerably higher in the control group than RA patients, demonstrating that that GC genotype and G allele have a protective effect for healthy individuals (GC vs CC; OR: 0.29; 95%CI [0.12,0.67] and G vs C; OR: 0.42; 95%CI [0.23,0.78]). However, no significant correlation was confirmed between allele and genotype frequencies of rs531564 with SLE risk (p>0.05). However, the G allele in rs531564 polymorphism was associated with serum level of C-reactive protein (CRP), erythrocyte
sedimentation rate (ESR), anti-dsDNA antibody, C3, C4, and creatinine, and frequency of renal involvements in SLE patients ($p<0.05$). Moreover, in RA patients, the G was correlated with lower concentration ESR and CRP ($p<0.001$).

Our findings propose a considerable association between rs531564 polymorphism in the pri-miR-124 gene with susceptibility and clinical characteristics of RA and SLE in the Iranian population.

Keywords: Rheumatoid arthritis; Single nucleotide polymorphism; Systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are complex multifactorial autoimmune diseases. These disorders have various systemic manifestations, ranging from fever, weight loss, skin lesions, oral ulcers, and arthritis to life-threatening complications such as kidney damages, cardiovascular disease, severe hematological abnormalities, and neurological symptoms. In most cases, the exact etiology of SLE and RA is unclear but in numerous studies multiple genetic, epigenetic, and environmental risk factors have been reported. Over the two last decades, genome-wide association studies (GWAS) have emerged as powerful tools for the investigation of new risk loci and single nucleotide polymorphisms (SNPs) which are associated with the risk of complex diseases such as autoimmune diseases in various populations with different ancestries.

Many research studies described that microRNAs (miRNAs) dysregulation, as a part of epigenetic changes, plays an important role in the pathogenesis of autoimmune diseases such as SLE and RA. MiRNAs are a class of small non-coding RNAs (containing about 18 to 28 nucleotide long) that are involved in post-transcriptional gene regulation by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs and inhibition of gene expression. Relevantly, these molecules have been proved as master regulators in many aspects of cellular biology such as the immune system and inflammation. Researchers have demonstrated that the genetic, epigenetic, and environmental elements can affect miRNAs and cause autoimmune diseases. As far as we know, miRNAs and SNPs play important roles in the risk and development of autoimmune disease especially RA and SLE.

SNPs as the most abundant form of allelic variations exist once almost in every 300 nucleotides with appreciable frequency (>1%) and could be associated with the risk of multifactorial autoimmune diseases. These SNPs can be located in the 3'-UTR sequence of target genes and dysregulate the expression of the target gene by disrupting the binding site of miRNAs. Importantly, SNPs can be situated in pre-miRNAs sequences and may influence the transcription and various processing steps in miRNA biogenesis and consequently alter the function of miRNAs.

MiR-124 is known as a key regulator of immune function and inflammation by regulating development and function of different immune cells. Some studies reported that this miRNA is dysregulated in immune and inflammatory diseases. For instance, a study demonstrated that this molecule is strikingly downregulated in RA and another study represented that overexpression of miR-124 inhibits adjuvant-induced arthritis (AIA) in an animal model and therefore decreases synoviocyte proliferation, leukocyte infiltration, and finally cartilage/bone destruction. On the other hand, studies on SLE patients demonstrated that miR-124 is decreased in peripheral blood samples and especially CD4$^+$ T cells of SLE patients compared with healthy controls.

Qi et al with using a functional analysis indicated that rs531564 (C>G) can affect the processing of pri-miRNA for miR-124 and subsequently change the expression of mature miR-124 form. They demonstrated that the existence of the G allele can increase the expression of miR-124 by changing the predicted secondary structure of the pri-miRNA. Therefore, with regards to these data, this polymorphism could influence the expression of miR-124 and finally modulate the risk of autoimmune diseases especially SLE and RA. In the current study, we set out to evaluate the plausible correlation between rs531564 and the risk of RA and SLE diseases in the Iranian population. Besides, we assessed the association between this variant and the clinical characteristics of these diseases.
MATERIALS AND METHODS

