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Original Article

The relationship between preeclampsia risk and SENCR rs555172 gene polymorphism and expression

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Article Info

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Abstract

Preeclampsia, the more severe manifestation of gestational hypertensive disorders, is a major cause of maternal and perinatal morbidity and mortality worldwide. Genetic polymorphisms in long non-coding RNAs (lncRNAs) are considered as potential genetic preeclampsia. This study aimed to explore the association between SENCR rs555172 SNP and PE risk in healthy pregnant women compared to women with preeclampsia. A total of 140 healthy pregnant women and 130 preeclampsia cases were included in the study. The rs555172 genotype was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the expression of the SENCR gene was analyzed in 40 placenta tissue samples from both groups. Various statistical approaches were employed to assess the genotypic and allelic frequencies. The results showed no significant difference in the frequency of the rs555172 polymorphism between healthy pregnant women and those with preeclampsia in terms of the dominant (p=0.82), recessive (p=0.39), and over-dominant (p=0.42) models. Additionally, the analysis of SENCR relative expression revealed no significant difference between the two groups (p=0.48). In conclusion, the LncRNA SENCR rs555172(G/A) seems not associated with an increased risk of Preeclampsia in pregnant women.

Keywords: SENCR, Iran, Preeclampsia, Variants, Single nucleotide polymorphism.

1. Introduction

PE (PE) is the leading cause of maternal and perinatal mortality, accounting for 3-5% of pregnancies. In PE, High blood pressure and endothelial dysfunction can cause damage to tissues such as the liver, kidneys, and brain [1]. The risk of death will be increased if PE is accompanied by severe medical conditions such as elevated liver enzyme activity, thrombocytopenia, or hemolysis. The diagnostic criteria for PE, according to the American College of Obstetricians and Gynaecologists, are the measurement of hypertensive thresholds (i.e., after 20 weeks, systolic and diastolic blood pressures equal or more than 140 and 90 mmHg, respectively, occurring twice, four hours apart), along with either proteinuria or, in the absence of proteinuria (a) thrombocytopenia; (b) renal insufficiency; (c) abnormal liver function; (d) pulmonary edema; or (e) cerebral or visual symptoms [2]. despite its high prevalence The aetiology of PE is not fully understood; however, there is evidence of heterogeneity in pathogenesis [3]. Genetics is one of the factors affecting PE susceptibility [4]. About 55% of PE cases are estimated to have a genetic link, and both mother and fetus genetic background may affect PE risk [5].

Long non-coding RNAs have more than 200 nucleotides long and are involved in various physiological processes, including cell differentiation and proliferation,

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homeostasis and metabolism, repair, and inflammation [6]. LncRNAs, as a new class of epigenetic regulators, play an essential role in epigenetic regulation and generally regulate gene expression at the transcription level by controlling histone acetylation or DNA methylation modifications [7]. Some lncRNAs control or impact the cell cycle, altering trophoblast cell proliferation, invasion, migration, and apoptosis, which results in placental malfunction and PE. Furthermore, The abnormal expression of lncRNAs in plasma or placenta samples taken from women with PE has been documented in several studies [8].

LncRNA SENCR is abundantly expressed in endothelial cells, smooth muscle, and aortic tissue. Recent research has revealed that high levels of lncRNA SENCR enhance the growth of endothelial cells via the induction of genes that stimulate endothelial cell development [9]. SENCR gene (gene id: 100507392) overlaps with the endothelial growth regulator companion leukemia integrating virus 1 (FLI1) gene. Boulberdaa et al. found that SENCR helps regulate the endothelial differentiation of pluripotent cells and regulates the angiogenic potential of HUVECs. These data provide new insights into the regulatory mechanisms involved in endothelial development and function [10]. As a transcriptional lncRNA, SENCR also supports the linkage integrity of endothelial cells [11].

Based on the primary role of the SENCR on endothelial cell function and differentiation, we aim to evaluate the effect of the SENCR rs555172 gene polymorphism and its expression on PE development risk.

2. Material and methods

In this case – controls were conducted on 140 healthy pregnant women and 130 preeclamptic women. The SENCR gene expression was analyzed in 40 placenta tissues of two groups. The inclusion and exclusion criteria were described previously [12].

2.1. Genotyping

PCR-RFLP technique was used for genotype analysis. DNA extraction from 500 µL blood containing EDTA using the salting-out method. The quality of the extracted DNA was approved by nano-drop and agarose gel electrophoresis. A 242 bp PCR product was amplified using the following primers: Forward primer: 5'- ACAGGGATTG-GCATGGCAG-3', and reverse primer: 5'- CACCAACA-CACACACTAAAGGC- 3'. Then, The PCR product was digested using the HinfI restriction enzyme. The digested fragment was separated on the 3% agarose gel electrophoresis (GG genotype: 172+ 70 bp, and CC genotype: 242 bp).

