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# Association of Keap1 (rs11085735) polymorphism and lncRNA MEG3 hypermethylation status with the risk of preeclampsia

Maryam Zangeneh<sup>1†</sup>, Sara Heydarian<sup>1†</sup>, Zahra Seifi<sup>1†</sup>, Maryam Kohsari<sup>2†</sup> and Zohreh Rahimi<sup>2,3\*</sup> 

## Abstract

**Background:** Preeclampsia (PE) is one of the complications of pregnancy. The pathogenesis of PE has not been completely understood. The aims of the present study were to investigate the role of Keap1 (rs11085735) variants and the methylation status of long non-coding RNA (lncRNA) MEG3 in the risk of PE.

**Methods:** In a case–control study, 150 pregnant women, including 75 PE patients and 75 healthy pregnant women recruited from Western Iran with Kurdish ethnic background, were studied for Keap1 variants using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The methylation status of lncRNA MEG3 was investigated using methylation-specific PCR (MSP) among 50 preeclamptic patients and 50 controls.

**Results:** The frequency of Keap1 A allele was significantly lower (5.3%) in preeclamptic patients compared to controls (12.7%,  $p = 0.024$ ). The frequencies of hemimethylated (UM) and full methylated (MM) lncRNA MEG3 were 94 and 6% ( $p = 0.04$ ), respectively, in all patients, 86.4, and 13.6% ( $p = 0.04$ ), respectively, in patients with severe preeclampsia and 98 and 0% in controls. The frequency of full methylated lncRNA MEG3 was 14.3% in early-onset preeclampsia and 2.8% in late-onset preeclampsia ( $p = 0.12$ ). Patients with PE had significantly higher levels of liver biomarkers (including ALT, AST, ALP, and total bilirubin) and lower PLT counts compared to healthy pregnant women.

**Conclusion:** The present study suggests the presence of hypermethylation status of lncRNA MEG3 in preeclampsia that might contribute to the pathogenesis and development of preeclampsia. Also, Keap1 rs11085735 polymorphism might be involved in the risk of preeclampsia.

**Keywords:** Preeclampsia, Keap1 (rs11085735), lncRNA MEG3, Methylation, Western Iran

## Introduction

Preeclampsia (PE) is the most challenging disorder of pregnancy [1]. The pathogenesis of PE is not well understood [2, 3]. Evidence suggested several theories in this regard as trophoblast cell invasion dysfunction into the

myometrium could play a leading role in PE incidence [4–6]. Endothelial cells dysfunction by trophoblast invasion and fluctuations in blood flow causes oxidative stress in trophoblast cell. Oxidative stress, a homeostatic imbalance dysregulation between oxidants and antioxidants, occurs in the early-onset PE and makes it progress [4]. Pregnant women with trophoblast pathologic invasion had a lower total antioxidant capacity [7]. One of the cytoprotective pathways against oxidative stress is the Nrf2-Keap1 pathway. Oxidative stress disrupts the KEAP1-NFE2L2 complex, with consequences of Nrf2

\*Correspondence: [zrahimi@kums.ac.ir](mailto:zrahimi@kums.ac.ir); [rahimizus@yahoo.com](mailto:rahimizus@yahoo.com)

<sup>†</sup>Maryam Zangeneh, Sara Heydarian, Zahra Seifi and Maryam Kohsari have equal role in preparing the manuscript

<sup>2</sup> Department of Clinical Biochemistry, Kermanshah University of Medical Sciences, Daneshgah Avenue, P.O. Box 67148-69914, Kermanshah, Iran  
Full list of author information is available at the end of the article

translocation to the nucleus, which acts as a regulatory factor and promotes the expression of antioxidant response element-dependent genes. Kelch-like ECH-associated protein 1 (Keap1) is a cysteine-rich-protein and critical negative regulator of Nrf2; therefore, it acts as a sensor for oxidative and electrophile stress. In normal conditions (not under oxidative stress), Keap1 directs NRF2 through ubiquitination to proteasomal degradation to preserve cellular hemostasis [8].

