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EXPRESSION PROFILES OF miR-155-5P AND miR-518B MICRORNAS IN CIRCULATING LEUKOCYTES OF THE PREGNANT PATIENTS WITH PREECLAMPSIA AND POLYCYSTIC OVARY SYNDROME

M. Hocaoglu[1,*], S. Demirer[2], I. Loclar Karaalp[1], E. Kaynak[2], E. Attar[3], A. Turgut[1,4], E. Komurcu Bayrak[2]

[1]Goztepe Prof Dr. Suleyman Yalcin City Hospital Affiliated to Istanbul Medeniyet University, Department of Obstetrics and Gynecology, [2]Aziz Sancar Institute of Experimental Medicine, Istanbul University, Department of Genetics, [3]Yeditepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, [4]Istanbul Medeniyet University, Faculty of Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey

Abstract

Context. Polycystic ovary syndrome (PCOS) is associated with increased prevalence of preeclampsia (PE); microRNAs (miRs) could play an important role in the pathogenesis of PE and PCOS.

Objective. To investigate the expression levels of miRs 155-5p and 518b in blood leukocytes of patients with PE and PCOS.

Design. Using real-time quantitative PCR method, miR-155-5p and miR-518b were examined from PE, PCOS, PE+PCOS, and controls.

Subjects and Methods. The relative expression of the target miRs in patient samples was compared to control samples. The results were calculated as relative quantification values.

Results. Confounding variables were controlled using analyses for covariance. Significant differences were observed in miR-155-5p (p=0.008) and miRNA-518 (p=0.016) expression levels among the groups. miR-155-5p (p=0.014) and miR-518b (p=0.036) were upregulated in PCOS patients and miR-518b (p=0.028) were increased in cases with PCOS+PE. Near significant difference was found (p=0.06) in miR-518b expression levels in cases with PE, compared to controls. miR-518b was observed to be positively correlated with alanine transaminase in cases with PE (r=0.80; P=0.017) and PE+PCOS (r=0.80, p=0.017).

Conclusions. Our preliminary findings suggested that expression profiling of miR-155-5p and miR-518b in blood leukocytes were upregulated in pregnant women with PCOS. Moreover, miR-518b was found to be related to PE in cases with PCOS.

Keywords: preeclampsia, polycystic ovary syndrome, pregnancy, microRNA, maternal blood leukocytes.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the multifactorial endocrinopathy in reproductive-age women. The principal clinical symptoms of PCOS include ovulation disorders as well as clinical or laboratory test indicators of androgens excess and polycystic ovarian morphology (1). Preeclampsia (PE) is a major hypertensive disorder specific to pregnant women and the leading cause of maternal morbidity worldwide. The disease is characterized by new appearance of hypertension and proteinuria after 20 weeks of gestation (2). A systematic review indicated that the pregnant women with PCOS are four times more likely to develop PE when compared with controls (3).

MicroRNAs (miRNAs) are endogenous, 20-22 nucleotides long, noncoding RNA molecules, which negatively regulate the gene expression at posttranscriptional level (4). MicroRNA-155 (miR-155) is a miRNA involved in the regulation of expression of eNOS, endothelium-dependent vasorelaxation and the pathogenesis of diabetes and its complications (5). Furthermore, miR-155 has been recently identified as a nuclear factor kappa B (NF-κB) -dependent microRNA, involving in the development of inflammation and immune responses (6). Whereas, among the numerous proposed mechanisms involved in the pathogenesis of diabetes and its complications, NF-κB has been identified as a master switch (7).

MiR-518 is identified as pregnancy-associated microRNA and its target gene EGR1 may regulate

*Correspondence to: Meryem Hocaoglu MD, Goztepe Prof Dr. Suleyman Yalcin City Hospital Affiliated to Istanbul Medeniyet University, Department of Obstetrics and Gynecology, Istanbul, 34720, Turkey, E-mail: dr.meryemtaskiran@gmail.com

cell proliferation, angiogenesis, and cell apoptosis to impact early embryonic development (8, 9). Studies have also shown that miR-155 (10) and miR-518 (11) could play an important role in the pathogenesis of PE. Furthermore, a recent study has suggested that miR-155 might have a role as a non-invasive biomarker in hyperandrogenic nonpregnant PCOS patients (1). However, up to now, no currently published studies have reported a link between miRNAs and PE and PCOS.

