

Diagnostic Significance of MiR-27a and MiR-938G>A Polymorphisms in the Risk Assessment of Recurrent Pregnancy Loss in Iraqi Populations

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ABSTRACT

Background: Recurrent pregnancy loss (RPL), defined as two or more consecutive failed clinical pregnancies confirmed by ultrasonography or histopathology, remains a significant clinical challenge. MicroRNAs (miRNAs) are critical regulators of gene expression during the preimplantation phase and may influence pregnancy outcomes. **Purpose:** This study aimed to explore the association between genetic polymorphisms in miRNAs (miR-27a and miR-938G>A) and recurrent pregnancy loss in Iraqi females. **Methods:** The study involved forty women diagnosed with idiopathic RPL at the departments of infertility, gynecology, and obstetrics in Baghdad Hospital City from February to October 2022, along with a control group of 40 healthy individuals. Genotyping of miR-27a and miR-938G>A was performed using polymerase chain reaction (PCR) on samples of whole blood. **Results:** Our findings revealed a significant correlation between the miR-27a GG and AG genotypes and a reduced risk of RPL. Conversely, the miR-938G>A polymorphism was strongly associated with an increased risk of RPL (odds ratio for G/A vs. GG = 36; 95% confidence interval: 9.89 - 130.9). Individuals possessing both miR-27a A/G and miR-938 G/A genotypes were found to have a higher likelihood of experiencing recurrent pregnancy losses. **Conclusion:** The study confirms that specific single nucleotide polymorphisms (SNPs) in miR-27a and miR-938 are associated with the risk of RPL. These miRNAs could serve as valuable biomarkers for predicting RPL, thereby aiding in the risk assessment and management of potentially affected women. Further research is encouraged to validate these findings and explore their potential diagnostic utility in clinical settings.

Keywords: Recurrent Pregnancy Loss, miRNA-27a A/G, and miRNA-938 G/A

INTRODUCTION

RPL is a prevalent and consequential pregnancy concern affecting a mere 1% to 3% of couples attempting to conceive. It is characterized by a sequence of two to three miscarriages following one another that occur prior to the twentieth week of pregnancy gestation [1]. Roughly, 2%–5% of couples have experience with RPL [2]. Several studies all over the world investigate the prevalence and etiological causes of RPL, in a study conducted by the Iraqi Ministry of Health found that the rate of recurrent spontaneous abortion increased from 11% to 22% in the last recent years and this is a serious indicator, globally 97% of recurrent spontaneous miscarriage cases occur in Africa, Asia and Latin America [3]. Genetics, anatomical abnormalities, endocrine dysfunction, Placental abnormalities, infections, and tobacco use. The use of alcohol excessively, environmental variables, trauma of psychology, and stress are some of the elements that contribute to the etiology of RPL. Almost 50% of cases are idiopathic [4]. RPL has been associated with several single nucleotide polymorphisms (SNPs), and genetic variation has been proposed as one of the major causes causing RPL [5]. Short (about 22 nt), non-coding RNA molecules as microRNAs (miRNAs) helps in controlling the post-transcriptional expression of target genes by messenger RNA degradation or translational repression [6]. Limited number studies revealed the correlations between RPL and miRNA polymorphisms. at the human genome, SNPs at the miRNA binding sites represent novel functional polymorphisms. These variations may influence the expression of target genes or miRNAs [7]. Numerous miRNAs, including genes involved in miRNA machinery, have been linked to an increased challenge of RPL [8]. miR-27a and miR-938G>A gene polymorphism

in particular. These miRNAs, which are thought to be significant through period of pregnancy, were selected in this investigation due to increased expression (miR-27a). Pregnancy losses have been reported to be avoided and folate levels maintained throughout [9]. Previously a link has been shown between miR-938 to the TGF-signaling pathway and many members of the TGF-superfamily are important in reproductive system of female. In particular, these proteins have a vital function in controlling all phases of ovarian follicle growth, starting with the formation of the corpus luteum to the generation of oocytes. Ovulation, and luteinization, as well as the bringing in embryonic follicles and the propagation of granulosa and luteinizing cells [10].

