BENHA MEDICAL JOURNAL bmj

Print ISSN 1110-208X.

Online ISSN 2357-0016

Original article

MicroRNA-155 Expression and Endothelial Nitric Oxide Synthase (eNOS) Gene Variants in Egyptian Patients with Preeclampsia

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Received:

Accepted:

Abstract

Background: A significant cause of maternal and perinatal morbidity and mortality is preeclampsia, a pregnancy complication that has recently been responsible for 10 to 15% of fatalities among pregnant women globally. **Purpose:** to clarify the role of microRNA-155 and endothelial Nitric Oxide Synthase (eNOS) gene variants as diagnostic and predictive biomarkers in patients with preeclampsia. **Patients and Methods:** This case-control study was conducted at the Departments of Gynecology & Obstetrics and Medical Biochemistry & Molecular Biology, Faculty of Medicine, Benha University, during the period from January 2024 to January 2025. The study was carried out on 100 pregnant women who were categorized into 3 groups: control group: included 30 normotensive pregnant women, gestational hypertension group: 30 pregnant women with gestational hypertension and preeclampsia group: 40 pregnant women who were clinically diagnosed with preeclampsia. MicroRNA-155 expression and the frequency of eNOS gene variants were measured in all groups. **Results:** In comparison to the control group, the expression level of microRNA-155 was markedly increased in both the gestational hypertension and preeclampsia groups. Median expression values increased progressively across the groups: 0.99 in controls, 1.40 in gestational hypertension, and 7.0 in preeclampsia. Regarding eNOS gene polymorphism (G894T), the frequency of the T allele was higher in the preeclampsia group (31.25%) as compared to gestational hypertension (26.7%) and controls (21.7%). **Conclusions**: microRNA-155 may be a promising diagnostic and predictive biomarker for preeclampsia. Preeclampsia is more common in people who carry the eNOS 894T allele.

Keywords: microRNA-155, eNOS gene, Polymorphism, Preeclampsia

Introduction

Preeclampsia is a complex multisystem disease, diagnosed by sudden-onset hypertension (>20 weeks of gestation) and at least one other associated complication, including proteinuria, maternal organ dysfunction or uteroplacental dysfunction [(1)]

The condition of preeclampsia is life-threatening and is a significant cause of morbidity and mortality in both mothers and neonates. Pregnant women with preeclampsia have a lower life expectancy and are more likely to have diabetes, heart disease, and stroke. In addition, infants born to preeclamptic mothers are at an increased risk of developing cardiovascular and metabolic diseases, neurodevelopmental disabilities, perinatal mortality, and preterm birth. [(1)], As a result, maternal and neonatal complications can be averted by implementing early diagnosis and expeditious management. [(2)]

Preeclampsia is a multifactorial disease; however, the etiopathogenesis is still not fully understood [(3)], It is prevalent among women who have a history of preeclampsia, have been hypertensive for an extended period, and have not received systematic antihypertensive treatment. [(4)]

For more targeted prevention and treatment of preeclampsia, the biomarker-based classification of this illness is preferable [(5)]**,** The release of factors into the maternal bloodstream by a dysfunctional placenta is the primary cause of preeclampsia, which results in pervasive maternal endothelial dysfunction and systemic inflammation[(1)]**.**

MicroRNAs are single-stranded non-coding RNAs that vary in length from 20 to 24 nucleotides and are responsible for regulating the expression of mRNA (messenger RNA). Due to their widespread presence in all body fluids, microRNAs have proven to be useful biomarkers in numerous diseases. The reason behind this is because changes in microRNA expression can affect cellular homeostasis by altering the relative levels of mRNA**.** [(3)]

Elevated microRNA-155 levels in the placentas or circulation of preeclamptic women have been reported [(5)], For this reason, preeclampsia may develop if microRNA-155 were continuously released into the mother's bloodstream, stimulating the vascular endothelium. Consequently, these discoveries offered the potential to accurately identify the preeclampsia subgroup prior to delivery and even prior to the clinical manifestation. [(5)]. In the investigation of the potential contribution of upregulated microRNA-155 to hypertension, they discovered that microRNA-155 upregulation could induce hypertension by inhibiting the dilation of arterioles due to the suppression of eNOS [(5)]**.**

In preeclamptic women, endothelial dysfunction is regulated by many factors. eNOS which is one of these factors [(6)] is an important modulator of vascular tone by converting L-arginine to L-citruline to produce nitric oxide (NO), a step that normally reduces uteroplacental resistance [(7)]**.** Polymorphisms in eNOS reduce the availability of NO, which is essential for the dilation of the arteries of the mother during pregnancy, thereby increasing the risk of preeclampsia [(7)].

There are 28 exons in the gene that encodes eNOS, which is situated on chromosome 7 (7q36.1). One of the most extensively identified single nucleotide polymorphisms (SNPs) on this gene is the rs1799983 (G894T) variant. Nucleotide 894 is the site of this polymorphism, which results in the substitution of glutamate with aspartate in codon 298 [(8)]. Most recently, research has been conducted to investigate the correlation between this variation and the likelihood of developing preeclampsia [(8)].

