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Impact of miR-155 (rs767649 A>T) and miR-146a (rs57095329 A>G) polymorphisms in System Lupus Erythematosus susceptibility in an Egyptian cohort

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Abstract. – OBJECTIVE: Systemic Lupus Erythematosus (SLE) is an autoimmune inflammatory disease. miR-155 and miR-146a were expressed in many autoimmune diseases such as rheumatoid arthritis. The aim of this study was to examine miR-155 rs767649 and miR-146a rs57095329 polymorphisms in SLE susceptibility in an Egyptian cohort and to investigate the correlation between them and clinical data and disease activity.

PATIENTS AND METHODS: The two SNPs were analyzed in 120 patients with SLE and 100 healthy controls using RT-PCR.

RESULTS: The TT genotype and T allele of miR-155 rs767649 were associated with a significant increase in the risk of SLE, particularly in females. On the other hand, miR-146a (rs57095329) polymorphism was not associated with SLE risk. The AT/TT genotypes of miR-155 rs767649 showed higher distributions among patients with higher SLEDAI and nephritis.

CONCLUSIONS: This study had demonstrated for the first time the association between miR-155 rs767649 and the risk of development of SLE in an Egyptian cohort, mostly in females.

Key Words:
Systemic lupus, miR-155 rs767649, miR-146a rs57095329, Polymorphism, Quantitative PCR.

Introduction

Systemic Lupus Erythematosus (SLE) is considered a complicated autoimmune inflammatory disease. It is characterized by an increased autoantibody production which is directed towards nuclear and cytoplasmic components. These autoantibodies cause the inflammatory process, which in turn causes the deposition of immune complex in tissues[1]. T cells have a fundamental role in SLE pathogenicity by stimulating B cells producing the autoantibody[2]. Furthermore, it increases the pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF-α), type I interferon (IFN), IL-1β, IL-6, IL-8, and B cell activating factor of the TNF-family (BAFF) causing lupus-related inflammation[3].

Women of childbearing age are mainly affected by this auto destructive disease. The cause of SLE is still obscure. Yet, environmental, hormonal, genetic involvement and epigenetic modifications, histone alteration and micro-ribonucleic acid expression are well known factors that trigger the disease[4-9].

The microRNAs (miRNAs) are a group of endogenous small non-coding RNAs molecules that

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are approximately 21–25 nucleotides in length. They regulate gene expression through promoting degradation of mRNA or through translational repression at multiple sites in the three prime untranslated regions (3'UTR) of target genes[10].

Many studies involving patients with SLE revealed that some miRNAs are pathogenic. However, other miRNAs are found to be protective[11-13]. MiR-146 are present in humans in two forms; miR-146a and miR-146b. miR-146a is greatly expressed in T lymphocytes, mononuclear cells, and macrophages. MiR-146 has a vital role in regulating the immune response via targeting genes with the IRAK1 (IL-1 receptor-associated kinase l)/ TRAF6 (TNF receptor-associated factor 6) TRAF6/NF-κB axis[14].

miR-155 are overexpressed in lupus regulatory T cells[15]. Their expression is altered in SLE B cells. Overexpression of miR-155 causes the enhancement of B cell survival. This causes an increased titer of IgG autoantibodies[16-18].

Single Nucleotide Polymorphisms (SNPs) are variations in a single nucleotide that happen at a particular site in the DNA[19]. miR-155 and miR-146a polymorphisms were observed to make immune-system dysfunctions and affect the susceptibility to various autoimmune diseases by affecting the miRNA target expression[20-22]. The selected miRNA polymorphisms are located in the regulatory regions of their miRNAs and may thus affect their expression levels[23].

Because of their role in immune response and the probable effect of both polymorphisms on their expression levels, we assume that they could play a role in SLE susceptibility. We aimed to determine if these polymorphisms are associated with susceptibility of SLE in the Egyptian population and to study the correlation between these polymorphisms with the clinical data and the disease activity.

Patients and Methods

This case-control study was done on one hundred and twenty Egyptian patients diagnosed with SLE. All patients were diagnosed according to the 2012 SLE International Collaboration Clinics (SLICC) classification criteria[24]. Patients were recruited from the Rheumatology and Rehabilitation Department, Fayoum University Hospital. Patients underwent history taking, with full clinical examinations. Clinical manifestations of SLE were assessed in all patients. Patients with Lupus nephritis were diagnosed by renal biopsy. SLE disease activity was evaluated according to the Systemic Lupus Disease Activity Index (SLEDAI)[25].