MATERIALS AND METHODS

Study Design and Population

This work is case-control study on a study group during the first of 2022 to the end of 2022. The participants who were included in this research were divided into two distinct categories. The initial s comprised forty females who had been identified as idiopathic RPL patients and had experienced a minimum of two pregnancies one after the other losses prior to the 20th week (aged (20- 44) years old); were subdivided into two sub-groups, two baby loss and three baby loss. They were regularly attending to the infertility and gynecological and obstructs department in Baghdad Hospital City during the period of (February /2022–October/ 2022). We screened patients to find any recurrent pregnancy loss due to karyotypes, scan for abnormalities in anatomy in the tract of reproduction, hormonal assays for

progesterone and thyroid functions, immunological assays for autoantibodies like anticardiolipin antibody, anti-nuclear antibodies and antipaternal complement-dependent antibodies (APCA). The tests revealed negative results in those who are patients. Also, the chosen patients had no history of thrombotic disease or prothrombotic and coagulation mutation factors. In addition, TORCH screening test were normal and the same is with diabetes mellitus and hypertension. The other groups included forty age-matched apparently healthy pregnant females were kindly recruited as a control group in this study at least a successful pregnancy with a regular menstrual cycle and a live child. The control had no miscarriage history, ectopic gestation, pre-eclampsia, preterm birth, and more deformities.

Sampling Criteria

Three milliliters of blood specimens were obtained through vein puncture using single-use syringes under sterile conditions. The collected specimens were then placed into EDTA tubes and promptly frozen at -20°C to minimize the necessity for repetitive frozen and thawed cycles during polymerase chain reaction amplification and recognition of miR-27a and miR-938G>A (Tetra- ARMS-PCR) technique. All individuals gave verbal informed approval, and the study complied with the ethics of the infertile, gynecological, and obstructions departments of Baghdad Hospital City.

Methodology

DNA extraction Genotyping

We isolated the genomic DNA of frozen blood specimens by the gSYAN extraction of DNA kit from Geneaid, USA, based on directions of the manufacturer.

Preparing T-ARMS-PCR master mix

The T-ARMS-PCR master mix preparation was (GoTaq® G2 Green Master Mix kit) and by two reactions for each sample based on company directions. Then, these PCR master mix components of the table were transferred to Exispin vortex centrifuge at 3000rpm for 3 minutes followed by placing them in PCR Thermocycler

PCR Thermocycler Conditions

PCR thermos cycle conditions for each gene independent as following tables was performed.

Genotyping of miR-27a and miR-938G>A

Genotyping was performed by the Tetra- ARMS-PCR Method Amplification-refractory mutation system (ARMS-PCR) method. The rs895819A/G variants in the miRNA-27a gene by primer 4 (table 4) and the miR-938G>A variant, by primer 4 are in table 2.

Table 1: Standard T-ARMS-PCR Reaction Mix

T- ARMS-PCR Master Mix	DNA Template	Forward Inner Primer (Wild Allele) (10pmol)	Reverse Inner Primer (Mutant Allele) (10pmol)	Forward Outer Primer (10pmol) 1µl	Reverse Outer Primer (10pmol) 1µl	G2 Green Master Mix	Nuclease Free Water 3.5µl	Total Volume
Volume	5µl	1µl	1µl	1µl	1µl	12.5µl	3.5µl	25µl

Table 2: ARMS-PCR Thermocycler Conditions for miRNA27A> G

ARMS-PCR step	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Hold
Temp	95°C	95°C	55°C	72°C	72°C	4°C
Time	5min.	30 sec.	30 sec.	30 sec.	5 min	Forever
Repeat	1		35 cycle		1	-

Table 3: ARMS-PCR Thermocycler Conditions for miRNA938G >A

ARMS-PCR step	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Hold
Temp	95°C	95°C	65°C	72°C	72°C	4°C
Time	5min.	30 sec.	30 sec.	30 sec.	5 min	Forever
Repeat	1		35 cycle		1	-

Table 4: Tetra-ARMS-PCR Primers for miR-27A>G rs895819 Gene Polymorphism with their Sequence and Amplicon Size

T-ARMS-PCR Primer	Forward inner primer (A allele):	Reverse inner primer (G allele):	Forward outer primer	Reverse outer primer
Sequence (5'-3')	GCTGCTTGTGAGCAGGGTCCCA	CTTAGCCACTGTGAACAGACTTTGC	GGTTCCTGGGGATGGGATTTCCTT	GGCTTGACCCCTGTTCCTGCTGA
Product size	223bp	197bp		371bp

Table 5: Tetra-ARMS-PCR Primers for miR-938G>A rs12416605 Gene Polymorphism with their Sequence and Amplicon Size