2.2. SENCR gene expression analysis

Expression of the SENCR gene in placental tissue was evaluated by the qRT-PCR method. After total RNA extraction by Trizol (ThermoFisher Scientific, UK), the cDNA was synthesized by using Easy™ cDNA Synthesis Kit (Parstous company, Iran). The sequences of used primers were: SENCR; forward primer: 5'- CACCATG-GCTAGGTTTCTCTT- 3', and reverse primer: 5'- GTA-TAGGAATGTTCGGGTGACTAC- 3'. GAPDH; forward primer: 5'-TCCCATCACCATCTTCCAGG-3', reverse primer: 5'- TGATGATCTTGAGGCTGTTGTCA-3'. PCR efficiency of SENCR and GAPDH genes was calculated in 2013.x version of LinRegPCR software [13]. The Pfaffl

method was used to calculate the relative expression of the SENCE gene in placental tissue [14].

2.3. Statical analysis

Statistical and demographic differences between groups were tested using the independent student's t-test or Fisher's exact test, CHI-SQUARE as appropriate. The test of normality for the distribution of variables was done by the Shapiro-Wilk test. Logistic regression analysis was used to calculate the independent effect of polymorphism on PE risk after adjusting for age. All statistical analyses were performed using SPSSV20. A P-value less than 0.05 (P<0.05) was considered significant.

3. Results
3.1. Demographic and Clinical of PE and control groups.

As described in Table 1, two groups were matched based on age variables. The mean age was 26.9 ± 6.6 and 27.9 ± 5.7 in PE and control groups, respectively (P= 0.256). The Gestation age was significantly higher in the control group than in the PE group (38.3 ± 1.36 and 37.3 ± 3.1, respectively, P= 0.001). Also, there were significant differences in birth weight, and systolic and diastolic blood pressure in women with and without PE.

3.2. Genotypic and allelic frequency of SENCR rs555172 gene polymorphism in PE and control groups.

As shown in Table 2, the frequency of GG, GA, and AA genotypes were 30.8%, 56.2%, and 13% in the PE group, and 32.3%, 51.5%, and 16.2% in the control group. We found no significant difference between the two groups regarding the allelic and genotypic frequency of the rs555172. Furthermore, the genetic models also found similar results.

3.3. Relative SENCR gene expression in PE and control groups.

Along with allele and genotype analyses, we investigated relative SENCR expression in PE and control groups. As shown in Figure 1, the relative SENCE gene in PE and control groups were 11.84±4.4 and 5±2.2, respectively and there was no significant difference between two groups (P=0.478).

4. Discussion
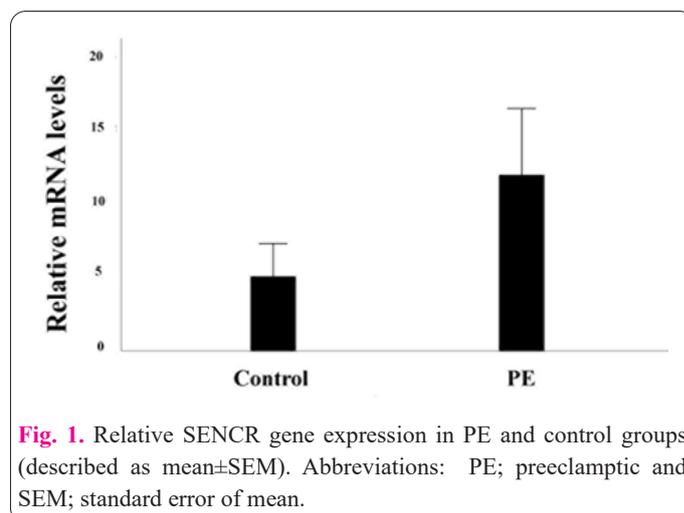
A body of evidence emphasizes on impact of LncR-

Fig. 1. Relative SENCR gene expression in PE and control groups (described as mean±SEM). Abbreviations: PE; preeclamptic and SEM; standard error of mean.

Table 1. Demographic and clinical characteristics of preeclampsia patients and controls.

Variable	Controls (N=130)	PE (N=130)	P-value
Maternal age (mean ± SD, years)	26.9 ± 6.6	27.9 ± 5.7	0.256
Gestation age (mean ± SD, weeks)	38.3 ± 1.36	37.3 ± 3.1	0.001
Birth weight (mean ± SD, g)	3108.3 ± 389	2942.4 ± 594	0.026
SBP (mean ± SD, mmHg)	108.7 ± 7.2	143.7 ± 21.8	<0.0001
DBP (mean ± SD, mmHg)	68.6 ± 7.3	94.1 ± 11.7	<0.0001
Family history (n, %)		48 (36.9)	
Proteinuria (n, %)			
Trace		14 (3.1)	
1+	-	33 (15.3)	
2+	-	50 (11.3)	
3+	-	27 (14.7)	
4+	-	6 (4.6)	
history of hypertension in previous pregnancy	6(3.6)	32 (31.9)	
Severity			
Mild (n, %)		50 (38.4)	
Severe (n, %)		80 (61.4)	

Table 2. The genotypic and allelic distribution of SENCR rs555172 gene polymorphism in preeclampsia (PE) and control groups.

