GENE EXPRESSION AND SINGLE NUCLEOTIDE POLYMORPHISM (rs1140713) OF MICRORNA-126

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ABSTRACT

MicroRNAs are non-coding gene regulators that may serve as systemic lupus erythematosus biomarkers for diagnosis or prognosis. This study was aimed to identify the single nucleotide polymorphism (rs1140713) of microRNA-126 and its expression by using PCR and qRT-PCR techniques. This research involved 100 womenwith SLE with a mean age of 32.85±9.992 years they were obtained from Baghdad city (medical city, Baghdad Hospital)/Iraq, along with 100 healthy controls with a mean age of 31.68±8.308 years. The mean score on the SLE disease activity index (SLEDAI-2k) was 10.860.±3.275 and a disease duration was 9.00 years, (Erythrocyte sedimentation rate, C-reactive protein, urea, and creatinine were all significantly higher in SLE patients compared to controls, while haemoglobin, white blood cells, complement 3, and complement 4 were significantly lower in contrast to controls (*p*-value=< 0.0001)). According to the examination of anti-nuclear antibodies (ANA) in patients, 89% of them have ANA and 95% have anti-double strand DNA (anti-dsDNA) antibodies. Single nucleotide polymorphism analysis for subjects involved in this study revealed a significant increased risk association between miRNA-126 (rs1140713) heterozygote (CT)(OR=20.64; *p*≤0.001) and homozygote mutant (TT) (OR = 16.35; *p*= 0.001) genotypes and SLE incidence. Moreover, the results showed a substantial decline in microRNA-126 expression with fold change mean (0.639±2.931) in comparison to controls' fold change mean (1.00±0.00). The present investigation found no indication of a relationship between changes in microRNA-126 folds and demographic or laboratory studies of the disease. The results suggest the microRNA-126 might consider as biomarkers in SLE pathogenesis.**

keywords: autoimmune disease, rs1140713, SLE, single nucleotide polymorphisms.

التعبير الجيني وتعدد الشكل الوراثي النيوكليوتيدي المفرد(rs1140713) في المياكرو رنا 126

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المستخلص

يعتبر المايكرو رنا من المنظمات الجينية غير المشفرة والتي ممكن ان تعمل كمؤشرات حيوية لمرض الذئبة الحمراء الجهازي. هدفت الدراسة الحالية إلى التعرف على تعدد أشكال النوكليوتيدات المفردة (rs1140713) للمايكرو رنا −126 وتعبيره باستخدام تقنيات ال PCR و qRT−PCR . ضم البحث 100 امرأة مصابة بمرض الذئبة الحمراءالجهازي بمتوسط عمر 32.85 ± 9.992 سنة والتي حصلنا عليها من مرضى مستشفى بغداد/مدينة الطب إلى جانب 100امرأه صحيحه استخدمت كعنصر سيطره بمتوسط عمر 31.68 ± 8.308 سنة. كان متوسط الدرجة على مؤشر نشاط مرض الذئبة الحمراء (SLEDAI−2k) 10.860±3.275ومدة المرض 9.00 سنوات، كان معدل ترسيب كرات الدم الحمراء، والبروتين التفاعلي C، واليوريا، والكرياتينين أعلى بكثير في مرضى الذئبة الحمراء مقارنة بالافراد الاصحاء بينما الهيموجلوبين وخلايا الدم البيضاء والمتمم 3 والمتمم 4 كانت أقل بشكل واضح مقارنة بالافراد الاصحاء **p−value=< 0.0001 . ووفقًا لفحص الأجسام المضادة للنواة (ANA) في المرضى، فإن 89 ٪ منهم كان لديهم أجسام مضادة لـ ANA و 95 ٪ لديهم أجسام مضادة لـ (anti−dsDNA). كشف تحليل تعدد الأشكال للنيوكليوتيدات المنفردة عن ارتباط خطروجود الانماط الجينية للمايكرو رنا −126 (rs1140713) متغاير الزيجوات CT(OR = 20.64 ؛ $p \leq 0.001$) و متماثل الزيجوات الطافرTT(=OR 16.35 ؛0.001=p) بالتسبب في حدوث مرض الذئبة الحمراءبشكل كبير. علاوة على ذلك، أظهرت النتائج انخفاضًا كبيرًا في تعبير المايكرو رنا − 126 مع متوسط تغيير الطية (0.639 ± 2.931) مقارنةً بمتوسط تغيير طيات الضوابط (1.00 ± 0.00). لم يجد البحث الحالي أي مؤشر على وجود علاقة بين التغييرات في طيات المايكرو رنا −126 والدراسات الديموغرافية أو المختبرية للمرض. هذا يشير إلى أن المايكرو رنا −126ربما يعتبر كعلامة حيوية في تشخيص مرض الذئبة الحمراء.

