BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2021, VOL. 78, NO. 4, 206–210
https://doi.org/10.1080/09674845.2021.1889157

MicroRNA variants in endometriosis and its severity

M Farsimadan[a], M Ismail Haje[b], C Khudhur Mawlood[c], I Arabipour[d], A Emamvirdizadeh[e], S Takamoli[a], M Masumi[a] and H Vaziri[a]

[a]Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran; [b]Medical Education Depatrment, College of Medicine, Hawler Medical University, Erbil, Kurdistan Region-Iraq; [c]Gynicology and Obstitrict Department, College of Medicine, Hawler Medical University, Erbil, Kurdistan Region-Iraq; [d]Department of Biotechnology, Science and Research Branch, Islamic Azad University, Tehran, Iran; [e]Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

ABSTRACT

Background: MicroRNAs (miRNAs) are naturally occurring posttranscriptional regulatory molecules that potentially play a role in endometriotic lesion development.

Method: We evaluated the prevalence of miRNAs variants miR-146a rs2910164, miR-149 rs2292832, miR-196a2 rs11614913, and miR-499 rs3746444 in endometriosis in 260 cases and 260 controls. DNA was extracted and genotyping of the SNPs was carried out by PCR.

Results: There was a significant association of rs2910164 and rs2292832 with increased rates of endometriosis under the dominant ($p < 0.001$), recessive ($p < 0.05$), co-dominant ($p < 0.001$), and allelic model ($p < 0.001$). Also, rs3746444 showed a borderline association with endometriosis under the recessive model ($p = 0.05$); however, rs11614913 was not linked to endometriosis. Further analysis indicated the significant association of miR-146a rs2910164 polymorphism genotypes (GG, GC, and CC) and miR-149 rs2292832 genotypes (CC and CT) with endometriosis severity in patients ($p < 0.001$). Additionally, haplotype frequency in cases compared to controls and Linkage disequilibrium (LD) between the mentioned SNPs was appraised.

Conclusion: MiR-146a, miR-149 and miR-499 may have a role in the pathogenesis of endometriosis.

ARTICLE HISTORY
Received 19 January 2021
Accepted 5 February 2021

KEYWORDS
Endometriosis; microRNAs; polymorphism

Introduction

Infertility affects about 15% of couples throughout the world and female infertility constitutes 37% of all infertility cases [1–3]. Endometriosis, defined as the presence and growth of endometrial tissue outside the uterus, is a leading cause of infertility, affects 10–15% of women of reproductive age and may even reach to 35–50% in women with pelvic pain and/or infertility [4]. Women with this syndrome display clinical symptoms including irregular menstrual bleeding, menstrual pain, pain during sexual intercourse, and pelvic pain [5]. The aetiology of endometriosis is not well-established; however, a number of factors including obesity, genetic, endocrine, and environmental factors may contribute to the development of this disease.

MicroRNAs (miRNAs) are naturally occurring posttranscriptional regulatory molecules that potentially play a role in endometriotic lesion development [6,7]. Different miRNAs have been shown to be potential biomarkers and a new approach for the diagnosis of endometriosis [8,9]. Studies have shown that single miRNA might regulate the synthesis of proteins encoded by hundreds of genes and play significant roles in the different biological function such as cell differentiation and proliferation, cell migration, and myogenesis [10,11] that occur in endometriosis; therefore, the identification of human disease-linked miRNAs would help to understand the pathogenesis of endometriosis.

Some single nucleotide polymorphisms (SNPs) in miRNAs may potentially interfere with miRNA-mediated regulation of cellular functions and influence the generation or function of miRNAs [12]. Many studies have investigated the association of SNPs with various female reproductive disorders [13–15], however, little is known about the effect of miRNAs variants in endometriosis. Thus, we hypothesized links between miRNA variants miR-146a rs2910164, miR-149 rs2292832, miR-196a2 rs11614913, and miR-499 rs3746444 in endometriosis.

Methods

We tested our hypothesis in a case-control study of 260 patients and 260 controls. The patient group consisted of women who submitted to laparoscopic surgery for the evaluation of pelvic pain and/or

CONTACT H Vaziri drhamidrezavaziri00@gmail.com Associate Professor in Cell and Molecular Biology, Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

infertility, and with histological confirmation of endometriosis and classification of the disease according to the Revised American Society for Reproductive Medicine [16]. The control group included endometriosis-free patients who underwent laparoscopic examination for indications other than infertility, such as prolapsed uterus, ovarian cyst, or urinary incontinence. Exclusion criteria for both groups were presence of systematic inflammation diseases and infections, pregnancy, history of pregnancy in the last 3 months, gynaecological cancer, adenomyosis and other major systemic diseases. The study has been approved by the high graduate committees of the Faculty of Medicine/University of Guilan, Rasht/Iran. The informed consent documents were obtained from all subjects prior to sampling.

