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Genetic and epigenetic analysis of the *BAX* and *BCL2* in the placenta of pregnant women complicated by preeclampsia

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Abstract

The current study examined the effects of *BAX* and *BCL2* polymorphisms and methylation as well as mRNA expression on susceptibility to PE. After delivery, the placentas were collected from 92 women with PE, as well as 106 normotensive pregnant women. The *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms were genotyped by the PCR-RFLP method. Methylation-specific PCR (MSP) was used for analysis of promoter methylation. mRNA expression was assayed by Quantitative RT-PCR. In addition, in silico analysis was performed by bioinformatics tools. There was no relationship between PE and placental *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms. The groups were not significantly different regarding the promoter methylation of *BAX* gene. Nonetheless, the MM status of *BCL2* promoter had a significantly higher frequency in the PE group and was associated with 2.7-fold higher risk of PE (OR = 2.7, 95% CI = 1.3–5.6; P = 0.01). The relative mRNA expression of *BCL2* was decreased in the placentas of PE women (P < 0.0001). The expression of *BAX* gene was not significantly different between the two groups. There was no association between placental *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms and mRNA expression levels. In silico analysis indicated that *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms were located in the core recognition site of different transcription factors and these substitutions of wild allele resulted in the loss and/ or change of these binding sites and subsequently may alter *BCL2* and *BAX* expression. This study showed that the *BAX* and *BCL2* polymorphisms and *BAX* promoter methylation were not associated with PE risk. The *BCL2* promoter methylation was associated with lower *BCL2* expression and higher PE susceptibility.

Keywords Apoptosis · Expression · Methylation · Preeclampsia · Polymorphism

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Introduction

Preeclampsia (PE) is a complicated disorder characterized by high blood pressure after 20 weeks of pregnancy, which is associated with proteinuria. Evidence showed that 3–5% of all pregnancies suffer from PE leading to 60,000 maternal deaths worldwide [1]. The certain pathological mechanism of PE is still undetermined. However, several mechanisms have been suggested as the major causes of PE including immunologic intolerance, angiogenic imbalance, and oxidative stress [2, 3].

It is commonly assumed that PE is initiated from the placenta, and placenta removal is its endpoint; therefore, PE initiates from this organ [4]. The higher rate of apoptosis and decrease in placental trophoblast cell invasion and migration has been proposed in the placentas, which was complicated by PE. Increased rate of apoptosis was indicated in total preeclamptic placentas as well as particular cell types of this organ [5–8]. In this condition, the inadequate blood flow of utero placental as the result of low trophoblast invasion of the uterine spiral arteries encourages hypoxic conditions in trophoblast tissue. The hypoxia condition may amplify the placental oxidative stress and increase apoptosis in trophoblast cells. Consequently, the above-mentioned cascade may induce the occurrence of PE [8].

Bcl-2-associated X protein (Bax), as a death-promoting protein, stimulates cell apoptosis by formation of a heterodimer with B-cell lymphoma-2 (Bcl-2) protein [9]. The *BAX* gene have six exons and a promoter region with four p53 binding sites and is mapped on chromosome 19 [10]. Bcl-2 as a member of Bcl2 family prevents apoptosis despite Bax protein. This protein is located in mitochondrial outer membrane and inhibits the action of pro-apoptotic proteins such as Bax. The *BCL2* gene is situated on chromosome 18 consisting of 2 promoters and 3 exons as well as 2 introns [11].

Evidences showed that maternal and fetal genetic factors as well as environmental factors play main roles in PE susceptibility [12]. The effects of environmental factors have been established on epigenetic alterations including DNA methylation [13]. Therefore, the relationship between genetic and epigenetic variations has been investigated in the maternal/placental genes, which are involved in diverse pathways in PE pathogenesis [14, 15]. DNA methylation is the best-known epigenetic regulatory mechanism that plays a key role in the regulation of transcription; thus, it may involve in pathogenic process of a disease. Previous investigations proposed that DNA methylation is altered during development and affected by environmental stress [16]. Altered methylation of placental genes has been observed to affect gene expression, which subsequently may damage the function of the placenta [17]. Therefore, this study investigated the possible effects of the placental *BAX* and *BCL2* genes polymorphism and methylation on PE susceptibility. Moreover, we aimed to evaluate the association of *BAX, BCL2* variants, and methylation with *BAX* and *BCL2* expression with respect to in silico analysis.

