Predictive value of microRNA-210 in preeclampsia: a prospective cohort study

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Aim
To assess the usefulness of circulating miR-210 as a non-invasive molecular biomarker for early prediction of preeclampsia (PE) in high-risk pregnant women.

Methods
A total of 40 pregnant women between 14 and 26 weeks of gestation associated with any risk factors for PE were enrolled in the study. MicroRNA-210 was detected using real-time polymerase chain reaction (Q-PCR) in the plasma sample. Follow-up of the pregnant women in antenatal clinic was done. The prediction of PE among the study group was evaluated, and also the level of microRNA-210 at which PE occurred was detected.

Results
Of 40 cases of high-risk pregnant women, 8 cases developed PE, where 2 had pregestational diabetes mellitus (DM), 1 was primigravida with pregestational DM, 1 had chronic hypertension with pregestational DM, and 4 were primigravida alone. The plasma miR-210 was significantly higher in high-risk pregnant women with PE (n = 8), with a mean ± SE of 19.23 ± 6.95 and median of 15.48 compared with those who did not develop PE (n = 32), with mean ± SE of 4.29 ± 1.36 and median of 1.51 (P = 0.001). At a cutoff value of 2.28-fold change, plasma miR-210 was 87.5% sensitive and 68.8% specific for prediction of PE in risk factor pregnant women, with area under the curve of 0.852.

Conclusion
Plasma miR-210 levels were significantly elevated in preeclamptic women compared with those without PE in high-risk pregnant women, so miR-210 may have possible pathophysiological role in PE.

Keywords:
markers, microRNA-210, preeclampsia

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Introduction

Preeclampsia (PE) is a systematic disease that is associated with hypertension and proteinuria; it occurs beyond 20 weeks of gestation and may lead to several maternal and fetal complications. Several studies have reported that dysfunction of placenta plays an important role in the etiology of PE [1]. PE is frequently seen in the third trimester of pregnancy. Several factors have a role to increase the risk for development of PE such as nulliparity, diabetes mellitus, obesity, and family history [2]. PE may occur early in pregnancy less than 34 weeks or may happen later on beyond 34 weeks of pregnancy [3]. However, several research studies have focused on PE, but there is still no accessible way to stop the severity of the disease, and the only existing treatment is to improve the symptoms [4].

Various studies have been done to detect PE by recognizing several markers such as plasma microRNAs (miRNAs), which play as gene regulators [5]. Although it was found that mRNAs are linked with pathophysiology of PE, these findings are still not clear [6]. MiRNAs consist of a single non-coding RNA of 19-23 nucleotides and are responsible for expression of gene by prevention of action of mRNA degradation and translation [4]. Many studies have found that some miRNAs that appeared in maternal circulation are associated with increased incidence of PE, such as miRNA-210 and miRNA-155 [7].

Previous studies have demonstrated that hypoxia occurs in PE, and miR-210 during hypoxia is produced by hypoxia-inducible factor-1α, so this makes an association between PE and miRNA-210 [8]. Therefore, miRNA-210 can be used as a possible marker for early detection and diagnosis of PE [9]. It

was found that miR-210 appears in the circulation, so it is easily evaluated in plasma [10].

Therefore, the current study aimed to evaluate the usefulness of circulating miR-210 as a non-invasive molecular biomarker for prediction of PE in pregnant women with risk factors for PE.

Patients and methods

This was a prospective cohort study conducted in Assiut University hospitals during the period from December 2018 to March 2020. The study protocol was approved by Assiut Faculty of Medicine Ethical Review Board (IRB.no 17200034). All participants signed an informed written consent form before inclusion.

The study included pregnant women between 14 and 26 weeks of gestation recruited from the antenatal care clinic. We included women with risk factors for development of PE such as primigravida, as well as women having pregestational or gestational diabetes mellitus, renal disease, chronic hypertension, and previous history of PE. The exclusion criteria were women aged more than 40 years and those living in distant cities with unreliable follow-up.

All the study participants were subjected to a structured interview using a pre-designed questionnaire that included assessment of personal and obstetric history. Clinical examination, blood pressure measurement, and body mass index estimation were performed.

Intervention

Determination of plasma miR-210 in the participants was done by quantitative reverse transcription polymerase chain reactions (qRT-PCR) using the 7500 fast real-time PCR (Applied Biosystems, USA).