SLEDAI is a measure of systemic lupus activity. It is composed of 24 items, 16 of which are clinical, such as visual disturbance, psychosis, organic brain syndrome, seizure, other neurological manifestations, new rash, arthritis, and mouth sores. Eight of the 24 items are laboratory results such as urinalysis testing, increased anti-DNA antibody levels, complement levels, neutropenia, and thrombocytopenia. The score is formulated on the existence or absence of these manifestations in the previous 10 days. A score of 6 or more was shown to be considered an active disease. SLEDAI scores are as follow: patients with SLEDAI = 0 are considered to have no activity, patients with SLEDAI from 1 to 5 are considered to have mild activity, patients with SLEDAI from 6 to 10 are considered to have moderate activity, patients with SLEDAI from 11 to 19 are considered to have high activity, very high activity is for SLEDAI score more than 20[26].

Patients were excluded if they had other autoimmune diseases like rheumatoid arthritis, scleroderma, overlapping syndrome and diabetes mellitus.

Controls involve one hundred apparently healthy individuals from the same geographical region, with matched age and sex, and negative family history of SLE or any other autoimmune disease.

The present study was approved by the Faculty of Medicine Fayoum University Ethics Committee. All participants delivered informed written consent. All procedures executed in the study were per the Ethical Declaration of Helsinki.

Blood samples were collected to perform CBC, ESR, CRP, Antinuclear Antibody (ANA), Anti-ds DNA, anticardiolipin, lupus anticoagulant, C3, C4. Urine samples were obtained to detect proteinuria. Another portion of blood was assembled in tubes containing EDTA and was stored at -20C until DNA extraction.

DNA Extraction and Genotype Detection

DNA was extracted by DNA extraction kit (QIAamp® Whole Blood Genomic DNA Purification Mini Kit (50) Venlo, The Netherlands) according to the manufacturer's procedure. Quantitation of DNA was done utilizing the NanoDrop1-1000 spectrophotometer (NanoDrop Innovations, Inc., Wilmington, DE, USA).

Genotyping was accomplished by Real-time polymerase chain reaction using the TaqMan allelic

discrimination assay (Applied Biosystems, Foster City, CA, USA) via predesigned primer/probe sets for miR-155 rs767649 (A/T)[C_2212229_10] and miR-146a rs57095329 (A/G) [C_90078480_10], (Applied Biosystems, Foster City, CA, USA). miR-155 rs767649 forward, 5'-ATATAACA-CATTATCAAAAACACTG-3' and reverse, 5'-CACTTTTCTGAGTGCTCTAATCAGG-3'; and miR-146a rs57095329 forward 5'-CCCCG-CGGGGCTGCGGAGAGTACAG-3' and reverse, 5'-CAGGAAGCCTGGGGACCCAGCGCCT-3'.

DNA amplification was performed in a 25 µl volume containing 12.5 µl TaqMan genotyping master mix, 1.25 µl primer/probe, 1 to 20 ng of purified genomic DNA, and 11.25 µl H2O. Thermal cycling conditions of PCR included denaturation at 95°C for 10 min, PCR reaction was carried out for 45 cycles at 92°C for 15 s then annealing and extension at 60°C for 90 sec. Real-time PCR was performed using the Applied Biosystems™ Real-Time PCR system (Foster City, CA, USA).

Statistical Analysis

SPSS version 25 (SPSS Inc., Armonk, NY, USA) was used for the statistical methods. For quantitative data, mean and standard deviation were used. For categorical data frequency and relative frequency (percentage) were used. ANOVA post-hoc Tukey's test and an unpaired t-test were used in the comparison between groups. Chi-square (c^2) test was used for categorical data. When the expected frequency is less than 5 the Fisher's Exact Test was used. Genotype and allele frequencies were done in cases and controls. Unadjusted and adjusted logistic regression models were used to calculate the odds ratio (OR) with 95% confidence intervals[27]. Univariate and multivariate regression analyses were used in order to calculate the odds ratio (OR) with 95% confidence interval (CI) and adjusted for age and sex as possible confounders. Regression analysis was used to define the independent predictors of SLE. *p*-values less than 0.05 reflected statistically significant.