Recently, two studies investigated the effect of the Keap1-Nrf2 pathway on the biological function of trophoblast cells (HTR8/SVneo cells) in the oxidative stress model at the cellular level. Their results demonstrated the expression level of Keap1 in the placenta of patients with PE was slightly lower than the normal placenta [3, 6]. Another important factor is maternally expressed gene 3 (MEG3), a long non-coding RNA (lncRNA) member, which contributes to apoptosis, proliferation, and growth. LncRNA MEG3 is typically produced by placenta cells. It has been suggested that a reduction of MEG3 in placental samples of patients with preeclampsia accounts for increased apoptosis and decreased migration that might have a role in remodeling failure of uterine spiral and PE incidence [9]. Also, Yu et al. proposed decreased MEG3 via downregulation of epithelial-mesenchymal transition (EMT) by the TGF- $\beta$  pathway inhibitor, which can be involved in the occurrence of PE [5].

There are no available reports related to the role of Keap1 polymorphism and MEG3 methylation status in the pathogenesis of preeclampsia. To find the effect of Keap1 variants and MEG3 methylation status in the risk of PE, we aimed to investigate the frequency of Keap1 (rs11085735) variants and the lncRNA MEG3 methylation status in preeclamptic patients compared with healthy individuals in a population from Western Iran with Kurdish ethnic background.

## Methods

In a case–control study, 150 women consisted of 75 women with PE and 75 women with normal pregnancy as controls were investigated. All participants were selected from women who referred to the obstetric clinic of Imam Reza Hospital of Kermanshah University of Medical Sciences (Kermanshah, Iran). The methylation status of lncRNA MEG3 was studied in 100 women. The number of healthy individuals in both analyses was equal to patients.

Preeclampsia was defined as systolic and diastolic blood pressure equal to or higher 140 and 90 mmHg, the presence of proteinuria confirmed by excreting more than 300 mg protein in a 24-h urine test or detecting 30 mg/dl or more in a randomized urine sample (1 + reaction on dipstick), the urine protein to creatinine

ratio higher than 0.3. Severe preeclampsia was defined as having one or more of the following criteria, blood pressure equal or higher than 160/110 mmHg after twice measurement and 6 h apart, while the patient was resting on the bed. Proteinuria > +3 on 2 random urine samples collected at least 4 h apart, elevated serum level of creatinine and transaminases, thrombocytopenia, visual disturbances, upper abdominal pain, headache, and fetal growth restriction [10]. Early-onset preeclampsia was defined as preeclampsia before 34 weeks of gestation [11] that was detected in 20 patients. Patients with diabetes, gestational diabetes, infection, premature rupture of the bladder, oligohydramnios, polyhydramnios, valvular heart disease, chronic hypertension, and chronic kidney disease were excluded from the study.

Six ml blood samples were obtained from each participant. Two ml of each sample was transferred to a falcon tube containing a 0.5 mM EDTA and used for DNA extraction and further genetic analysis. The remaining blood sample without anticoagulant was centrifuged at 300g for 10 min. Serum separated and restored in cryotubes at  $-20^{\circ}\text{C}$ . The serum concentration of blood urea, creatinine, total and direct bilirubin, and the activity of liver enzymes (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) were measured by an enzymatic colorimetric assay kit (Pars Azmon kit, Tehran, Iran) according to the manufacturer's instructions and performed by automated RA-1000 (Technicon, CA).

Genomic DNA was extracted from peripheral blood leukocytes using the phenol–chloroform method [12]. The Keap1 polymorphism (rs11085735) was detected using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Using the forward primer of 5' CTC AGC CTC CCA AAG TCC CT 3' and the reverse primer of 5' CTC CCA CGG CTG CAT CCA C 3' a fragment with 354-bp was amplified. The obtained 354-bp PCR product was digested with HinfI restriction enzyme [13].

Methylation-specific PCR (MSP). For bisulfite treatment of extracted DNA, 30  $\mu\text{L}$  of melted 2% low melt agarose was added to about 200 ng DNA and was incubated at  $65^{\circ}\text{C}$  for a few minutes. To denature DNA strands, it was incubated with NaOH at  $80^{\circ}\text{C}$  for 15 min and re-solidifies the agarose bead by chilling on ice. By adding 10 mM hydroquinone and 40.5% sodium bisulfite at  $50^{\circ}\text{C}$  for about 4 h, treatment was done. To stop bisulfite reaction and desulfonation, the beads were washed with Tris–EDTA buffer, 0.2 M NaOH, 1 M hydrochloric acid, and pure water, respectively. The washed beads were diluted in 30  $\mu\text{L}$  deionized water, heated at  $80^{\circ}\text{C}$  and aliquoted for PCR reaction. The MSP method was used to determine the methylation status of the lncRNA MEG3

gene promoter. After bisulfite treatment, methylation status was detected using two pairs of specific primers, unmethylated primers (forward GAGGATGGTTAGTTA TTGGGGT and reverse CCACCATAACCAACACCC TATAATCACA) and methylated primers (forward GTT AGTAATCGGGTTTGTTCGGC and reverse AATCAT AACTCCGAACACCCGCG). In MSP one, primer pairs are specific for detection of methylated status of gene and the other pairs are used for identifying the unmethylated template [14].

