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The relationships between maternal and placental polymorphisms of miR-196a2 and miRNA-499 genes and preeclampsia

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ABSTRACT

Background: miRNAs are small non-coding RNAs with potential roles in the complications of pregnancy. We hypothesised links between polymorphisms in miRNA-196a2 and miRNA-499 in maternal blood and the placentas of patients with preeclampsia.

Methods: The blood of 315 women with preeclampsia and 317 controls and the placentas of 103 PE and 133 healthy women were collected. The genotyping of both polymorphisms was performed by PCR-RFLP.

Results: The maternal blood rs11614913 was unrelated to preeclampsia in genotype and allele models, but in placental tissue, the CT (odds ratio [95% CI] 0.5 [0.3–0.9, p = 0.018) and TT (0.4 [0.2–0.9] p = 0.033) genotypes alone and together (CT+TT v CC 0.5 [0.3–0.8] p = 0.009), and the T allele (0.6 [0.4–0.9], p = 0.019) were associated with lower risk of preeclampsia. The maternal blood rs3746444 CC genotype was more frequent in preeclampsia (2.2 [1.2–3.8] p = 0.008) and the recessive model (CC v TC+TT) was also significant (1.9 [1.1–3.3], p = 0.018), as was the C allele (1.4 [1.1–1.7] p = 0.014). In placental tissue, the increase in the frequency of the CC genotype was marginally significant (2.4 [1.0–5.8] p = 0.046).

The maternal or placental miRNA-196a2 rs11614913 and miRNA-499 rs3746444 polymorphisms were unrelated to the severity of preeclampsia.

Conclusion: The placental but not maternal miRNA-196a2 rs11614913 variant could be a protective factor for preeclampsia predisposition in all models except the recessive model. The maternal/placental rs3746444 CC genotype was in association with higher preeclampsia risk.

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Introduction

Preeclampsia is a serious disorder of gestation that affects ~ 2–5% of all pregnancies and is an important cause of maternal and perinatal morbidity and mortality [1]. Although several pathophysiologic mechanisms are involved in preeclampsia, the precise underlying reason for its onset are unclear but may include abnormal spiral artery remodelling, endothelial dysfunction, oxidative stress, immune-system alterations, and systemic inflammation [2]. In a healthy pregnancy, the syncytotrophoblasts invade and remodel the spiral arteries in the myometrium and lead to an important increase in placental blood flow. In preeclampsia, these processes fail and placentation is disturbed [3]. The insufficient blood flow in the preeclamptic placenta affects the placental oxygenation and results in hypoxia and hyperoxia events which activate oxidative stress, inflammation and necrosis [4,5]. Aberrant expression of different genes in preeclampsia placentas have been shown, and epigenetics is a potential mechanism in the regulation of gene expression [5].

Micro RNAs (miRNAs) are a group of single-stranded non-coding RNAs that affect gene expression by mRNA degradation or translational inhibition of mRNA [6]. Evidence suggests roles for miRNAs in biological processes, including angiogenesis, apoptosis, growth, proliferation and differentiation [7]. Numerous miRNAs have been identified in placentas at different stages of gestation, and have different expression profile in preeclamptic and normal placentas [8,9]. Therefore, altered miRNA levels may result in dysregulated expression of their target genes, abnormal function of the placenta and subsequent complications such as preeclampsia [8].

MiRNA-196 (miR-196) is linked with various disorders such as cancer, arthritis and cardio-cerebrovascular disease. Evidence of the oncogenic properties of the miR-196 family includes stimulating cell proliferation, migration and invasion [10]. Since placental growth shows similar features with cancer progression including rapid expansion, invasive growth, and active angiogenesis, miR-196 may be a candidate marker in pregnancy complication [11].

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MiRNA-499(miR-499) has potential roles as an inflammation suppressor by targeting genes involving in inflammatory responses and that may play a regulatory role in hypoxic-ischaemic conditions [12,13]. The relation between genetic variants in miRNAs and numerous diseases has been established, including effects on pregnancy complications such as preeclampsia [14–16].

