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ARTICLE

The effect of the placental *DROSHA* rs10719 and rs6877842 polymorphisms on PE susceptibility and mRNA expression

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Abstract

Evidence showed that microRNA biosynthesis plays the main role in pathogenesis of several diseases including Preeclampsia (PE). Therefore, microRNA processing enzymes may involve in PE predisposition. The aim of the present study was to evaluate the relation between *DROSHA* rs10719 and rs6877842 polymorphisms and mRNA expression in the placenta of PE women and controls. This study recruited 110 PE women and 115 age matched normotensive pregnant women for genotyping of *DROSHA* polymorphisms and analyzing of mRNA expression. There was no association between alleles and genotypes of placental *DROSHA* rs10719 and rs6877842 polymorphisms and PE susceptibility. However, placental *DROSHA* rs10719 was associated with increased PE risk in the recessive model. The combination of CC/GG genotypes of *DROSHA* rs10719 and rs6877842 polymorphisms was associated with higher risk of PE. The frequency of C-G haplotype was higher in PE women, but the difference was not significant. The *DROSHA* mRNA expression was downregulated in the placenta of PE women. There was no relation between *DROSHA* mRNA expression and rs6877842 polymorphism, however, it was decreased in the placenta of women with rs10719CC genotype. The placental *DROSHA* rs10719 but not rs6877842 polymorphism could be a risk factor for PE susceptibility only in the recessive model. The combination of CC/GG genotypes could be risk factors for PE susceptibility. The *DROSHA* expression downregulated in the preeclamptic placentas and those carrying rs10719CC genotype.

Introduction

Preeclampsia (PE), described as a heterogeneous pregnancy-related disorder, is identified by maternal hypertension after 20 weeks of gestation and is associated with maternal multi-organ dysfunction [1]. The exact mechanism involves in PE pathogenesis is not completely understood, however, the insufficient trophoblast cell invasion and dysfunction of uterine spiral artery remodeling are leading pathological characteristics of PE, which may be the result of placental ischemia and oxidative stress [2]. Today, it is well known that the placenta certainly contributes to the pathogenesis of PE, as its removal eliminates the clinical presentations of this disorder; however, PE is considered to be a result of interactions between maternal and fetal genetic factors as well as environmental factors [2, 3]. Therefore, numerous studies have been performed on the association between variations in maternal or placental genes involving in various pathways in PE pathogenesis [4, 5]. Recently miRNAs (MicroRNAs) are investigated in association with pathogenesis of various diseases [6]. The

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placenta is a pregnancy-specific organ with a major miRNA expression and the altered of differentially expressed miRNAs have been observed in placentas complicated with PE [7]. The role of these miRNAs in the regulation of trophoblast cell invasion and placental immune activation has been established, therefore, miRNAs play significant physiological and developmental roles in the maintenance of pregnancy [8].

MicroRNAs (miRNAs; ~22 nucleotides) are defined as small non-coding RNAs, which contribute to the regulation of gene expression by the post-transcriptional repression. Overall, miRNA attaches to a region at 3′-Untranslated Region (UTR) of mRNA that downregulates its expression, therefore the miRNAs regulate gene expression [9].

According to important role of miRNAs in normal pregnancy and abnormal levels of miRNAs in various pregnancy-specific conditions such as PE, the miRNA biosynthesis machinery may play key role in these complications [7]. The miRNA biosynthesis, which consists of different miRNA biogenesis enzymes, takes place in several steps. In the nucleus, RNA polymerase II generates large primary miRNA transcripts (500–3000 nucleotides), which are processed by a protein complex that contains Drosha to create precursor miRNA (pre-miRNA) hairpins. Then RanGTPase (Ran) and exportin 5 (Xpo5) transfer pre-miRNAs to the cytoplasm, and a and a polymerase II enzyme, Dicer1 converts it to miRNA. After unwinding the double-stranded miRNA duplex, a mature, single-stranded miRNA (18–24 nucleotides) is formed [6].

Drosha is a member of the ribonuclease III super-family plays key role in miRNA processing. Several variants have been introduced in *DROSHA* gene, which their association with various diseases have been investigated [10]. Regarding to the crucial role of miRNAs in the development of placenta and the function of Drosha in miRNAs biogenesis, therefore, in this study for the first time we evaluated possible effects of placental *DROSHA* polymorphisms and *DROSHA* gene expression on PE susceptibility.