In this prospective study, we aimed to investigate the association between selected miRNAs and PE and PCOS. We measured the expression levels of miR-155-5p and miR-518b in peripheral blood leukocytes of the pregnant patients with PE and PCOS.

MATERIALS AND METHODS

Subjects

This prospective study was conducted on 64 Caucasian women. From October 2018 to January 2019, the participants were selected from antenatal clinic, labor ward, and antenatal ward of Department of Obstetrics and Gynecology, Goztepe Prof Dr. Suleyman Yalcin City Hospital during the third trimester of pregnancy. Eligible women were classified into four groups: (I) PE (n=24), (II) PCOS (n=9), (III) PE and PCOS (n=8), (IV) healthy controls with no obstetrical or medical complications (n=23). PE group (Group I) consisted of 6 patients with early-onset mild PE, 6 patients with early-onset severe PE, 5 patients with late-onset mild PE, and 6 patients with late- onset severe PE.

The diagnostic and the severity criteria for PE were made on the basis of the American College of Obstetricians and Gynecologists (ACOG) bulletin (12). Preeclampsia was defined by the onset of hypertension (blood pressure ≥140/90 mm Hg) and proteinuria (≥0.3 g of protein in the urine within a 24-hour period or a protein/ creatinine ratio of at least 0.3) during the second half of pregnancy (>20 weeks). In the absence of proteinuria, the diagnosis of PE was made based on hypertension with the presence of any of the following: thrombocytopenia, impaired liver function, renal insufficiency, pulmonary edema, or cerebral or visual disturbances (12). PE occurring at less than 34 weeks of gestation was identified as early- onset PE (EOPE), whereas PE that occurred at 34 weeks or later was labeled late-onset PE (LOPE), irrespective of the gestational week at delivery (12, 13).

The women with PCOS who were diagnosed before pregnancy were included in the study according to the hospital patient registry and a prior diagnosis of PCOS made by a doctor (self-reported medical diagnosis). The diagnosis of PCOS was made according to the Rotterdam criteria in the presence of at least two of the following three criteria: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and PCO morphology as examined by trans-vaginal ultrasound (14). Gestational age (GA) was determined from the last menstrual period and verified during the routine first-trimester ultrasound measurement of the fetal crown-rump length. Corticosteroids were used in 18 of 24 preeclampsia cases and 1 of 8 cases of the women with PE and PCOS to accelerate lung maturation (two doses of betamethasone administered intramuscularly 24 h apart). The exclusion criteria for all groups were as follows: under 18 years of age; multiple pregnancies; gestational or chronic hypertension, with or without superimposed PE; pre-gestational or gestational diabetes, and any other confounding pathologies (including intrahepatic cholestasis of pregnancy, cardiovascular, autoimmune, renal and hepatic diseases); premalignancies or malignancies; drug/alcohol use; becoming pregnant through the use of Assisted Reproduction Techniques (ART).

Study patients were followed from inclusion in the study up to delivery with respect to the outcome of the pregnancy, GA at delivery, infant birth weight and fetal sex.

Sample collection and RNA isolation

Maternal non-fasting peripheral blood samples were collected from cases and controls into 10 mL Vacuette K2EDTA tubes (BD, Franklin Lakes, NJ) during the third trimester of pregnancy in Department of Obstetrics and Gynecology, Goztepe Prof Dr. Suleyman Yalcin City Hospital. The isolation of total RNA from peripheral leukocytes was described in detail in the previous study (15).

Quantification of candidate miRNAs

Real-time quantitative PCR was performed to measure the expression levels of miRNAs with the miScript Primer Assays (Qiagen, Valencia, CA, USA). Reverse transcription of 1 μg total RNA was performed according to the miScript II RT kit protocol (Qiagen). The cDNA samples were diluted in nuclease-free water as 40x and amplified using the miScript SYBR® Green PCR kit (Qiagen), the provided miScript Universal