الكلمات المفتاحية: أمراض المناعة الذاتية، الرنا الميكروي 126، rs1140713، داء الذئبة الحمراءالجهازي, تعدد اشكال النيوكليوتيدة المفرده.

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INTRODUCTION

Systemic lupus erythematous (SLE) is an autoimmune inflammatory disease with a wide variety of clinical manifestations, characterized by the inability to tolerate self-antigens, activation of dysregulated autoreactive B cells and T cells, production of autoantibodies, and unsettling cytokine activities (9). About 50% of SLE patients develop potentially fatal consequences include vasculitis, nephritis, pulmonary hypertension, cerebral stroke, and interstitial lung disease (17). There is no clear evidence of the exact DNA transcription and translation mechanisms as they are influenced by many factors such as transposons, ribosomes, inhibiting factors and epigenomics (10). MicroRNAs, a form of non-coding RNA that are only 22 nucleotides long, regulate genes after transcription has already taken place. By inhibiting mRNA translation, they play important roles in the biological processes, including metabolism, cell proliferation, differentiation and death. Research have shown that aberrant microRNA expression is linked to the development of several diseases, including tumours (24),inflammatory diseases (14, 16), and autoimmune diseases (25,14). MicroRNA-126 is a significant regulator that targets a set of genes involved in the immune response. The human microRNA-126 gene is located in intron 7 of the epidermal growth factor like-domain, multiple7 (EGFL7) gene on chromosome 9 (35). MicroRNA-126 has been linked to the development of several disorders, one of these are autoimmune disorders (19). MicroRNA-126 may serve as a biomarker for diabetes since its expression is downregulated in autoimmune disorders like diabetes mellitus(20).Single nucleotide polymorphism SNPs are genetic variations that may affect a protein's structure or functionality (36, 38, 40). Also, circulating miRNA-126 has been reported as a potential biomarker for nonalcoholic fatty liver disease (1). Blood samples can be used as specimens for measuring gene expression levels of genes expressed by the immune cells to evaluate differences in expression levels between patients and healthy controls and to study the role of these genes in the development of diseases (2). The aim of this study is evaluate the single nucleotide polymorphism (rs1140713) and gene expression of micrRNA-126 in Iraqi women with systemic lupus erythematosus.

MATERIALS AND METHODS

Subjects: One hundred SLE women from Baghdad City in Iraq (Medical City, Baghdad Hospital) participated in research. The rheumatologists at the unit clinic made the diagnosis of the disease by using the analytical criteria for SLE developed by the European League Against Rheumatism and American College of Rheumatology (30). Both the clinical and analytical evaluations were made. Additionally, each patient's (SLEDAI-2k) score was directly established by the physician via a blood sample. After obtaining clinical and demographic data including age, sex and another assays. Before participating in the research, all individuals gave their informed consent in writing. This study had obtained approval from the local Ethics Commission (CSEC/0223/0015) gives its approval and acquired written consent from each participant (informed consent). The University of Baghdad group carried out the investigation in Baghdad, Iraq, under the supervision of medical experts at (City of Medical, Baghdad Hospital). The healthy controls group samples, which included 100, were obtained from National Blood Transfusion Centre.