Blood samples were collected in tubes containing EDTA and stored at −20 °C. Genomic DNA was extracted from white blood cells with the Blood and Cell Culture DNA kit (Sinacolon, Iran) according to the manufacturer's protocol. DNAs were analysed by electrophoresis on 2% agarose gels with ethidium bromide staining. Concentrations of extracted DNAs were determined by Nanodrop. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the polymorphism of miR-146a rs2910164 G > C, miR-149 rs2292832 T > C, miR-196a2 C > T and miR-499 T > C. Table 1 shows information about the position, types of selected SNPs, the primers sequences, enzymes, and the product length for each SNP. The PCR conditions started with an initial denaturation at 94 °C for 5 minutes, 35 cycles of denaturation of molecules at 94 °C for 1 minute, annealing at 61 °C for rs2910164, 65 °C for rs2292832, 60 °C for rs11614913, and 57 °C for rs3746444 for 1 minute, and the products were under extension at 72 °C for 40se, and then a final extension at 4 °C for 5 minutes in Biorad Thermocycler. PCR products were separated on agarose gel and visualized by ethidium bromide staining.

Statistical analyses were done using both SNPAlyze software (ver.8.1, Dynacom, Japan) and SPSS (ver.22). Allele and genotype frequencies of the SNPs among control and case groups were compared and checked using Pearson χ2 statistic. Moreover, deviations from Hardy–Weinberg equilibrium (HWE) were tested using a χ2 goodness-of-fit test. Analyses were also performed assuming recessive, codominant, and dominant models of inheritance and crude odds ratio (OR), their 95% CIs. Haplotype analyses were done using rs2292832, rs2910164, rs11614913, and rs3746444, variants for all study samples according to the maximum-likelihood method with an expectation–maximization algorithm. In addition, pairwise linkeage disequilibrium (LD) coefficients of |D'| and r2 were assessed using SNPAlyze software version 8.1 (DYNACOM, Japan). LD analyses were done based on Hardy-Weinberg equilibrium model. The significance level of the statistical tests was <0.05.

Results

Cases and controls were matched for age (mean/SD: 34.1 ± 4.1 years vs. 32.9 ± 3.65, p = 0.12) and BMI (27.2 ± 4.7 kg/m^2 vs. 28.1 ± 5.0, p = 0.22). Of the 260 endometriosis patients 33% had no history of pregnancy and 67% had one or more than one successful pregnancy, while 25% of controls indicate no history of pregnancy and 75% had one or more than one successful pregnancy (p = 0.027). The number of caesarean (61% vs. 58%) and natural (39% vs. 42%) deliveries were matched (p = 0.42 and p = 0.17 respectively). However, a significant association was found between the frequency of previous miscarriages in cases compared with controls (28% vs. 16%) (p = 0.001).

Statistical analyses indicated that the frequencies of SNP alleles in the control group are in accordance with the Hardy–Weinberg equilibrium, except for rs2292832 (Table 2).

Allele and genotype distribution frequencies of each miRNA SNPs are shown in Table 2. rs2910164 and rs2292832 had a significant association with increased rates of endometriosis incidence under the dominant, recessive, co-dominant, and allelic model. Also, rs3746444 showed a significant association with endometriosis under the recessive model, and rs11614913 was not linked to endometriosis. A subgroup analysis was performed to evaluate the frequency and links between rs2910164 and rs2292832 genotypes and 148 patients with mild or severe endometriosis. Significant differences were observed in GG, GC and CC genotype prevalent among the subgroups regarding miR-146a rs2910164. The results suggest that GC genotype may protect from endometriosis severity. Our results also revealed a significant association of CC and CT

Table 1. General information of selected SNPs.