Sample Selection and Characteristics
In this case-control study, a total of 115 RA patients and 110 SLE patients were enrolled according to diagnostic criteria created by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) (2019). All patients and 120 age and sex frequency-matched healthy controls were recruited from the rheumatology clinics and inpatient wards at Al-Zahra Hospital of Isfahan University of Medical Sciences. This study was approved by the Semnan university research ethics committee (Approved number: IR.SEMUMS.REC.1399.314) and all the subjects provided written informed consent. All subjects in this study were unrelated and controls had no symptoms or personal and family history of RA and SLE, or other autoimmune conditions. Demographic and clinical manifestation data of all subjects were collected. These data were gender, age, blood pressure (systolic blood pressure (SBP) and diastolic blood pressure (DBP)), height, and weight to calculate body mass index (BMI, calculated as weight [kg] divided by height [m] squared), family history of RA, SLE and other autoimmune disorders and clinical symptoms such as the existence of skin lesions, neurological disorders, hematological manifestations, oral ulcer, arthritis, and renal disorders. Similarly, laboratory features such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), anti-dsDNA antibodies, complement component 3 (C3), complement component 4 (C4), white blood cell (WBC) count, hemoglobin, creatinine, platelet count test (PLT), blood urea nitrogen (BUN), fasting blood sugar (FBS), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were documented. Ultimately, about 5 ml of peripheral blood was collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes from each participant and stored at −20°C until genotyping by real-time polymerase chain reaction high-resolution melting (HRM) method.

HRM method was used to determine rs531564 polymorphism genotypes. HRM was performed using Type-it HRM PCR kit containing HotStarTaq plus DNA polymerase and EvaGreen dye (Qiagen Germany), and analysis carried out with Rotor-Gene 6000™ (Corbett Research, Mortlake, New South Wales, Australia). The forward and reverse primer sequences for the 213bp fragment that spanned the rs531564 in the pri-miR-124 gene were GAGGTGTGCAGCCTGTGAC and GGAGGAAGGTGTTGACCCAC, respectively. The thermal program of the reaction is as follows: 5 min denaturation at 95°C, 30 cycles of 95°C for 10 s, 59°C for 30 s, and 72°C for 20 s. The melting curve is generated by increasing between 60°C and 95°C at 0.1°C/s. Finally, melting curves were normalized between the two temperatures to determine the specimens with known genotypes as a standard. For using sample genotypes in HRM analysis as a standard, some samples with different melt curves were subjected to direct Sanger sequencing and their exact genotypes were specified.

Statistical Analyses
The SPSS 25 (Armonk, NY: IBM Corp) was performed for statistical analyses. Genotype distribution in patients and controls were assessed for Hardy-Weinberg equilibrium using the $\chi^2$ test. Logistic regression analysis was performed to examine the correlation between genotypes in case and controls and compute specific odds ratios (ORs), 95% confidential intervals (CIs), and $p$ values. For demographic, clinical, and laboratory characteristics, $P$ values were calculated using the independent Pearson $\chi^2$ test for categorical variables and t-test for continuous variables test with the significance level of <0.05.

RESULTS

Subject Characteristics
Individuals in the present study consisted of 110 SLE patients (25 males and 85 females with a mean age at the sampling time of 43.62±13.41) and 115 subjects as RA patients (33 males and 82 females with a mean age at the sampling time of 47.40±10.45). The participants in the control group consisted of 120
persons (39 males and 81 females with mean age of 45.39±12.73). The mean age of onset in the SLE and RA case groups was 26.23±2.34 and 41.12±10.39, respectively. There was no considerable difference between the patients and control groups in terms of age ($P_{\text{Controls vs SLE}}: 0.305$, $P_{\text{Controls vs RA}}: 0.187$) and gender ($P_{\text{Controls vs SLE}}: 0.107$, $P_{\text{Controls vs RA}}: 0.527$), representing that for these features matching was satisfactory. Table 1 listed the features of the patients and healthy controls. Concerning BMI, there was a remarkable difference between the patients and participants in the control group ($p<0.001$). Similarly, there was a remarkable difference between the SLE subjects and the control group in terms of systolic blood pressure (SBP) ($p<0.001$) and diastolic blood pressure (DBP) ($p<0.001$). However, there was not any difference between the RA patients and the control group in terms of SBP and DBP ($p>0.05$). In the SLE cases group, twenty (18.2%) subjects and in RA cases 19 (16.52%) patients had a family history of SLE and RA or other autoimmune diseases.

Of all SLE patients, 24.5% with neurological symptoms, 43.6% with renal involvement, 50.9% with hematological manifestations, 63.65% with skin lesions, 76.4% with oral ulcers, and 89.1% with arthritis were documented. Based on laboratory test results, the concentration of ESR, CRP, anti-dsDNA antibody, creatinine, BUN, and was meaningfully higher in SLE subjects than healthy controls ($p<0.05$). However, the mean concentration of hemoglobin, PLT, C3, and C4 levels was expressively lower in SLE patients compared with the control group ($p<0.05$). However, there was not a meaningfully significant difference between SLE cases and controls in terms of white blood cell count, FBS, HDL, LDL, and TG ($p>0.05$). On the other hand, patients with RA had a higher level of ESR, CRP, white blood cell count, and creatinine and lower level of hemoglobin than control subjects ($p<0.05$). The laboratory features of cases (SLE and RA) and controls are documented in Table 2.