### Statistical analysis

The frequencies of Keap1 variants and the lncRNA MEG3 methylation status in preeclamptic patients and controls were investigated using the  $\chi^2$  test. The Student *t* test was used to compare the quantitative data. Quantitative data are presented by mean  $\pm$  SD and qualitative data are demonstrated by frequency and percentage. The SPSS version 16.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Statistical significance level was considered at the  $p < 0.05$ .

### Results

Characteristics of patients with PE and healthy controls are represented in Table 1. At the time of sampling, gestational age was  $37.5 \pm 2.1$ ,  $35.4 \pm 2.4$  ( $p < 0.001$ ), and  $35.2 \pm 2.2$  ( $p < 0.001$ ) weeks, in controls, mild-, and severe preeclampsia, respectively. All preeclamptic patients had significantly higher levels of liver function biomarkers (ALT, AST, ALP, and total bilirubin) compared to the healthy controls. There were 28 patients with severe

preeclampsia. The mean and the range of platelets (PLT) levels were  $187.2 \pm 47.8$  (128–299),  $153.1 \pm 38.1$  (92–277), and  $152.2 \pm 33.6$  (104–220)  $\times 10^3/\mu\text{L}$  in controls, in mild-, and in severe preeclampsia, respectively. Preeclamptic patients had a significantly lower level of PLT compared to controls. In all preeclamptic patients and also in mild PE, the BMI values before pregnancy ( $26.3 \pm 6.2$ ,  $p = 0.008$ , and  $26.5 \pm 5.4$ ,  $p = 0.037$   $\text{kg}/\text{m}^2$ , respectively) and after pregnancy ( $30.2 \pm 7.2$ ,  $p = 0.004$ , and  $30.4 \pm 6.6$   $\text{kg}/\text{m}^2$ ,  $p = 0.024$ , respectively) were significantly higher than those in controls ( $22.8 \pm 9.2$  and  $25.4 \pm 11.9$   $\text{kg}/\text{m}^2$ , respectively).

Distribution of Keap1 variants and lncRNA MEG3 methylation status in both patients and controls is demonstrated in Table 2. There was no significant difference in the frequency of Keap1 genotypes comparing PE patients with controls. However, the frequency of Keap1 A allele was significantly lower (5.3%) in preeclamptic patients compared to controls (12.7%,  $p = 0.024$ ). The unmethylated lncRNA MEG3 (UU) was not detected among patients. Further, the lncRNA MEG3 full methylated (MM) was not found in controls. The frequencies of hemi methylated (UM) and full methylated lncRNA MEG3 were 94 and 6% ( $p = 0.04$ ), respectively, in all patients, 86.4, and 13.6% ( $p = 0.04$ ), respectively, in patients with severe preeclampsia and 98 and 0% in controls (Table 2). In Table 3, the frequencies of Keap1 genotypes and lncRNA MEG3 methylation status have been compared between patients with early-onset preeclampsia with late-onset preeclampsia. The frequency of Keap1 genotypes was not significantly different