SNP	Position	Change	Primer sequences	Enzyme	Products length (bp)
miR-146a rs2910164	chr5:160485411	NR_029701.1:n.60 C > G	CATGGGTTGTGTCAGTGTCAGAGCT TGCCTTCTGTCTCCAGTCTTCCAA	SacI	147, 120, 27
miR-149 rs2292832	chr2:240456086	NR_029702.1:n.86 T > C	CTCTGGCTCCGTGTCTTCACTC CCTGCAGGTTCTGAGGGGC	PvuII	225, 154, 71
miR-196a rs11614913	chr12:53991815	NR_029617.1:n.78 C > T	CTTACCCACCCAGCAACCC CCCCACTCACAGCTTGTCC	HpyCH4III	360, 218, 142
miR-499 rs3746444	chr20:34990448	NR_039912.1:n.25 T > G	GCCCCTTGTCTCTATTAGCTG ACTTTTGCTCTTTCACTCTCAT	Tsp451	416, 232. 184

Table 2. Allele and genotype distribution frequencies of SNPs in the case and control groups and in the severity of endometriosis.

	Population			
SNP Model	Case (n,%)	Control (n,%)	OR (95% CI)	Adjusted P-value
miR-149 rs2292832				
Allele C	334(64.3)	408(78.5)	2.20 (1.66-2.91)	p<0.001
Allele T	186(35.7)	112(21.5)		
Co-dominant (CT v CC+TT)	148(57.0)	96(37)	2.25 (1.58-2.20)	p<0.001
Dominant (CC v CT+TT)	93(35.7)	156(60)	2.99 (2.09-4.28)	p<0.001
Recessive (TT v CC+CT)	19(7.3)	8(3)	2.49 (1.07-5.80)	0.05
	P[a]0<0.01	P[b]=0.13		
miR-146a rs2910164				
Allele G	354(68)	416(80)	1.89 (1.42-2.51)	p<0.001
Allele C	166(32)	104(20)		
Co-dominant (GC v GG+CC)	104(40)	74(28.4)	1.67 (1.16-2.41)	p<0.001
Dominant (GG v GC+CC)	125(48)	171(65.7)	2.09 (1.47-2.98)	p<0.001
Recessive (CC v GG+GC)	31(12)	15(5)	2.22 (1.16-4.22)	0.03
	P[a]=0.19	P[b]=0.07		
miR-196a2 rs11614913				
Allele C	353(67.8)	323(62.1)	0.78 (060-1.00)	0.080
Allele T	167(32.1)	197(37.8)		
Co-dominant (CT v CC+TT)	119(45.7)	135(52)	0.78 (0.55-1.10)	0.137
Dominant (CC v CT+TT)	117(45)	94(36)	0.69 (0.49-0.99)	0.080
Recessive (TT v CC+CT)	24(9)	31(12)	0.75 (0.43-1.32)	0.320
	P[a]= 0.42	P[b]=0.09		
miR-499 rs3746444				
Allele T	294(56.5)	327(62.8)	1.33 (1.04-1.71)	0.24
Allele C	226(43.4)	193(37.1)		
Co-dominant (TC v TT+CC)	130(50.6)	131(50.3)	1.00 (0.70-1.41)	0.080
Dominant (TT v TC+CC)	82(34.8)	98(37.6)	1.39 (0.97-2.00)	0.086
Recessive (CC v TT+TC)	48(14.5)	31(12)	1.69 (0.99-2.63)	**0.05**
	P[a]=0.77	P[b]=0.20		
	Mild endometriosis (N=112)	**Severe endometriosis** (N=148)		
miR-149 rs2292832				
Allele C	130 (58)	204 (69)	1.62 (1.13-2.34)	0.011
Allele T	94 (42)	92 (31)		
Co-dominant (CT v CC+TT)	84 (75)	64 (43)	3.93 (2.30-6.73)	p<0.001
Dominant (CC v CT+TT)	23 (20.5)	70 (47)	3.63 (2.05-6.40)	p<0.001
Recessive (TT v CC+CT)	5 (4.5)	14 (9)	0.45 (0.15-1.29)	0.13
miR-164a rs2910164				
Allele G	145 (64.7)	219 (74)	1.57 (1.07-2.29)	0.022
Allele C	79 (35.2)	77 (26)		
Co-dominant (GC v GG+CC)	63 (56)	31 (21)	4.85 92.81-8.36)	p<0.001
Dominant (GG v GC+CC)	41 (36.6)	94 (63.5)	3.09 (1.58-5.19)	p<0.001
Recessive (CC v GG+GC)	8 (0.07)	23 (15.5)	0.42 (0.18-0.93)	0.006

[a]HWB p-value for cases. [b]HWB p-value for controls.

genotypes in miR-149 rs2292832 with severity in endometriosis among patients. A significant protective effect was found between CT genotype and disease severity.