Primer and target-specific miScript Primer Assays for hsa-miR-518b (Assay ID: MS00004466), hsa-miR-155-5p (Assay ID: MS00031486), RNU6B (Assay ID: MS00033740). A total of 0.1 ng template cDNA was used per well reaction volume. The reactions were performed in duplicate in 96-well plates on a LightCycler 480 instrument (Roche, San Francisco, CA) using manufacturer-recommended cycling conditions: 95°C for 15 min with a ramping rate of 4.4°C/s, 45 cycles of 94°C for 15 s, 55° C for 30 s, 70° C for 30 s with a ramping rate of 1.0°C/s, and a final melt-curve analysis. Threshold cycle number was used to calculate the relative expression between the samples. We used the ΔΔCt (cycle threshold) method in which relative expression = 2-ΔΔCt, where ΔΔCt = (ΔCt of test sample) - (ΔCt of calibrator). The data were normalized using the human RNU6B (RNU6-2) as endogenous control. The arithmetic mean Ct of the control samples (n=23) was used as the calibrator. The relative expression of the target miRNAs in patient samples was compared to the calibrator and the results were calculated as Relative Quantification (RQ) values.

Statistical analysis

All statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). The characteristics of the study subjects were compared using the analysis of variance (one-way ANOVA) test among four groups. The significance of maternal leukocytes miRNA levels was determined by Kruskal Wallis test among four groups. One-way ANOVA (analysis unadjusted for confounding variables) and ANCOVA (analysis of covariance for adjusted with GA, body mass index (BMI) and, administration of antenatal corticosteroids along with Bonferroni's post-hoc test for pairwise comparisons were utilized to analyze the relative expression levels of miRNAs among the study groups. Power analysis and sample size calculations were performed using the G*Power statistics software (16). In total sample size of 64 individuals of four groups for miRNAs expression levels of this study. The power analysis (α=0.05) with ANOVA test and ANCOVA test with three covariates (effect size for both test, d=0.55) were calculated as 86% and 85%, respectively. Statistical significance was found for the P values which are less than 0.05.

RESULTS

Baseline characteristics of the participants

Significant differences were observed in gestational age (p<0.0001), systolic blood pressure (p<0.0001), diastolic blood pressure (p<0.0001), serum albumin level (p<0.001), pre-pregnancy BMI (p<0.001), BMI at enrolment (p<0.0001), and in terms of administration of antenatal corticosteroids (p<0.0001) between the four groups. The main features of the studied women per the study group are shown in Table 1.

miRNA expression levels in patients with PE and PCOS compared to control subjects

We compared miR-155-5p and miR-518b expression levels in maternal blood leukocytes among four groups. The comparisons of miRNA expression levels are demonstrated on Table 2 and Figure 1 between four groups. While there is significant difference in expression levels of miRNA-155-5p (p=0.012), we found no significant difference in expression levels of miRNA-518b (p = 0.817) between four groups (Table 2; Figure 1A-C). Whereas, we adjusted for potential confounding variables due to the fact that the factors such as GA, BMI, and administration of antenatal corticosteroids at enrollment may influence miRNA expression in leucocytes. After adjustment, significant differences were observed in miR-155-5p (p = 0.014) and miRNA-518b (p = 0.018) expression levels between PE, PCOS, PE+PCOS, and the control groups (Figure 1B-D). To further compare the expression levels of miR-518b and miR-155-5p between the two groups, the effects of GA, BMI at blood drawn and, administration of antenatal corticosteroids were adjusted. miR-155-5p was found to be upregulated in the cases with PCOS (RQ value = 21.43 ± 5.79, p = 0.009), compared to the control group (RQ value = -1.45 ± 4.21). No significant differences were found in the expression levels of miR-155-5p in the patients with PE and PE+PCOS *versus* controls, respectively (RQ value = 2.36 ± 4.36 vs RQ value = -1.45 ± 4.21, p = 1; RQ value = 8.82 ± 7.49 *vs.* RQ value = -1.45 ± 4.21, p = 0.13). We observed significant increase in the expression levels of miR-518b in cases with PE+PCOS *versus* controls (RQ value = 14.08 ± 4.02 *vs.* RQ value = -1.55 ± 2.38, p = 0.025). Furthermore, no significant differences were found in the expression levels of miR-518b in the patients with PE and PCOS cases *versus* controls, respectively (RQ value = 3.63 ± 2.34 *vs.* RQ value = -1.55 ± 2.38, p =1; RQ value = 7.53 ± 3.11 *vs.* RQ value-1.55 ± 2.38, p = 0.09)

