Differential expression of *Hsa-miR-517a/b* in placental tissue may contribute to the pathogenesis of preeclampsia

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Abstract

Objective: Preeclampsia (PE) is a pregnancy hypertensive disorder that affects both maternal and fetal health. Many studies have investigated possible mechanisms in the pathogenesis of PE although the role of the placenta is undeniable. Evaluation of placental-specific microRNAs may provide additional data about the pathogenic mechanism of PE. This study compared the expression levels of *Hsa-miR-517a/b* in placental tissues obtained from PE patients and healthy controls.

Material and Methods: One hundred tissues were obtained from fetal and maternal sides of the placenta of PE patients and healthy controls. Expression analysis was performed using quantitative real-time polymerase chain reaction.

Results: *Hsa-miR-517a/b* level was significantly decreased in PE compared to controls (expression ratio: 0.40; p=0.007). Down-regulation of *Hsa-miR-517a/b* was also detected in fetal-side placental samples when compared to maternal-side in PE (expression ratio: 0.33; p=0.04). Furthermore, decreased expression of *Hsa-miR-517a/b* was detected in fetal-side tissue from PE cases compared to fetal-side samples from healthy pregnancies (expression ratio: 0.36; p=0.03). In maternal-side placental samples the expression level did not differ between PE and healthy pregnancies (p=0.1).

Conclusion: These results demonstrate a differential expression of *Hsa-miR-517a/b* within placentas in pregnancies affected by PE and between placentas from PE and healthy pregnancies. Further studies are required to investigate a possible role for *Hsa-miR-517a/b* in the pathogenesis of PE. (J Turk Ger Gynecol Assoc 2021; 22: 273-8)

Keywords: Preeclampsia, microRNAs, expression analysis, placenta, *Hsa-miR-517a/b*

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Introduction

Preeclampsia (PE) is one of the most frequent complications of pregnancy and is characterized by high blood pressure and proteinuria after 20 weeks of gestation. About 2-8% of all pregnancies are affected by PE, which increases morbidity and mortality of fetus and mother (1,2). Despite much research the cause of PE remains unclear. However, different possible mechanisms have been proposed, including abnormality in trophoblast invasion, inappropriate placental implantation, ischemia, endothelial dysfunction, and imbalance between pro-angiogenic and anti-angiogenic factors (3-5). Imbalance in the components of the angiogenesis pathway in placental tissue is reported to be involved in PE pathogenesis (3). Vascular

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endothelial growth factor (VEGF) binds to its receptors, such as FMS-like tyrosine kinase 1, which results in the initiation of the angiogenesis pathway (6). Alterations in the mRNA levels of this ligand and its receptor have been previously investigated in PE (7-9). MicroRNAs (miRNAs) may play an important role in the regulation of expression of the genes related to the angiogenesis pathway (10). By binding to the 3' untranslated region of mRNAs, miRNAs regulate gene expression at the post-transcriptional level (11). MiRNAs transcribed from the chromosome 19 microRNA cluster (C19MC) are suggested to have a specific expression in trophoblast cells, and also in term and preterm preeclamptic placental tissue (12,13). Dysregulation of these placental-specific miRNAs could result in pregnancy-associated disorders, including PE (14). The *Hsa-miR-517* family contains three isoforms, including *Hsa-miR-517a*, *Hsa-miR-517b* and *Hsa-miR-517c*, all of which are transcribed from C19MC cluster. Due to the very close sequence similarity between *Hsa-miR-517a* and *b*, these two isoforms have been merged and are known as *Hsa-miR517a/b* (15,16). Previous studies regarding the expression level of the *Hsa-miR-517* family in the preeclamptic placental tissue showed inconsistent findings (12,17-19). As a fetomaternal organ, the placenta has fetal and maternal sides, and it is suggested that the expression of miRNAs may be different on each side (20,21). This characteristic of placental tissue has not usually been considered in previous studies. The aim of this study was to compare the expression levels of *Hsa-miR-517a/b* between preeclamptic and normal placenta. Moreover, the differential expression of *Hsa-miR-517a/b* was assessed in both the fetal-side and maternal-side of the placenta between both PE cases and healthy controls.

Material and Methods

Samples were collected from an equal number of PE patients and women with normal pregnancies. Placental tissues were collected from both the fetal-side and maternal-side of the placenta, up to ten minutes after delivery. On the fetal-side small placental tissue pieces were obtained, after separating the embryonic membranes, from just below the membranes and to a depth of less than 0.5 cm. On the maternal side, small biopsies of placenta were cut out from the cotyledons, also to a depth of less than 0.5 cm. The maternal-side specimens were obtained from the center of the cotyledons as far as possible from the calcified areas. All samples were washed with normal saline solution to remove debris and blood. The tissue samples were kept in RNA later solution (Ambion, Austin, Texas, USA) and stored at -20 °C until RNA extraction.