Patients and Methods

Study Design:

This Case-Control study was conducted at the Departments of Gynecology & Obstetrics and Medical Biochemistry & Molecular Biology, Faculty of Medicine, Benha University during the period from January 2024 to January 2025.

Patients:

The study was carried out on 100 pregnant women who were categorized into 3 groups: Control group: included 30 normotensive pregnant women, gestational hypertension group: included 30 pregnant women with gestational hypertension and preeclampsia group: included 40 pregnant women who are clinically diagnosed with preeclampsia. Micro-RNA155 expression and eNOS gene variants were measured in the three studied groups.

Inclusion criteria for preeclampsia (diagnostic criteria): [(9)]

- new-onset hypertension after 20 weeks of gestation in pregnant women (with previously normal blood pressure):
  Blood pressure ≥140/90 mmHg, measured on two occasions at least 4 hours apart.
- In addition to at least one of the following:
  a. Proteinuria: (≥300 mg in a 24-hour urine collection, or Protein/creatinine ratio ≥0.3, or Dipstick reading of ≥1+ (only if quantitative methods unavailable)).
  b. Platelet count <100,000/μL.
  c. Serum creatinine >1.1 mg/dL or doubling of baseline.
  d. Elevated liver transaminases (twice the normal concentration).
  e. Pulmonary edema.
  f. New-onset cerebral or visual disturbances (not due to alternative diagnoses).

Exclusion criteria: Presence of pre-existing chronic hypertension**,** diabetes mellitus**,** twins or multiple pregnancy and elevated liver enzymes.

Methods:

- **Sampling**:

Venous blood samples were collected from pregnant women at 20-36 weeks of gestation. Blood samples were stored at -80°C for further investigation.

- **Estimation of microRNA-155 expression level according to manufacturer's instructions:**

1. **RNA Extraction:**

RNA was isolated from blood samples using ABT(Applied Biotechnology) Total and MicroRNA Mini Extraction Kit , Catalog number.EX02,(Ismailia, Egypt), following the manufacturer's instructions. The quantity of the eluted RNA was measured using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).

2. cDNA Synthesis and PCR Amplification:

The 1st step PCR (complementary DNA synthesis) was performed using 2X RT mix 50rxn (Solarbio® LIFE SCIENCES, Beijing, China) In a Veriti thermocycler (Gene Technologies Ltd, United Kingdom). cDNA served as a template for real-time PCR analysis using HERA PLUS SYBR® Green qPCR Kit (Willowfort, Birmingham, England)

The 2nd step using ABI Step One real-time PCR system, (Applied Biosystem, Waltham, MA), The conditions were as following: initial activation at 95°C for 2 minutes, cycling which is 40 cycles of (denaturation at 95°C for 10 seconds, annealing at 60°C for 1 minute and extension at 95°C for 15 seconds), using primers' sequences as shown in [**Table 1]**

Data analysis

Because the relative quantities of the microRNA-155, they are normalized against the relative quantities of endogenous control (GAPDH). Fold expression changes are calculated using the equation $2^{-\Delta\Delta}$ CT and expressed as relative units (RU) **(10).**

According to the Stepone software v2.2.2, the data were produced as sigmoid-shaped amplification curves in which the number of cycles was plotted against normalized reporter fluorescence (Rn) **[Figure 1]**. microRNA-155 gene expression levels in the apparent healthy control group were set to 1. The relative quantitation of target gene expression was normalized to that of human GAPDH. Gene expression fold changes were calculated using the equation 2-ΔΔCT **(10)**. ΔCt values were determined by subtracting the threshold cycle (Ct) value of GAPDH from the Ct value of microRNA-155. ΔΔCt was determined by subtracting the ΔCt of controls from ΔCt of diseased groups. The melting curve for microRNA-155 is shown in **[Figure 2]**.

- **Genotyping of eNOS single nucleotide polymorphism (G894T):**

Genotyping of G894T SNP was detected by polymerase chain reaction – based restriction fragment length polymorphism (PCR-based RFLP) on 3 steps:

1. **DNA extraction:** DNA was extracted from 200 µl blood sample; using ABT (Applied Biotechnology) Genomic DNA Mini Extraction Kit, Catalog number. EX01 (Ismailia, Egypt) according to manufacturer's instructions. The extracted DNA concentration was measured by Nanodrop Spectrophotometer 2000 (Thermo-Fisher Scientific, Wilmington, USA).

2. Genomic DNA amplification:

DNA amplification was done using primers' sequences of eNOSG894T as shown in [**Table 1].** Amplification was done in EntiLink™PCR Master Mix(red), (Wuhan, China).

3. **Digestion by Eco (241) BanII restriction enzyme:** Digestion was done for eNOSG894T by Eco24I (BanII) (10 U/μL), Catalog number: ER0281 (Waltham, Massachusetts, USA). DNA fragments (10 μl) and 100 bp ladder (5 μl) were separated on 3% agarose gel stained with 0.3ug/ml ethidium bromide. The bands (pre and post-digestion) were visualized using UV transilluminator (254 nm) and imaged with a digital camera 8 mega pixel. The image was analyzed by computer software (Alpha InoTech Gel Documentation System). Predigestion bands were visualized at 206 bp. Post-digestion; the G allele (uncut) gave one fragments (206 bp), while the T allele was (cut) gave 2 fragments (119 bp&87 bp) **[Figure 3]**.The success rate was 95%. The failed PCR were rerun by the same conditions.