Results

The present study involved 120 patients with SLE (114 female, 6 male) whose mean age is 31.68± 8.52 years, with a duration of disease 15.3±4.7y, and 100 healthy control subjects (93 female, 7 male) with a mean age of 31.44± 8.70 years; age and gender of both groups were matched. SLEDAI of patients was 5.20± 4.79. 50% of patients have a skin rash, 45% have an oral ulcer, 37.5% have photosensitivity, 100% have ANA, 82.5% have Anti-ds DNA and 40% have low C4.

Frequency Distribution Regarding the Genotypes and Alleles of miR-155 (rs767649) in Patients with SLE and Healthy Controls

On comparing the SLE group of patients to healthy individuals, there was a significant difference in the frequency of miR-155(rs767649) genotypes in SLE cases ($p < 0.001$). The incidences of TT, AT, and AA genotypes were 12.5%, 80%, and 7.5%, respectively, in patients, and were 10.0%, 65%, and 25.0%, respectively, in healthy control subjects.

Considering AA genotype as a reference group in the genotypic and dominant model, the frequency of the TT, AT and AT+TT genotypes were significantly elevated in SLE patients than the control group ($p = 0.011$ and 0.001, respectively), and these genotypes were associated with a significant increase in the risk of SLE after adjusting age and sex (adjusted OR, 4.153%; 95% CI, 1.355-12.723; $p = 0.013$, adjusted OR, 4.085, 95% CI, 1.777-9.392, $p = 0.001$, adjusted OR, 4.093%; 95% CI, 1.793-9.344, $p = 0.001$, respectively) (Table I).

Furthermore, in the additive and allelic model where AA or A allele were used as a reference group, TT in additive and T allele in the allelic model showed significant differences, with increased distribution in SLE patients compared with the controls (p =0.011, 0.037, respectively). The TT genotype and T allele were associated with significant increase in the risk of SLE (adjusted OR, 5.238, 95% CI, 1.539-17.821, p = 0.008, adjusted OR, 1.480, 95% CI, 1.013- 2.163; $p = 0.043$) (Table I).

Regarding miR-146a (rs57095329); the GG, AG, and AA genotypes were not significant risk factors for SLE. Moreover, miR-146a (rs57095329); polymorphism was not associated with SLE under the dominant and additive models. In addition, no significant difference was detected regarding allele frequency of miR-146a (rs57095329) among SLE cases relative to healthy subjects ($p = 0.180$).

The haplotype effect of miR-155rs767649 and miR-146a rs57095329 polymorphisms in patients with SLE relative to the control group was also examined. The carriage of combinations of rs767649 T Allele +rs57095329 G Allele was significantly associated with SLE (p =0.041).

Table I. Genotype and allele frequencies of miR-146a (rs57095329) and miR-155(rs767649) polymorphisms in SLE and healthy controls.

Genotype and allele		Control n (%)	Systemic Lupus Erythematosus n (%)	*p*-value	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)
rs767649(155) A/T	TT	10 (10.0%)	15 (12.5%)	0.011*	4.167 (1.380-12.578)	0.013*	4.153 (1.355-12.723)
	AT	65 (65.0%)	96 (80.0%)	0.001*	4.103 (1.799-9.356)	0.001*	4.085 (1.777-9.392)
	AA	25 (25.0%)	9 (7.5%)	1		1	
rs767649(155) A/T Dominant model	TT+AT	75 (75.0%)	111 (92.5%)	0.001*	4.111 (1.817-9.300)	0.001*	4.093 (1.793-9.344)
	AA	25 (25.0%)	9 (7.5%)	1		1	
rs767649(155) A/T Recessive model	TT	10 (10.0%)	15 (12.5%)	0.561	1.286 (0.551-3.003)	0.588	1.266 (0.539-2.977)
	AA+AT	90 (90.0%)	105 (87.5%)	1		1	
rs767649(155) A/T Additive model	TT	10 (28.6%)	15 (62.5%)	0.011*	4.167 (1.380-12.578)	0.008*	5.238 (1.539-17.821)
	AA	25 (71.4%)	9 (37.5%)	1	1		
rs767649(155) A/T Alleles	Allele T	85 (42.5%)	126 (52.5%)	0.037*	1.495 (1.025-2.182)	0.043	1.480 (1.013-2.163)
	Allele A	115 (57.5%)	114 (47.5%)	1		1	
rs767649 T Allele + rs57095329 G Allele		50 (25%)	81 (33.8%)	0.041*	1.603(1.02-2.520)	0.055	1.569 (0.9912.484)