Materials and methods

This study was approved by the ethics committee of the Zahedan University of Medical Sciences and carried out according to the Declaration of Helsinki (2013) by the World Medical Association. PE was described as blood pressure ≥ 140/90, as well as proteinuria ≥ 0.3 g/24 h (or dipstick result ≥ 1+), after 20 weeks of gestation. Placental tissues of 110 PE patients (mean aged 29.1 ± 7.4 years) and 115 normotensive women (mean aged of 27.7 ± 6.5 years old) accumulated after delivery and prepared as previously described [4].

DNA extraction kit (DynaBio, Takapoozist, Iran) was used to extract DNA from all subjects. Genotyping of DROSHA rs10719 and rs6877842 gene polymorphisms was performed by polymerase chain reaction fragment length polymorphism (PCR-RFLP) method as previously described [11].

Total RNA extraction was performed using RNX-Plus (Sinaclon, Iran). The quality of extracted RNA checked via agarose gel electrophoresis and its concentration determined by the Biowave II, Biochrom WPA, Cambridge. The first-strand cDNA synthesis kit (Takara Bio, Shiga, Japan) was applied to synthesize cDNA. The qRT-PCR (quantitative real-time PCR) method was applied to determine mRNA expression of the target and housekeeping genes (*DROSHA* and β-*ACTIN* respectively). The primers used for mRNA expression have been reported previously by Chong et al. [12]. Amplification was performed in total volume of 20 µl, including 10 µLSYBR Green/High ROX (Amplicon, Denmark), 2µl of the cDNA solution, 10pmol of each primer and 6µl dH_2O. The $2^{-\Delta\Delta Ct}$ statistical method was used to determine relative Gene expression.

For statistical analysis, SPSS version 21 (SPSS Inc., Chicago, IL) was used. Fisher exact test and Student's *t*-test were applied for comparison of clinical and demographic characteristics of two groups whenever appropriate. The odds ratio (OR) and 95% confidence interval (95% CI) were measured to determine relationships between PE and alleles or genotypes, based on the logistic regression analysis. Haplotypes analyses of two SNPs were conducted using SHEsis software [13]. The Bonferroni correction was performed to adjust the P-values for multiple comparisons. $P < 0.05$ was statistically significant.

Significant differences were determined via two-way ANOVA and *t*-tests with Bonferroni-Holm correction

Results

The characteristics of the study participants are shown in Table 1. The maternal age and BMI were not statistically different between two groups. Gestational age and birth weight of neonatal were lower in the PE group. The primiparity was associated with PE.

The frequency of *DROSHA* rs10719 TC genotype was not different between two groups. The frequency of rs10719CC genotype was higher in PE women (19.1 vs 9.6%), but the differences was not significant ($P = 0.07$). The *DROSHA* rs10719 was associated with PE risk in recessive model ($P = 0.04$, OR = 2.2, 95% CI = 1–4.9), however, there was no association between this polymorphism and PE in dominant model. There was no association between *DROSHA* rs6877842 polymorphism and PE risk neither in recessive nor in dominant and allelic models (Table 2).

Table 1 Demographic characteristics of PE women and controls

Variable	Controls ($n = 115$)	PE ($n = 110$)	*P*-value
Maternal age(mean ± SD, years)	27.7 ± 6.5	29.1 ± 7.4	NS
Gestation age(mean ± SD, days)	270 ± 12	255 ± 23	<0.0001
Birth weight (mean ± SD, g)	3088 ± 261	2871 ± 340	<0.0001
SBP(mean ± SD, mmHg)	116 ± 9.4	151 ± 24.8	<0.0001
DBP(mean ± SD, mmHg)	71.4 ± 11.5	95.7 ± 13.5	<0.0001
BMI (kg/m^2)	26.8 ± 2.6	27.6 ± 4.1	NS
Primiparity, *n* (%)	35(30)	51 (46)	0.02

NS not significant, *SD* standard deviation

Table 2 Allelic and genotypic frequency of placental *Drosha rs10719 and rs6877842* polymorphisms in PE women and control group

	PE ($N = 110$)	control ($N = 115$)	*P*-value	OR (95% CI)
Drosha rs10719				
TT, *n* (%)	49 (44.5)	55 (47.8)		1
TC, *n* (%)	40 (36.4)	49 (42.6)	0.8	0.9 (0.5–1.6)
CC, *n* (%)	21 (19.1)	11 (9.6)	0.07	2 (0.9–4.9)
Dominant (TC + CC vs TT)			0.6	1.1 (0.7–1.9)
Recessive (CC vs TC + TT)			0.04	2.2 (1–4.9)
Allele				
T, *n* (%)	138 (63)	159 (69)		1
C, *n* (%)	82 (37)	71 (31)	0.16	1.3 (0.9–2)
Drosha rs6877842				
GG, *n* (%)	94 (85.5)	93 (80.9)		1
GC, *n* (%)	15 (13.6)	19 (16.5)	0.5	0.8 (0.4–1.6)
CC, *n* (%)	1 (0.9)	3 (2.6)	0.3	0.3 (0.03–3.2)
Dominant (GC + CC vs GG)		0.4	0.7 (0.4–1.5)	
Recessive (CC vs GC + GG)		0.4	0.3 (0.04–3.3)	
Allele				
G, *n* (%)	203 (92)	205 (89)		1
C, *n* (%)	17 (8)	25 (11)	0.3	0.7 (0.4–1.3)