Materials and methods

Placentas were delivered from 92 PE women (mean age of 28.5 ± 6.8 years) and 106 normotensive pregnant women (mean age of 29.7 ± 6.4 years) at the hospital affiliated with the Ali-ibn-Abi Taleb Educational Hospital. All of the participants have filled informed consent form. The study protocol was approved by the Ethics Committee of Zahedan University of Medical Science. The main criteria for PE diagnosis were: SBP $\geq$ 140 or DBP $\geq$ 90 on two occasions at least 4 h apart; and proteinuria on dipstick $\geq$ 1 or $\geq$ 30 mg/24 h after 20 gestational weeks. SBP $\geq$ 160 mmHg or DBP $\geq$ 110 mmHg on two occasions at least 4 h apart while the patient is on bed rest defined as severe PE criteria [18].

Sample preparation

Immediately after delivery, placental tissues were gained from standard localization. After washing the tissue samples in PBS buffer at 4 °C to eliminate blood, They were stored at − 80 °C for RNA and DNA extraction.

BAX and *BCL2* polymorphisms analysis

Genomic DNA was extracted from the placenta of all the subjects by a DNA extraction kit (DynaBio, Takapoozist, Iran) according to the instruction recommended by the manufacturer. In order to identify *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms, the PCR-RFLP method was utilized. The sequence of primers was used to amplify the DNA fragments published by Chen et al. [19]. The PCR programs included primary denaturation step at 95 °C for 5 min, followed by 30 cycles with next denaturation at 95 °C for 30 s, annealing at 59 °C for rs2279115 or 56 °C for rs4645878 for 30 s, primary extension at 72 °C for 30 s, and last cycle of extension step at 72 °C for 5 min. The PCR products were digested by *Msp*I and *Bcc*I restriction enzymes (*BAX* and *BCL2*, respectively), separated by Agarose gel electrophoresis, and visualized by safe stain (Sinaclon, Iran) under ultraviolet light.

Quantitative real-time PCR

Total RNA was extracted using RNX-Plus (Sinaclon, Iran) following the instructions provided by the manufacturer. Then, PrimeScript 1st strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan) was employed to transcribe RNA reversely. Sequences of the primers have been described previously [20]. Quantitative RT-PCR was analyzed in triplicate using SYBR Green/High ROX (Amplicon) in ABI PRISM 7500 Real-Time PCR System, Applied Biosystems. The relative mRNA expression levels of target (*BAX* and *BCL2*) and housekeeping (*β-Actin*) genes were analyzed using $2^{-\Delta\Delta Ct}$ method.

Bisulfite modification

Methylation status of CpG islands in promoter regions of *BAX* and *BCL2* genes were assessed by methylation-specific PCR (MSP) with the Thermal Cycler System. EpiTect Bisulfite Kit (Qiagen, Germany) was employed to treat DNA with sodium bisulfite. The forward and reverse primers were used to analyze methylated and un-methylated alleles as previously described [21]. MSP amplification was performed in a final volume of 15 µL containing 7 µL PCR Master Mix, 1 µL of each primer, 1 µL template bisulfite DNA, and 5 µL deionized water. PCR conditions for both methylated and un-methylated primers were as follows: five min of denaturation at 95 °C, followed by 35 cycles with 30 s of next denaturation at 95 °C; 35 s of annealing at 60 °C; and 35 s of initial extension at 72 °C, followed by a last extension at 72 °C for 10 min.

In silico analysis

Some bioinformatics tools were used to evaluate the potential biological functions of two SNPs, *BAX* − 248 G > A and *BCL2* − 938 C > A [22, 23]. The promoteric DNA sequences contained both SNPs that were screened to detect the core promoter motifs by the in silico prediction tools EPD [24], and ElemeNT [25]. Moreover, bioinformatics tools, SNPnexus [26], and PROMO [27], were utilized to predict the potential transcription factor binding sites in the promoter sequences of the *BAX* and *BCL2* genes containing − 248 G > A and − 938 C > A polymorphisms. The impact of different alleles *of BAX* − 248 G > A and *BCL2* − 938 C > A polymorphisms on methylation statues of a CpG island were evaluated using the DataBase of CpG islands and Analytical Tool: DBCAT [28, 29]. Finally, GoldenPath [30] and WebLogo [31] bioinformatics tools were employed to predict the conservation of the DNA sequences containing *BAX* − 248 G > A and *BCL2* − 938 C > A polymorphisms.

Statistical analysis

SPSS-V22 was used to data analysis. All of the continuous and categorical data was analyzed by Student's *t* test or Fisher's exact test. Moreover, chi-squared test was applied to compare the distributions of allele and genotype frequencies. The association among polymorphisms and PE was obtained by calculating odds ratio (OR) and 95% confidence interval (95% CI) from logistic regression analyses. The *P*-value < 0.05 was established as significant difference.