Correlation of miRNA expression levels with clinical parameters

A significantly positive correlation between hematocrit levels and miR-155-5p expression levels (r=0.45, p=0.027) was observed in the cases with PE. Also, we found a strong positive correlation between miR-518b expression levels and the hepatic enzyme alanine aminotransferase (ALT) (r=0.80; P=0.017) in the PE group. A strong positive correlation was observed between miR-518b and ALT levels

Table 1. The main characteristics of the enrolled study subjects

Characteristics	PE (n=24) Mean ± SD	PCOS (n=9) Mean ± SD	PE + PCOS (n=8) Mean ± SD	Control (n=23) Mean ± SD	P value
Age (years)	31.4 ± 4.8	30.2 ± 4.9	28.1 ± 4.9	27.5 ± 5.3	0.063
Nulliparous[a] n (%)	6(33.3)	2(11.1)	4(22.2)	6(33.3)	0.53
Gestational age at sampling (weeks)	30.4 ± 3.5	29.5 ± 5.2	36.7 ± 2.4	29.1 ± 0.6	< 0.0001*
Pre-Pregnancy BMI, kg/m^2	26.8 ± 5.3	30.0 ± 6.1	30.2 ± 6.3	23.5 ± 3.2	0.001*
BMI at enrollment, kg/m^2)	30.6 ± 5.3	33.6 ± 5.4	34.6 ± 5.4	26.3 ± 3.0	< 0.0001*
Systolic blood pressure at sampling (mmHg)	152.5 ± 14.2	106.7 ± 8.7	151.8 ± 14.5	101.3 ± 4.6	< 0.0001*
Diastolic blood pressure at sampling (mmHg)	94.8 ± 9.3	63.3 ± 7.1	91.6 ± 7.8	60.4 ± 4.7	< 0.0001*
Total serum protein (g/dL)	6.3 ± 0.7	6.7 ± 0.5	6.2 ± 0.4	6.8 ± 0.5	0.058
Serum albumin (g/dL	3.3 ± 0.3	3.6 ± 0.3	3.2 ± 0.2	3.5 ± 0.2	0.001*
AST (IU/l)	70.8 ± 193.2	12.6 ± 4.9	28.9 ± 28.5	15.0 ± 4.4	0.387
ALT (IU/l)	79.7 ± 220.1	9.9 ± 3.1	39.6 ± 58.0	11.4 ± 5.9	0.341
LDH (IU/l)	338.9 ± 403.1	171.2 ± 54.3	267.3 ± 120.0	178.3 ± 31.5	0.142
Creatinine, mg/dl	0.59 ± 0.11	0.54± 0.1	0.57 ± 0.13	0.5 ± 0.05	0.026*
Hb (g/dl)	11.8 ± 1.6	11.7 ± 0.8	11.6 ± 1.8	11.5 ± 2.1	0.939
Hct (%)	35.3 ± 4.4	34.9 ± 1.9	35.3 ± 5.6	33.6 ± 4.7	0.560
WBC, (x10^3/ ul	12.3 ± 4.1	10.1 ± 1.7	10.7 ± 4.5	10.3 ± 2.3	0.184
Platelets (x10^3/ ul)	220.2 ± 66.2	230.4 ± 59.2	207.0 ± 48.1	223.4 ± 50.9	0.861
Spot urine protein/creatinine ratio	2.4 ± 3.7	-	1.4 ± 2.0	-	0.005*
Smoking n (%)	4(66.7)	-	-	2(33.3)	0.35
Antenatal corticosteroids used[a,b] n (%)	18(94.7)	-	1(5.3)	-	< 0.0001*
Fasting glucose (mg/dl) at sampling	77.8 ± 6.5	77.3 ± 8.9	80 ± 12.5	72.3 ± 7.4	0.05
GA at delivery,weeks	33.6 ± 4.1	38.8 ± 0.69	37.47 ± 0.7	39.3 ± 1.1	< 0.0001*
Infant birth weight, g	1825± 976.4	3265.5 ±400.3	2806.2 ±584.6	3446 ±389.9	< 0.0001*
Fetal sex (male)[a] n (%)	10(34.5)	4(13.8)	1(3.4)	14(48.3)	0.11

Abbreviations: SD, standard deviation; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, Lactate dehydrogenase; GA, Gestational Age. Data presented as mean ± SD and compared using ANOVA. a p value was presented as a result of Chi-Square test. c Corticosteroids were used to accelerate lung maturation (two doses of betamethasone administered intramuscularly 24 h apart). *p < 0.05 was considered statistically significant.