Table 3 indicated the effect of combined genotypes of *DROSHA* rs10719 and rs6877842 polymorphisms on PE susceptibility. The CC/GG combined genotype was more frequent in PE women compared to control women (19.1% vs 7%) and was associated with 2.8-fold increased risk of PE ($P = 0.03$, OR = 2.8, 95% CI = 1.1–7). Moreover, haplotype analysis showed that the C-G haplotype was more frequent in PE women, however, the difference was marginally non-significant [OR = 1.5, 95% CI: (1–2.2); $P = 0.06$, Table 4].

The relative mRNA expression of the *DROSHA* in the placentas of pregnant women with PE was lower than of that in controls (0.2-fold, Fig. 1). Therefore, the expression of this gene was five times higher in the placentas of normotensive pregnant women ($P < 0.0001$). The relative mRNA expression of *DROSHA* gene did not differ between rs10719 CC, TC and TT genotypes neither in PE nor in control group ($P = 0.5$ and $P = 0.1$ respectively, Fig. 2). However, the relative mRNA expression of *DROSHA* gene was significantly lower in the placentas of women with rs10719CC genotype compared to TT and CC genotypes in total studied women ($P = 0.009$ and $P = 0.017$, Fig. 2). There was no significant association between relative mRNA gene expression of *DROSHA* gene and different

Table 3 The combination effects of placental *DROSHArs6877842* and *rs10719* polymorphisms on PE risk

rs10719	*rs6877842*	PE ($N = 110$)	Control ($N = 115$)	*P*-value	OR (95% CI)
TT	GG	42 (38.2)	45 (39.1)		1
TT	GC	6 (5.5)	7 (6.1)	0.9	0.9 (0.3–3)
TT	CC	1 (0.9)	3 (2.6)	0.4	0.4 (0.04–3.6)
TC	GG	31 (28.2)	40 (34.8)	0.6	0.8 (0.4–1.6)
TC	GC	9 (8.2)	9 (7.8)	0.9	1.1 (0.4–3)
TC	CC	0 (0)	0 (0)	–	–
CC	GG	21 (19)	8 (7)	0.03	2.8 (1.1–7)
CC	GC	0 (0)	3 (2.6)	1	–
CC	CC	0 (0)	0 (0)	–	–

Table 4 Haplotypes frequency of placental *Drosha* rs10719 and rs6877842 polymorphisms in PE women and control group

Drosha rs10719	*Drosha rs6877842*	PE	Control	*P*-value	OR (95% CI)
T	G	121 (55)	140 (60.9)		1
T	C	17 (7.7)	18 (7.8)	0.86	1.1 (0.5–2.2)
C	G	82 (37.3)9	64 (27.8)	0.06	1.5 (1–2.2)
C	C	0 (0)	8 (3.5)	0.06	–

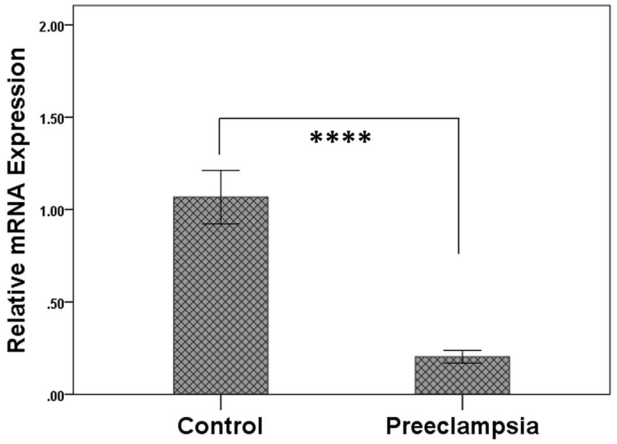

Fig. 1 The relative mRNA expression of the *DROSHA* gene in the Placenta of PE women and Control group. ****$P < 0.0001$

genotypes of rs6877842 in PE women, control group and total population (Fig. 3).