Ethical consideration:

The Institutional Review Board- Faculty of Medicine, Benha University- Research Ethics Committee granted ethical approval for this study (Ms.40.11.2023). Written informed consents were submitted by all participants. The research followed the principles laid out in the Declaration of Helsinki by the World Medical Association.

Statistical analysis: The sample size was calculated using Epi-Info (Epidemiological information package) software version 7.2.5.0. Based on the prevalence rate of preeclampsia in Egypt according to a study which reported prevalence of 15-19% [(11)]. So, with expected frequency 15% and acceptable margin of error (MOE) of 5% and level of confidence of 80%, the total sample size will be 84 patients. The sample size will be increased to 100 patients to increase validity.

The data was entered into a spreadsheet using the Windows version of Microsoft Excel 2016, which is included in the 2016 Microsoft Office suite. Software giant Microsoft of the United States designed and programmed the spreadsheet. For this analysis, we consulted IBM's statistical package SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). We validated the normality of the distribution using the Kolmogorov-Smirnov test. The median, interquartile range, and mean ± standard deviation were the metrics that we used for continuous data. Categorical data was represented by percentages and numbers. What this meant for us was that the p-value was far less than 0.05.

Results

Our results regarding maternal age, gravidity and family history of hypertensive disorders showed a significant difference between the three groups ($P<0.001$) as women in preeclampsia group had significantly older age, higher proportion of primigravida and, higher proportion with positive family history compared to gestational hypertension and control groups **[Table 2].** Otherwise, no significant difference between the three groups regarding personal history or recurrence of hypertensive disorders) ($P>0.05$) **[Table 2].**

In the preeclampsia group, frequencies of genotypes and alleles weren`t significantly

different from controls, but the frequency of the T allele was higher in the preeclampsia group (31.25%) compared to controls (21.7%) (OR: 1.64; 95% CI: 0.76–3.57; *p*=0.250) **[Table 3 & Figure 4].**

There was statistically significant differences between the three groups regarding RQ by fold change for microRNA-155 expression (p<0.001). The expression level of microRNA-155 was significantly elevated in both the gestational hypertension and preeclampsia groups compared to controls. Median expression values increased progressively across the groups: 0.99 in controls, 1.40 in gestational hypertension, and 7.0 in preeclampsia **[Table 4].**

In preeclampsia group, there was a significant association between genotypes of the eNOS polymorphism with family history (p=0.007), personal history/recurrence (p=0.014), proteinuria/albuminuria (*p*=0.002), preterm labor (p<0.001), and placental abruption (p=0.020) as TT genotype showed higher proportions of positive family and personal histories, severe proteinuria, and adverse outcomes like preterm labor and placental abruption. While, there was no significant association was found between genotypes of the eNOS polymorphism with gravidity, IUGR (intrauterine growth restriction) and mode of delivery (*p*>0.05) **[Table 5]**.

In ROC (Receiver Operating Characteristic) curve, for controls vs. gestational hypertension and controls vs. preeclampsia, microRNA-155 showed excellent AUCs(Area Under the Curve) of 0.858 and 0.894, indicating strong discriminatory power (p<0.001 for both). In contrast, differentiation between preeclampsia and gestational hypertension was had a lower AUC of 0.668. Overall, microRNA-155 appears to be a promising biomarker for early discrimination between the three groups **[Table 6&Figure 5]**.

Table (1): Primers' sequences of (human microRNA-155 as a target gene & endogenous control genes) and (eNOSG894T).

Sequence name	5′ to 3′ sequence forward	5′ to 3′ sequence reverse	Reference
microRNA-155	5′ TGCTAATCGTGATAGGGG3′	5′ GAACATGTCTGCGTATCTC3′	[12].
GAPDH*	5′TGTTGCCATCAATGACCCCTT3′	5'CTCCACGACGTACTCAGCG3′	[13].
eNOSG894T	5′AAGGCAGGAGACAGTGGATGGA3′	5′CCAGTCAATCCCTTTGGTGCTCA3′	[14].

***Glyceraldehyde 3-phosphate dehydrogenase**

Table (2): The mean ± standard deviation of age & subjects' number and percentage of gravidity, family history & personal history of previous hypertensive disease in control, gestational hypertension and preeclampsia groups.