T-ARMS-PCR Primer	Forward Inner Primer (A Allele):	Reverse Inner Primer (G Allele):	Forward Outer Primer	Reverse Outer Primer
Sequence (5'-3')	GGCATGTTGGAAGGTGTACCATTTA	GCACTGGGTTACCTTTAAGTGC	TTCTTCCCCCAATCATTTCTGG	GGAGCTATTGGCAACTCTAAACACCT
Product size	226bp	200bp		378bp

T-ARMS-PCR

In accordance with the company's directions, a master mix consisting of two actions for all samples was put together by the GoTaq® G2 Green Master Mix kit. Subsequently, the components of the PCR master mix were in an Exispin vortex centrifuge and spun for three minutes at 3000 rpm. Inserted in a BioRad (USA) PCR thermocycler after that. The following conditions were used for the PCR: 5 minutes of first denaturation at 95°C, 35 cycles of 30s at 94°C, 30s of annealing (at 55°C and 65°C for miR-27a and miR-938G>A,

in respective), extension 30s at 72°C, and the last 5 minutes of 72°C extension.

T-ARMS-PCR product analysis

The T-ARMS-PCR was tested the use of agarose gel electrophoresis: Preparing the 2% Agarose gel by 1X TBE dissolved in water at 100 °C ver a quarter of an hour, then left to cool 50°C. Later there was an addition of 3µL of ethidium bromide stain into agarose gel solution followed by pouring solution in tray fixing the comb in proper position. It is then left to solidify over a quarter of an hour at room temperature,

followed by gently removing the comb from the tray whose gel tray was fixed in electrophoresis chambers which 1X TBE buffer, 10µl PCR product fills in every comb well and 3µl of (100bp Ladder) in the first well. At 100 volts and 80 mA, we conducted the electric current for 1 hour and the T-ARMS-PCR was visualized by UV trans illuminator.

Statistical Analysis

SPSS (statistical application for social science version 26) was used on an IBM computer analyze the data. So, categorical parameters were: percentage and frequency, for continuous variables, the notation "mean $\pm$ standard deviation (SD)" was used. A comparison was made between the occurrences of diverse alleles and genotypes in separate sets by the chi-square test. In order to find aberrations from the Hardy-Weinberg equilibrium (HWE), allele frequencies were measured). Chi-square test showed the link between any two categorical variables. Using logistic regression analysis, and 95% confidence intervals (CIs),

the odds ratio (OR) for the genetic risk in RPL were computed. The comparison of means was conducted using the independent Student t-test. The study took A P value less than 0.05 to show the significance of the statistic.

Results

Table 6 displays the RPL demographic of patients and controls. The mean patients' age was (32.72 ± 4.82) and the controls was (33.16 ± 4.63) years, and this mean disparity did not exhibit statistical significance. ($P = 0.701$). Based on BMI ($P = 0.289$), frequency distribution between sufferers and standard subjects did not differ significantly. The present findings—zero significant differences in the age and BMI distributing persons in both groups—is a necessary condition to prevent age-related bias in case control studies of this kind.

Table 6: The Demographic Profile of Women with RPL and Control-Healthy Participants

Characteristic	Patients N = 40	Healthy Control N = 40	P
Age (years)			
Mean $\pm$ SD	32.72 ± 4.82	33.16 ± 4.63	0.701
Range	22 – 42	23 – 42	†
			NS
< 30, n (%)	11 (27.5%)	8 (20.0%)	0.685
30 - 39, n (%)	24 (60.0%)	28 (70.0%)	¥
≥ 40 , n (%)	5 (12.5%)	4 (10.0%)	NS
Body mass index (BMI) kg/m ²			
Mean $\pm$ SD	22.46 ± 1.49	22.79 ± 1.34	0.289
Range	19.00 – 24.60	20.60 – 24.00	†
			NS

n: number of cases; *SD*: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P > 0.05$

Distribution of MiRNA-27a (rs895819) A/G polymorphism had been detected by T-ARMS-PCR technique. Lane (AA) of the rs895819 genotyping for miR-27a>G wild type homozygotes contained only the A allele at 223bp in the PCR product. Figure (1) illustrates that the

lane (GG) mutant homozygote showed solely the G allele at 197bp in the PCR product. In contrast, the lane (AG) heterozygote displayed both the A and G alleles at 223bp and 197bp in the T-ARMS-PCR products and the outer internal control was detected at 371bp.