To evaluate the association of these variants with endometriosis, haplotype analyses were done using, rs2910164, rs2292832, rs11614913, and rs3746444 variants. Haplotype analyses were done according to the maximum-likelihood method with an expectation–maximization algorithm. P values were assumed by comparing haplotype frequencies between case and control subjects based on 10,000 replications. We found significant negative associations between five haplotypes and endometriosis status. The C-G-C-T corresponding to rs2292832, rs2910164, rs11614913, and rs3746444 was the most prevalent haplotype among cases and controls, whereas the T-C-T-C haplotype in cases and T-C-T-T and T-C-T-C in controls were the least prevalent haplotypes (Table 3). There was no LD between the SNPs based on the measured D′ and r^2 parameters (Table 4).

Discussion

Our principal result is a significant association of rs2910164 and rs2292832 with endometriosis; however, rs11614913 did not show any link to increased rates of endometriosis, and rs3746444 was associated with borderline increased levels of endometriosis incidence among patients under the recessive model.

The significant role of different miRNAs in the pathogenesis of endometriosis has been investigated in many studies. Teague et al. suggested 22 different miRNAs and their cognate mRNA target sequences constitute pathways that would promote endometriosis and introduced these miRNAs as potential therapeutic targets [11]. Similarly, Cosar et al. identified several miRNAs in the serum of endometriosis women distinguishing them from healthy controls. They showed that miR-125b-5p had the greatest potential as a single diagnostic biomarker [17]. Others revealed that miR-31 expression levels were significantl down-regulated in stage 1,2,3 and 4 in

Table 3. Distribution of haplotype blocks in endometriosis patients and controls.

rs2292832	rs2910164	rs11614913	rs3746444	Case frequency%	Control frequency%	OR (95% CI)	P-value
C	G	C	T	17	25	0.66 (0.50–0.88)	0.021
C	G	C	C	12	13	1.00 (0.62–1.60)	0.98
C	G	T	T	9	15	0.73 (0.52–1.03)	0.16
C	G	T	C	5	10	0.60 (0.39–0.92)	0.05
T	G	C	T	8	6	1.16 (0.68–2.00)	0.82
C	C	C	T	7	6	1.37 (0.68–2.77)	0.59
T	G	C	C	8	4	1.64 (1.04–2.59)	0.09
C	C	C	C	6	3	0.90 (0.53–1.52)	0.84
C	C	T	T	4	4	1.00 (0.56–1.79)	0.98
T	G	T	T	3	4	0.55 (0.18–1.66)	0.48
T	C	C	C	4	1	2.99 (1.79–5.01)	<0.001
T	G	T	C	3	3	1.20 (0.36–3.98)	0.85
T	C	C	T	3	1	2.79 (0.88–8.84)	0.16
C	C	T	C	2	1	1.00 (0.20–4.99)	0.84
T	C	T	T	3	0	3.26 (1.29–8.23)	0.008
T	C	T	C	1	0	-	-

Table 4. Evaluation of linkage disequilibrium between SNPs.

	D′ statistic[a]				r statistic[b]			
SNPs	rs2292832	rs2910164	rs11614913	rs3746444	rs2292832	rs2910164	rs11614913	rs3746444
rs2292832	-	0.06	0.15	0.08	-	0.003	0.005	0.004
rs2910164	-	-	0.01	0.03	-	-	<0.001	<0.001
rs11614913	-	-	-	0.07	-	-	-	0.002

a D′ represents a relative measure of disequilibrium (D) compared to its maximum
b r represents r^2 (the square of the correlation coefficient between two indicator variables)

patients with endometriosis, whereas the expression level of miR-145 was significantly up-regulated in stage 1 or 2 [7]. The role of different miRNAs including miR-449b [8], miR-205, miR-183, miR-503, miR-152, and miR-92a [18–22] as novel diagnostic biomarker and therapeutic target for endometriosis treatment has been indicated in different studies. Zhang et al. evaluated the expression of miR-146b and variants on endometriosis and its associated pain symptom and reported higher rates of miR-146b expression in endometriosis compared with controls and indicated the strong association of miR-146b rs1536309 with the risk of pain [23]. Sepahi and colleagues studied 157 endometriosis patients and 252 healthy women, showing that the frequency of miR-126 rs4636297 significantly differed between the two groups, the authors speculating that this miRNA plays an important role in individual's susceptibility to endometriosis and its severity [24]. In addition, Chang et al. indicated that genetic variants in miR-196a2 (rs11614913) was strongly associated with endometriosis and related clinical phenotypes, such as infertility [25].