**Table 1** Characteristics of patients and controls

Variables	All patients <i>n</i> = 75	Severe preeclampsia <i>n</i> = 28	Mild preeclampsia <i>N</i> = 47	Controls <i>n</i> = 75
Age, year	$31.5 \pm 6.6$ ; $p = 0.018$	$29.6 \pm 6.9$ ; $p = 0.8$	$32.6 \pm 6.2$ ; $p = 0.008$	$29 \pm 6.1$
Gestational age, weeks	$35.3 \pm 2.2$ ; $p < 0.001$	$35.2 \pm 2.1$ ; $p < 0.001$	$35.4 \pm 2.4$ ; $p < 0.001$	$37.5 \pm 2.1$
Weight(before pregnancy), kg	$71.2 \pm 10.7$ ; $p = 0.09$	$70.9 \pm 13.4$ ; $p = 0.5$	$71.5 \pm 9.1$ ; $p = 0.26$	$68 \pm 12$
Weight ( after pregnancy), kg	$81.3 \pm 12.7$ ; $p = 0.4$	$80.3 \pm 14.4$ ; $p = 0.9$	$81.9 \pm 11.8$ ; $p = 0.66$	$79.7 \pm 12.7$
BMI ( before pregnancy), $\text{kg}/\text{m}^2$	$26.3 \pm 6.2$ ; $p = 0.008$	$25.9 \pm 7.5$ ; $p = 0.1$	$26.6 \pm 5.4$ ; $p = 0.037$	$22.8 \pm 9.2$
BMI ( after pregnancy), $\text{kg}/\text{m}^2$	$30.2 \pm 7.2$ ; $p = 0.004$	$29.9 \pm 8.4$ ; $p = 0.1$	$30.4 \pm 6.6$ ; $p = 0.024$	$25.4 \pm 11.9$
Systolic blood pressure, mm Hg	$152 \pm 16.3$ ; $p < 0.001$	$165 \pm 17.5$ ; $p < 0.001$	$144.4 \pm 9.4$ ; $p < 0.001$	$113.3 \pm 7.5$
Diastolic blood pressure, mm Hg	$93.3 \pm 15.2$ ; $p < 0.001$	$102.6 \pm 11.9$ ; $p < 0.001$	$87.8 \pm 14.3$ ; $p < 0.001$	$73.5 \pm 5.4$
Platelet count $\times 10^3/\mu\text{L}$	$152.7 \pm 36.3$ ; $p = 0.026$	$152.2 \pm 33.6$ ; $p = 0.02$	$153.1 \pm 38.1$ ; $p = 0.015$	$187.2 \pm 47.8$
Urea, mg/dL	$26.2 \pm 7.3$ ; $p < 0.001$	$26.8 \pm 7.4$ ; $p < 0.001$	$25.8 \pm 7.3$ ; $p = 0.001$	$19.6 \pm 6.5$
Creatinine, mg/dL	$0.7 \pm 0.2$ ; $p = 0.3$	$0.8 \pm 0.2$ ; $p = 0.5$	$0.75 \pm 0.2$ ; $p = 0.38$	$1.4 \pm 3.8$
AST, U/L	$39.2 \pm 45.5$ ; $p = 0.001$	$38.3 \pm 47.3$ ; $p = 0.1$	$39.7 \pm 45$ ; $p = 0.046$	$19.9 \pm 7.1$
ALT, U/L	$28.5 \pm 27.5$ ; $p < 0.001$	$28.7 \pm 25$ ; $p = 0.05$	$28.5 \pm 29$ ; $p = 0.028$	$15.3 \pm 8$
ALP, U/L	$350.5 \pm 131$ ; $p = 0.006$	$352.6 \pm 132.8$ ; $p = 0.05$	$349.3 \pm 131.4$ ; $p = 0.036$	$280.1 \pm 108.2$
Direct bilirubin, mg/dL	$0.31 \pm 0.13$ ; $p = 0.2$	$0.2 \pm 0.08$ ; $p = 0.9$	$0.33 \pm 0.16$ ; $p = 0.21$	$0.2 \pm 0.1$
Total bilirubin, mg/dL	$0.76 \pm 0.29$ ; $p = 0.001$	$0.6 \pm 0.2$ ; $p = 0.03$	$0.77 \pm 0.3$ ; $p = 0.006$	$0.57 \pm 0.26$

**Table 2** Distribution of Keap1 and Lnc RNA MEG3 genotypes in patients with preeclampsia, severe- and mild preeclampsia patients and controls