Table 2. Relative expression levels of miRNA-155-5p and miRNA-518b among the study groups

miRNA-155-5p				
Study Groups	**Unadjusted mean ± S.D. (95% CI)**	**p***	**Adjusted mean ± S.E. (95% CI)**	**p****
PCOS (n=9)	21.24 ± 40.43 (-9.83-52.32)	0.012	21.43 ± 5.79 (9.84-33.02)	0.014
PE + PCOS (n=8)	2.51 ± 4.72 (-1.43-6.45)		8.82 ± 7.49 (-6.17-23.81)	
PE (n=24)	1.91 ± 4.02 (0.21-1.71)		2.36 ± 4.36 (-6.38-11.09)	
Control (n=23)	1.28 ± 1.00 (0.84-1.72)		-1.45 ± 4.21 (-10.30-7.40)	
miRNA-518b				
Study Groups	**Unadjusted mean ± S.D. (95% CI)**	**p***	**Adjusted mean ± S.E. (95% CI)**	**p****
PCOS (n=9)	5.14 ±9.28 (-1.99-12.27)	0.817	7.53 ± 3.11 (1.31-13.76)	0.018
PE + PCOS (n=8)	9.59 ± 21.53 (-8.41-27.59)		14.08 ± 4.02 (6.03-22.14)	
PE (n=24)	3.26 ± 4.70 (1.27-5.25)		3.63 ± 2.34 (-1.06-8.32)	
Control (n=23)	1.34 ±0.80 (0.99-1.68)		-1.55 ± 2.38 (-6.30-3.21)	

Abbreviations: PE, preeclampsia; PCOS, polycystic ovary syndrome; SD, standard deviation; SE, standard error; CI, confidence interval. p * values, Kruskal-Wallis Test was performed for the associations of miRNAs expression levels among study groups. p ** values, Analysis of Covariance (ANCOVA) was performed to adjust with gestational age, body mass index and, administration of antenatal corticosteroids at enrollment for the associations of miRNAs expression levels among study groups. p < 0.05 was considered statistically significant.

(r=0.80, p=0.017) in the patients with PE+PCOS. Interestingly, significantly inverse correlation was observed between miR-518b expression levels and pre-pregnancy BMI in cases with PE+PCOS (r=-0.79, p=0.021). While miR-518b was moderately strong positively correlated with aspartate transaminase (AST) and ALT levels, respectively (r=0.53, p=0.009; r=0.45, p=0.033), infant birth weight was found to be negatively correlated with miR-518b (r=-0.48, p=0.021) in the control group. Furthermore, we found positive correlation between miR-155-5p and hematocrit levels (r=-0.79, p=0.021) in the control group.