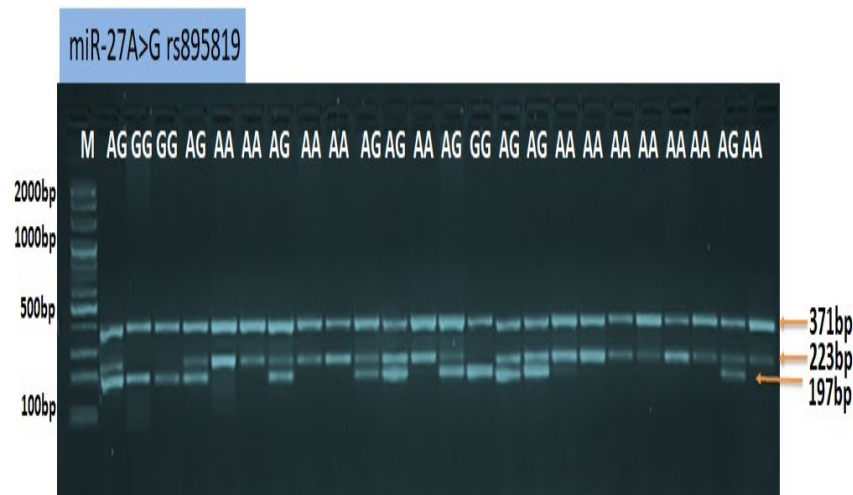


Figure 1: ARMS-PCR Product Agarose gel Electrophoresis Image of MiRNA-27a (rs895819) A/G Gene Polymorphism.

Across all research groups, the genotype distribution of miR-27a>G (rs895819) remained similar to from the Hardy-Weinberg equilibrium. The data presented in Table7 illustrates a robust the miR-27a A>G polymorphism link to a reduced likelihood of developing RPL (OR = 0.06; 95% CI = 0.01-0.22; A/G vs. AA: OR =

0.34; 95% CI = 0.08-1.37). The miR-27a A>G polymorphism correlation to d reduced risk of RPL was in the RPL=3 subgroup (GG vs. AA: OR = 0.541; AG vs. AA: OR = 0.562) when categorizing RPL patients based on RPL=3 vs. RPL women with RPL=2, as shown in Table 8.

Table 7: The MiRNA-27a (rs895819) A/G POLY Genotype Frequency in those with RPL and Healthy Pregnancy

MiRNA-27a (rs895819)		Patients N = 40	Control N = 40	P	OR	95% CI	PF	EF
Co-Dominant	GG	8 (20.0%)	28 (70.0%)	< 0.001	0.06	0.01-0.22	0.822	
	A/G	13 (32.5%)	8 (20.0 %)	¥ HS	0.34	0.08 -1.37	0.438	
Dominant	AA	19 (47.5 %)	4 (10.0 %)			Reference		
	GG+ A/G	21 (52.5 %)	36 (90.0 %)	0.002		Reference		
Recessive	AA	19 (47.5 %)	4 (10.0 %)	¥ S	8.14	2.44-27.1		0.416
	GG	8 (20.0%)	28 (70.0 %)	< 0.001	0.11	0.03-0.29	0.625	
Alleles	A/G +AA	32 (80.0%)	12 (30.0 %)	¥ HS		Reference		
	G	29 (36.3%)	64 (80.0 %)	< 0.001	0.142	0.07-0.28	0.686	
	A	51 (63.7 %)	16 (20.0%)	¥ HS		Reference		0.686

¥: Chi-square test; EF: etiologic fraction; PF: Protective factor; S: significant at $P < 0.05$; HS: significant at $P \leq 0.001$

Table 8: Association between Number of Pregnancy Loss in Women with RPL and ARMS-PCR (MiRNA-27a A/G Polymorphism) Results

MiRNA-27a A/G		Pregnancy Loss		p-Value	OR
		Three (18)	Two (22)		
Genotypes	GG	3 (16.7%)	5 (22.7%)	0.472	0.541
	A/G	5 (27.7%)	8 (36.4%)	0.430	0.562
	AA	10 (55.6%)	9 (40.9%)	Reference	
Allele	G	11 (30.6%)	18 (40.9%)	0.337	0.635
	A	25 (69.4%)	26 (59.1%)	Reference	

The T-ARMS-PCR approach was employed to identify the distribution of the miRNA-938 G/A polymorphism. The miR-938G>A (rs12416605) genotyping with lane (GG) wild homozygote was exposed only G allele at 200bp of PCR. The (GA) heterozygote was

shown as both G and A allele at 200bp and 226bp T-ARMS-PCR product, as well as the outside internal control was seen at 378bp PCR product, figure (2). The lane (AA) mutant type homozygote was shown to have only A allele at 226bp PCR.