miRNAs have also been studied in other diseases of the female reproductive system. miR-499 rs3746444 is reported to have a potential function in reducing the risk of endometrial cancer [26]. However, no significant relationship was demonstrated between the SNPs of miR-146a and miR-196a-2 and ovarian cancer although the limited number of cases and controls in this study might have influenced the results [27]. Alipour et al investigated miR-146a C > G, miR-149 T > C, miR-196a2 T > C, and miR-499 A > G in 120 women with a history of two or more unexplained miscarriages and 90 matched healthy women, showing a link between the higher rate of miR-149 and miR-499 and pregnancy loss (RPL) and concluded that the presence of both SNPs were the genetic determinant for the risk of idiopathic RPL [28]. A link between miRNA-196a2, miRNA-499, miR-146a, and miR-149 polymorphisms to preeclampsia has also been investigated [29,30].

The results of our study demonstrate SNPs of different miRNAs miR-146a rs2910164, miR-149 rs2292832, and miR-499 rs3746444 to be linked to endometriosis. This work therefore represents an advance in biomedical science because it shows a link between endometriosis and miRNAs variants.

Summary table

What is known about this subject

- The importance of certain miRNAs in endometriosis.
- The significant association of four different miRNAs variants including, miR-146a rs2910164, miR-149 rs2292832, miR-196a2 rs11614913, and miR-499 rs3746444 with various female reproductive disorders has been shown.

What this paper adds

- Our study showed a significant association of rs2910164, rs2292832, and rs3746444 with increased rates of endometriosis.
- The rs2910164 and rs2292832 variants indicated a significant link with severity of endometriosis.
- The miR-196a2 rs11614913 did not indicate any association with endometriosis.

Disclosure statement

The authors declare no conflict of interest.

ORCID

M Farsimadan http://orcid.org/0000-0001-5917-7845
H Vaziri http://orcid.org/0000-0001-6325-8349

References

[1] Farsimadan M, Motamedifar M. Bacterial infection of the male reproductive system causing infertility. J Reprod Immunol. 2020;103183. DOI:10.1016/j.jri.2020.103183

[2] Farsimadan M, Motamedifar M. The effects of human immunodeficiency virus, human papillomavirus, herpes simplex virus-1 and −2, human herpesvirus-6 and −8, cytomegalovirus, and hepatitis B and C virus on female fertility and pregnancy. Br J Biomed Sci. 2021;78:1–11.

[3] Siahpoosh Z, et al. KISS1R polymorphism rs587777844 (Tyr313His) is linked to female infertility. Br J Biomed Sci. 2021;78:98–100.

[4] Zhang M, et al. Downregulated circular RNA hsa_circ_0067301 regulates epithelial-mesenchymal transition in endometriosis via the miR-141/Notch signaling pathway. Biochem Biophys Res Commun. 2019;514:71–77.

[5] Wang F, et al. Serum miR-17, IL-4, and IL-6 levels for diagnosis of endometriosis. Med (Baltimore). 2018;97.

[6] Jia S-Z, et al. Plasma miR-17-5p, miR-20a and miR-22 are down-regulated in women with endometriosis. Hum Reprod. 2013;28:322–330.

[7] Bashti O, et al. miR-31 and miR-145 as potential non-invasive regulatory biomarkers in patients with endometriosis. Cell J. 2018;20:84.

[8] Liu Y, Chen J, Zhu X, et al. Role of miR-449b-3p in endometriosis via effects on endometrial stromal cell proliferation and angiogenesis. Mol Med Rep. 2018;18:3359–3365.

[9] Petracco R, et al. Evaluation of miR-135a/b expression in endometriosis lesions. Biomed Rep. 2019;11:181–187.

[10] Sørensen AE, Udesen PB, Wissing ML, et al. MicroRNAs related to androgen metabolism and polycystic ovary syndrome. Chem Biol Interact. 2016;259:8–16.

[11] Ohlsson Teague EMC, Van der Hoek KH, Van der Hoek MB, et al. MicroRNA-regulated pathways associated with endometriosis. Mol Endocrinol. 2009;23:265–275.