Data are expressed as n: (%). SLE: SLE, OR: Odds Ratio, CI: Confidence Interval. *Indicates statistical significance ($p < 0.05$).

Stratification of Genotype and Allele Frequencies According to Sex in SLE Cases and Control Groups

Both polymorphisms were further stratified according to sex in cases and controls as shown in Table II. As regards miR-155 rs767649 polymorphisms, our results showed that TT and AT genotypes in the genotypic model, TT+AT in the dominant model, T allele in the allelic model all were associated with increased risk of SLE in female subjects (p =0.004, <0.001, <0.001, 0.027, respectively). As regards miR-146a rs57095329, the GG, AG, and AA genotypes were not associated with the risk of SLE in female subjects.

Logistical Regression Analysis

Univariate and multivariate logistical regression analyses were performed to predict the genotype which is associated with increased risk of SLE (Table III). Both TT and AT genotypes of miR-155 rs767649 are positively associated with the risk of SLE in both univariate and multivariate analyses. On the other hand, miR-146a rs57095329 genotypes were not associated with risk of SLE in either of the two Analyses.

Association Between miRNA-155 (rs767649) Genotypes and miRNA-146a (57095329) and the Clinical Data of SLE Patients

Regarding miRNA-155 (rs767649), a significant relationship was documented among patients carrying the AT and TT genotypes *vs.* AA genotype. Regarding oral ulcers (p = 0.007); 60% of patients carrying the mutant TT genotype had oral ulcer while 100% of patients carrying the wild AA genotype had no ulcer.

Regarding anti-ds-DNA, 100% and 81.3% of patients carrying the TT and AT genotype respectively, had positive anti-dsDNA, whereas 60% of patients carrying the AA genotype had positive anti-dsDNA.

The TT genotype of miR-155 rs767649(A/T) polymorphism was associated with Low C4; about 60% of patients carrying this genotype had low C4.

The AT/TT genotypes of miR-155 rs767649 were significantly associated with higher SLEDAI values. 60% of patients carrying TT genotype had high to a very high score for active disease. On the other hand, 66.7% of patients carrying the AA genotype had no active or mild activity disease (p =<0.001).

Moreover, the percentage of SLE patients with nephritis was considerably higher in patients with AT and TT genotypes relative to the AA genotype as well as in patients carrying AT genotype compared with TT genotype (p =0.009, and < 0.001, respectively). As regards the miRNA-146 (rs57095329), thrombocytopenia and nephritis were higher in patients carrying AG and GG genotypes compared with AA carriers (p = 0.001 and 0.002 respectively), Table IV. Hardy Weinberg equilibrium was shown in Table V.

Table II. Stratification of genotype and allele frequencies according to sex in SLE cases and control groups.

Parameters	Females cases/controls, %	Adjusted OR, (95% CI)	*p*-value	Males cases/controls, %	Adjusted OR, (95% CI)	*p*-value
rs767649 (155) A/T						
TT	13.2/10.8	5.750 (1.726-19.150)	0.004	0.0	–	–
AT	81.6/64.5	5.942 (2.286-15.445)	< 0.001	50.0/71.4	0.400 (0.040-3.955)	0.433
AA	5.3/24.7	1	50/28.6	1		
Dominant model						
TT+AT	94.7/75.3	5.914 (2.293-15.254)	< 0.001	50.0/71.4	0.400 (0.040-3.955)	0.433
AA	5.3/24.7	1	50.0/28.6	1		
Recessive model						
TT	13./10.8	1.258 (0.537-2.947)	0.598	0.0%	–	–
AA+AT	86.8/89.2	1	100.0/100	1		
Alleles						
Allele T	53.9/43.0	1.552 (1.051-2.292)	0.027	25.0/35.7	0.600 (0.109-3.296)	0.557
Allele A	46.1/57.0	1	75.0/64.3	1		
Allele A	56.6/62.4	1	100.0/100.0	1		

Table III. Logistical regression analysis.