### Table 1. Baseline characteristics of patients (systemic lupus erythematosus and rheumatoid arthritis) and control subjects who participated in the study.

| Characteristics                  | Controls | SLE       | $P_{\text{Controls vs SLE}}$ | RA        | $P_{\text{Controls vs RA}}$ |
|----------------------------------|----------|-----------|-------------------------------|-----------|-----------------------------|
| Total number                     | 120      | 110       | 115                           |           |                             |
| Age at sampling time (mean±SD)   | 45.39±12.73 | 43.62±13.41 | 0.305                        | 47.40±10.45 | 0.187                       |
| Gender n (%)                     | Male: 39(32.5%) | 25(22.7%) | 0.107                        | 33(28.7%) | 0.527                       |
|                                  | Female: 81(67.5%) | 85(77.3%) | -- 41.12±10.39 | 82(71.3%) |                             |
| Age of onset (mean±SD)           | 23.14±3.31 | 25.80±2.34 | $<0.001^*$                   | 26.20±2.50 | $<0.001^*$                  |
| SBP (mean±SD)                    | 120.92±9.74 | 125.50±16.00 | 0.009*                       | 122.43±12.45 | 0.300                       |
| DBP (mean±SD)                    | 78.80±8.30 | 82.64±5.90 | $<0.001^*$                   | 78.50±7.80 | 0.796                       |
| Positive family history n (%)    | 0        | 20(18.2%) | -- 19 (16.52%) | --         |                             |
| Neurological symptoms n (%)      | 0        | 27(24.5%) | -- 0                         | --         |                             |
| Skin manifestations n (%)        | 0        | 70(63.6%) | -- 0                         | --         |                             |
| Hematological manifestations n   | 0        | 56(50.9%) | -- 0                         | --         |                             |
| (%)                              | Oral ulcers n (%) | 0        | 84(76.4%) | -- 0 | --                           |
| Arthritis n (%)                  | 0        | 98(89.1%) | -- 63(100%)                  | --         |                             |
| Renal involvement n (%)          | 0        | 48(43.6%) | -- 0                         | --         |                             |

* $p$ value $<0.05$. BMI: Body mass index; SD: Standard deviation; SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.
rs531564 Polymorphism: Association with Autoimmune Diseases

Table 2. Laboratory characteristics of patient and control groups

|                     | Controls (120) | SLE (110) | P Controls vs SLE | RA (115) | P Controls vs RA |
|---------------------|---------------|-----------|-------------------|----------|-----------------|
| **ESR (mm/h)**      | 15.60±6.92    | 41.33±22.81| <0.001*           | 37.10±22.54| <0.001*         |
| **CRP (mg/L)**      | 4.60±2.82     | 16.30±9.70 | <0.001*           | 16.30±13.11| <0.001*         |
| White blood cell (10^9/L) | 6578.50±1378.10 | 6820.91±1780.71 | 0.253           | 7319.60±2176.30 | 0.002*         |
| Hemoglobin          | 14.33±1.60    | 11.90±1.40 | <0.001*           | 12.43±1.10 | <0.001*         |
| **PLT (10^9/L)**    | 251.03±66.80  | 225.91±63.30| 0.004*           | 261.70±71.90 | 0.241           |
| Creatinine (mg/dL)  | 0.90±0.18     | 1.02±0.24  | <0.001*           | 1.02±0.19  | <0.001*         |
| BUN                 | 16.12±4.10    | 19.60±11.90| 0.004*           | 17.12±4.70 | 0.081           |
| FBS                 | 92.92±22.01   | 89.70±12.71| 0.168             | 96.44±15.80 | 0.160           |
| HDL                 | 50.41±11.10   | 51.10±8.90 | 0.628             | 49.40±7.60 | 0.395           |
| LDL                 | 107.03±31.30  | 102.74±26.15| 0.262           | 110.01±29.10 | 0.456           |
| Anti-dsDNA (IU/mL)  | 10.91±4.35    | 198.91±181.70| <0.001*       | --        | --              |
| C3 level (mg/dL)    | 141.53±35.12  | 50.30±36.03| <0.001*           | --        | --              |
| C4 level (mg/dL)    | 19.90±5.84    | 10.52±7.16 | <0.001*           | --        | --              |

* p<0.05. Data are mean±SD, or n (%). SD: Standard deviation; SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; BUN: Blood urea nitrogen; PLT: Platelet; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; FBS: Fasting blood sugar; C3: Complement component 3; C4: Complement component 4; dsDNA: Double-stranded DNA.