		Controls (N= 30)	Gestational hypertension (N= 30)	Preeclampsia (N= 40)	Test value	P-value
Age (years)	**Mean± SD**	23.67± 2.41	26.97± 2.06	29.48± 3.3	F= 44.07	<0.001** P_1<0.001 P_2<0.001 P_3<0.001
	Range	19- 29	24- 31	19- 36		
Gravidity	**Multigravida N (%)**	21 (70.0%)	13 (43.3%)	13 (32.5%)	X^2= 1.183	<0.001** P_1=0.068 P_2=0.004 P_3=0.498
	Primigravida N (%)	9 (30.0%)	17 (56.7%)	27 (67.5%)		
Family history	**Negative N (%)**	29 (96.7%)	24 (80.0%)	29 (72.5%)	X^2= 41.24	0.032* P1=0.103 P2=0.020 P3=0.234
	Positive N (%)	1 (3.3%)	6 (20.0%)	11 (27.5%)		
Personal history/ Recurrence	**Negative N (%)**	30 (100.0%)	25 (83.3%)	38 (95.0%)	X^2= 6.81	0.539MC
	Positive N (%)	0 (0.0%)	5 (16.7%)	2 (5.0%)		

p>0.05 is non-significant, *p≤0.05 is significant, **p≤0.01 is highly significant, SD: standard deviation, F: One-Way ANOVA Test, X^2: Chi-Square Test, MC: Monte-Carlo correction, P_1: Comparison between group controls & Gestational hypertension, P_2: Comparison between controls & preeclampsia, P_3: Comparison between Gestational hypertension & preeclampsia

Table (3): The frequency of genotypes and alleles of eNOS gene among control, gestational hypertension and preeclampsia groups.

		Controls (N= 30)	Gestational hypertension (N= 30)	Preeclampsia (N= 40)	OR1	95% CI	P value	OR2	95% CI	P value
Genotypes	GG	19 63.3%	17 56.7%	20 50.0%	0.76	0.27–2.13	0.792	0.58	0.22–1.52	0.334
	GT	9 30.0%	10 33.3%	15 37.5%	1.17	0.39–3.47	1.000	1.40	0.51–3.84	0.614
	TT	2 6.7%	3 10.0%	5 12.5%	1.56	0.24–10.05	1.000	2.00	0.36–11.10	0.690
Recessive model	GG+GT	28 93.3%	27 90.0%	35 87.5%	1.56	0.24–10.05	1.000	2.00	0.36–11.10	0.690
	TT	2 6.7%	3 10.0%	5 12.5%						
Dominant model	GG	19 63.3%	17 56.7%	20 50.0%	1.32	0.47–3.72	0.792	1.73	0.66–4.54	0.334
	TT+GT	11 36.7%	13 43.3%	20 50.0%						
Allele	G	47 78.3%	44 73.3%	55 68.75%	1.31	0.57–3.04	0.670	1.64	0.76–3.57	0.250
	T	13 21.7%	16 26.7%	25 31.25%						

p>0.05 is non-significant, *p≤0.05 is significant, **p≤0.01 is highly significant, X^2: Chi-Square Test, MC: Monte-Carlo correction, OR: Odds ratio, FET: Fischer Exact Test, OR^1: for gestational hypertension, OR^2: for preeclampsia

Table (4): Comparison of RQ by fold change for microRNA-155 expression among control, gestational hypertension and preeclampspia groups.

		Controls (N= 30)	Gestational hypertension (N= 30)	Preeclampsia (N= 40)	Test value	*P-value*
microRNA-155 expression	Median (IQR)	0.99 (0.98- 1.01)	1.40 (1.36- 1.43)	7.0 (6.66- 7.27)	Kw= 87.33	<0.001** P1<0.001 P2<0.001 P3<0.001
	Range	0.94- 1.04	1.27- 1.5	6.22- 8.04		

p>0.05 is non-significant, *p≤0.05 is significant, **p≤0.01 is highly significant, SD: standard deviation, IQR: Interquartile range, KW: Kruskal Wallis Test, X^2: Chi-Square test, P_1: Comparison between group controls & Gestational hypertension, P_2: Comparison between controls & preeclampsia, P_3: Comparison between Gestational hypertension & preeclampsia

Table (5): Association between genotypes of the eNOS polymorphism and different parameters in preeclampsia group.

		GG (N=20)	GT (N= 15)	TT (N=5)	Chi-Square Test	
					Test value	P-value
Gravidity	Multigravida	6 30.0%	5 33.3%	2 40.0%	0.190	>0.999MC
	Primigravida	14 70.0%	10 66.7%	3 60.0%		
Family history	Negative	18 90.0%	10 66.7%	1 20.0%	10.24	0.007**MC P1=0.088 P2=0.001 P3=0.069
	Positive	2 10.0%	5 33.3%	4 80.0%		
Personal history/ Recurrence	Negative	20 100.0%	15 100.0%	3 60.0%	14.737	0.014*MC P1=NA P2=0.003 P3=0.01
	Positive	0 0.0%	0 0.0%	2 40.0%		
IUGR	No	16 80.0%	12 80.0%	4 80.0%	0.00	>0.999MC
	Yes	4 20.0%	3 20.0%	1 20.0%		
proteinuria /albuminuria	+	17 85.0%	10 66.7%	0 0.0%	16.951	0.002**MC P1=0.179 P2<0.001 P3=0.001
	++	2 10.0%	4 26.7%	2 40.0%		
	+++	1 5.0%	1 6.7%	3 60.0%		
Preterm labor	No	19 95.0%	9 60.0%	0 0.0%	18.33	<0.001**MC P1=0.010 P2<0.001 P3<0.001
	Yes	1 5.0%	6 40.0%	5 100.0%		
Placental abruption	No	20 100.0%	12 80.0%	3 60.0%	7.086	0.020*MC P1=0.037 P2=0.003 P3=0.371
	Yes	0 0.0%	3 20.0%	2 40.0%		
Mode of delivery	Cesarean section	17 85.0%	12 80.0%	5 100.0%	1.176	0.728MC
	Vaginal	3 15.0%	3 20.0%	0 0.0%		

p>0.05 is non-significant, *p≤0.05 is significant, **p≤0.01 is highly significant, X^2: Chi-Square Test, MC: Monte-Carlo correction, P1: Comparison between GG & GT, P2: Comparison between GG & TT, P3: Comparison between GT & TT