	B	S.E.	*p*-value	OR	95% C.I.	
					Lower	Upper
Univariate analysis						
rs767649(155) A/T (TT)	1.427	0.564	0.011	4.167	1.380	12.578
rs767649(155) A/T (AT)	1.412	0.421	0.001	4.103	1.799	9.356
rs57095329(146a) A/G (GG)	0.511	0.468	0.276	1.667	0.665	4.174
rs57095329(146a) A/G (AG)	0.427	0.295	0.148	1.533	0.859	2.736
Multivariate analysis						
rs767649(155) A/T (TT)	1.249	0.580	0.031	3.486	1.119	10.855
rs767649(155) A/T (AT)	1.433	0.423	0.001	4.190	1.827	9.607
rs57095329(146a) A/G (GG)	0.528	0.505	0.295	1.696	0.631	4.563
rs57095329(146a) A/G (AG)	0.454	0.313	0.147	1.574	0.853	2.905

Discussion

Because of the diversity of the proposed pathogenic mechanisms, SLE is considered the prototype of autoimmune diseases[28,29]. Recently, variations in miRNA expression have been found in certain autoimmune diseases, such as SLE, rheumatoid arthritis (RA), and primary Sjogren's syndrome (pSS)[30,31]. Therefore, these molecules may be considered as new biomarkers that are specific for diverse autoimmune disorders. Additionally, it has been proposed that miRNA-targeting treatment could be more selective than other therapeutic regiments in these diseases[32]. MiRNA Polymorphisms, involving miR-155 and miR-146a, were observed to play an important role in susceptibility to various autoimmune diseases by affecting the miRNA targets expression[20]. The present study investigated the genotype effects of rs767649 of miR-155 and rs57095329 of miR-146a on SLE predisposition, clinical manifestations, and disease activity in Egyptian patients with SLE.

The current study is considered the first study to introduce miR-155 rs767649 (A>T) polymorphism as a possible predisposing factor for SLE in the genotypic, dominant, additive and allelic models. In accordance with our study, many studies had demonstrated the role of MiR-155 and its polymorphism in autoimmune diseases; a recent study showed that MiR-155 expression

Table IV. Effect of miR-146a (rs57095329) and miR-155 (rs767649) on clinical features in SLE patients.

		miR-146a (rs57095329)				miR-155(rs767649)			
		GG n (%)	AG n (%)	AA n (%)	*p*-value	TT n (%)	AT n (%)	AA n (%)	*p*-value
SLEDAI score	No activity	3 (20)	18 (26.1)	12 (33.3)	0.764	6 (40)	21 (21.9)	6 (66.7)	< 0.001[a,b,c]
	Mild activity	3 (20)	21 (30.4)	9 (25)		0 (0)	33 (34.4)	0 (0)	
	Moderate activity	6 (40)	18 (26.1)	12 (33.3)		0 (0)	33 (34.4)	3 (33.3)	
	High activity	3 (20)	9 (13)	3 (8.3)		6 (40)	9 (9.4)	0 (0)	
	Very high activity	0 (0)	3 (4.3)	0 (0)		3 (20)	0 (0)	0 (0)	
Nephritis	No	0	9 (13)	9 (25)	0.003	0	18 (18.8)	0	0.003[a,b,c]
	Class II	0	3 (4.3)	9 (25)		0	12 (12.5)	0 (0)	
	Class III	6 (40)	12 (17.4)	6 (16.7)		6 (40)	18 (18.8)	0 (0)	
	Class IV	6 (40)	30 (43.5)	9 (25)		6 (40)	36 (37.5)	3 (33.3)	
	Class V	3 (20)	15 (21.7)	3 (8.3)		3 (20)	12 (12.5)	6 (66.7)	

[a]: Significant difference between TT *vs*. AT or Significant difference between GG *vs*. AG. [b]: Significant difference between TT *vs*. AA or Significant difference between GG *vs*. AA. [c]: Significant difference between AT *vs*. AA or Significant difference between AG *vs*. AA.