Association of rs531564 (C>G) Polymorphism with SLE

The genotype distribution of rs531564 in the SLE case and control groups was in agreement with those predicted via Hardy–Weinberg equilibrium. Among the SLE cases, the frequency of the CC, GC, and GG was 82.7%, 10.9%, and 6.4%, respectively. In the control group, the frequency of the rs531564 genotype was 77.5% for CC, 15.0% for GC, and 7.5% for GG. Furthermore, the frequencies of C and G alleles were 88.2% and 11.8% in cases, and 85.0% and 15.0% in the control group, respectively. Considering these distributions, our analysis revealed that there was not an important difference between SLE subjects and healthy controls in terms of genotypes and allele frequency (p>0.05).

Our assessment showed that patients with G allele (GG + GC genotypes) and CC genotypes had 32.84±11.23 and 24.85±10.30 mean age of onset, respectively. This finding uncovered that the G allele was connected with higher age of onset (p=0.003).

Association of rs531564 (C>G) Polymorphism with RA

Based on our analysis, significant association was indicated between GC genotype and decreased risk of RA (p=0.003). The frequency of CC, GC, and GG genotypes were 89.6%, 7.0%, and 3.5% in cases and
75.0%, 20.0%, and 5.0% in controls, respectively. Our evaluations on different models of inheritance for this polymorphism demonstrated that the genotype frequency of rs531564 polymorphism was markedly different under the dominant model between RA patients and the control group ($p$: 0.004). The frequency of the G allele in controls and cases was, respectively, 15.0% and 7.0% and the frequency of C allele in healthy control was 85.0% and in cases was 93.0%. Concerning allele distribution, the G allele was more frequent in the healthy individuals’ group than in cases ($p$:0.008) (Table 4). Moreover, our investigation revealed that the mean concentration of ESR in patients with G allele (GG + GC genotypes) was significantly

Table 3. Association between genotypes and allele frequency of miR-124 polymorphism (rs531564) with systemic lupus erythematosus risk.

| Genotype group | Patients (n = 110) n (%) | Controls (n = 120) n (%) | OR (95%CI) | p  |
|----------------|------------------------|-------------------------|------------|----|
| Genotype       |                        |                         |            |    |
| CC             | 91(82.7%)              | 93(77.5%)               | Reference  | ---|
| GC             | 12(10.9%)              | 18(15.0%)               | 0.67(0.31,1.43) | 0.336|
| GG             | 7(6.4%)                | 9(7.5%)                 | 0.76(0.28,2.22) | 0.661|
| Allele         |                        |                         |            |    |
| C              | 194(88.2%)             | 204(85.0%)              | Reference  | ---|
| G              | 26(11.8%)              | 36(15.0%)               | 0.76(0.43,1.23) | 0.318|
| Dominant inheritance |         |                         |            |    |
| CC             | 91(82.7%)              | 93(77.5%)               | Reference  | ---|
| GG+GC          | 19(17.3%)              | 27(22.5%)               | 0.71(0.37,1.39) | 0.322|
| Recessive inheritance |       |                         |            |    |
| CC+GC          | 103(93.6%)             | 111(92.5%)              | Reference  | ---|
| GG             | 7(6.4%)                | 9(7.4%)                 | 0.83(0.30,2.33) | 0.735|

*p <0.05.

Table 4. Association between genotypes and allele frequency of miR-124 polymorphism (rs531564) with rheumatoid arthritis risk

| Genotype group | Patients (n = 115) n (%) | Controls (n = 120) n (%) | OR (95%CI) | P value |
|----------------|------------------------|-------------------------|------------|---------|
| Genotype       |                        |                         |            |         |
| CC             | 103(89.6%)             | 90(75.0%)               | Reference  | ---     |
| GC             | 8(7.0%)                | 24(20.0%)               | 0.29(0.12,0.67) | 0.003* |
| GG             | 4(3.5%)                | 6(5.0%)                 | 0.58(0.16,2.0) | 0.409   |
| Allele         |                        |                         |            |         |
| C              | 214(93.0%)             | 204(85.0%)              | Reference  | ---     |
| G              | 16(7.0%)               | 36(15.0%)               | 0.42(0.23,0.78) | 0.008* |
| Dominant inheritance |         |                         |            |         |
| CC             | 103(86.6%)             | 90(71.4%)               | Reference  | ---     |
| GG+GC          | 16(13.4%)              | 36(28.6%)               | 0.38(0.20,0.75) | 0.004* |
| Recessive inheritance |       |                         |            |         |
| CC+GC          | 111(96.5%)             | 114(95.0%)              | Reference  | ---     |
| GG             | 4(3.5%)                | 6(5.0%)                 | 0.67(0.19,2.5) | 0.563   |

*p <0.05.
lower than patients with homozygote CC genotype (23.50±6.25 vs 38.70±23.23; p>0.001). Likewise, RA patients with GG + GC genotypes have a considerably lower concentration of CRP (3.20±1.61) compared with RA-subjects with CC genotypes (18.03±13.01) (p=0.001). Also, the concentration of creatinine was slightly lower in cases with GG + GC genotypes than the CC genotype (p= 0.040). Moreover, the genotype frequencies in the case and control groups were in agreement with those predicted via Hardy–Weinberg equilibrium. The summary of the stratification analysis is shown in Table 6.