Both groups were Iranian with a common ethnic-geographic origin and were age-matched. Inclusion and exclusion criteria were considered based on the criteria defined by the American College of Obstetricians and Gynecologists for the diagnosis of PE (22). The PE women had systolic blood pressure above 140 mmHg and diastolic blood pressure above 90 mmHg, along with new-onset proteinuria, and no prepregnancy history of hypertension. Subjects with a history of hypertension, renal disease, and/or preexisting proteinuria, were not included in the study. All subjects signed written informed consent, and the Ethics Committee of the Shahid Beheshti University of Medical Sciences approved the study protocol (approval number: IR.SBMU.MSP.REC.1399.25).

According to the origin of biopsy samples, the placental tissues grouped into maternal preeclamptic (MP), fetal preeclamptic (FP), maternal control (MC), and fetal control (FC) samples. Using a RiboEx total RNA solution (GeneAll, Korea), the placental RNA was extracted according to the manufacturer's protocol. The High-Capacity complementary (cDNA) Reverse Transcription Kit (ABI, Cat. 4368814) was used to synthesize cDNA from 4 µg of isolated RNA. The stem-loop primer was designed using sRNAPrimerdb online software (available from; http://www.srnaprimerdb.com). The nucleotide sequence for the designed stem-loop primer was: GTCGTATCCAGTGCAGGG TCCGAGGTATTCGCACTGGATACGACACACTC. The synthesized cDNAs were stored at -70 °C.

Quantitative real-time PCR (qRT-PCR) was carried out to assess *Hsa-miR-517a/b* expression levels in the placental tissues using SYBR Green I Master Mix PCR (BioFACT™, Cat. DQ383-40h, Daejeon, Korea) in an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA). Thermal cycling parameters were: denature at 94 °C for 15 minute, and subsequent 50 amplification cycles including, 94 °C for 5 sec and then 60 °C for 34 sec. To evaluate the specificity of the PCR products, melting curve analysis and 2% agarose gel electrophoresis were performed. The primers' sequences were as follows: AAGCACATCGTGCATCCCT as the forward primer and GTCGTATCCAGTGCAGGGT as the universal reverse primer. *SNORD48* was used as the housekeeping gene. Specific primers used for amplification of *SNORD48* were: AACAGAAGAAGTGATGATGACCCCAGGTA as the forward and AATAATAATGTCAGAGCGCTGCGGTGAT as the reverse primer.

Statistical analysis

LinRegPCR software, version, 2017.1 (Academic Medical Center, Amsterdam, Netherlands) was used to specify the efficiency and the cycle threshold values for each qRT-PCR reaction. REST 2009 software (Qiagen, Hilden, Germany) was used to compare the expression level of the *Hsa-miR-517a/b* gene between the PE patients and healthy subjects. The *Hsa-miR-517a/b* expression was also compared between FP, FC, MP, and MC samples. The experimental data were analysed using Mann-Whitney U and Kruskal-Wallis tests in GraphPad Prism

software version 8.0 (GraphPad, La Jolla, CA, USA). A $p<0.05$ considered statistically significant.

Results

A total of 100 placental samples were examined, 50 from PE patients and 50 from women with normal pregnancies. Table 1 shows the clinical characteristics of the PE and control groups. Patients in the PE group had significantly higher systolic and diastolic blood pressure, and their babies had lower fetal birth weight and were born earlier. No significant differences were observed regarding mean age, mean body mass index, family history of hypertension and pregnancy loss between patients and controls.

Hsa-MiR-517 a/b was down-regulated in preeclamptic tissues compared to the control samples (expression ratio: 0.40; $p=0.007$) (Figure 1). A significant reduction was observed in the expression of *Hsa-miR-517a/b* in FP tissues compared to FC tissues (expression ratio: 0.36; $p=0.03$) (Figure 2). *Hsa-miR-517a/b* was also down-regulated in FP tissues compared to MP tissues (expression ratio: 0.33; $p=0.04$) (Figure 2). The similar difference was found when comparing the *Hsa-miR-517a/b* expression levels between FP and MC tissues (expression ratio: 0.189; $p=0.0002$) (Figure 2). The expression level was not statistically different between MP and MC tissues ($p=0.1$).

Discussion

The current study has shown that *Hsa-miR-517a/b* was down-regulated in the preeclamptic placenta compared to normal tissue. Moreover, it was observed that the dysregulation of *Hsa-miR-517a/b* was confined to the fetal side of the preeclamptic tissue. These result suggests that placenta, as a fetomaternal tissue, exhibits differential expression of genes on the fetal-side and maternal-side of the tissue.