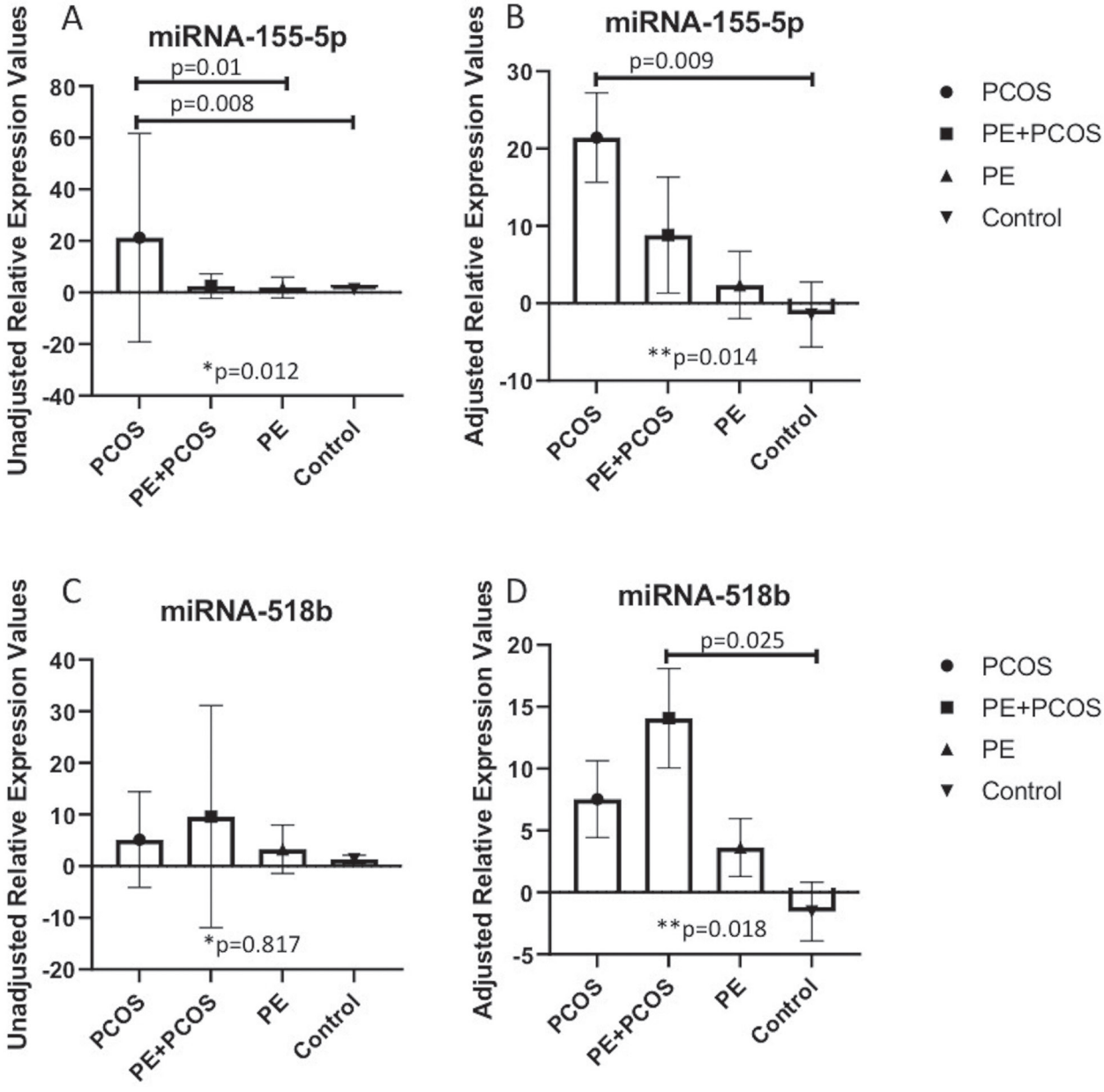

Figure 1. Bars of relative miR-155-5p and miR-518b expression levels in preeclampsia (PE), polycystic ovary syndrome (PCOS) + PE, PCOS, and healthy control samples. Kruskal-Wallis test (*) was used to compare the difference of the two miRNAs expression levels among four groups. There was significant difference in expression levels of miR-155-5p (p=0.012) and no significant difference in expression levels of miR-518b (p=0.817) among four groups (Figure 1 A and C). After adjustment of confounding variables such as gestational age, body mass index, and administration of antenatal corticosteroids at enrollment (Figure 1 B and D) by using an ANCOVA (**), significant differences were observed in expression levels of miR-155-5p between PCOS cases and controls (p=0.009) and between four groups (p=0.014). There were significant differences in expression levels of miRNA-518b between PE+PCOS cases and controls (p=0.025) and among four groups (p=0.018). The p values of pairwise comparisons given above the bars were obtained by Bonferroni's post-hoc test.