Table 5. Association of miR-124 polymorphisms (rs531564) with various parameters of systemic lupus erythematosus (110 Patients)

| Genotype group       | GG+GC (n=19) | CC (n=91) | P value |
|----------------------|--------------|-----------|---------|
| Age of onset         | 32.84±11.23  | 24.85±10.30 | 0.003*  |
| ESR (mm/h)           | 28.74±13.60  | 44.10±23.51 | <0.001* |
| CRP (mg/L)           | 10.10±5.90   | 17.60±9.90 | <0.001* |
| C3 level (mg/dL)     | 90.80±30.22  | 41.82±31.20 | <0.001* |
| C4 level (mg/dL)     | 18.27±6.60   | 8.90±6.17  | <0.001* |
| Anti-dsDNA (IU/mL)   | 24.90±10.24  | 235.24±179.53 | <0.001* |
| Creatinine (mg/dL)   | 0.90±0.18    | 1.10±0.24  | 0.012*  |
| Hemoglobin (HB)      | 11.95±1.51   | 11.85±1.40 | 0.780   |
| Neurological symptoms | 2(10.5)     | 25(27.5)  | 0.118   |
| Skin manifestations  | 12(63.15)    | 58(63.73) | 0.856   |
| Hematological manifestations | 13(68.4) | 43(47.3) | 0.093   |
| Oral ulcers (%)      | 14(73.7)     | 70(76.9)  | 0.762   |
| Arthritis (%)        | 18(94.7)     | 80(87.9)  | 0.385   |
| Renal involvement (%)| 2(10.5)      | 46(50.5)  | 0.001*  |

Data are mean±SD, or n (%). * p<0.05. SD: Standard deviation; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; C3: Complement component 3; C4:Complement component 4; dsDNA: Double-stranded DNA.

Table 6. Association of miR-124 polymorphisms (rs531564) with various parameters of rheumatoid arthritis (115 Patients)

| Genotype group       | GG+GC (n=12) | CC (n=103) | P value |
|----------------------|--------------|-----------|---------|
| Age of onset         | 38.30±9.80   | 41.50±1.50 | 0.314   |
| ESR (mm/h)           | 23.50±6.25   | 38.70±23.23 | <0.001* |
| CRP (mg/L)           | 3.20±1.61    | 18.03±13.01 | <0.001* |
| Creatinine (mg/dL)   | 0.92±0.20    | 1.04±0.20  | 0.040*  |
| Hemoglobin (HB)      | 13.10±0.82   | 12.38±1.08 | 0.126   |

Data are mean±SD, or n (%). * p<0.05. SD: Standard deviation; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

DISCUSSION

Generally, miR-124 is expressed in various immune cells and organs, such as peripheral blood mononuclear cells (PBMCs), lymph nodes, the thymus, and bone marrow (https://www.genecards.org/cgi-bin/carddisp.pl?gene=MIR124-1). Several studies have reported that miR-124 is dysregulated in immune and autoinflammatory diseases.28,33 For instance, Koukos and colleagues reported that this miRNA is reduced in children with ulcerative colitis (UC) because of promoter hypermethylation and stimulates inflammation by inducing the expression and activity of STAT3 in colon tissues.34 Similarly, in a study by...
Zhou et al, it was revealed that the miR-124 promoter is hypermethylated and subsequently its expression is downregulated in RA patients than the control group. They indicated that downregulation of miR-124 is correlated with higher synovocyte proliferation. Reduced expression of miR-124 was also reported in the other studies in RA patients and they represented that this downregulation is correlated with increased chemokine expression of fibroblast-like synovial cells. Furthermore, Zhang et al reported that miR-124 expression is remarkably decreased in SLE patients with active lupus nephritis than subjects with non-active lupus nephritis and patients without kidney involvements. Also, they indicated that downregulation of miR-124 is associated with increased serum levels of IL-6, IL-1β, TRAF6, and TNF-α. Interestingly, they characterized that miR-124 had an inhibitory effect on the inflammation of renal mesangial cells via binding to 3′-UTR of TRAF6 mRNA. Chen et al demonstrated that miR-124 is reduced in CD4⁺ T cells of SLE patients and functional assays revealed that overexpression of miR-124 represses the immune activity of isolated CD4⁺ T cells of SLE patients. On the other hand, Jin et al unveiled that miR-124 is diminished in serum samples of patients with SLE, RA, UC, and Sjogren’s syndrome (SS) and could be used as an appropriate biomarker in the diagnosis of these diseases.