Results

The case and control groups were not significantly different regarding maternal age. Lower birth weight and gestational age were shown in the PE group. The PE group had significantly higher diastolic and systolic blood pressures, compared to the controls. Primiparity was also related to the risk of PE (Table 1).

Placental *BAX* and *BCL2* polymorphisms and PE risk

The placental *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms were not deviated from Hardy Weinberg equilibrium. The frequency of placental *BAX* rs4645878GA and AA genotypes were not significantly different between PE women and normotensive pregnant women as controls. Moreover, placental *BAX* rs4645878 polymorphism was not associated with PE in neither recessive and dominant nor allelic models. There was no association between placental *BCL2* rs2279115AC and CC genotypes and PE. The *BCL2* rs2279115 polymorphism was not associated with PE in recessive, dominant, and allelic models (Table 2). The

Table 1 The demographic and clinical characteristics of PE women and controls

	PE (N = 92)	Control (N = 106)	P-value
Maternal age (mean ± SD, years)	28.5 ± 6.8	29.7 ± 6.4	NS
Gestation age (mean ± SD, weeks)	36.3 ± 2.7	38.1 ± 1.8	< 0.0001
Birth weight (mean ± SD, g)	2837 ± 404	3085 ± 392	< 0.0001
SBP (mean ± SD, mmHg)	152 ± 15	106 ± 13	< 0.0001
DBP (mean ± SD, mmHg)	98 ± 11	68 ± 9	< 0.0001
Primiparity, n(%)	39 (42)	27 (27)	0.015

Table 2 Allelic and genotypic frequency of placental *BAX* and *BCL2* polymorphisms in PE women and control group

	PE (N = 92)	Control (N = 106)	P-value	OR (95% CI)
BAX rs4645878				
GG, n(%)	65 (70.6)	71 (67)		1
GA, n(%)	25 (27.2)	30 (28.3)	0. 8	0.9 (0.5–1.7)
AA, n(%)	2 (2.2)	5 (4.7)	0.3	0.4 (0.1–2.3)
Dominant			0.6	0.8 (0.5–1.5)
Recessive			0.4	0.5 (0.1–2.4)
Allele				
G, n(%)	155 (84)	172 (81)		1
A, n(%)	29 (16)	40 (19)	0.5	0.8 (0.5–1.4)
BCL2 rs2279115				
AA, n(%)	52 (56.5)	58 (54.7)		1
AC, n(%)	36 (39.1)	42 (39.6)	0. 9	1 (0.5–1.7)
CC, n(%)	4 (4.3)	6 (5.7)	0.7	0.7 (0.2–2.8)
Dominant			0.8	0.9 (0.5–1.6)
Recessive			0.7	0.8 (0.2–2.8)
Allele				
A, n(%)	140 (76)	158 (75)		1
C, n(%)	44 (24)	54 (25)	0.7	0.9 (0.6–1.5)

placental *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms had no synergistic effect on PE risk. No significant difference was found between mild and severe PE regarding *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms.

The relative mRNA expression of placental *BAX* and *BCL2* genes

As it is shown in Fig. 1a, the relative mRNA expression of the placental *BAX* gene was 1.4-fold higher in PE women compared to normotensive pregnant women, but this difference was not significant (P = 0.6). However, the relative mRNA expression of the *BCL2* gene was significantly decreased (Fig. 1b) in the placentas of PE women (0.1-fold, P < 0.0001). The relative mRNA expression of *BAX* and *BCL2* genes was not different between mild and severe preeclamptic women.

The effects of *BAX* and *BCL2* polymorphisms on mRNA expression

Among all studied women, *BAX* rs4645878GG, GA, and AA genotypes were not significantly different regarding the relative mRNA expression of *BAX* gene (Fig. 2a). Moreover, the mRNA expression of *BAX* gene was not associated with *BAX* rs4645878 polymorphism in PE and control groups separately. The relative mRNA expression of *BCL2* gene was not associated with *BCL2* rs2279115 neither in total studied women (Fig. 2b), nor in PE and control groups.

Promoter methylation of *BAX* and *BCL2* genes and PE risk

The frequency of *BAX* UM and MM statuses were not different between PE and control groups. Therefore, the promoter methylation of *BAX* gene was not associated with PE susceptibility (Table 3). The frequency of UM status in the promoter region of placental *BCL2* gene was lower in PE women compared to controls, but the difference was not significant. However, the frequency of MM status was higher in PE women (29.4 vs. 13.2%) and associated with 2.7-fold higher risk of PE (OR 2.7, 95% CI 1.3–5.6; P = 0.01, Table 3). However, no association was observed between promoter methylation status of *BAX and BCL2* genes and PE severity.