Parameters	All preeclamptic patients <i>N</i> = 75	Severe preeclampsia patients <i>N</i> = 28	Mild preeclampsia patients <i>N</i> = 47	Controls <i>N</i> = 75
<i>Keap1</i> genotypes				
AA	5 (6.7%)	1 (3.6%)	4 (8.5%)	7 (9.3%)
AC	0 (0.0%) $\chi^2 = 2.9$ ; $p = 0.08$	0 (0.0%) $\chi^2 = 0.67$ ; $p = 0.4$	43 (91.5%)	5 (6.7%)
CC	70 (93.3%) $\chi^2 = 0.5$ ; $p = 0.4$	27 (96.4%) $\chi^2 = 1.1$ ; $p = 0.2$	0 (0)	63 (84.0%)
<i>Keap1</i> alleles				
A	8 (5.3%)	2 (3.6%)	8 (8.5%)	19 (12.7%)
C	142 (94.7%) $\chi^2 = 5.1$ ; $p = 0.02$	54 (96.4%) $\chi^2 = 3.7$ ; $p = 0.055$	86 (91.5%) $\chi^2 = 2.3$ ; $p = 0.006$	131 (87.3%)
	<i>N</i> = 50	<i>N</i> = 22	<i>N</i> = 28	<i>N</i> = 50
<i>Lnc RNA MEG3</i> genotypes				
UU	0 (0.0%)	0 (0.0%)	0 (0%)	1 (2.0%)
UM	47 (94.0%) $\chi^2 = 0.95$ ; $p = 0.3$	19 (86.4%) $\chi^2 = 0.38$ ; $p = 0.5$	28 (100)	49 (98.0%)
MM	3 (6.0%) $\chi^2 = 4.0$ ; $p = 0.04$	3 (13.6%) $\chi^2 = 4.0$ ; $p = 0.04$	0 (0)	0 (0.0%)

\*Overall  $\chi^2$  comparing Keap1 genotypes between all preeclamptic patients and controls was 5.7;  $p = 0.05$

\*\*Overall  $\chi^2$  comparing Keap1 genotypes between severe preeclamptic patients and controls was 0.3;  $p = 0.2$

\*\*\*Overall  $\chi^2$  comparing Keap1 genotypes between mild preeclamptic patients and controls was 3.3;  $p = 0.18$

\*\*\*\*Overall  $\chi^2$  comparing Keap1 genotypes between mild- and severe- preeclampsia was 0.68;  $p = 0.4$

# Overall  $\chi^2$  comparing LncRNA MEG3 genotypes between all preeclamptic patients and controls was 4.0;  $p = 0.1$

## Overall  $\chi^2$  comparing LncRNA MEG3 genotypes between severe preeclamptic patients and controls was 7.4;  $p = 0.02$

### Overall  $\chi^2$  comparing LncRNA MEG3 genotypes between mild preeclamptic patients and controls was 0.57;  $p = 0.45$

#### Overall  $\chi^2$  comparing LncRNA MEG3 genotypes between mild- and severe preeclampsia was 4.1;  $p = 0.044$

**Table 3** Distribution of Keap1 and LncRNA MEG3 genotypes in early-onset and late-onset preeclampsia

Parameters	Early-onset preeclampsia <i>N</i> = 16	Late-onset preeclampsia <i>N</i> = 59
<i>Keap1</i> genotypes		
AA	1 (6.3%)	4 (6.8%)
AC	0	0
CC	15 (93.8%)	55 (93.2%)
	<i>N</i> = 14	<i>N</i> = 36
<i>LncRNA MEG3</i> genotypes		
UU	0	0
UM	12 (85.7%) $\chi^2 = 0.1$ ; $p = 0.6$	35 (97.2%)
MM	2 (14.3%)	1 (2.8%)

\*Overall  $\chi^2$  comparing Keap1 genotypes between early-onset preeclamptic patients and late-onset preeclamptic patients was 0.006;  $p = 0.94$

\*\*Overall  $\chi^2$  comparing LncRNA MEG3 genotypes between early-onset preeclamptic patients and late-onset preeclamptic patients was 2.4;  $p = 0.12$

comparing early-onset with late-onset preeclampsia. The frequency of full methylated LncRNA MEG3 was 14.3% in early-onset and 2.8% in late-onset preeclampsia ( $p = 0.12$ ). Among early-onset preeclampsia, 12 out

of 14 patients (85.7%) had BMI before pregnancy  $> 25$  kg/m<sup>2</sup> compared to 21 out of 36 (58.3%) in late-onset preeclampsia ( $\chi^2 = 3.36$ ,  $p = 0.066$ ). Also, the methylation status of LncRNA MEG3 according to the BMI indicated

the absence of full methylated lncRNA MEG3 among patients with BMI before pregnancy  $\leq 25$  kg/m<sup>2</sup>. However, its frequency was 9.1% among patients with BMI before pregnancy  $> 25$  kg/m<sup>2</sup> ( $\chi^2 = 1.64$ ,  $p = 0.2$ ).