In recent years, investigation into obstetrical and gynecological disorders, including PE, has increased. However, there are limited studies regarding the effect of miRNAs on gene expression regulation in this area (12,23). Placental-specific miRNAs are expressed uniquely in the placental tissue and have the potential to predict and act as biomarkers for placental conditions in both normal and adverse obstetrical pregnancy outcomes, including in PE (23). *Hsa-miR-517a/b* is a member of the C19MC miRNAs that locates on 19q13.42 and is considered placental-specific (14). Na et al. (18) reported that *Hsa-miR-517a/b* was down-regulated in the hydatidiform mole placenta when compared with the normal placenta. Preeclampsia and hydatidiform mole

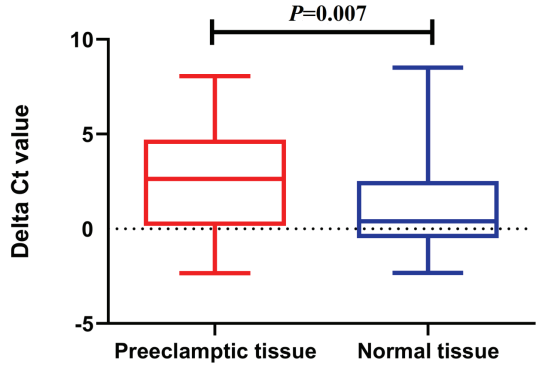

Figure 1. Comparison of the *Hsa-miR-517a/b* expression levels between preeclamptic tissues and normal tissues. The box plot represents the relative expression of *Hsa-miR-517a/b* normalized to *SNORD48* in preeclamptic placental tissues compared to normal placental tissues by Mann-Whitney U test. The Y and X axes show delta Ct (Ct_{target} gene-$Ct_{reference}$ gene) and studied groups, respectively. Boxes show the extent of the IQR and the central line is the median value. The whiskers show the full range of results. The median (IQR) values are 2.6 (0.2-4.7) and 0.4 (-0.5-2.5) in preeclamptic and normal tissues, respectively.

IQR: Interquartile range, Ct: Cycle threshold

Table 1. Basic characteristics and clinical features of the patients and controls

	PE patients	Controls	OR (CI)	p
Age (years)	32.4±5.0 (22-43)	33.2±6.9 (26-48)	-	0.14
Body mass index (kg/m²)	33.1±3.9 (25-40.1)	31.5±5.8 (20.8-41.7)	-	0.32
Gestational age at birth (weeks)	34.9±3.7 (32-39)	38.1±0.9 (36-40)	-	0.0005
Fetal weight (kg)	2.6±0.9 (1.9-3.9)	3.2±0.5 (1.8-3.9)	-	0.03
Systolic blood pressure (mmHg)	161±6 (120-190)	111±7 (90-120)	-	<0.0001
Diastolic blood pressure (mmHg)	92±9 (80-120)	73±8 (60-90)	-	<0.0001
Family history of hypertension (%)	44	24	2.5 (0.7-8.4)	0.14
History of pregnancy loss (%)	32	16	2.5 (0.6-9.6)	0.19
Preeclampsia				
Mild (%)	64	-	-	-
Severe (%)	36	-	-	
PE: Preeclampsia, OR: Odds Ratio, CI: Confidence interval				

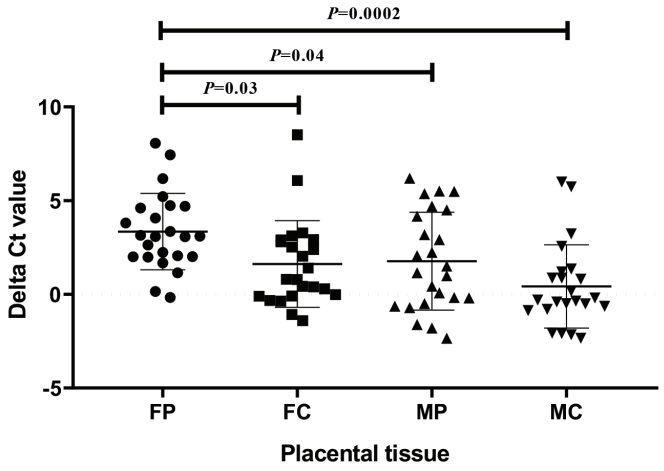

Figure 2. Comparison of *Hsa-miR-517a/b* expression levels between the fetal side of preeclamptic tissues (FP), fetal side of control tissues (FC), maternal side of preeclamptic tissues (MP) and maternal side of control tissues (MC). The Y and X axes show delta Ct (Ct_{target} gene-$Ct_{reference}$ gene) and placental tissues, respectively. The median (IQR) values are 3.1 (2-4.7), 0.8 (-0.1-2.9), 1.5 (-0.5-4.6), and -0.3 (-0.8-1.2) in FP, FC, MP, and MC groups, respectively.