[12] Króliczewski J, Sobolewska A, Lejnowski D, et al. microRNA single polynucleotide polymorphism influences on microRNA biogenesis and mRNA target specificity. Gene. 2018;640:66–72.

[13] Haqiqi H, Farsimadan M, Abiri A, et al. Association of FSHR missense mutations with female infertility, in silico investigation of their molecular significance and exploration of possible treatments using virtual screening and molecular dynamics. Anal Biochem. 2019;586:113433.

[14] Farsimadan M, et al. Association analysis of KISS1 polymorphisms and haplotypes with polycystic ovary syndrome. Br J Biomed Sci. 2021;78:In press.

[15] Jodeiryzaer S, et al. Association of oestrogen receptor alpha gene SNPs Arg157Ter C> T and Val364Glu T> A with female infertility. Br J Biomed Sci. 2020;1–3.

[16] Canis M, et al. Revised american society for reproductive medicine classification of endometriosis: 1996. Fertil Steril. 1997;67:817–821.

[17] Cosar E, Mamillapalli R, Ersoy GS, et al. Serum microRNAs as diagnostic markers of endometriosis: a comprehensive array-based analysis. Fertil Steril. 2016;106:402–409.

[18] Zhou C-F, Liu M-J, Wang W, et al. miR-205-5p inhibits human endometriosis progression by targeting ANGPT2 in endometrial stromal cells. Stem Cell Res Ther. 2019;10:287.

[19] Shi X-Y, GU L, CHEN J, et al. Downregulation of miR-183 inhibits apoptosis and enhances the invasive potential of endometrial stromal cells in endometriosis. Int J Mol Med. 2014;33:59–67.

[20] Hirakawa T, Nasu K, Abe W, et al. miR-503, a microRNA epigenetically repressed in endometriosis, induces apoptosis and cell-cycle arrest and inhibits cell proliferation, angiogenesis, and contractility of human ovarian endometriotic stromal cells. Hum Reprod. 2016;31:2587–2597.

[21] Tsuruta T, Kozaki K-I, Uesugi A, et al. miR-152 is a tumor suppressor microRNA that is silenced by DNA hypermethylation in endometrial cancer. Cancer Res. 2011;71:6450–6462.

[22] Li M, et al. miR-92a promotes progesterone resistance in endometriosis through PTEN/AKT pathway. Life Sci. 2020;242:117190.

[23] Zhang Z, Li H, Zhao Z, et al. miR-146b level and variants is associated with endometriosis related macrophages phenotype and plays a pivotal role in the endometriotic pain symptom. Taiwanese J Obstetrics Gynecol. 2019;58:401–408.

[24] Sepahi N, Kohan L, Jahromi AR, et al. mir-126 rs4636297 and TGF β RI rs334348 functional gene variants are associated with susceptibility to endometriosis and its severity. Gynecological Endocrinol. 2017;33:429–432.

[25] Chang C-Y-Y, Lai M-T, Chen Y, et al. Up-regulation of ribosome biogenesis by MIR196A2 genetic variation promotes endometriosis development and progression. Oncotarget. 2016;7:76713.

[26] Liu X, Xu B, Li S, et al. Association of SNPs in miR-146a, miR-196a2, and miR-499 with the risk of endometrial/ovarian cancer. Acta Biochim Biophys Sin. 2015;47:564–566.

[27] Lukács J, Soltész B, Penyige A, et al. Identification of miR-146a and miR-196a-2 single nucleotide polymorphisms at patients with high-grade serous ovarian cancer. J Biotechnol. 2019;297:54–57.

[28] Alipour M, Abtin M, Hosseinzadeh A, et al. Association between miR-146a C > G, miR-149 T > C, miR-196a2 T > C, and miR-499 A > G polymorphisms and susceptibility to idiopathic recurrent pregnancy loss. J Assist Reprod Genet. 2019;36:2237–2244.

[29] Salimi S, Eskandari F, Rezaei M, et al. The effect of miR-146a rs2910164 and miR-149 rs2292832 polymorphisms on preeclampsia susceptibility. Mol Biol Rep. 2019;46:4529–4536.

[30] Asadi-Tarani M, Saravani M, Teimoori B, et al. The relationships between maternal and placental polymorphisms of miR-196a2 and miRNA-499 genes and preeclampsia. Br J Biomed Sci. 2020;77:191–195.