**Figure 2:** An Agarose Gel Electrophoresis Image Illustrating the miRNA-938 G/A Gene Polymorphism as Determined by ARMS-PCR Product Analysis

In every study group, the genotype distribution of miRNA-938 showed no divergence from Hardy-Weinberg equilibrium. Table (9) shows a significant correlation between the miRNA-938 variation in form and an increased incidence of RPL (G/A vs. GG: OR = 36; 95% CI

= 09.89 -130.9). Table (10) shows that there was a correlation between the miRNA-938 polymorphism and higher RPL risk in the RPL=3 subgroup (G/A vs. GG: OR = 1.47) when RPL patients were categorized based on frequency RPL (RPL= 3 vs. RPL females with RPL=2).

Table 9: The miRNA-938 G/A POLY Genotype Frequency in women with Recurrent Pregnancy Loss and Healthy Pregnancy

MiRNA-938 G/A	Patients n = 40	Control n = 40	P	PF	EF	OR	95% CI
Genotype frequency							
G/A	32 (80.0 %)	4 (13.3 %)	< 0.001		0.777	36	9.89 -130.9
GG	8 (20.0 %)	36 (86.7 %)	¥ HS	0.777		Reference	
Allele frequency							
A	32 (40.0%)	4 (5.7 %)	< 0.001		0.368	12.7	4.2-38.1
G	48 (60.0 %)	76 (94.3%)	¥ HS	0.368		Reference	

¥: Chi-square test; EF: etiologic fraction; PF: Protective factor; HS: significant at $P \leq 0.001$

Table 10: Association between Pregnancy Loss Number in Recurrent Pregnancy Loss Females and ARMS-PCR (miRNA-938 G/A Polymorphism) Results

	miRNA-938 G/A	Pregnancy Loss		p-Value	OR
		Three (18)	Two (22)		
Allele	G/A	15 (83.3%)	17 (77.3%)	0.633	1.47
	GG	3 (16.7%)	5 (22.7%)	Reference	
	A	15 (41.7%)	17 (38.6%)	0.783	1.14
	G	21 (58.3%)	27 (61.4%)	Reference	

In present study, determined the relationship between various combinations of the two miRNA polymorphisms and recurrent pregnancy loss to conduct a genotypes combination analysis, table (11). From this analysis, the current results perceived a statistically significantly linked miRNA-27a A/G, to miRNA-938 G/A combination and increased RPL (OR, 11.25; 95% CI, 0.90–139.5; $P = 0.033$).

Among the combination analyses models of the one polymorphic allele, the AG-GG genotypes (OR = 0.50; 95% CI = 0.04–5.15) and two polymorphic alleles the GG-GG genotypes (OR = 0.055; 95% CI = 0.006–0.44) was connected to a declining recurrent pregnancy loss risk, whereas three polymorphic alleles, GG-GA (OR = 4.5; 95% CI = 0.33–60.1) were linked to a rising, recurrent pregnancy loss risk.

Table 11: Combination Analyses of miRNA-27a A/G, and miRNA-938 G/A Polymorphism in Patients and Controls

Genotype	Patients n = 40	Control n = 40	P	OR	95% CI
Genotype Frequency					
AA-GG	4 (10.0%)	3 (7.5%)		Reference	
AA-GA	15 (37.5%)	1 (2.5%)	0.033	11.25	0.90–139.5
AG-GG	2 (5.0%)	6 (15.0%)	0.558	0.5	0.04–5.15
AG-GA	11 (27.5%)	2 (5.0%)	0.176	4.125	0.49–34.4
GG-GG	2 (5.0%)	27 (67.5%)	0.001	0.055	0.006–0.44
GG-GA	6 (15.0%)	1 (2.5%)	0.237	4.5	0.33–60.1

χ²: Chi-square test; NS: not significant at $P > 0.05$; S: significant at $P \leq 0.05$