Laboratory investigations

From each patient's medical file, several clinical manifestations and demographic data were gathered. To assess the standard and other laboratory tests, laboratory investigations were carried out. At the time of the sample collection, the complete blood count (CBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were all assessed. According to manufacturer's recommendations, automated Fujifilm measurements of blood urea and serum creatinine were made. Conferring to the manufacturer's recommendations, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in blood taken from patients and controls for biochemical assays. Radial Immunodiffusion (RID) was used to estimate complement (C3) and complement (C4). The enzyme-linked

immunosorbent assay (ELISA) technology (Human Company, Germany) was used for the analytical testing of the anti-nuclear autoantibody (ANA) and anti-dsDNA.

Extraction and analysis of DNA

The genomic DNA was extract directly from whole blood using the Norgen® blood DNA extraction kit from both patient and healthy control (Norgen, Canada). The Qubit 4.0 was used to evaluate DNA concentration and purity, which were given as a ranged between (18-50) ng/µl, and 260/280 respectively. The genomic DNA was kept at (-20°C) for future use. The primers used in the PCR reaction were designed and provided by Macrogen (Korea). The sequence of forward and reverse primers are shown in Table (1).

Table 1. The sequence of forward and reverse primers for (rs1140713).

Name of SNP	Sequence 5' to 3'	Product size	Reference
rs1140713	F- GACACTTCAAACTCGTACCG R- TGCACTTCTTCCTTCATTGC	327 (bp)	Newly Designed

SNP: single nucleotide polymorphism, F: forward, R: reference

Molecular sequencing of microRNA-126 (rs1140713): Amplification of microRNA-126 by using set of primers which mentioned in Table 1 to amplify (327) bp for being utilize in sequencing analysis, and target SNPs will be concerned in this area. According to the manufacturers instruction, fifty microliter of PCR amplify reaction contain 25 µl (OneTaq® 2X Master MixKit, New England Biolabs (NEB®, England)), 8µl of DNA sample, 4µl (10 pmol/µl) from each primer and 9µl of free nuclease water was prepared. The reaction was carried on the optimal PCR condition as shown in Table (2). More than 40µl PCR product for each sample have been sent to Macroegen Korea for sequencing by Sanger method to recognize the SNP.

Table 2. The optimum conditions of detection *microRNA-126* gene

Cycle No.	Stage	Temperature	Time
1	Initial denaturation	94 ºC	5min.
	Denaturation	94 ºC	30 sec.
30	Annealing	51 ºC	45 sec.
	Extension	72 ºC	30 sec.
1	Final extension	72 ºC	7 min.

Total RNA extraction: total RNA was isolated from both patient and healthy control from whole blood by Trizol RNA isolation kit (Invitrogen, USA with Catt. No. 15596026). The Qubit™ RNA HS Assay Kit (Q32852) was used to quantify total RNA in samples (Thermo Fisher, USA). RNA samples stored at (-80°C) until process to downstream application.

Primer design for *microRNA-126 gene* expression: The National Center for Biotechnology Information (NCBI) Gene Bank database was used to get the cDNA sequences of the *microRNA-126* gene, with microRNA-U6 as a housekeeping gene. Table (3) summarizes the sequences of the primers utilize in this study.

Table 3. The primers of microRNA-126 forward and reverse, used in the study

Primers of microRNA	Sequence 5' to 3'
microRNA-126 Forward primer	(GGGCATTATTACTTTTGG)
microRNA-126 Reverse Primer	(TGCGTGTCGTGGAGTC)
microRNA-126 Reverse Transcription Primer	(GTCGTATCCAGTGCGTGTCGTGGAG TCGGCAATTGCACTGGATACGACCGCATT)
U6 Forward primer	(GAGAAGATTAGCATGGCCCCT)
U6 Reverse Primer	(ATATGGAACGCTTCACGAATTTGC)

Synthesis of cDNA from microRNA: ProtoScript® First Strand cDNA creation Kit (E6300S) was used. The conditions for cDNA reverse transcription in a thermal cycler steps, an incubation step at 25°C for 5 min, Incubation the 20µl of cDNA creation reaction at 42°C for one hour, and inactivate the enzyme at 80°C for 5 minutes. The product of cDNA must be stored at –20°C for downstream PCR amplification.