Venous blood was drawn from all pregnant women (2 ml) and collected in an ethylenediaminetetraacetic acid (EDTA) tube. The blood samples were centrifuged at 1000 rpm for 10 min, and the supernatant layer of the plasma was separated into RNase-free tube for extraction of RNA and was stored at – 80°C until analysis.

Thawing of samples and kit components was done at room temperature for 30 min to extract miRNAs from maternal plasma. RNA extraction was carried out using miRNeasy Mini Kit (Cat. No. 217004; Qiagen, Hilden city, Germany), according to the manufacturer's instructions. Then, extracted RNA was subjected to RNA quantification and purity assessment using NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

Reverse transcription of miRNAs into complementary DNA (cDNA) was done by using miScript® II RT Kit (Cat. No. 218161; Qiagen, Germany), according to the manufacturer' instructions. To synthesize the cDNAs, the RT mix was incubated for 60 min at 37°C, followed by 5 min incubation at 95°C.

Then, miRNA quantification was done by using miScript SYBR® Green QRT-PCR Kit (Cat. No. 218073; Qiagen, Germany) according to the manufacturer' instructions. Target-specific miScript Primer Assay (forward primers) for the selected miRNAs was used in this step, Hs_miRNA-210 (Cat. No. MS00003801; Qiagen, Germany), in addition to the housekeeping gene (HK gene) Hs-RNU6-2_11 (Cat. No. MS00033740; Qiagen – Germany), which is used as an endogenous control for data normalization and relative quantification.

The quantification was done by Rotor Gene Q 72-well rotor (Qiagen, Germany) which was set by the following steps: incubation at 95°C for 15 min as a preliminary activation step for HotStarTaq DNA polymerase (included in 2× QuantiTect SYBR Green PCR Master Mix), followed by 40 amplification cycles, and each cycle is achieved by three consecutive steps of DNA denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 70°C for 30 s.

Calculation of the results was done after completing qRT-PCR cycles; specific amplification of miRNA in each sample was ensured through analyzing the melting curves. Cycle threshold (Ct) values were automatically calculated using the Rotor Gene® Q software 2.1 (Qiagen, Germany). According to the Ct value for each targeted miRNA, the data were normalized through relative expression to the reference gene Hs-RNU6 using ΔCt method, where Ct values of Hs-RNU6 were subtracted from Ct values of the target miRNAs. This was done for all pregnant women with risk factors for PE. Then, ΔΔCt values were calculated by subtracting ΔCt values of the mean of control samples from ΔCt values of pregnant women with risk factors for PE. Then, the fold change (FC) of expression or relative quantification for the targeted miRNAs was calculated using 2–ΔΔCt method. If the FC (expression ratio) was positive, it means that the gene is upregulated; if the FC was negative, it means it was downregulated. So, the results were expressed as FC compared with the control sample which was considered the normal value and assumed to equal 1.

Follow-up

Follow-up of the participants was done every month, and then once the patients were suspected during antenatal care, they were admitted to the hospital and diagnosed as a PE case according to the ACOG guidelines 2013. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation. Proteinuria was defined as the urinary excretion of ≥0.3 g protein in a 24-h specimen, or 0.3 g/l, or 2+ readings on dipstick in a random urine determination with no evidence of the urinary tract infection.

Study outcomes

The study outcomes were to evaluate the occurrence of PE among the study group and also to detect the level of microRNA-210 at which PE occurred.

Statistical analysis

Software (SPSS, Chicago, IL, USA) version 23.0 was used for data analysis. Mean ± SE, with median and range when appropriate, was used to describe quantitative data of pregnant women with risk factors of PE. Numbers with percentages were used to describe qualitative data of pregnant women with risk factors of PE. All variables were tested before evaluation if they were parametric data or not by estimating the test of normality Kolmogorov–Smirnov test. Two-sample *t*-test was used for comparison between parametric dependent continuous variables in two groups, and Mann-Whitney *U* test was used for nonparametric variables. Categorical variables were compared using Chi-square test.

Receiver operating characteristic (ROC) analysis of miR-210 in pregnant women with risk factors of PE was done for evaluation of the area under curve (AUC) as well as the sensitivity and specificity. *P* value was considered significant if less than 0.05.