Analysis of concomitant presence of methylated and unmethylated lncRNA MEG3 with Keap1 genotypes indicated the lack of a significant difference between patients with PE and controls (Table 4).

## Discussion

The present study investigated the biochemical and hematological parameters and also the frequency of Keap1 variants and lncRNA MEG3 methylation status in preeclamptic patients compared with women with normal pregnancy in a population from Western Iran. In patients with PE, a significant difference in liver function tests (ALT, AST, ALP, and total bilirubin) was detected compared with healthy pregnant women. Patients with PE had higher serum levels of liver function tests except for direct bilirubin. During pregnancy, hormonal changes cause dysfunction of hepatocytes with a slight increase in ALP level. On the other hand, the serum concentration of ALT and AST usually remains normal. Evaluation of women with PE indicated dramatically increased serum levels of ALT, AST, ALP, and total bilirubin in these patients compared to women with normal pregnancy that might be involved in the PE development. However, the direct bilirubin level did not significantly increase in patients compared to controls [15–17]. Also, elevated liver enzymes eventuate with emerging of HELLP syndrome. HELLP syndrome is a pregnancy disorder with clinical emersion hemolysis, elevated liver enzymes, and low PLT count and generally is considered as a type of severe PE [18]. Studies demonstrated HELLP can be overlapped with early-onset preeclampsia [19]. Consistent with reports, our results showed a significantly lower PLT level in all PE, severe-, and mild PE subjects than the healthy pregnant women. Platelet and ALP levels have been suggested to have potential role in the prediction of PE and its severity [20]. Evaluation of renal function can

effectively diagnose gestational hypertension, as elevated urea and creatinine levels might be diagnostic and predictive biomarkers of PE [21, 22]. We found a higher level of blood urea in patients than in healthy subjects.

Previous studies have represented obesity as a risk factor for PE [23]. Our study demonstrated that mothers in the PE group before pregnancy were overweight and had BMI above 25 kg/m<sup>2</sup>.

In the present study, we investigated the frequency of Keap1 variants. The Nrf2-Keap1 pathway is one of the leading mechanisms against oxidative stress. Keap1 acts as an adaptor protein for Cul3-based E3 ubiquitin ligase; therefore, via Nrf2 proteasomal degradation regulates its function negatively. The expression of Nrf2 downstream target genes is regulated by genetic variations in the Keap1 or NFE2L2 gene [8]. There is no available study related to the frequency of Keap1 genotypes in preeclampsia and their influence on the risk of preeclampsia. Some studies established that the rs11085735 polymorphism of Keap1 is correlated with several diseases. Siedlinski et al., in a prospective study, suggested that rs11085735 in Keap1 might be associated with the risk of chronic obstructive pulmonary disease [24]. The minor allele of Keap1 (A) was associated with lower Keap1 and higher nuclear Nrf2 expression and reduced the overall survival in breast cancer patients treated with radiotherapy and tamoxifen [25]. Testa et al. demonstrated that the rs110857735 polymorphism of the Keap1 gene strongly predicted the incidence of cardiovascular events in chronic kidney disease patients [26]. Studies on trophoblast cell line (HTR8 / SVneo) obtained from PE patients showed decreased Keap1 and increased Nrf2 expression in HTR8 / SVneo cells [3, 6]. We did not find an association between the rs11085735 Keap1 genotypes with the risk of PE. However, significantly lower frequency of Keap1 A allele was detected in patients with PE than controls. The minor A allele of Keap1 is associated with lower Keap1 and higher Nrf2 expression. The Nrf2 overexpression (Keap1 knockdown) might have a protective role in some diseases such as diabetic neuropathy. However, some studies suggested increased Nrf2 expression exacerbated the obesity and insulin resistance phenotypes [13]. Our findings suggest lower frequency of Keap1 A allele in patients with PE compared to healthy pregnant women that might result in higher Keap1 and lower Nrf2 levels and increased PE risk.

lncRNA is a transcript with more than 200 nucleotides that plays role in biological regulations such as DNA methylation, cell-cycle regulation, apoptosis, and angiogenesis and with the incidence and the progression of numerous diseases. Recent observations illustrated the role of lncRNA in trophoblast motility and migration. The lncRNA MEG3 is one of the long non-coding RNAs