A functional study unraveled that rs531564 polymorphism in pri-miR-124 can influence miRNA biogenesis and consequently alter the expression of mature miR-124 by affecting the ring-shaped structure of the pri-miRNA of miR-124. Expression analysis demonstrated that the expression of miR-124 is significantly higher in patients with the GC genotype than samples with other genotypes. Also, individuals with the GG genotype had tremendously higher expression compared with people with the CC genotype. Hence, in the light of these data, rs531564 polymorphism could influence the expression of miR-124 and finally modulate the risk of autoimmune diseases especially SLE and RA. However, most association studies on rs531564 in pri-miR-124 were carried out on non-autoimmune disorders such as cancers.

To the best of our knowledge, the current study is the first report that investigates the correlation between pri-miR-124 polymorphism, rs531564, with SLE and RA risk. Our findings discovered that no significant correlation exists between allele and genotype frequencies of rs531564 polymorphism and SLE risk in the Iranian population. Neither combined genotypes i.e. TG + GG genotypes nor CC genotype affected the risk of SLE. Nevertheless, this polymorphism was significantly correlated with the production level of some serum proteins and clinical presentations in SLE patients which reflect disease activity and severity. Because some studies demonstrated that level of these factors was associated with disease activity and severity. The G allele in rs531564 polymorphism was associated with a lower level of ESR, CRP, anti-dsDNA antibody, and creatinine, as well as, higher level of C3 and C4 and lower frequency of renal involvements in SLE patients. However, our analysis demonstrated that the frequency of GC genotype and G allele is substantially lower in the control group than RA patients, demonstrating that GC genotype and G allele have a protective effect for healthy individuals (GC vs CC; OR: 0.29; 95%CI [0.12, 0.67] and G vs C; OR: 0.42; 95%CI [0.23, 0.78]). Similarly, healthy subjects with the G allele (GG + GC genotypes) had a lower risk of RA in the population under study (OR: 0.38; 95%CI [0.20, 0.75]). These results were inconsistent with previous studies which reported GC genotype and G allele were correlated with higher expression of miR-124 and also higher expression of these miRNA was associated with lower risk of autoimmune disease. Furthermore, stratification analysis demonstrated that patients with GC and GG genotypes had lower concentration ESR and CRP, two serum proteins that inform disease activity.

In concordance with our results, Li et al represented that the minor allele (G) in this polymorphism might protect against type 2 diabetes (T2DM) in a Han Chinese population. The other study in the Chinese population discovered that rs531564 polymorphism was meaningfully associated with the reduced risk of colorectal cancer. In the meanwhile, Ciccacci et al reported that rs531564 is not correlated with the risk of Crohn’s disease (CD) but was associated with colon location in patients with CD in an Italian population. Chuanyin et al demonstrated that this variant was associated with cervical intraepithelial neoplasia (CIN) and cervical cancer in the Chinese population. Although, in the Iranian population, it was revealed that rs531564 is not correlated with breast cancer risk. However, the other studies reported have the association of this polymorphism with other cancers.
such as gastric cancer and esophageal squamous cell carcinoma.45

On the one hand, the inflammatory pathways have an important role in cancers, inflammatory diseases, and also autoimmune disorders such as SLE and RA. On the other hand, by considering our findings and the results from previous studies about the significance of miR-124 in the immune system and inflammation, the influence of rs531564 in miR-124 biogenesis, and association with several diseases, we speculate that rs531564 polymorphism has a substantial role in the pathogenesis and modulation of SLE and RA risk.

Finally, in this work, probably, some possible limitations in the statistical validity of our results such as small population size exist, so further association studies in larger sample size would help to confirm the suggested correlations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

We would like to appreciate any support provided by Semnan and AJA University of Medical Sciences.