Quantitative Real Time PCR (qRT- PCR) for microRNA: qRT-PCR was used to estimate the levels of microRNA expression. Luna Universal qPCR MasterMix, (qPCR

MasterMix is an optimized 2X reaction mix for qRT-PCR detection and quantification of target microRNA using the SYBR®/FAM channel on the majority of qRT-PCR instruments. It is formulated with a unique passive reference dye that is compatible with Hot Start Taq DNA Polymerase (New England Biolabs, UK). The reaction mix composed from component with their quantity shows in Table 4.

Table 4. Quantitative real-time PCR components utilized in a gene expression experiment

Components	20 ul Reaction
Luna Universal qPCR Master Mix	10 ul
Forward primer (10 µM)	1ul
Reverse primer (10 µM)	1ul
Template DNA	5ul
Nuclease-free Water	3ul

Table (5) shows the RT-PCR protocol was configured using the thermocycling protocol specified.

Table 5. The profile of gene expression temperature.

Cycle Step	Temperature	Time	Cycle
Initial Denaturation	95°C	60 seconds	1
Denaturation Extension	95°C	15 seconds	40-45
	60°C	30 seconds	
Melt Curve	60-95°C	40 minute	1

Statistical Analysis

The frequencies of genotypes were checked for their approval with (HWE) Hardy Weinberg equilibrium and a considerable difference between the frequencies of genotype in both (observed and expected) which was estimated by (Pearson's Chisquaretest)from(https://wpcalc.com/en/equilibriumhardy-weinberg/(34). In this study, data was analysed using the Statistical Package for the Social Sciences (SPSS) version 27. Quantitative parametric results have been estimated using the inter quartile range and mean (median), whereas qualitative non-parametric data has been calculated using means and standard deviation. Additionally, the Spearman association test and the Pearson Chi-square tests were employed to compare results and determine the correlation between the various study parameters. *p*-value or less than to 0.05 at a 95% confidence level indicates to significance. Receiver operating characteristic (ROC) analysis was adopted to estimate sensitivity and specificity of a parameter, as well as its area under curve (AUC) that predicts the parameter significance (23).

RESULTS AND DISCUSSION

Characteristics of the study population

This case-control study included 100 SLE women with mean age (32.85 ± 9.992) years and 100 healthy controls women with mean age (31.68 ± 8.308) years as show in Table (6). The majority of patients (91%) have no family history of SLE. There were significant differences in (BMI) between patients (23.106 ± 3.791) and control groups (20.345 ± 1.879). The median(IQR) of disease duration for patients was 9.00 (6.75). The mean score of the SLEDAI-2 was10.860 ± 3.275. Table (6) lists all of the laboratory parameters of the women with SLE and healthy controls who participated in this study. There were high significant differences (p<0.0001) in ESR and CRP in patients compared to controls. complete blood count showed high significant variation (p<0.0001) in Hemoglobin levels and white blood cells counts between patients and healthy control group while, there was no significant platelet count (p =0.373). Also the biochemical tests revealed highly significant difference in urea and creatinine (p < 0.0001) between patients compared to healthy control groups. While non-significant differences were found in ALT (p = 0.754) and AST (p = 0.617) between the two groups. The immunological analysis showed high significant variation in C3 and C4 levels in patient groups compared with control groups (p <0.0001). The analysis of ANA and anti-dsDNA in patients showed that 89% of patients have ANA while 95% have anti-dsDNA.