Discussion

Any couple may experience the terrible event of recurrent pregnancy loss affecting 5% of couples. Seldom do couples receive a clear explanation for why they are losing pregnancies once more. Owing to advancements in the medical field, prevalent causes of RPL are now better understood and treated. The search for the underlying reasons of idiopathic RPL is urgently needed these days [11, 12]. The mean age of women experiencing repeated pregnancy loss was 32.72 ± 4.82 years, whereas it's of healthy control subjects was 33.16 ± 4.63 years. The mean age disparity between the two cohorts was not statistically significant. ($P = 0.701$) and that mean of patients with recurrent pregnancy loss and healthy control was 34.08 ± 4.52 vs. 33.9 ± 3.84 , respectively, according to Ezat and Haji's [13] results, which are similar to ours ($p = 0.22$). Additionally, a study by Fatema and Akbay [3] indicated that maternal age was substantially connected with the decision to have a recurrent pregnancy loss and that the abortion rate in the Kurdistan Region of Iraq was 27.7%. When compared control group of pregnant women in good health, no statistically significant variation was observed in age at first pregnancy loss. The current research reveals that the age group between 30–39 years had the most frequent distribution of patients with recurrent pregnancy loss, with 24 (60.0%) followed by the age group less than 30 years 11 (27.5%). However, the frequency distribution fell at the age group 40 and older, with 5 (12.5%). This group was more prone to experiencing repeated miscarriages, which is consistent with the study's findings that patients who were between the ages of 25 and 34 had the highest number of miscarriages [14]. The trend among women today is to delay childbearing until their thirties or even forties due to the development of effective contraception; nevertheless, the risk of miscarriage varies depending on the specified maternal age [15]. The probability increases in a nearly linear fashion beyond the age of 30, reaching 54% for individuals aged 45 and above. This is

accompanied by a significant decline in both the quality and quantity of eggs, as well as a greater likelihood of DNA in mitochondria mutation and meiosis error [16]. The current findings indicate that there were no significant variations in BMI between cases as opposed to controls ($P = 0.289$). The findings confirm Rah et al. [17], who demonstrated a lack of significant variation in BMI between RPL patients and controls (21.68 ± 3.45 vs 21.49 ± 3.84) ($P = 0.668$). The current findings confirmed Ezat and Haji [13], which shown that the body mass index (BMI) of female patients did not differ statically from the controls (28.11 ± 4.49 vs. 27.96 ± 4.89) ($p = 0.31$). Humadi et al. [18] reported that the average BMI was 23.8 ± 3.9 kg/m² for control cases and 22.3 ± 4.2 kg/m² for study cases in the respective. Prior research has indicated that individuals with a BMI bigger than or equal to 25 have a 1.2-fold increased likelihood of experiencing recurrent pregnancy loss in the general population, as opposed to those with a normal BMI [19]. However, the present findings contradict a previous meta-analysis that incorporated two studies and reported a higher likelihood of subsequent recurrent pregnancy loss in obese women (OR 1.75, 95% CI 1.24–1.70) as opposed to those with a healthy body mass index (BMI) [20]. Female reproductive health is profoundly affected by obesity; a higher body mass index (BMI) is correlated with infertility, unfavorable results following fertility interventions, and miscarriage. Possible explanations include an unfavorable endometrial milieu that is linked to obesity, as well as the potential for oocyte abnormalities in obese women [21, 22].

In addition, miRNAs are of paramount importance in the regulation of numerous biological processes. Over 5000 human genes are targeted by over 1500 microRNAs regulating normal pregnancy [23]. The microRNAs (miRNAs) use as prospective biomarkers for diagnosing diverse ailments, such as reproductive disorders, has garnered growing attention from scientists in the last ten years. Multiple studies have established a correlation between different variants of microRNAs and the risk of developing reproductive