REFERENCES

1. Pabón-Porras MA, Molina-Ríos S, Flórez-Suárez JB, Coral-Alvarado PX, Méndez-Patarroyo P, Quintana-López G. Rheumatoid arthritis and systemic lupus erythematosus: Pathophysiological mechanisms related to innate immune system. SAGE Open Medicine. 2019;7(2):2050312119876146.
2. Signorini V, Elefante E, Zucchi D, Trentin F, Bortoluzzi A, Tani C. One year in review 2020: systemic lupus erythematosus. Clin Exp Rheumatol. 2020;38(0):592-601.
3. Kim J-W, Suh C-H. Systemic Manifestations and Complications in Patients with Rheumatoid Arthritis. J Clin Med. 2020;9(6):2008-14.
4. Kamen DL. Environmental influences on systemic lupus erythematosus expression. Rheum Dis Clin North Am. 2014;40(3):401-9.
5. Orozco G, Eyre S, Hinks A, Bowes J, Morgan AW, Wilson AG, et al. Study of the common genetic background for rheumatoid arthritis and systemic lupus erythematosus. Annals Rheumatic dis. 2011;70(3):463-8.
6. Ceccarelli F, Agmon-Levin N, Perricone C. Genetic Factors of Autoimmune Diseases. J Immunol Res. 2016;2016:3476023.
7. Lettré G, Rioux JD. Autoimmune diseases: insights from genome-wide association studies. Hum Mol Genet. 2008;17(R2):116-21.
8. Kochi Y. Genetics of autoimmune diseases: perspectives from genome-wide association studies. Inter Immunol. 2016;28(4):155-61.
9. Moran-Moguel MC, Petarra-del Rio S, Mayorquin-Galvan EE, Zavala-Cerna MG. Rheumatoid Arthritis and miRNAs: A Critical Review through a Functional View. J Immunol Res. 2018;2018:2474529.
10. Mazzone R, Zwerger C, Artico M, Taurone S, Ralli M, Greco A, et al. The emerging role of epigenetics in human autoimmune disorders. Clin Epigenetics. 2019;11(1):34-9.
11. Ehtesham N, Mosallaei M, Karimzadeh MR, Moradikazerouni H, Sharifi M. microRNAs: key modulators of disease-modifying therapies in multiple sclerosis: Pancreatic cancer is one of the lethal malignant tumours in the world. In this study, we investigated the CAR T-Cell therapy of pancreatic cancer. International reviews of immunology. 2020;39(6):264-79.
12. Catalanotto C, Cogoni C, Zardo G. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. Int J Mol Sci. 2016;17(10):1712.
13. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol. 2018;9(402):258-63.
14. Simonian M, Sharifi M, Nedaenia R, Mosallaei M, Khosravi S, Avan A, et al. Evaluation of miR-21 inhibition and its impact on cancer susceptibility candidate 2 long noncoding RNA in colorectal cancer cell line. Adv bio Res. 2018;7(14(2)):15-9.
15. Raisch J, Darfeuille-Michaud A, Nguyen HTT. Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol. 2013;19(20):2985-96.
16. Long H, Wang X, Chen Y, Wang L, Zhao M, Lu Q. Dysregulation of microRNAs in autoimmune diseases: Pathogenesis, biomarkers and potential therapeutic targets. Cancer Lett. 2018;428(41):90-103.
17. Moszyńska A, Gebert M, Collawn JF, Bartoszewski R. SNPs in microRNA target sites and their potential role in human disease. Open Biol. 2017;7(4):48-54.
18. Gregersen PK, Olsson LM. Recent advances in the genetics of autoimmune disease. Annu Rev Immunol. 2009;27(5):363-91.
19. Ehtesham N, Alani B, Mortazavi D, Azhdari S, Kenarangi T, Esmailzadeh E, et al. Association of rs3135500 and rs3135499 Polymorphisms in the MicroRNA-binding Site of Nucleotide-binding Oligomerization Domain 2 (NOD2) Gene with Susceptibility to Rheumatoid Arthritis. Iran J Allergy, Asthma Immunol. 2021;20(2):178-87.

20. Ricaño GPonce I, Zhernakova DV, Deelen P, Luo O, Li X, Isaacs A, et al. Refined mapping of autoimmune disease associated genetic variants with gene expression suggests an important role for non-coding RNAs. J Autoimmun. 2016;68(4):62-74.

21. Kim Y, Kang C, Min B, Yi G-S. Detection and analysis of disease-associated single nucleotide polymorphism influencing post-translational modification. BMC Med Genomics. 2015;8(2):S7-9.

22. Karimzadeh MR, Zarin M, Ehtesham N, Khoosravi S, Soosanabadi M, Mosallaei M, et al. MicroRNA binding site polymorphism in inflammatory genes associated with colorectal cancer: literature review and bioinformatics analysis. Cancer Gene Therapy. 2020;27(10):739-53.

23. Simonian M, Mosallaei M, Khoosravi S, Salehi R. rs12904 polymorphism in the 3'-untranslated region of ephrin A1 ligand and the risk of sporadic colorectal cancer in the Iranian population. J cancer Res Therapeutics. 2019;15(1):15-26.

24. Mosallaei M, Simonian M, Esmailzadeh E, Bagheri H, Miraghaian M, Salehi AR, et al. Single nucleotide polymorphism rs10889677 in miRNAs Let-7e and Let-7f binding site of IL23R gene is a strong colorectal cancer determinant: Report and meta-analysis. Cancer genetics. 2019;239:46-53.

25. Wang Y, Ru J, Jin T, Sun M, Jia L, Sun G. An Approach to Identify Individual Functional Single Nucleotide Polymorphisms and Isoform MicroRNAs. BioMed Res Interna. 2019;2019:6193673.