Table (6): Validity (AUC) for RQ by fold change for microRNA-155 expression in differentiation between groups.

RQ by fold change for MicroRNA-155 expression	PPV	NPV	AUC	95%CI	P-value
Controls Vs G. hypertension	90 %	90%	0.858	0.743 to 0.935	<0.001**
Controls Vs Preeclampsia	77.1 %	91.4 %	0.894	0.743 to 0.923	<0.001**
Preeclampsia Vs G. hypertension	64.1%	83.9%	0.668	0.546 to 0.776	0.013*

AUC: Area Under a Curve, NPV: Negative predictive value, PPV: Positive predictive value, *: Statistically significant at p ≤ 0.05

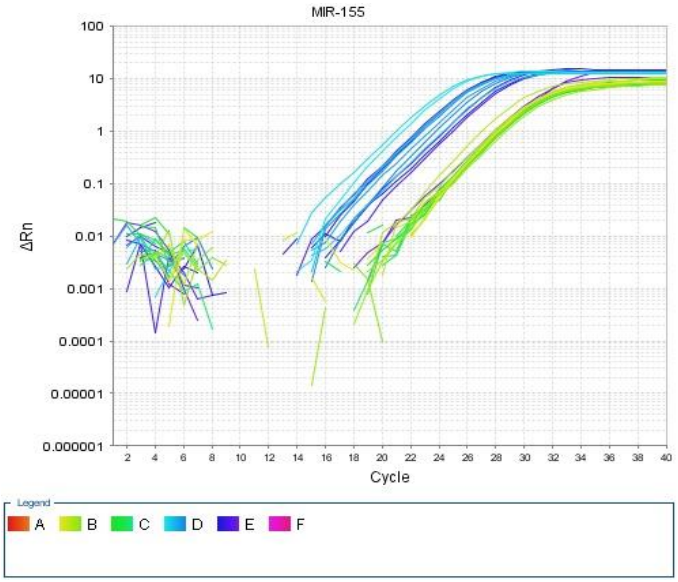

Figure (1): Amplification plot of microRNA-155 gene

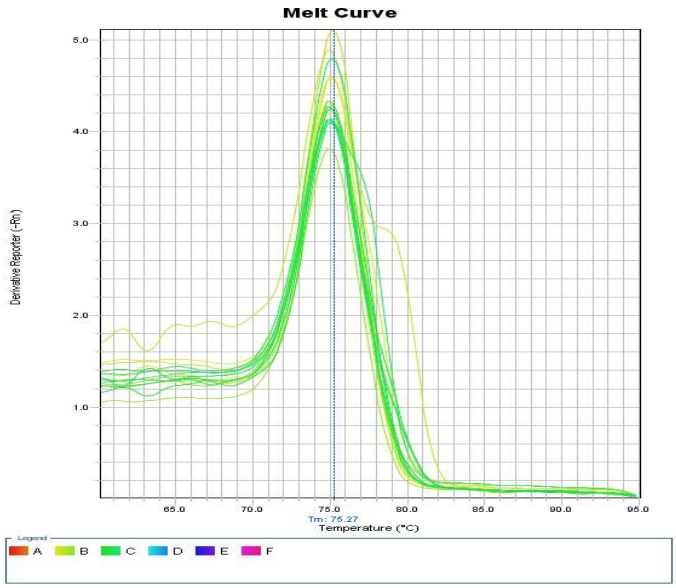

Figure (2): Melt curve for microRNA-155

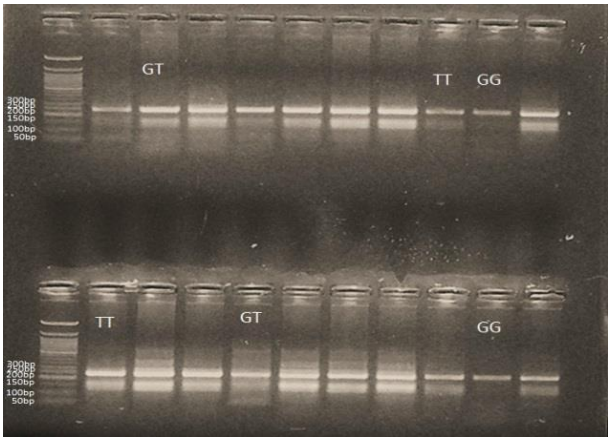

Figure (3): PCR-based RFLP of rs1799983 after digestion by BanII restriction enzyme [(GG → 206 bp), (TT → 119 bp & 87 bp) , (GT → 206, 119 & 87 bp)] on the gel.