Discussion

MicroRNAs, as short and endogenous single-stranded non-coding RNAs, are known to control the expression of many genes at post-transcription level [6]. miRNAs bind to 3′-UTR of target mRNAs and affect gene expression epigenetically which leads to inhibition of protein translation or stimulation of transcript degradation. In general, miRNAs influence cell proliferation, growth, differentiation, angiogenesis, apoptosis, and inflammation mechanisms [14]. In addition, histone modifications and DNA methylation are regulated by miRNAs, with possible effects on miRNA expression [15]. It is belived that miRNAs can be potential biomarkers for different diseases, including, immunologic and inflammatory disorders, cardiovascular diseases, cancer and pregnancy-associated diseases [14, 16]. Evidence showed that alteration in miRNA expression during pregnancy is depend on different stages of placental development, therefore, regulation of miRNAs are related to gestational development and during the pregnancy, miRNAs exhibit stage-specific functions [17]. Various proteins and enzymes are involved in miRNA biogenesis including DROSHA, XPO5, and Dicer, which play key roles in conversion of the pri-miRNA into miRNA. Several investigations demonstrated the existence of these molecules in trophoblast cells, which showed the activation miRNA machinery synthesis in human placenta [10, 18]. Various genes with altered expression have been found in the transcriptome of PE-affected placentas in microarray and conventional approaches in comparison with healthy normotensive term deliveries. This finding shows that PE pathogenesis is associated with differential gene expression [3].

It is well known that the origin of PE is the placenta and PE symptoms diminishes after delivery or placentas removal [19]. Altered expression of several genes in the placenta of pregnancy complicated with PE has been described which may contribute in pathogenesis of this condition [20–22]. Amongst them the study about the miRNA biosynthesis machinery genes and PE is rare [23]. In the current study we found no association between placental *DROSHA* rs6877842 polymorphism and PE risk, however, *DROSHA* rs10719 polymorphism could increase the risk of PE in recessive model. Moreover, we observed higher frequency of CC/GG combined genotypes in PE women. The *DROSHA* mRNA expression was significantly decreased in the placentas complicated with PE. Moreover, the *DROSHA* mRNA expression was decreased in the placentas carrying rs10719CC genotype. There was no relation between *DROSHA* rs6877842 polymorphism and mRNA expression. To best of our knowledge this is the first report on the relation between placental *DROSHA* polymorphisms and expression in PE women.

In our previous study we observed the relation between maternal *DROSHA* rs10719(TC and CC genotypes) but not rs6877842 polymorphism and PE. In addition, consistent to findings of current study the maternal C-G haplotype and CC/GG combined genotype were associated with increased PE risk [11]. In the study of Eskandari et al., no association was found between maternal *DICER1* rs3742330 polymorphism as another miRNA biosynthesis machinery and PE or PE severity, but, the placental *DICER1* rs3742330 AG genotype was related to increased risk of PE and severe PE. No significant difference was observed regarding to the placental *DICER1* expression, but the *DICER1* expression was reduced in the placentas of the women carrying rs3742330 AG + GG genotypes [23].

Although, the studies about the association between polymorphisms/ expression of *DROSHA*, *DICER1*, and

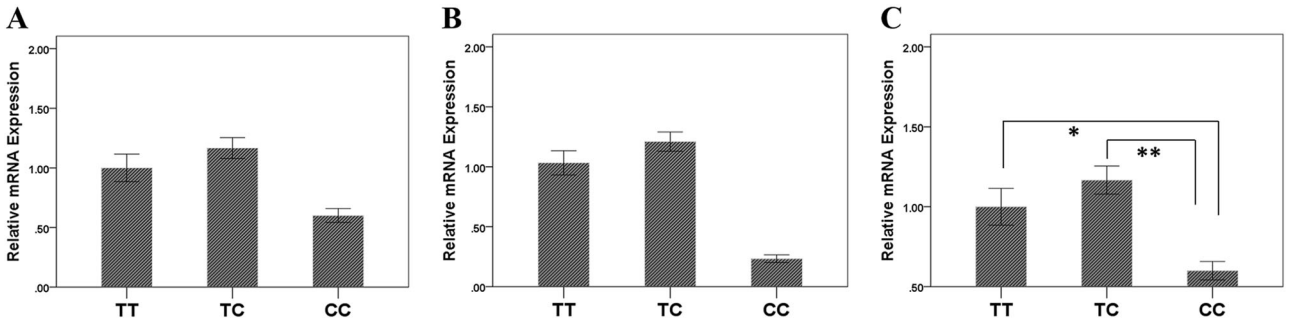

Fig. 2 The association of relative mRNA expression and placental *DROSHA* rs10719 polymorphism in **a** PE women, **b** normotensive pregnant women and **c** total pregnant women. $^{**}P < 0.01$, $^{*}P < 0.05$