Table 6. Baseline characteristics of SLE patients and healthy control

Parameters	Patients N=100 mean±SD,N% or median(IQR)	Control N=100 mean±SD,N% or median(IQR)	*P*-value
Ages(years)	32.85 ±9.992	31.68 ± 8.308	0.369NS
BMI	23.106±3.791	20.345±1.879	< 0.0001**
Disease duration	9.00(6.75)	-	-
SLEDI-2K	10.860±3.275	-	-
ESR mm/h	50.00(33.00)	10.00(4.00)	< 0.0001**
CRP mg/dl	16.70(1.78)	0.650(0.80)	< 0.0001**
Hb g/dl	8.40(1.75)	13.00(2.10)	< 0.0001**
WBC x10^9/L	3.500(1.30)	6.800(2.17)	< 0.0001**
PLTx10^9/L	250.500(147.00)	250.500(134.00)	0.373(NS)
Urea mg/l	58.00(43.75)	33.00(16.00)	< 0.0001**
Creatinine mg/l	1.550(0.70)	0.700(0.30)	< 0.0001**
ALT U/L	34.00(30.50)	34.00(31.00)	0.754 (NS)
AST U/L	23.00(22.85)	23.00(22.05)	0.617 (NS)
C3 mg/dl	74.00(8.00)	130.00(42.00)	< 0.0001**
C4 mg/dl	10.00(4.00)	13.50(11.00)	< 0.0001**
ANA positivily	89%	-	-
Anti-dsDNA positivily	95%	-	-

NS: non-significant, **p-value≤0.01, N: number in each parameter, %: percentage and mean ± SD, BMI: body mass index, The abbreviations are; WBC: white blood cell, Hb: hemoglobin, S: serum.

SLE is an autoimmune disorders, which characterized by production of autoantibodies against antigens of self and manifestations of variable clinical with unpredictable of totally flares course (31). Inflammation various markers measured in patients of SLE, such (CRP) (32). Our results agreed with (32) which showed a level of CRP can be elevated or normal in patients of SLE as an inflammatory response a sign. White blood cell deficiencies were observed in 29% of SLE patients in a research from China(21), 25.03% of SLE patients in Pakistan(18), 22.5% of patients in Oman(26), 23.5% of patients in the UAE(27), and 51% of SLE patients in the UAE according to other studies(27). The major difference that has been shown between SLE patients and a healthy control group was a decrease in complement proteins (C3 and C4). This agree with a previous study conducted in Iraq (5). Complement proteins decrease in SLE as a consequence of excessive production of autoantibodies and cytotoxicity mediation (22). Also our results agree with (6) that demonstrated the ANA and Urea were significantly higher in SLE patient than control group whereas Hemoglobin levels were significantly lower in SLE Patient than control group. In the second assessment of previous immunological factors for the same Iraqi patients after a time from their diagnosis and undergoes for treatment, the results showed that ANA-positivity is lost over time. This seroconversion in ANA positivity was previouslyassumed (8).The anti-dsDNA states the most decrease in the autoantibodies frequency with the second assessment among the ANA and complements proteins. An observation that has been corroborated by previous studies performed in Brazil(11) and Sweden(12). All studies shared the same suggestion, despite the differences in the measurement methods and the populations involved in this studies(8).This transformation in anti-dsDNA to negative after treatment suggested it can be used for SLE disease monitoring (15). The complement proteins (C4 and C3) level in the SLE patient's serum also become typical in most of them after time. Research reported a similar finding and suggested that this is due to the decrease in the number of autoantibodies after treatment (22).

Micro-RNA126 Polymorphism

The sequencing results of rs1140713 SNP were illustarted in Figure 1 which had C/T genotypes located on chromosome 9.