We hypothesised links between maternal and placental rs11614913 and rs3746444 polymorphisms in miR-196a2 and miR-499 with preeclampsia.

Material and methods

The protocol of the study was approved by the Research Ethics Committee of Zahedan University of Medical Sciences, and participants were recruited from the Ali-ibn-AbiTaleb hospital. The maternal blood of 315 patients and 317 healthy women and the placentas of 103 preeclampsia and 133 controls were collected. Both groups were matched for age. The inclusion and exclusion criteria for the study participants have been presented in our previous study [17]. The blood and samples of placental were collected after delivery. After washing with phosphate buffered saline (PBS), the placenta tissues were stored at – 80 °C for future use.

The DNA was separated from whole blood using the salting out method. A DNA extraction kit (DynaBio, Takapoozist, Tehran, Iran) was used to extract the DNA from the placenta. The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) was used for genotyping. The primers sequences and details of PCR-RFLP are presented in Table 1 [18,19]. The PCR reaction contained 150 ng/µl genomic DNA and 10 mM of each forward and reverse primers in a final 20 µl PCR reaction volume for both polymorphisms. The reactions of restriction digestion were done with 10 U of the restriction enzyme (Fermentas, Waltham, Mass, USA) was incubated at 37 °C overnight. Digested fragments were electrophoresed on a 2.5% agarose gel containing 3 µl safe stain and observed under a UV light.

Statistical analysis was performed using SPSS v23. The demographic parameters were compared with Student's t-test and Fisher's exact test. Links between the SNPs and preeclampsia are presented as odds ratio (OR) and 95% confidence interval (95% CI) by logistic regression. Allele frequencies were compared by Fisher's exact test. $P < 0.05$ was considered significant.

Results

Clinical and demographic data on cases and controls are shown in Table 2. The women were matched for age. As expected, cases were more likely to be having their first child, and with a higher BMI and blood pressure, and delivering an infant sooner and of less weight. The maternal blood rs11614913 was unrelated to preeclampsia in genotype and allele models (Table 3), but in placental tissue, the CT and TT genotypes alone and together (CT+TT v CC), and the T allele were associated with lower risk of preeclampsia (Table 4). The maternal blood rs3746444 CC genotype was more frequent in preeclampsia and the recessive model (CC v TC+TT) was also significant, as was the C allele (Table 3). In placental tissue, the increase in the frequency of the CC genotype was marginally significant (Table 4).

Discussion

We hypothesised links between variants of two miRNAs and preeclampsia, finding a relation between the placental miRNA196a2 rs11614913 CT and TT genotypes (alone and in combination), and the T allele, and a lower likelihood of preeclampsia. However, there were no such links in maternal samples. Both maternal and placental miR-499 rs3746444 CC genotypes were associated with preeclampsia. The aetiology of preeclampsia is complex and develops over months [1–4], and accordingly we cannot, with our simple

Table 2. Demographic and clinical characteristics of PE patients and controls.

Variable	Controls n = 317	Preeclampsia n = 315	P-value
Maternal age (years)	27.2 ± 6.6	27.9 ± 7.3	0.21
BMI (kg/m^2)	26.7 ± 3.3	27.1 ± 4.1	0.03
Gestation age (days)	268 ± 17	253 ± 27	<0.0001
Birth weight (Kg)	3.1 ± 0.4	2.90 ± 0.5	<0.0001
SBP (mmHg)	116 ± 9.4	154 ± 24.9	<0.0001
DBP (mmHg)	71.5 ± 11.5	98 ± 15	<0.0001
Primiparity, n(%)	88 (27.8)	136 (43.2)	<0.001

Data mean [SD] or n (%).