**Table 4** Analysis of concomitant presence of Keap1 and lncRNA MEG3 alleles in patients with preeclampsia compared to controls

Keap1 alleles	lncRNA MEG3 methylation status	Haplotype frequency in patients %	Haplotype frequency in controls %
A	Unmethylated	5	8
C	Unmethylated	42	43
A	Methylated	5	4
C	Methylated	48	45

Overall  $\chi^2 = 0.95$ ,  $p = 0.81$



that located in chromosome 14q. The lncRNA MEG3 downregulation is correlated with PE through suppression of migration, invasion, and epithelial-mesenchymal transition of placental trophoblast cells [27]. Zhang et al. indicated downregulation of MEG3 triggered apoptosis and repressed cell migration in trophoblast cells in vitro; consequently, it correlated with uterine spiral artery remodeling failure and participated in PE pathogenesis. The lncRNA MEG3 provoked apoptosis of trophoblast cells via NF- $\kappa$ B and Bax/Bcl2 signaling pathways [9]; also, Yu et al. declared that the lncRNA MEG3 downregulation is involved in the pathogenesis of PE through suppression of migration, invasion, and EMT of placental trophoblast cells by TGF- $\beta$  pathway inhibitors [5]. Epigenetic changes such as DNA methylation are influenced by environmental factors including life style [28].

In the current study, we found a significant difference between patients with PE and also severe PE with the healthy group regarding methylation status of lncRNA MEG3. Patients with PE had a higher frequency of lncRNA MEG3 methylation than the healthy group. Also, among patients with preeclampsia, women with early-onset PE had a higher frequency of lncRNA MEG3 methylation than late-onset preeclampsia. Hypermethylation of this gene decreases its expression and the lncRNA MEG3 downregulation is involved in PE pathogenesis [5]. So, it seems hypermethylation of this gene in preeclamptic patients especially in severe preeclampsia could be associated with the risk of preeclampsia and its severe form. Also, the frequency of lncRNA MEG3 full methylated was higher among overweight patients compared to normal weight ones. Further, higher frequency of overweight was detected among patients with early-onset preeclampsia compared to late-onset preeclampsia. So, there might be a role for obesity in the pathogenesis of severe form of preeclampsia through lncRNA MEG3 methylation that needs to be elucidated. We did not detect a synergism between Keap1 variants and lncRNA MEG3 methylation in the risk of preeclampsia.

Animal and cell line studies suggested that lncRNA MEG3 acts as a competitive endogenous RNA for Nrf2 by sponging miR-34a. They reported that the lncRNA MEG3 through upregulation of Nrf2 protected hepatic tissue of mice against ischemia reperfusion injury [29].

## Conclusions

We found biochemical and hematological parameters such as ALT, AST, ALP, total bilirubin, urea, and PLT levels were significantly different in patients with PE compared to the healthy pregnant women. Findings of the present study indicated the implication of biochemical and hematological alterations in the pathophysiology of PE and could help in clinical practice for diagnosis and

monitoring of preeclampsia using laboratory tests. The present study detected increased hypermethylation status of lncRNA MEG3 in patients with PE compared to controls that could be associated with the risk of preeclampsia and its severe form. Also, the role of obesity in the pathogenesis of preeclampsia through gene methylation should be elucidated. Also, Keap1 rs11085735 polymorphism might be involved in the risk of preeclampsia.

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## Authors' contributions

MZ diagnosed patients and provided samples. SH and ZS diagnosed patients and involved in doing experiments. MK wrote the first draft of the manuscript. ZR designed the study and critically revised the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

Data are available from the authors upon reasonable request.

## Declarations

### Ethical approval and consent to participate

Informed consent was obtained from all human adult participants under approval by the Ethics Committee of Kermanshah University of Medical Sciences (the research project code 990654). The study was under the Declaration of Helsinki II principles.

### Consent for publication

Informed consent related to publishing results of serum and blood parameters of studied individuals was obtained from all human adult participants.

### Competing interests

The authors declare that they have no competing interests.

### Author details

<sup>1</sup>Department of Obstetrics and Gynecology, Kermanshah University of Medical Sciences, Kermanshah, Iran. <sup>2</sup>Department of Clinical Biochemistry, Kermanshah University of Medical Sciences, Daneshgah Avenue, P.O. Box 67148-69914, Kermanshah, Iran. <sup>3</sup>Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

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