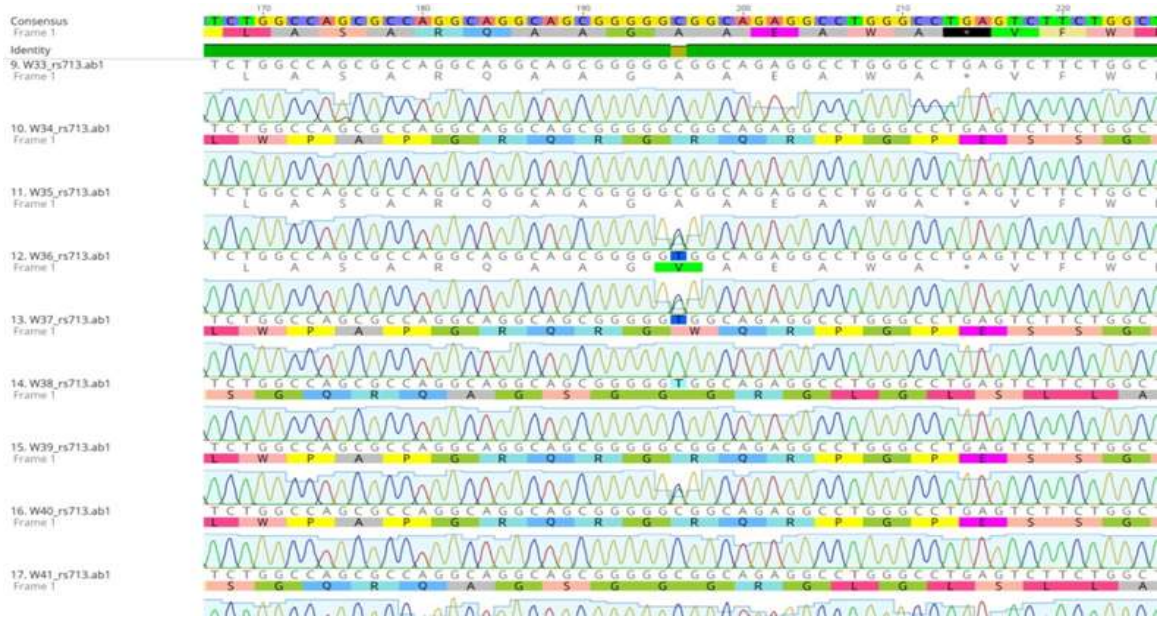

Figure1. DNA sequence chromatogram of *microRNA-126*gene SNP (C/T: rs1140713) showing three genotypes: CC,CT & TT

This research studied the alleles and genotypes frequency of microRNA-126 SNP (rs1140713) among Iraqi patients with SLE and healthy controls. More heterozygotes have been discovered than expected. This shows that (rs1140713) SNP of microRNA-126 may have some importance for the development of SLE. There are many increased heterozygotes mutant type and homozygote mutant type (CT and TT) respectively of (rs1140713) SNP in SLE compared with healthy subjects which showed no homozygote mutant type (TT) of this SNP. The distributions of genotype frequencies and allele of (rs1140713) SNP is reported in Table 7. We found there were a greatly significant differences between genotypes CT and TT in SLE patients in comparison with control groups ($p \leq 0.001$ and $p \leq 0.001$) respectively. The frequency of CT genotype in SLE was 40.75% and the odd ratio was 20.64 (95%CI=6.83 - 82.37) while the frequency of CT genotype in control group was (3.92%).The estimated frequencies of TT genotype in SLE was (8.12%), while the frequency of TT genotype in control group was (0.04%) and the odd ratio was 16.35(95%CI=3.43 - 78.09). The significance analysis was higher in CT genotypes which were (p>0.000). The significance of such an relationship was assess by Fisher`s Exact possibility. An assessment is preferred because it allows for correction of possibility and is not affected by little numbers (less than 5). It seems that the disease can occur when the patients have heterozygote CT odd ratio (OR) of (CT) was 20.64 and less than when patients have homozygote of mutant TT (odd ratio was (16.35). The SNP's T allele was detected in (28.5%) of SLE patients and (2%)of healthy controls with significant differences in frequency between the two groups (OR = 19.53, 95%CI = 6.95 - 75.37, $p \leq 0.001$. The distributions of (rs1140713) SNP did not depart substantially from HWE (p>0.05) as show in Table 7.