Table 1. The primer sequences, annealing temperatures and PCR products for analysis of rs11614913 and rs3746444 polymorphisms.

polymorphism	Primer Sequence 5′ → 3′	PCR Product (bp)	Tm °C	RE[a]	Fragments sizes (bp)
miRNA-196a2 (rs11614913)	Forward: CCCCTTCCCTTCTCCTCCAGATA Reverse: CGAAAACCGACTGATGTAACTCCG	149	57	MspI	CC: 125 + 24 CT: 149 + 125 + 24 TT: 149
miRNA-499 (rs3746444)	Forward: CAAAGTCTTCACTTCCCTGCCA Reverse: GATGTTTAACTCCTCTCCACGTGATC	146	57	*BclI*	CC: 146 CT: 146, 120, 26 TT: 120, 26

[a]RE, Restriction Enzyme

Table 3. The alleles and genotypes frequency of miR-196a2 rs11614913 and miR-499 rs3746444 polymorphisms in maternal blood of preeclampsia women and controls.

	Control n = 317	Preeclampsia n = 315	OR (95% CI)	P-value
miR-196a2 rs11614913				
Genotype				
CC	96 (30.3)	114 (36.2)	1 (reference)	-
CT	166 (52.4)	159(50.5)	0.8 (0.6–1.1)	0.226
TT	55 (17.4)	42 (13.3)	0.6 (0.4–1.04)	0.074
Dominant (CT+TT vs. CC)			0.8 (0.6–1.1)	0.115
Recessive (TT vs. CT + CC)			0.7 (0.5–1.1)	0.162
Allele				
C	358 (56.5)	387 (61.4)	1 (reference)	-
T	276 (43.5)	243 (38.6)	0.8 (0.7–1.0)	0.073
miR-499 rs3746444				
TT	146 (46.1)	121 (38.4)	1 (reference)	-
TC	148 (46.7)	153 (48.6)	1.3 (0.9–1.7)	0.190
CC	23 (7.3)	41 [13]	2.2 (1.2–3.8)	0.008
Dominant (TC+CC vs. TT)			1.4 (1–1.8)	0.052
Recessive (CC vs. TC+TT)			1.9 (1.1–3.3)	0.018
Allele				
T	440 (69.4)	395 (62.7)	1 (reference)	-
C	194 (30.6)	235 (37.3)	1.4 (1.1–1.7)	0.014

OR, odds ratio; CI, confidence interval. Data are n (%).

Table 4. The alleles and genotypes frequency of placental miR-196a2 rs11614913 and miR-499 rs3746444 polymorphisms in PE women and controls.

	Control n = 133	Preeclampsia n = 103	OR (95% CI)	P-value
miR-196a2 rs11614913				
Genotype				
CC	35 (26.3)	44 (42.7)	1 (reference)	-
CT	76 (57.1)	48 (46.6)	0.5 (0.3–0.9)	0.018
TT	22 (16.5)	11 (10.7)	0.4 (0.2–0.9)	0.033
Dominant (CT+TT vs. CC)			0.5 (0.3–0.8)	0.009
Recessive (TT vs. CT + CC)			0.6 (0.3–1.3)	0.201
Allele				
C	146 (55)	136 (66)	1 (reference)	-
T	120 (45)	70 (34)	0.6 (0.4–0.9)	0.019
miR-499 rs3746444				
TT	57 (42.9)	34(33)	1 (reference)	-
TC	65 (48.9)	53 (51.5)	1.4 (0.8–2.4)	0.273
CC	11 (8.38)	16 (15.5)	2.4 (1–5.8)	0.046
Dominant (TC+CC vs. TT)			1.5 (0.9–2.6)	0.124
Recessive (CC vs. TC+TT)			2.0 (0.9–4.6)	0.087
Allel				
T	179 (67.3)	121 (58.7)	1 (reference)	-
C	87 (32.7)	85 (41.3)	1.5 (1–2.1)	0.068

OR, odds ratio; CI, confidence interval. Data are n (%).