Table V. Hardy Weinberg equilibrium for the studied groups

		Controls		Cases	
		N	%	N	%
rs767649(155) A/T	TT	10	10.0%	15	12.5%
	AT	65	65.0%	96	80.0%
	AA	25	25.0%	9	7.5%
	PHW	0.001		< 0.001	
rs57095329(146a) A/G	GG	10	10.0%	15	12.5%
	AG	50	50.0%	69	57.5%
	AA	40	40.0%	36	30.0%
	PHW	0.323		0.041	

and its polymorphism MiR-155 rs767649 contribute significantly in susceptibility to rheumatoid arthritis[22]. Additionally, the mutant genotypes of miR-155 (rs767649 A>T) were shown to be related to the multiple sclerosis risk[33]. It has shown that miR-155 is related to autoimmunity and inflammation[34].

The results of this study might be explained by the increased expression level of miR-155 caused by the presence of the mutant allele of miR-155(rs767649 A>T) through increased transcriptional activity of that mic-RNA[35,36].

To understand the role of miRNA-155 in SLE requires the identification of critical miRNA-155 targets. Overexpression of miR-155 could downregulate the suppressor of cytokine signaling 1[37] and src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1)[38] which is an inhibitory signal transducer upon activation causes suppression of activation of B cell[39]. Thus, it plays a critical function in the immune response. Furthermore, overexpression of miRNA-155 induces the activation of Signal Transducer and Activator of Transcription 3 (STAT3) and production of inflammatory cytokines, interferon, interleukin-6, suggesting that miRNA-155 may serve as a bridge between inflammation and development of SLE[40].

Additionally, miR-155 increases the risk of multiple sclerosis (MS) in Experimental Autoimmune Encephalomyelitis (EAE); a mouse model of MS[41]. Furthermore, miR-155 increased in macrophages of the synovial membrane and synovial fluid in Rheumatoid Arthritis patients[42]. miR-155 was shown to prompt the growth of Th1 and Th17 cells ensuring the increase in the production of proinflammatory cytokines[43]. Accumulated evidence revealed that miRNA-155 is frequently upregulated in SLE[44,45]. From the previous studies, we expected that miR-155 rs767649 polymorphism could play a role in the increased risk of SLE.

As regard to the miR-146a rs57095329 (A>G) polymorphism, we couldn't find any of the genotypes; the GG, AG, or AA genotypes to have a significant association with SLE. However, the combination of miRNA-155 rs767649 T Allele and miR-146a rs57095329 G Allele was significantly associated with SLE (p =0.041, OR=0.5).

Our results were in accordance with Lee and Bae[46] who demonstrated in a meta-analysis of 2013 patients and 2555 controls that the miR-146a polymorphism is not associated with susceptibility to SLE or Rheumatoid arthritis in any of the dominant or recessive models or homozygote contrast. They revealed in this Meta-analysis that there was no association between SLE and the miR-146a G allele with OR for the G allele = 1.007, 95% CI = 0.910–1.114, p = 0.888). Furthermore, Li et al[47] demonstrated that this mutant G allele was not associated with the development of MS.

However, our results contradict the findings of Luo et al[48] who demonstrated that patients having the mutant G allele had a low expression level of miR-146a noticed in SLE. These differences could be related to ethnic variability in the different populations.

SLE is more common in females, with a female to male ratio of 9:1[49]. Thus, this study estimated the effect of both polymorphisms on the risk of SLE in both sexes. Our study showed that miR-155 rs767649 A>T polymorphism was associated with the risk of SLE in females in the genotypic, dominant, and allelic models. However, microRNA-146a polymorphism was not associated with the risk of SLE in both sexes. To explain these findings, Zhang et al[50] demonstrated the relationship between estrogen and miR-155. They denoted that treatment with estrogen was shown

to increase miR-155 expression. Moreover, many studies had demonstrated overexpression of miRNA-155 in breast cancer[51-53]. Thus, females carrying miR-155 rs767649 polymorphism could be liable to SLE in comparison to males having the same polymorphism.