FP: Fetal preeclamptic, FC: Fetal control, MP: Maternal preeclamptic, MC: Maternal control, Ct: Cycle threshold

placenta originate from an inappropriate invasion of trophoblast cells (24-26). Zhu et al. (19) reported the differential expression of miRNAs in placental tissues from PE patients vs normal controls. They showed that several miRNAs located at 19q13.42, including *Hsa-miR-517*, *Hsa-miR-518b*, and *Hsa-miR-519e* were expressed differentially in preeclamptic placentas (19). Anton et al. (12) also reported that *Hsa-miR-517a/b* and *Hsa-miR-517c* play an important role in PE development via the regulation of placental and trophoblastic function. In a recent study, Hromadnikova et al. (17) investigated the association between differential expressions of 15 different C19MC miRNAs in the placenta and pregnancy-related complications, including PE, fetal growth restriction, and gestational hypertension. They showed that *Hsa-miR-517* was down-regulated in all these pregnancy complications, including PE (17). They previously reported that *Hsa-miR-517-5p* was increased in the maternal plasma of preeclamptic women, and therefore suggested that up-regulation of C19MC miRNAs serves as a characteristic phenomenon of established PE (27). Down-regulation of *Hsa-miR-517a/b*, as shown in the present study, could contribute to the dysregulation of predicted target genes and, therefore, to the pathogenesis of PE. According to the TargetScan database, *STAT1*, *FOXC1,* and *HOXA5* are predicted as *Hsa-miR-517a/b* target genes (28). These genes are involved in the conduction of important biological pathways that includes angiogenesis. In some cases, PE is associated with inhibition of the angiogenesis pathway due to the imbalance between pro-angiogenic and anti-angiogenic factors (29). Signal transducer and activator of transcription 1 (STAT1), as a transcription factor, regulates the expression of several genes via the interferon-gamma (IFN-γ)/STAT1 pathway, involved in inflammation and angiogenesis, the main features of PE. A bioinformatics analysis conducted by Luo et al. (30) proposed STAT1 as a hub gene in the protein-protein interaction network related to PE. Zhang et al. (31) showed that *STAT1* is expressed under conditions of hypoxia and affects the expression of *VEGF-A* and *HIF1a* in glioma cells. In a similar fashion to these tumor cells, altered expressions of placental *VEGF* and *HIF1a* are associated with the pathogenesis of PE. Moreover, in a recent study, it was suggested that IFN-γ/STAT1 promotes the expression of erythropoietin-producing hepatocellular receptor B4 that regulates endothelial activation in PE pathogenesis (32).

The Forkhead box C1 (FOXC1) belongs to the FOX transcription factor family and plays a vital role in embryonic development. A previous study suggested that FOXC1 affects angiogenesis by regulating the balance between anti- and pro-angiogenic pathways. It was shown that *FOXC1*-null mutations in mice result in over-expression of *sFlt1* as an inhibitor of angiogenesis (33). Løset et al. (34), in a genome-wide transcriptional profiling study, confirmed the differential expression of *FOXC1* between the preeclamptic and normal decidual tissues. *HOXA5* is an anti-angiogenic homeobox gene. Increased expression of *HOXA5* is associated with decreased expression of pro-angiogenic genes such as *VEGFR2*, and increased expression of *thrombospondin-2* (*TSP2*) as an angiogenesis inhibitor (35). In most previous gene expression studies in preeclamptic tissues, it is unclear whether the tissue is obtained from the maternal or fetal side of the placenta. Therefore, these discrepant results may be due to tissue sampling from different placental zones.

In the present study, it was hypothesized that gene expression levels might differ between the maternal and fetal sides of the placenta, due to the effects of the maternal and fetal genomes, respectively. To the best of our knowledge, there are very few articles regarding this issue. Sahay et al. (36) showed the differential VEGF and VEGFR1 protein levels in different regions of the placenta. The present results provide robust evidence of the differential expression of *Hsa-miR-517a/b* in the maternal and fetal sides of the placenta.

Conclusion

PE was associated with down-regulation of *Hsa-miR-517a/b*. Furthermore, *Hsa-miR-517a/b* expression level was different only in the fetal side of placental tissue when comparing between PE patients and healthy controls. The results of the present study may help to understand the possible mechanisms

involved in the pathogenesis of PE. Moreover, the present results confirmed the importance of tissue sampling accuracy when undertaking gene expression studies in PE.

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Informed Consent: *All subjects were informed about the study and each gave written consent.*

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