DISCUSSION

Main findings

To the best of our knowledge, this is the first study providing preliminary data on expression levels of miR-155-5p and miR-518b in pregnant patients with PE and PCOS. In terms of adjusting the potentially confounding clinical variables, significant differences were seen in miR-155-5p (p=0.014) and miRNA-518b (p=0.018) expression levels between PE, PCOS, PE+PCOS, and the controls. When comparing the miRNA expression profile in cases with PCOS and controls, miR-155-5p was found to be over-expressed in cases with PCOS. Also, no significant differences were seen in the expression levels of miR-155 in PE and PE+PCOS groups *versus* controls. Furthermore, we observed significantly upregulated expression levels of miR-518b in cases with PE+PCOS *versus* controls. However, no significant differences were observed in the expression levels of miR-518b in the patients with PE and PCOS cases *versus* controls. We observed a strong positive correlation between miR-518b and ALT levels in PE and PE+PCOS groups. While miR-518b was moderately strong positively correlated with AST and ALT levels, infant birth weight was found to be negatively correlated with miR-518b in the control group.

Previous studies documented the association between pathogenesis of PCOS and PE. The underlying pathophysiological mechanisms involved are not completely understood; however, it is possible that the high androgen levels, hyperinsulinemia and subsequent diabetic and hypertensive susceptibilities in PCOS may act as co-factors (17). In this regard, miRNAs have been shown to target metabolic path- ways such as the insulin signaling pathways (AKT/PI3K pathway) in PE. Khaliq *et al.* demonstrated that low levels of miRNA-222 and high levels of miRNA-29a which are involved in angiogenesis and insulin resistance were found to be negatively correlated with AKT/PI3K in PE patients, reinforcing the presence of metabolic dysregulation in PE (18). Similarly, a recent study has suggested that circulating miR-222 levels that were related to metabolic syndrome, insulin resistance, and diabetes were found to be significantly lower in non-pregnant PCOS patients compared with the control group (19, 20).

miR-155 was previously demonstrated to interfere with trophoblast migration through miR-155-CYR61-VEGF pathway, which hinders placental angiogenesis, thus promoting PE pathogenesis (10). The recent studies investigating the expression levels of miR-155 in maternal plasma (21) and serum (22, 23) of the women with PE have found higher levels of miR-155 in PE patients than controls. In our study, no significant differences were seen in the expression levels of miR-155 in PE and PE+PCOS groups *versus* controls. Moreover, the findings of the current study are in contrast to our recent work in which decreased miR-155 expression levels were found in maternal blood leukocytes for women with PE (15). The explanation for this divergent result is not clear, but may be related to GA at blood drawn. GA at blood drawn was higher in the cases with PE and controls in the previous study than those in our current work.

miRNA-155 have been associated with inflammatory processes, adipogenesis and insulin signaling (24). Lopez *et al*. demonstrated that miR-155 was significantly reduced in the circulation of the subjects with prediabetes, compared to controls (25). Arancio *et al*. indicated that miR-155 was evaluated in non-pregnant women who suffered from PCOS (1). In our present work, we found significantly increased expression levels of miR-155-5p in cases with PCOS, compared to controls. Therefore, our findings may well reflect that the expression of miR-155-5p in the leukocytes of patients could be associated with metabolic dysregulation in PCOS during pregnancy.

miR-518b is a member of C19MC cluster and its expression is involved in reproductive system and placenta (26). miR-518b was observed to be upregulated in severe preeclamptic placentas, compared with normal placenta (27). Previous studies also have shown that increased plasma levels of miR-518b in severe PE patients (10, 28). In our recent work, we have found that the expression levels of miR-518b is upregulated in the early- onset PE (EOPE) and late -onset PE (LOPE) of groups compared to controls although there was no significant difference in the miR-518b expression level between total cases of PE and control. In addition, miR-518b was found to be positively correlated with ALT levels in EOPE (29). In line with our recent research, no significant differences were observed in the expression levels of miR-518b in patients with PE *versus* controls. The explanation of conflicting findings with previous studies is not clear, but they may reflect the differences in PE subgroups, particularly for severe PE. Another reason may be that the miRNA levels in tissue (placenta) or plasma may not be completely consistent with those in maternal blood leukocytes. Furthermore, miR-518b was found to be positively correlated with ALT levels in PE,

PE+PCOS, and control groups. While miR-518b was moderately strong positively correlated with AST and ALT levels, infant birth weight was found to be negatively correlated with miR-518b in the control group. These findings indicate that miR-518b may function as a placenta associated miRNA and also related with the clinical presentations of PE.