26. Qin Z, Wang P-Y, Su D-F, Liu X. miRNA-124 in immune system and immune disorders. FrontImmunol. 2016;7:406.

27. Han SM, Na HY, Ham O, Choi W, Sohn M, Ryu SH, et al. TCF4-targeting miR-124 is differentially expressed amongst dendritic cell subsets. Immune Network. 2016;16(1):61-9.

28. Nakamachi Y, Kawano S, Takenokuchi M, Nishimura K, Sakai Y, Chin T, et al. MicroRNA-124a is a key regulator of proliferation and monocyte chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Front Immunol. 2009;60(5):1294-304.

29. Nakamachi Y, Ohnuma K, Uto K, Noguchi Y, Saegusa J, Kawano S. MicroRNA-124 inhibits the progression of adjuvant-induced arthritis in rats. Ann Rheumatic dis. 2016;75(3):601-8.

30. Chen J, Peng L, Zhao Z, Yang Q, Yin F, Liu M, et al. HDAC1 potentiates CD4+ T cell activation by inhibiting miR-124 and promoting IRF1 in systemic lupus erythematosus. Cell Immunol. 2021;362:104284.

31. Qi L, Hu Y, Zhan Y, Wang J, Wang B-B, Xia H-F, et al. A SNP site in pri-miR-124 changes mature miR-124 expression but no contribution to Alzheimer's disease in a Mongolian population. Neuroscience lett. 2012;515(1):1-6.

32. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Front Immunol. 2019;71(9):1400-12.

33. Qin Z, Wang P-Y, Su D-F, Liu X. miRNA-124 in Immune System and Immune Disorders. Front Immunol. 2016;7:406.

34. Koukos G, Polytarchou C, Kaplan JL, Morley–Fletcher A, Gras–Miralles B, Kokkotou E, et al. MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. Gastroenterology. 2013;145(4):842-52.

35. Zhou Q, Long L, Shi G, Zhang J, Wu T, Zhou B. Research of the methylation status of miR-124a gene promoter among rheumatoid arthritis patients. Clin Develop Immunol. 2013;2013:524204.

36. Zhang L, Zhang X, Si F. MicroRNA-124 represents a novel diagnostic marker in human lupus nephritis and plays an inhibitory effect on the growth and inflammation of renal mesangial cells by targeting TRAF6. Int J Clin Exp Pathol. 2019;12(5):1578-88.

37. Jin F, Hu H, Xu M, Zhan S, Wang Y, Zhang H, et al. Serum microRNA profiles serve as novel biomarkers for autoimmune diseases. Front Immunol. 2018;9:2381.

38. Sjöwall C, Zickert A, Skogh T, Wetterö J, Gunnarsson I. Serum levels of autoantibodies against C-reactive protein correlate with renal disease activity and response to therapy in lupus nephritis. Arthritis Research & Therapy. 2009;11(6):R188.

39. Narayanan K, Marwaha V, Shanmuganandan K, Shankar S. Correlation between Systemic Lupus Erythematosus Disease Activity Index, C3, C4 and Anti-dsDNA Antibodies. Med J Armed Forces India. 2010;66(2):102-7.
40. Li Y, Zhang Y, Li X, Shi L, Tao W, Shi L, et al. Association study of polymorphisms in miRNAs with T2DM in Chinese population. I Int J Med Sci. 2015;12(11):875-80.
41. Gao X-r, Wang H-p, Zhang S-l, Wang M-x, Zhu Z-s. Pri-miR-124 rs531564 polymorphism and colorectal cancer risk. Sci Rep. 2015;5(1):1-7.
42. Ciccacci C, Politi C, Biancone L, Latini A, Novelli G, Calabrese E, et al. Polymorphisms in MIR122, MIR196A2, and MIR124A Genes are Associated with Clinical Phenotypes in Inflammatory Bowel Diseases. Mol Diagn Ther. 2017;21(1):107-14.
43. Chuanyin L, Xiaona W, Zhiling Y, Yu Z, Shuyuan L, Jie Y, et al. The association between polymorphisms in microRNA genes and cervical cancer in a Chinese Han population. Oncotarget. 2017;8(50):87914-9.
44. Danesh H, Hashemi M, Bizhani F, Hashemi SM, Bahari G. Association study of miR-100, miR-124-1, miR-218-2, miR-301b, miR-605, and miR-4293 polymorphisms and the risk of breast cancer in a sample of Iranian population. Gene. 2018;647(11):73-8.
45. Moazeni-Roodi A, Hashemi M. Association between miR-124-1 rs531564 polymorphism and risk of cancer: An updated meta-analysis of case-control studies. EXCLI journal. 2018;17:608-19.