Results

The study included 40 participants of 72 evaluated women at antenatal care clinic. At the follow-up visits, eight women (20%) were diagnosed as having PE according to the previously mentioned criteria. The demographic and clinical data of those women who developed PE or not were similar, with no significant difference in the age, body mass index, and blood pressure measurement at inclusion [Table 1].

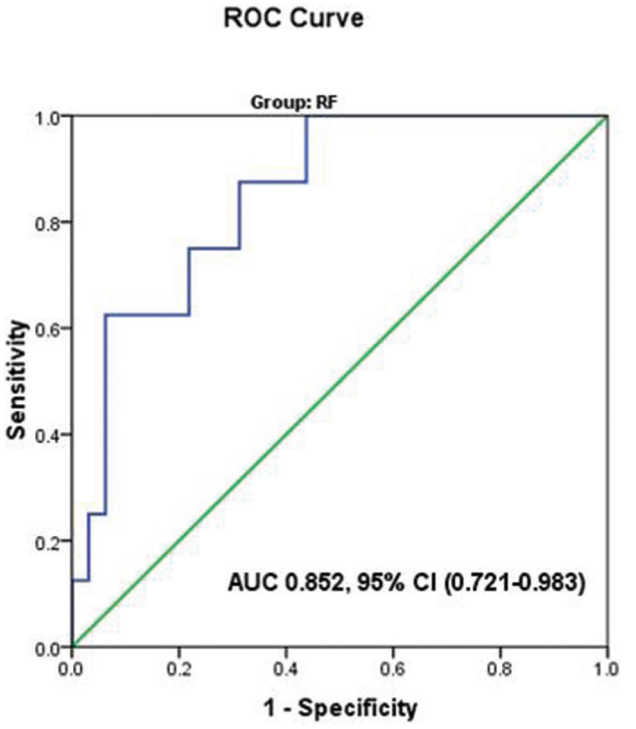
Plasma miRNA-210 was significantly higher in pregnant women with risk factors for PE who developed PE [mean ± SE (19.23 ± 6.95)], with median of 15.48, compared with those without PE [mean ± SE (4.29 ± 1.36)], with median of 1.51 (*P* = 0.001) [Table 2].

ROC curve was constructed for assessment of miRNA-210 as a predictor of PE in pregnant women with risk factors. At a cutoff value of 2.28-fold change, plasma miR-210 was 87.5% sensitive and 68.8% specific for prediction of PE in pregnant women with risk factors for PE, with AUC = 0.852 (Fig. 1).

Discussion

In the present study, we found that plasma miRNA-210 was significantly higher in pregnant women with risk factors for PE who subsequently developed PE compared with those who did not develop PE. Ghafari *et al.*, obtained plasma samples from 90 pregnant women from 26 to 40 weeks of gestation and divided into two groups: 48 being preeclamptic and 42 being control healthy pregnancies. They found that miR-210, 155, and 494 were significantly elevated in pregnant women who got preeclampsia compared with the control group [4]. In the present study, the results were matched with their results that miRNA-210 increased in preeclamptic women. On the contrary, they evaluated miRNA-210 in already preeclamptic women, as compared with the present

Figure 1



ROC curve of miRNA-210 in pregnant women with risk factors for PE (developed preeclampsia vs non-preeclampsia).

Table 1 Demographic and clinical data of pregnant women who developed PE vs those without PE

Variables	Study participants				*P*
	With preeclampsia (*n*=8)		Without preeclampsia (*n*=32)		
	Mean	SE	Mean	SE	
Age (years)	28.38	1.8	28.31	1.3	0.983
Weight (kg)	63.50	2.0	67.44	1.3	0.184
Height (cm)	157.62	2.2	157.19	1.0	0.753
BMI (Kg/m^2)	25.68	1.16	27.51	0.44	0.086
Systolic blood pressure (mmHg)	122.50	4.9	118.62	2.06	0.403
Diastolic blood pressure (mmHg)	73.75	3.7	75.94	1.4	0.630

BMI, body mass index; SE, standard error.