In our study, the AT/TT genotypes of miR-155 rs767649 were significantly associated with higher SLEDAI values. 60% of patients carrying the TT genotype had high to a very high score for active disease. On the other hand, 66.7% of patients carrying the AA genotype had no active or mild activity disease. This may be explained by the increase in the level of the expressed mic-RNA 155 which is affected by this polymorphism. T allele of miR-155 rs767649 was shown to cause an elevation in miR-155 expression[35,36], and Zununi et al[54] reported that miRNA-155 was related to SLEDAI scores.

The present study showed that miR-155 rs767649 (A/T) polymorphism was significantly associated with oral ulcer in patients with SLE. About 60% of patients carrying the TT genotype have oral ulcers. On the other hand, 100% of patients carrying the AA genotype have no ulcers. These findings could be explained by previous studies which revealed that oral mucosal lupus is associated with increased systemic disease activity[55,56].

The anti-dsDNA antibodies are a marker for SLE. Anti-dsDNA antibodies are positive in about 70–98% of patients with SLE[57]. The positivity of anti-dsDNA is usually associated with low C4 serum levels. Moreover, it was found to be associated with an increased incidence of renal involvement, authorizing the pathogenic role of anti-dsDNA in kidney affection[58]. This association confirms the relationship between complement and anti-dsDNA in patients with SLE and renal manifestations[59].

Now a new link appears to emerge between anti-dsDNA and miR-155 rs767649 (A>T) polymorphism: the present study showed that 100% and 81.3% of patients carrying the TT and AT genotypes respectively had positive anti-dsDNA. To understand this finding, critical attention should be paid towards the role of mic-RNA 155 in the development of anti-dsDNA Ab, it's noteworthy that miR-155 is encoded within a region known as the B cell integration cluster gene[60]. miR-155 was shown to regulate antibody production in SLE patients[30]. Furthermore, Bic/miR-155–deficient mice showed a decrease in B cell immunity[61]. Moreover, Zhenke et al[62] reported that the TLR2/ MyD88/ miR-155/Ets-1 pathway had an essential role in anti-dsDNA Ab induction. Yet, the exact role of mic-RNA155 rs767649 in the production of anti-dsDNA Ab is either to be due to the induction of mic-RNA 155 expression or it has a role in TLR2/ MyD88/miR-155/Ets-1 pathway itself.

In the current research, regarding miRNA-155 (rs767649), the percentage of SLE patients with nephritis was considerably higher in patients with AT and TT genotypes relative to the AA genotype as well as in patients carrying AT genotype comparing with TT genotype (p =0.009, and $<$ 0.001, respectively), the same observed with the miRNA-146 (rs57095329). Nephritis were higher in patients carrying AG and AA genotypes compared with GG carriers (p = 0.001 and 0.002, respectively), this unexpected result may be due to modifications in genetic liability and environmental issues. Inconsistent with these results, Tawfik et al[63] found an association between lupus nephritis and expression of miRNA-155 miRNA-146a and disagree with Hashad et al[64] who report down-regulation of miRNA-146a in lupus nephritis. Zununi et al[54] also had demonstrated the upregulation of miRNA-155 in patients with lupus nephritis. This could be explained by the inflammation which causes injury to the glomerular endothelial and fibrosis[65]. The upregulation of miRNA-146a and miRNA-155 was shown to be associated with in macrophage-mediated inflammation[66].

In summary, miR-155 polymorphism was associated with SLE susceptibility, disease activity, and nephritis. These findings may participate in the realization of the underlying molecular mechanisms of SLE. Moreover, this polymorphism was associated with an increased risk of SLE in female subjects.

This study has potential limitations. First, there was deficient data about other genetic risk factors associated with SLE susceptibility, and therefore gene-gene interactions could not be evaluated. Second, due to the small number of SLE patients, further studies should be applied to confirm the association between the two polymorphisms as well as other functional polymorphisms and SLE. Third, due to the nature of case-control studies, some recall and selection bias couldn't be avoided.

Conclusions

This study had demonstrated for the first time the association between miR-155 rs767649 and

the risk of development of SLE in an Egyptian cohort, mostly in females. On the other hand, miR-146a (rs57095329) polymorphism was not associated with SLE risk. Furthermore, it is the first study to investigate the role of the two SNPs in SLE susceptibility in Egyptian patients. These findings may expand our knowledge of the clinical consequence of the two polymorphisms in SLE susceptibility.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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