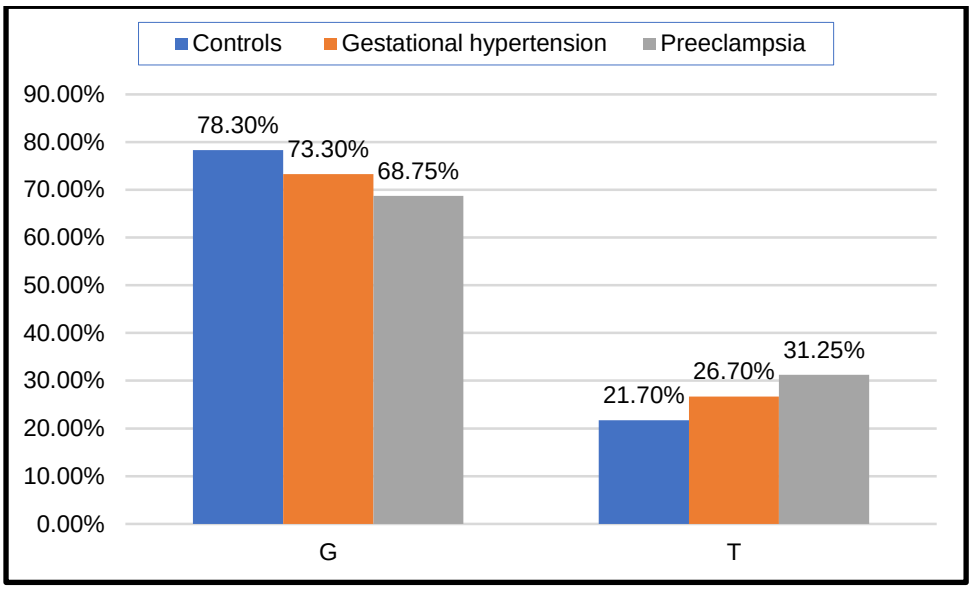

Figure (4): Comparison between the studied groups regarding alleles**.**

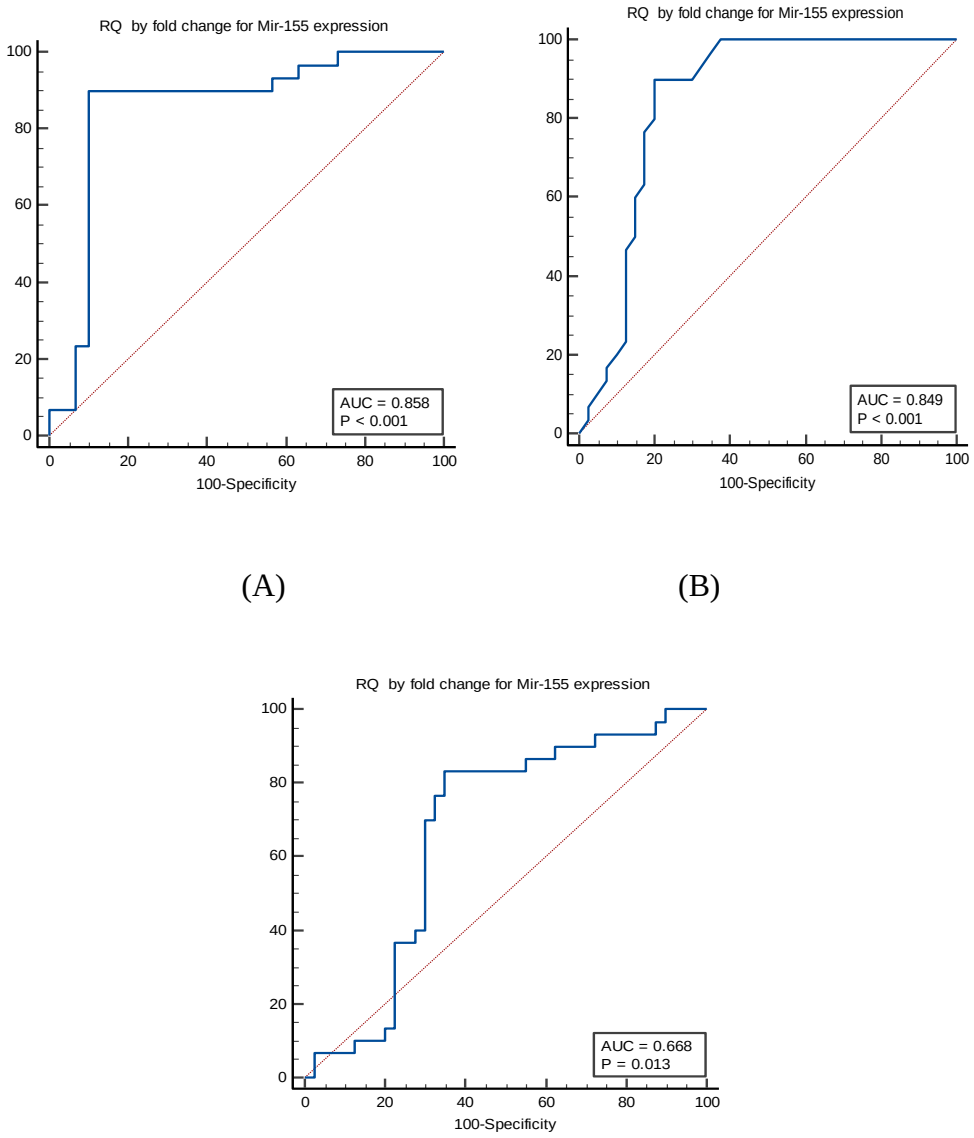

Figure (5): ROC curve of RQ by fold change for microRNA-155 expression in differentiation between: (A) Gestational hypertension group and controls, (B) preeclampsia group and controls and (C) preeclampsia and gestational hypertension groups.

Discussion

Worldwide, 3-6% of pregnancies are affected by preeclampsia, which is a significant cause of maternal mortality and morbidity. Proteinuria, new-onset hypertension, and end-organ dysfunction are the hallmarks of preeclampsia, which usually manifests after 20 weeks of gestation. **(15)**

Preeclampsia is a serious hypertensive disorder of pregnancy and a major contributor to maternal and neonatal death

and disability. Multiple pathological cascades work together to cause intravascular inflammation, oxidative stress, endothelial cell activation, and syncytiotrophoblast stress, all of which contribute to the disease's progression [16]

Although preeclampsia is a complex disorder with many potential causes, its exact origins are unknown. In order to decrease the incidence of preeclampsia and promote maternal and fetal well-being, it is crucial to understand the molecular mechanisms, explore suitable markers, implement targeted interventions, conduct comprehensive screening, and employ holistic strategies [16].

The small RNAs that are extremely conserved and usually between 20-24 nucleotides long are called microRNAs (miRNAs). The ability to recognize and bind to target genes in complementary or partially complementary ways is conferred upon mature microRNAs by their binding to messenger RNAs (mRNA), which in turn form RNA-induced silencing complexes. Organ development, cell proliferation, mRNA stability, and apoptosis are all impacted by this process, which also controls target gene expression and protein translation. [17]

Researchers found that pregnant women who were at risk of preeclampsia had higher levels of microRNA-155 in their blood or placentas. Therefore, our study focused on this [5].

Nitric oxide (NO) plays an important role during pregnancy by improving blood flow to the placenta and widening the mother's blood vessels, which helps deliver enough oxygen to the fetus. The placental arterioles of preeclamptic women relax in response to acetylcholine, but their impaired NO levels or function reduce this relaxation, leading to vasoconstriction and an increase in the mother's blood pressure and preeclampsia. In a complex biological setting, direct measurement of NO is very challenging because of NO's short half-life [7]. Because (NO) is quickly broken down into nitrite and nitrate