observational design, state whether the links with the miRNAs we report are causative or bring susceptibility, which can only be determined by following pregnancies from their earliest stages to determine true risk.

miRNAs are a group of non-coding RNAs with numerous potential roles in regulation of cellular growth, proliferation, invasion, apoptosis, autophagy, stress responses, death and tumorigenesis. Therefore, the abnormal expression or function of miRNAs is believed to contribute to conditions such as cancer [18–20], inflammatory and autoimmune disease, heart disease [6] and the complications of pregnancy [8,14–16]. Preeclampsia is a disorder of pregnancy with unknown aetiology but probably related to differences in maternal and foetal genetics [1–4]. With numerous potential roles in the regulation of trophoblast invasion and immune activation in the placentas, miRNAs may be involved in physiological and developmental processes in the normal pregnancy [21–23]. In a small study of 27 women with preeclampsia and/or small for gestational age infants, controlled by 9 women with spontaneous pre-term labour and delivery, Pineles et al found potential roles for placental miR-210 and miR-182 [24]. Demirer et al [25] reported a potential role for leukocytes miR-518b in 96 cases of early and late preeclampsia compared to 52 controls, whilst Lip et al [26] speculated pathophysiological links between plasma miR-574-5p and miR-1972 and preeclampsia that involve the tube-formation capacity of endothelial cells. Huang et al. [27] determined levels of hsa-miR-181a-5P in 20 preeclamptic placentas and 20 normal placentas, hypothesising raised expression of the miRNA and in vitro transfections to be directly related to trophoblast dysfunction. Similarly, Yang and Meng [28] also translated their data on raised miR-431 from 30 preeclamptic

placentas versus 30 normal placentas, alongside transfection data to conclude that the miRNA might give rise to preeclampsia by inhibiting the migration and invasion of trophoblastic cells.

The studies describe above have reported miRNAs on maternal blood or placental material, not both. In this respect, the well-powered study of Salimi et al. [14] has strength in that it compares blood and placental tissues, finding links between a variant of maternal and placental miR-146a genotypes in preeclampsia versus controls. Our data extend these findings, pointing to differences in maternal versus placental material. The question therefore arises as to which of these sources is better at detecting preeclampsia, and the potential input of paternal genetics into the aetiology of the preeclamptic placenta.

miR-499 is involved in inflammation suppression and targets genes which ameliorate the inflammatory damage to endothelial cells [12] but whether this is relevant in preeclampsia is unknown. Mohseni et al. [29] showed higher concurrent expression of miR-499-5p women with both preeclampsia and cardiac remodelling, suggesting a possible role in myocyte biology, whilst Alipour et al. [30] reported that miR-499, but not miR-196, is linked to recurrent pregnancy loss, some of which may have been due to preeclampsia. Jeon et al. [31,32] found that both miR-196a2 CC and miR-499 AG+GG (and their combination) are associated with idiopathic recurrent spontaneous abortions and foetuses. Although our results support these data, we recognised the limitation of relatively small numbers and that we cannot offer data on the levels of the miRNAs.

Our study represents an advance in biomedical science because it show links between placental but not maternal miR-196a2 rs11614913, and maternal miR-499 rs3746444 polymorphism and preeclampsia.

Summary Table

What is known about this subject:

- miRNAs are short non-coding RNAs with probable roles in different processes.
- Genetic variations in miRNAs may influence their biogenesis, their stability, and efficiency in regulation of target genes.
- There are links between altered miRNAs expression and preeclampsia.

What this paper adds:

- The placental but not maternal miRNA-196a2 rs11614913 polymorphism is associated with a lower risk of PE.
- The maternal and placental r miRNA-499 rs3746444 polymorphism is associated with higher risk of PE.
- miRNA-196a2 rs11614913 and miRNA-499 rs3746444 polymorphisms showed no effect on PE severity.

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Disclosure statement

The authors declare no conflicts of interest.

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