disorders in humans. Furthermore, numerous studies have consistently shown the direct impact of these factors on the occurrence of female fertility issues [24]. The expression and maturation of miRNAs can be modified by mutations in their coding regions, potentially impacting the incidence of diseases [25]. Through the use of partial base complementarity, microRNA bonds to various miRNA sites, thereby impeding translating and/or speeding up the breakdown of mRNA. Entirety represents a significant regulatory characteristic of the transcriptome. The expression of subsequent signaling events can also be influenced by miRNAs via co-expression with host genes. Different physiological and pathological processes, including embryogenesis, apoptosis, angiogenesis, and cellular differentiation, are profoundly and directly influenced by the expression of microRNAs (miRNAs). Dysregulation of miRNAs disrupts Embryogenesis, angiogenesis, apoptosis, and cellular difference. Furthermore, microRNAs (miRNAs) are important in maternal-fetal immune tolerance and modulate Expression of genes in the uterus is related to response to inflammation during period around implantation [26]. Polymorphisms in microRNAs may influence endometrial decidualization and cause abnormalities in the development of embryos, leading to successive miscarriages [27]. The current study identifies prevalent genetic variants in microRNAs raising the vulnerability of recurrent miscarriages in the Iraqi populace. Prior research has examined the functions of miR-499, miR-125, miR-196a2, and miR-423 polymorphisms in relation to pregnancy loss. These studies have obtained functional evidence that production of fully developed microRNA and its subsequent regulation of target genes. are disrupted by these polymorphisms [7]. Within a relatively brief period, numerous study teams have investigated the significance of miRNA polymorphisms and hypothesized a robust correlation with disease progression [28]. Consequently, the findings presented in the current study constitute an original replication and bolster the established correlation between miRNAs and pregnancy loss. Numerous SNPs are widely recognized to be present in miRNAs. Subsequently, genetic variants of MiRNA-27a (rs895819) were chosen and their genetic associations with the risk of pregnancy loss were assessed via ARMS-PCR analysis in order to determine whether they could serve as a genetic marker to predict the likelihood of experiencing recurrent pregnancy loss. Because of its heightened expression, specifically during pregnancy, miR-27a was selected. Gene and allele frequencies pertaining to MiRNA-27a (rs895819) for this study, polymorphism was assessed in forty women who experienced recurrent pregnancy loss and forty healthy control women (refer to figure 4). Due to distributing genotype frequencies for MiRNA-27a (rs895819), the groups ($P > 0.001$) differed significantly. According to this study, the miRNA-27a polymorphism is correlated with a reduced likelihood of experiencing recurrent miscarriage. In contrast to the present findings, Korean female study revealed that the A>G polymorphism of miR-27a (rs895819) significantly associated with recurrent pregnancy loss [17]. The current findings contradict Shaker et al. [11] documenting a significant link between the genotypes of miR-27a A>G polymorphism and the risk of RPL [29]. An additional Chinese study revealed that the study subjects had a greater the GG genotype and G allele of miR-27a rs895819 A/G than the controls. Also, both the GG genotype and G allele frequencies were significantly higher than in the controls (GG genotype: 15.9% vs. 9.2%, OR 2.357, $P = .008$; G allele: 37.7% vs. 27.1%, OR=1.625, $P = .008$; 29). Also, miR-27a rs895819 and the likelihood of experiencing recurrent spontaneous abortions and repeated implantation failures [30] significantly correlated. The inconsistent expression of microRNAs