Table 2 MiRNA-210 in pregnant women who developed PE vs those without PE

Item	Study participants										*P*
	With preeclampsia (*n*=8)					Without preeclampsia (*n*=32)					
	Mean	SE	Median	Min.	Max.	Mean	SE	Median	Min.	Max.	
MiRNA-210	19.23	6.95	15.48	1.65	60.11	4.29	1.36	1.51	0.04	37.01	0.001*

SE, standard error. **P* value is statistically significant at <0.05.

study, where we included women with risk factors for PE.

The findings of the present study, where miRNA-210 was increased in preeclamptic women, agree with Youssef and Marei, 2019, who included 30 pregnant women with PE and subdivided them into mild and severe PE and compared them with 20 individuals as a control group. Their results showed that there was significant elevation of miRNA-210 and miRNA-155 in pregnant women who got PE compared with the healthy group. Moreover, they found that pregnant women with severe PE had significantly higher expression of miRNA-210 than mild preeclamptic group [5].

The study of Zang *et al.* [11], reported that the level of plasma miRNA-210 in mild and severe preeclamptic Chinese pregnant women was significantly elevated in comparison with normotensive pregnant women. The results of the present study were similar, as miRNA-210 was seen to be increased in matched gestational age preeclamptic women. However, they evaluated miRNA-210 in already mild and severe preeclamptic women.

Another study was done by Jairajpuri *et al.* [12], which showed that the expression of plasma miRNA-210 was elevated in preeclamptic pregnant women in comparison with normotensive pregnant women. The present study finding was similar to their results, as miRNA-210 was increased in preeclamptic women.

Gunel *et al.* [13], found that plasma miR-210 was significantly higher in preeclamptic pregnant women from 24-40 weeks of gestation compared with normotensive pregnant women. Our results were similar; however, again they evaluate miR-210 at gestational age of 26-40 weeks in already preeclamptic women.

Receiver operating characteristic (ROC) curve was conducted for assessment of miRNA-210 as a predictor of PE in pregnant women with risk factors for PE. The results showed that at a cutoff value of 2.28-fold change, plasma miRNA-210 was 87.5% sensitive and 68.8% specific for prediction of PE in pregnant women with risk factors for PE, with AUC of 0.852.

Ura *et al.* [14], found that miRNA-210 increased in serum samples of pregnant women in early second trimester (12-14 weeks) who subsequently developed PE in comparison with pregnant women who remained normotensive at the end of pregnancy. In the present study, the results were similar that miRNA-210 can be used as a predictive marker for PE in the second trimester. On the contrary, we evaluated miRNA-210 in second-trimester pregnant women only with risk factors for PE, compared with their study, which included all pregnant women.

Anton *et al.* [15], found that miRNA-210 was overexpressed in pregnant women at gestational age of 15-20 weeks who subsequently developed hypertensive disorder in pregnancy (HDP) compared with those who did not have HDP. Our results coincide with their study that miRNA-210 can be used as a predictive marker for PE in the second trimester of pregnant women; however, their study differed from the present study, as we evaluated miRNA-210 in women only with risk factors for PE, whereas their study included pregnant women with HDP not only PE.

On the contrary, Luque *et al.* [16], disagreed with our results and reported that there is no value to measure serum miRNA of pregnant women at first trimester to

predict PE. This could be attributed to the use of serum samples at first trimester not the second trimester as the present study.

Adel *et al*. [8], reported that there is overexpression of miRNA-210 in placental samples obtained from preeclamptic primigravida when compared with normotensive primigravida. Another study was done by Xue *et al*. [17], and found that miR-210 extracted from placental samples of pregnant women with severe PE was overexpressed in comparison with normotensive pregnant women. Zhu *et al*.[18] showed that there was upregulation of miRNA-210 in placenta of severe preeclamptic pregnant women and downregulation in mild preeclamptic pregnant women when compared with normal pregnant women.

Small sample size owing to financial constrains was the main limitation of the present study. Further studies with larger sample size are needed to confirm the value of using miRNAs in prediction of occurrence of PE.

In conclusion, plasma miRNA-210 levels were significantly elevated in pregnant women with risk factors for PE who subsequently developed PE compared with those without PE. Therefore, miRNA-210 may have a possible pathophysiological role in PE. Moreover, plasma miRNA-210 can be used as a marker for prediction of PE in pregnant women with risk factors for PE.

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Conflicts of interest

There are no conflicts of interest.

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