	PE (N=130)	Control (N=130)	P-value	OR (95% CI)
SENCR rs555172				
GG, n (%)	40 (30.8)	42 (32.3)		1
GA, n (%)	73 (56.2)	67 (51.5)	0. 629	1.14 (0.66-1.9)
AA, n (%)	17 (13)	21 (16.2)	0.576	0.8 (0.36-1.7)
Dominant			0.822	1.06 (0.62-1.79)
Recessive			0.39	0.735 (0.36-1.48)
Overdominant			0.415	1.22 (0.75-2)
Allele				
G, n (%)	153 (58.9)	151 (80.9)		1
A, n (%)	107 (41.1)	109 (19.1)	0.929	0.96 (0.68-1.3)

NAs on PE development and clinical outcomes. According to previous studies, abnormal long non-coding RNA (lncRNA) expression may be associated with pathological changes leading to PE [15]. However, our results showed no association between SENCR gene expression and rs555172 polymorphism with PE risk.

LncRNAs gene expression alteration could influence the trophoblast cell. Normal endothelial function is required for successful placental implantation, maternal and fetal circulation networks [16] and also Line proliferation, migration, and invasion [17-19]. Dong et al. reported a correlation among lncRNA MIR193BHG with systolic and diastolic blood pressure and urine protein [20]. Wang et al. indicated that the lncRNA T-cell leukaemia/lymphoma 6 (TCL6) is elevated in the blood of preeclampsia patients. In addition, TCL6 was elevated in the group of patients with early-onset or severe symptoms of preeclampsia compared to other preeclamptic patients [21].

One main reason for abnormal placental implantation is endothelial dysfunction, followed by altered capillary permeability; this highlights the importance of a healthy fetal-mother circulation network [16]. SENCR is transcribed antisense from the first intron of the Friend Leukemia Integration virus 1 (FLI1) gene and has little effect on FLI1 gene expression regulation as sic element and versa [22]. FLI1 expression is one of the first processes in endothelial cell development. Burberda et al. found that SENSE helps control the endothelial differentiation of pluripotent cells and regulates the angiogenic potential of human umbilical endothelial cells (HUVECs) [10]. Numerous intricate physiological pathways control blood pressure, and the effects of environment, behavior, and heredity on each route have an impact on the blood pressure's actual levels and oscillations [23]. Myocardin and smooth muscle contractile genes are lost when SENCR is knocked down in vitro, whereas pro-migratory genes are increased. It was discovered that SENCR negatively regulated vascular smooth muscle cell (VSMC) migration [22].

For the first time, we found no significant association between the SENCR rs55172 polymorphism and the risk

SENCR expression and preeclampsia risk.

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of PE. Changes in nucleotide sequences can potentially modify the strength of interactions between transcription factors and regulatory elements, leading to abnormal gene expression. The minor allele rs555172 has a frequency (MAF) of 0.48, resulting from a G-to-A substitution in the upstream region of the gene. The potential impact of rs55712 on the probability of acquiring a disease has been examined in numerous studies. Shahmoradi et al. reported that SENCR gene polymorphisms do not contribute to coronary artery disease susceptibility in the Iranian population [24]. Elwazir et al. found no significant variation in allele or genotype frequency between coronary artery disease patients and controls about SENCR (rs12420823 C/T) and also, there was no evidence of an association with a predisposition to disease [25]. A significant association between susceptibility to Ewing sarcoma and four SENCR SNPs, specifically rs10893909, rs11221437, rs12420823, and rs4526784 polymorphisms, was discovered by Martinelli et al [26].

5. Conclusion

In conclusion, the genotype and allele frequency of SENCR rs55172 gene polymorphism did not show difference between the two groups. Furthermore, identical outcomes were also discovered by the genetic models. It is important to note that our study had limitations due to the small number of participants involved.

Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
The questionnaire and methodology for this study were approved by the Human Research Ethics Committee of Tehran University of Medical Sciences.

Informed consent
Informed consent was obtained from all individual participants included in the study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions
ZH and **MS** contributed to conceptualization; **MG, EK, SK** and **SR** contributed to patient management and sampling; **HD**, **RZ**, **SS** and **MN** contributed to data collecting, lab work and data analysis; **MS, HRC** and **ZH** contributed to Writing - original draft preparation; **MZD** and **MS** contributed to writing - review and editing; **ZH** contributed to Funding acquisition; and **ZH** and **MS** contributed to supervision.

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