may contribute to developing recurrent pregnancy loss, according to a number of studies [31]. However, our findings contradict the research conducted by Wang et al. [32] in the statistically significant link between the rs895819 A>G polymorphism of miR27a and the recurrent spontaneous abortion risk in any genetic scenario. The AA, AG, and GG genotypes frequencies among recurrent pregnancy loss cases and controls were 19, 13, 8, and 4, 18, 28, respectively ($P = 0.001$). A low risk was significantly linked to the miR-27a A/G polymorphism (GG vs AA: OR = 0.06; 95% CI = 0.01-0.22; AG vs AA: OR = 0.34; 95% CI = 0.08-1.37). The AA genotype was found in a greater proportion of 19 patients (47.5%) who experienced recurrent pregnancy loss, suggesting a substantial carrier rate for this allele in the present study. However, this study revealed a strong and statistically significant correlation (OR = 0.142; 95% CI = 0.07-0.28) between the miR-27a variant G allele and a reduced likelihood of experiencing recurrent pregnancy loss. An OR value of 0.774 was associated with the G allele and a reduced risk of recurrent pregnancy loss, similar to the present finding by Rah et al. [17]. However, our work contradicts Shaker et al. [11] and Wang et al. [32] regarding the risk allele for recurrent pregnancy loss. According to Shaker et al. [11], the G allele frequency showed a statistically significance among the experimental group, making it a risk allele. Wang et al. [32] said that the contrast between the alleles (G and A) did not indicate a significant risk allele for recurrent pregnancy loss, with an odd ratio of 1.53 (0.92-2.55). In relation to this, miR-938G>A (rs12416605) was also chosen, and its genetic associations with the risk of RPL were assessed via T-ARMS-PCR analysis in order to determine whether genetic variants in miR-938G>A (rs12416605) could serve as a genetic marker to predict the likelihood of experiencing recurrent pregnancy loss. The analysis of distributing two genotypes in the current work unveiled that the frequencies of GA and GG among controls were 4, and 36, respectively. In contrast, the frequencies of these genotypes were 32 and 8, respectively, among patients who experienced RPL (see figure 2). In summary, the findings of this study establish a highly significant link between the miR-938G>A polymorphism and the RPL risk ($p > 0.001$). In contrast to Cho et al. [10], our research incorporated 227 healthy controls and 388 patients diagnosed with recurrent pregnancy loss disease. So, the polymorphic genotype miR-938G>A was linked to a decreased risk of idiopathic RPL. Prior research has established a connection between miR-938 and the TGF- β signaling pathway, and various members of the TGF- β superfamily play vital roles in the reproductive systems of females [33]. More precisely, these proteins control every facet of ovarian follicle development—the recruitment of primordial follicles, the granulosa proliferation and theca cells, the gonadotropin receptor expression, the maturing process of oocytes, ovulation, luteinization, and the corpus luteum formation [34]. $P = 0.001$ indicates that the AA, AG, and GG genotype frequencies were as follows: 19, 13, 8, and 4, 18, 28 for recurrent pregnancy loss cases and controls, respectively. The miR-27a A/G polymorphism was in a significant link to a reduced likelihood of RPL (GG vs AA: OR = 0.06; 95% CI = 0.01-0.22; AG vs AA: OR = 0.34; 95% CI = 0.08-1.37). A significant proportion of the 19 patients (47.5%) who encountered recurrent pregnancy loss possessed the AA genotype, indicating a considerable carrier rate for this allele within the cohort under investigation in the present study. Nevertheless, the results of this research demonstrated a robust and statistically significant link (OR = 0.142; 95% CI = 0.07-0.28) between the miR-27a variant G allele to a decreased probability of recurrent miscarriage. G allele link with a lower risk of RPL was quantified by an OR value of 0.774, confirming Rah et al. [17]. In contrast, our results disprove the conclusions drawn

by Wang et al. [32] and Shaker et al. [11] concerning the risk allele associated with RPL. Shaker et al. [11] established that the study group exhibited a statistically significant frequency of the G allele, thereby designating it as a risk allele. According to Wang et al. [32] and with an odd ratio of 1.53 (0.92-2.55), the contrast between the alleles (G and A) did not suggest the presence of a risk allele for recurrent pregnancy loss that was statistically significant. Pertaining to this, the genetic associations between miR-938G>A (rs12416605) and RPL were evaluated using T-ARMS-PCR to determine whether genetic variants in miR-938G>A (rs12416605) could function as a prognostic genetic marker for the probability of encountering RPL. The present study's examination of the distribution of two genotypes revealed that the frequencies of GA (4 individuals) and GG (36 individuals) among the control group were, respectively.

On the contrary, as illustrated in Figure 2, the frequencies of these genotypes were 32 for patients who encountered recurrent pregnancy loss and 8 for those who did not. In brief, according to the results the miR-938G>A polymorphism is statistically and significantly correlated ($p > 0.001$) with the likelihood of experiencing RPL. Our research, contradicting Cho et al. [10], included 388 patients who had been diagnosed with recurrent pregnancy loss disease and 227 healthy controls. It was found that the polymorphic genotype miR-938G>A

exhibited a correlation with a reduced likelihood of experiencing idiopathic recurrent pregnancy loss. Previous studies have established a correlation between miR-938 and the TGF- β signaling pathway, and the female reproductive system is significantly influenced by numerous members of the TGF- β superfamily [33]. Specifically, these proteins control every developing ovarian follicle aspect—forming the corpus luteum, the recruitment of primordial follicles, and the proliferation of granulosa and theca cells; the expression of gonadotropin receptors; and the maturation of oocytes, ovulation, and luteinization [34, 35].

Conclusions

The present findings have demonstrated a connection between variations in individual nucleotides (SNPs) within microRNAs (miRNAs) and repeated miscarriages (RPL) progression. This correlation may have influenced potential function of miRNA by the peri-implantation stage with an early pregnancy. We suggest these SNPs might be used as prognostic biomarker in the future. Although the obtained findings are valuable, it is necessary to expand the data and study more SNPs in miRNA related with recurrent baby loss which is applicable in prognostic or therapeutic scope.

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