Table 7. Numbers and percentage frequencies ofrs1140713 SNP of microRNA-126, and their Hardy-Weinberg equilibrium (HWE) in SLE patients compared with control groups

Gene	SNPS	Models	genotypes	SLE Patients N=100	HWE *P*-value	Control N=100	HWE *P*-value	OR(95% CI)	*P*-value
MicroRNA-126	rs1140713	Genotypes	CC	50(51.12%)	0.5817	96(96.04%)	0.8383	Reference	-
			CT	43(40.75%)		4(3.92%)		20.64(6.83-82.37)	0.000**
			TT	7(8.12%)		0(0.04%)		16.35(3.43-78.09)	0.001**
		Alleles Frequencies	C	143 (71.5%)		196(98%)		Reference	-
			T	57(28.5%		4(2%)		19.53(6.95-75.37)	0.000**

Logistic regression analysis of the rs1140713SNP revealed that (CT and TT) genotypes were significantly associated with the hazard of SLE in patients compared to controls in all models under study (Codominant, dominant, over dominant and recessive)Table8, both genotypes (CT+TT and CT) under dominant and overdominant models of rs 1140713 SNP exhibited the same significant differences compared to controls ($p \leq 0.001$). While the genotype TT in the recessive model demonstrated less significant differences when compared to controls($p=0.014$).

Table 8. Logistic regression of rs1140713 SNP of microRNA-126 in SLE patients compared to control

Gene	Models	genotypes	SLE Patients N=100	control N=100	OR(95% CI)	*P*-value
MicroRNA-126	Codominant	CC	50	96	Reference	-
		CT	43	4	20.64(6.83 - 82.37)	0.000**
		TT	7	0	16.35(3.43 - 78.09)	0.001**
	Dominant	CC	50	96	Reference	-
		CT+TT	50	4	24.00(8.68 - 80.80)	0.000**
	Overdoinant	CC+TT	57	96	Reference	-
		CT	43	4	18.11(6.54 - 61.17)	0.000**
	Recessive	CC+TC	93	100	Reference	-
		TT	7	0	7.86(1.75 - 35.40)	0.014**

CI:confidence interval, OR: odds ratio, HWE: Hardy-Weinberg equilibrium. Most studies agree that SLE affects women more frequently than it does men, and the findings from the studies given show a risk effect for female sex and age in the aetiology of SLE (33,28,4). The interaction between sex hormones, the environment, and genotype during individual development, as well as the fact that all cases were adult, are the two most plausible explanations for the female predominance in SLE. This finding is consistent with earlier research that showed that females are more likely than males to develop SLE after puberty due to elevated levels of circulating oestrogen (4,29). The regulating role of microRNAs in the immune system occurs in a delicate tune. Numerous studies have demonstrated that the microRNAs gene's polymorphisms have an impact on its expression, synthesis, and maturation, which may be a significant risk factor in disease susceptibility, including carcinogenesis and inflammation (37). Our results agree with (13) which demonstrated the *microRNA-126* gene SNPs rs1140713 and rs4636297 are related with acute myocardial infarction AMI, probably by influencing the expression levels of the *microRNA-126* gene. Additionally, these findings agree with (7) which showed the susceptibility role of *IL*1 gene SNPs in SLE might be better understood in terms of specific allelic combinations between SNPs (haplotypes).

Expression levels of microRNA-126: Synthesis of cDNA was carried out as the initial step in RT-qPCR followed by amplification of target genes (39). Quantification real-time PCR was done to measure expression of microRNA-126. The microRNA-U6 gene was used as a house keeping gene to normalize the gene expression, also used Livak formula to quantify of the gene expression. The fold change mean of microRNA-126 gene was down regulated in SLE patients (0.639± 2.931) when compared to fold change mean in controls (1.00 ±0.00), with the significant differences($p \leq 0.001$). The results showed that microRNA-126gene was decreased in SLE patients when compared to fold change in healthy control, these results agree with (19) which reported the low level of microRNA-126 plays an essential role in the development and initiation of SLE. Also agree with (33)

which demonstrated low expression of microRNA-126 in the epithelial cells of patients with type 2 diabetes and also show that microRNA-126 is involved in the development and initiation of diabetes by promote the migration, proliferation, and apoptosis of the epithelial cells. However, the expression of microRNA-126 and its role in other autoimmune diseases, such as SLE, have rarely been reported. Determination of Pearson correlation coefficient between microRNA-126 and other parameters under study, is shown in Table 9.