when exposed to oxygen, measuring these two products of NO (called NOx) is a common way to gauge overall NO production. One big drawback of using nitrite/nitrate determination to estimate tissue NO level is that dietary nitrite and nitrate intake can have a significant impact on plasma NOx level. For example, nitrite can be found in cured meat and nitrate can be found in vegetables [7]. Therefore, eNOS genotyping is advised.

To better understand preeclampsia and find ways to manage or treat it, it would be helpful to identify different factors and candidate genes. Many populations have participated in genome wide association studies, which have shown the role of many genes and variations on those genes. Preeclampsia is also linked to endothelial dysfunction, the eNOS gene, and decreased NO production [7].

The aim of this study was to clarify the role of microRNA-155 and endothelial Nitric Oxide Synthase (eNOS) gene variants as diagnostic and predictive biomarkers in patients with preeclampsia.

In the present study, there was a significant in older age women in preeclampsia group when compared to gestational hypertension and control groups while gestational hypertension had

significantly older age compared to controls. These results were in line with **Gilboa et al [18],** It shown a strong correlation between the mother's age and the clinical symptoms and laboratory markers of severe preeclampsia, highlighting the need of tailoring therapy to the mother's age.

Kassa et al [19] findings indicate that preeclampsia is more common in mothers aged 35 and up, which may be attributable to a higher risk of vascular damage and an abnormally elevated lipid profile. Preexisting conditions, such as high blood pressure, diabetes, and obesity, are more common in the elderly and may contribute to this heightened risk.

Our study revealed a significant difference in gravidity between the preeclampsia and control groups (P<0.001), with a significantly higher proportion of primigravida women in the preeclampsia group compared to other groups. The results are corroborated by **Chang et al [20]**, who stated that preeclampsia risk can be tripled in cases of nulliparity, which has long been recognized as a risk factor. According to several theories, this could be because of immunological factors. One possible explanation is that preeclampsia develops because of impaired uteroplacental perfusion, which could be an indirect consequence of a mother's less-than-ideal response to fetal or paternal alloantigens.

In contrast **Chai et al [21]** found that multiparity was significantly associated with the risk of preeclampsia (P<0.05) and this may be due to different ethnicity, geographic location, and lifestyle.