Zhao *et al*. demonstrated a significant increase of miR-518d (part of the C19MC) concentration in GDM, compared to normoglycemic controls and a strong inverse correlation between miR-518d levels and the protein concentration of peroxisome proliferator-activated receptor-α (PPARα) in full-term placenta. PPARα was shown to be a direct target of miR-518d using a luciferase reporter assay (30, 31). As such, it might be expected that an increased production of miR-518b as another member of C19MC in placenta due to metabolic dysregulation would lead to an increased presence of miR-518b in maternal blood leukocytes. Notably, we observed upregulated expression levels of miR-518b in PE+PCOS, compared to controls. Also, a strong positively correlation was observed between miR-518b and ALT levels in the patients with PE+PCOS. But also, no significant differences were found in the expression levels of miR-518b in the patients with PCOS *versus* controls. These findings indicate that miR-518b is related to PE in the cases with PCOS.

Clinical and research implications

Future studies that examine miR-518b and miR-155-5p expression levels during the first trimester of pregnancy in blood leukocytes of the patients with PCOS may provide further elucidation, its pathophysiology, prediction of PE, therapeutic interventions, and to improve clinical outcomes. During pregnancy, the placenta secretes various miRNAs, which may serve as either a marker of cell turnover or an active messenger between the placenta and the mother. Thus, miRNAs could serve as biomarkers for the prediction of pregnancy disorders (32). In particular, there is a need for further studies to assess miR-518b and miR-155-5p expression profiles in the placentas of the patients with PCOS and PE and to compare these findings with that miRNAs expression which released into the maternal circulation. In addition, it would be of great interest to determine miR-518b and miR-155-5p expression levels in pregnant women with PCOS according to specific PCOS phenotypes (33). Furthermore, recent studies have highlighted the role of imbalances in the endocannabinoid system (ECS) in the underlying pathophysiology of both PE and PCOS (34). Further investigations are needed to determined the exact relationship between ECS and miRs in patients with PE and PCOS.

Strengths and limitations

The main strength of the current study is identifying candidate miRNA expression levels from blood leukocytes of the patients with PE and PCOS, compared to controls. Thus, the abnormal pattern of several markers of low- grade chronic inflammation was demonstrated in non-pregnant women with PCOS (17, 35). Moreover, it is known that activated monocytes and neutrophils are present in the fetal and placental circulation under hypoxic conditions in PE (36).

There are, however, limitations of our research. This study explores the preliminary data in a limited population sample size. Thus, here, we have reported the preliminary findings and no definitive conclusion can be drawn from our data. However, this study provides interesting driving information for the future studies regarding the relationship between PE, PCOS and miRNAs, with a larger sample size. Another potential bias for this study is whether the administration of betamethasone is able to limit miRNA expression in the women. Therefore, we adjusted for administration of antenatal corticosteroids as a potential confounder due to the fact that it may influence the miRNA expression in leucocytes. However, the administration of corticosteroids is known to increase the number of circulating white blood cells by causing leukocyte extravasation from bone marrow (37). But also, previous studies have failed to find any significant effect of the use of betamethasone (in the doses used clinically) on maternal immune responses (38). The limitations of the current study also include our cases and controls were not matched for GA at the time of sampling by 1:1 paired matching. Blood samples were collected from cases and controls during the third trimester of pregnancy. There was statically significant difference in GA at enrollment among groups. Therefore, GA at blood drawn was adjusted for a potential confounding variable due to the fact that it may influence miRNA expression in maternal blood leucocytes. Finally, the diagnosis of PCOS was made on the basis of hospital patient register in some participants and a prior diagnosis of the PCOS made by a doctor (self-reported medical diagnosis) in others.

In conclusion, our preliminary results provide evidence for increased maternal blood leukocyte miR-

155-5p levels in the pregnant women with PCOS compared with controls. Moreover, our study suggests that miR-518b is related to PE in the cases with PCOS. We suggest further studies be carried out so as to confirm and extend our findings.

Conflict of interest

The authors declare that there is no conflict of interest. The author has no substantial direct or indirect commercial or financial incentive associated with publishing the article.

Ethical Approval

The study was approved by the Institutional Review Board of Istanbul Medical Faculty, Istanbul University. This study was performed in accordance with the ethical standards specified in the Declaration of Helsinki (2013). All participants were given written informed consent.

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