Table 9. Determination of Pearson correlation coefficient between microRNA-126 and parameters under study

Parameters	Folding of microRNA-126 in SLE patients	
	r_s	*p*-value
Ages(years)	**0.192**	**0.055**
BMI	**-0.085**	**0.359**
Disease duration	**0.080**	**0.428**
SLEDI-2K	**0.074**	
0.458		
ESR mm/h	**0.107**	**0.286**
CRP mg/dl	**0.069**	**0.490**
Hb g/dl	**-0.141**	**0.159**
WBC x10^9/L	**-0.103**	**0.307**
PLTx10^9/L	**0.097**	**0.335**
Urea mg/l	**-0.171**	**0.091**
Creatinine mg/l	**-0.061**	**0.545**
ALT U/L	**0.018**	**0.855**
AST U/L	**-0.047**	**0.641**
C3 mg/dl	**0.182**	**0.072**
C4 mg/dl	**-0.043**	**0.666**
ANA positivily	**0.029**	**0.775**
Anti-dsDNA positivily	**0.207**	**0.039**

The correlation study results showed a non-significant association between microRNA-126 and all parameters under study except age. A scarce number of research papers have adopted the association between the chosen microRNA-126 expression and studied parameters of disease. This findings are in agreement with (3) which showed that genetic and environmental factors may also contribute to the onset and progression of heart failure. According to these findings, microRNA-126 may serve as a new target or a potential biomarker for the SLE condition.

Receiver operating characteristic (ROC) curve: Analysis of receiver operating characteristic (ROC) curve was carried out to determine the diagnostic accuracy of microRNA-126 in distinguishing SLE patients from control participants. The analysis revealed that microRNA-126 at the ideal cut-off point was 0.126 which could significantly differentiate patients from control subjects (AUC = 0.914; 95 % CI = 0.8422-0.9859; $p<0.0001$ and with a sensitivity and a specificity of 87.50 % and 87.50%, respectively as shown in figure 2. This ROC curve was created to determine the predictive values of microRNA-126 expression as an acute phase marker in SLE. The ROC curves were substantially above the diagonal, indicating excellent sensitivity and specificity.

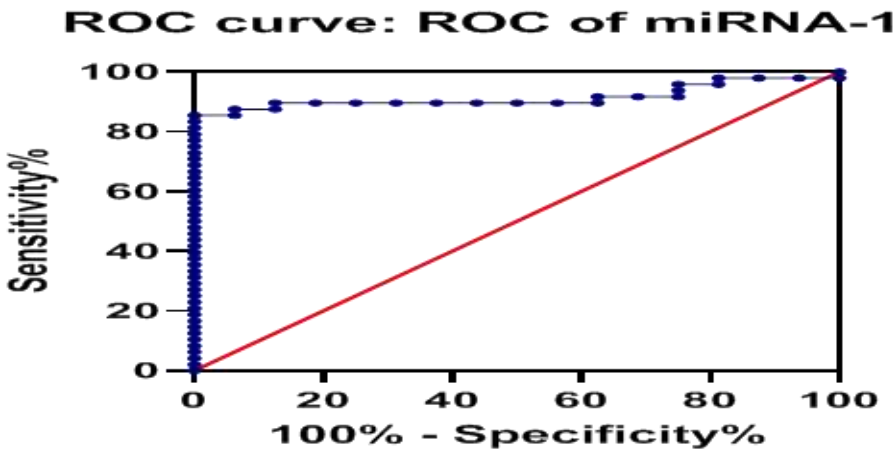

Figure 2. Receiver operating characteristic (ROC) curve analysis of microRNA-126 for differentiating SLE patients from healthy controls

In current study, the results suggest a risk effect of CT and TT genotypes of microRNA-126 SNP in SLE, according to the area under the curve of ROC curve could identify microRNA-126 as a strong biomarker for SLE disorders and we determined decreased in microRNA-126 expression in systemic lupus erythematosus patients. The low of microRNA-126 expression could give a disease indications recurrence suggesting patients a closer monitoring. This information provide improved understand the SLE pathogenesis and supports for further studies to determine whether such changes are regular across different populations and whether the recognized microRNA-126 may represent SLE novel biomarkers.

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