The current study showed that the number of patients with family history of hypertension or preeclampsia revealed a significant difference between the three groups (control group, gestational hypertension group and preeclampsia group) (P=0.032), with positive family history more common in the preeclampsia group (27.5%) compared to gestational hypertension group (20.0%) and controls (3.3%). These results are in parallel with **Wu et al [22]** They found that preeclampsia is highly hereditary. Their research also found that the risk of preeclampsia was 2.6 times higher for mothers whose families had a history of the condition than for mothers whose families did not. There is a 2.79-fold increased risk of gestational hypertension in pregnant women with a positive family history of the illness.

This finding is explained by **Kassa et al [19]** who reported that the higher risk of preeclampsia in those with a positive family history may be due to hereditary variables that heighten the physiological susceptibility to the condition.

Interestingly, placental abruption showed a statistically significant difference between the three groups (p= 0.014), with 12.5% of the preeclampsia group affected, while it was absent in the other groups. These results were in line with **Yang et al [23]** who reported that placental abruption is a severe complication of preeclampsia and it is an important cause of maternal and infant mortality. **Yang et al [23]** illustrated this finding since the exact reason behind placental abruption is still a mystery. Scientists believe that aberrant trophoblast cell invasion and remodeling problems of the spiral arteries are major contributors to its development.

In our study, the mode of delivery significantly differs among groups (p= 0.015), with cesarean sections (CS) more frequent in preeclamptic patients (85%) compared to gestational hypertension (70%) and controls (53.3%). These results supported by **Pasokpuckdee et al [24]** who reported that women with preeclampsia had an almost two fold increase in the overall rate of cesarean sections. Additionally, they clarified that the severity of the disease often leads to a high rate of CS, which in turn causes immediate delivery to be commonly recommended due to the potential for severe maternal complications and fetal compromises.

The present study showed that there was a significant increase in microRNA-155 expression in preeclampsia group as compared to gestational hypertension and control groups. These findings are supported by the findings of **Wang et al [5]**, who discovered that microRNA-155 levels were significantly higher in preeclampsia than in healthy pregnant women. Also, **Shoeib et al [25]** discovered that microRNA-155 levels were greater in pregnancies characterized by preeclampsia as compared to those without preeclampsia. The regulation of placental angiogenesis by microRNA-155, which in turn inhibits trophoblastic migration, is likely to blame for this phenomenon.

Preeclamptic women had significantly different levels of microRNA-155 in their blood than women whose pregnancies were not complicated. When comparing healthy pregnancies to those in which preeclampsia developed later in the pregnancy, researchers found that the latter group had higher levels of this microRNA [3]

In our study, we demonstrated that the frequency of TT genotype was higher in preeclamptic women (12.5%) as compared to controls (6.7%) and gestational hypertensive women (10%). Women with mutant TT genotype may have a higher risk of preeclampsia than the women having wild type GG genotype. The T allele frequency was higher in preeclamptic women (31.25%) as compared to controls (21.7%) so there is increased risk of PE associated with the T allele.

Such finding is illustrated by **Shaheen et al [7]** according to those, who have studied the genetic risk factors for preeclampsia and found that these variants of eNOS gene can cause functional changes that could affect NO bioavailability. Also, **Tamanna et al [26]** who found that one of the main causes of preeclampsia is endothelial dysfunction caused by low NO bioavailability and one possible factor that lowers NO bioavailability is variations in the eNOS gene.

Although the T allele was more frequent in preeclamptic women compared to controls, this difference did not reach statistical significance. Thus, further studies with larger sample sizes may be needed to clarify its potential association with preeclampsia risk. These results supported by **Rao et al [27]** who demonestrated that eNOS 894 G>T locus had no significant difference between normal control group and preeclampsia group. Also, these results of our study opposed by **Abbasi et al [28]** who found a significant association between the eNOS 894 G>T and preeclampsia risk and this may be due to the different ethnicity.

Our results revealed that, in preeclampsia group, there was a significant association between genotypes of the eNOS polymorphism with family history (p=0.007), personal history/recurrence (p=0.014), proteinuria/albuminuria (p=0.002), preterm labor (p<0.001), and placental abruption (p=0.020) as TT genotype showed higher proportions of positive family and personal histories, severe proteinuria, and adverse outcomes like preterm labor and placental abruption. These results were in line with **Shaheen et al** [7] who reported that among patients with moderate preeclampsia, 72.3% had the G allele and 27.7% the T allele; among those with severe preeclampsia, 71.4% and 28.6%, respectively; and among those in the control group, 82.25% and 17.75%, respectively, were the frequencies.

Conclusions:

It could be concluded that microRNA-155 may be a promising diagnostic and predictive biomarker for preeclampsia. The eNOS 894T allele carriers are at greater risk of preeclampsia, and this might be a factor of genetic susceptibility.

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To cite this article: Sania K.Elwia , Naglaa F.Alhusseini , Mohamed A.Mohamed , Esraa A.Mohammed , Neama E. Abdelmaksoud. MicroRNA-155 Expression and Endothelial Nitric Oxide Synthase (eNOS) Gene Variants in Egyptian Patients with Preeclampsia. BMFJ XXX, DOI: 10.21608/bmfj.2025.402452.2529