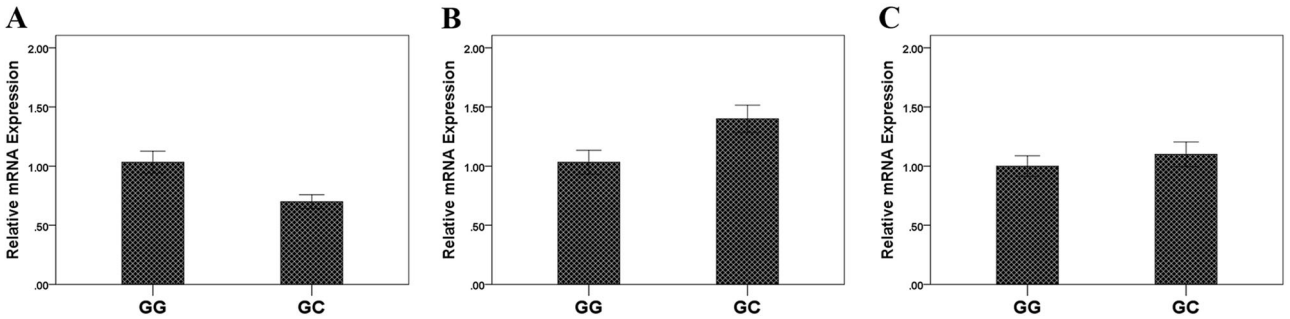

Fig. 3 The association of relative mRNA expression and placental *DROSHA* rs6877842 polymorphism in **a** PE women, **b** normotensive pregnant women and **c** total pregnant women

other miRNA biosynthesis machinery genes and PE are rare, there are some published reports about these molecules and other complications such as primary hypertension [24], idiopathic recurrent pregnancy loss [25], and recurrent spontaneous abortion [26]. In a study that performed on the relation between *DROSHA* rs10719, *DICER1* rs3742330, *RAN GTPase (RAN)* rs14035, and *exportin-5 (XPO5)* rs11077 by Jung et al. [25], there was the relation between *RAN* and the combination of DROSHA/DICER polymorphisms with idiopathic recurrent pregnancy loss.

Similarly, Fu et al. [26] revealed the combination effect of *DICER1*rs3742330 and DROSHA rs10719 variants on unexplained recurrent spontaneous abortion. The possible effect *DICER1* and *DROSHA* polymorphisms on azoospermia has been investigated by Moghbelinejad et al. [27] in an Iranian population and showed that *DICER1* rs1057035 polymorphism could affect idiopathic male infertility/ azoospermia. Among *DROSHA*, *DICER1*, *RAN* and *XPO5* polymorphisms, Rah et al. [28] found the association between *XPO5* rs2257082 T allele and primary ovarian insufficiency.

Regarding to *DROSHA* expression, Li et al. [29] indicated that the levels of *DROSHA* and *DICER1* expression were higher in placentas of neonates with macrosomia compared with normal controls. The findings of Rahimi et al. [30] showed the higher expression of *DROSHA*, *DICER1* and *DGCR8* genes in pregnant and gestational diabetes mellitus women compared to controls. The association of *DROSHA* rs10719 polymorphism and primary hypertension was described by Zhang et al. which could be due to disruption of the interaction between miR27b and *DROSHA* gene. Moreover, similar to finding of present study they showed that *DROSHA* expression was reduced in CC genotype compared to CT and TT genotypes [24].

Since, the investigations about the relation between polymorphisms of miRNA biosynthesis machinery genes and PE are rare, further studies on the placental and maternal variants are needed to confirm or refute our findings. In addition, experimental studies are necessary to assess the possible effects of *DROSHA* polymorphisms on *DROSHA* expression in different tissues. This work represents an advance in biomedical science because it shows that the placental *DROSHA* rs10719 polymorphism was associated with higher PE risk in the recessive model and the mRNA expression of *DROSHA* was significantly lower in the placentas of PE women and women with rs10719CC genotype.

Summary

What is known about this topic

- The placenta is a pregnancy specific organ with a major miRNA expression and the altered of differentially

expressed miRNAs have been observed in placentas complicated with PE.
- The role of these miRNAs in the regulation of trophoblast cell invasion, platelet aggregation and placental immune activation has been established.

What this study adds

- To best of our knowledge this is the first report on the relation between placental *DROSHA* polymorphisms and expression in PE women.
- The *DROSHA* mRNA expression was significantly decreased in the placentas complicated with PE.
- There was no relation between *DROSHA* rs6877842 polymorphism and mRNA expression.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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