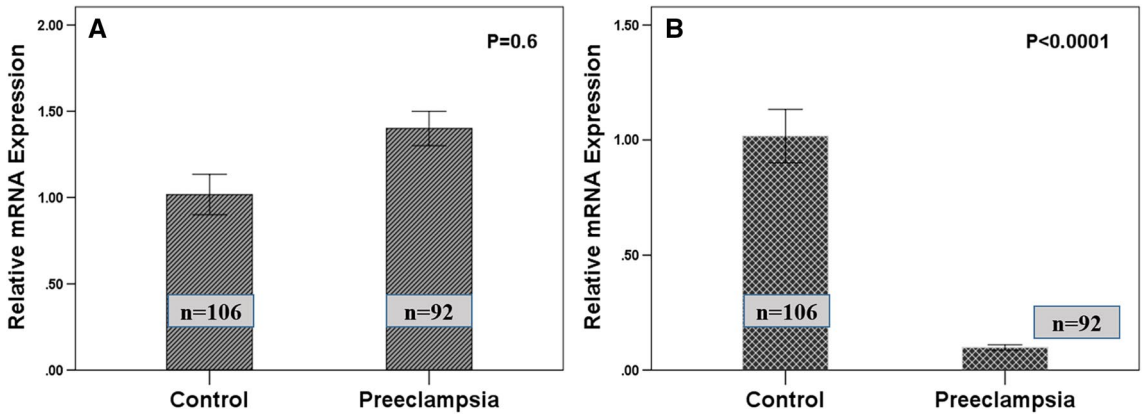


Fig. 1 The relative mRNA expression of **a** *BAX* and **b** *BCL2* gene in the placenta of PE and control women

The effects of promoter methylation of *BAX* and *BCL2* genes on mRNA expression

The promoter methylation of *BAX* gene was not associated with its mRNA expression either in the total population (Fig. 3a), or in the control or PE groups (Fig. 3b, c).

Women with the MM status had a significantly lower mRNA expression of *BCL2* gene, compared to women with UM and UU status (P = 0.004 and P = 0.001 respectively). However, no relationship was found between UM

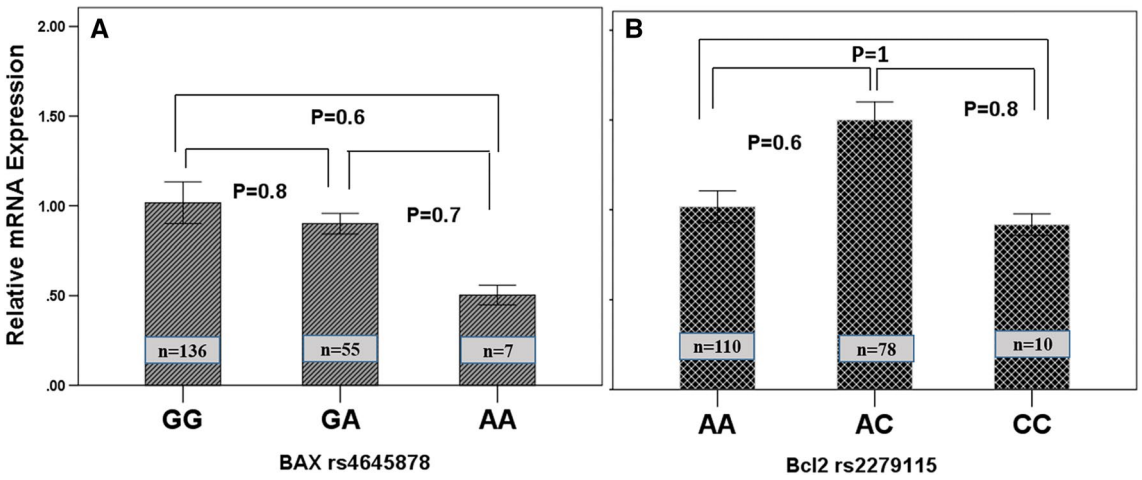


Fig. 2 The relative mRNA expression of placental **a** *BAX* gene between rs4645878 GG, GA and AA genotypes, **b** *BCL2* gene between rs2279115 AA, AC and CC genotypes

Table 3 Promoter DNA methylation of the *BAX* and *BCL2* genes in PE women and control group

	PE (N = 92)	Control (N = 106)	P-value	OR (95% CI)
Methylation status				
BAX				
UU, n(%)	64 (69.6)	66 (62.3)		1
UM, n(%)	22 (23.9)	31 (29.2)	0.34	0.7 (0.4–1.4)
MM, n(%)	6 (6.5)	9 (8.5)	0.5	0.7 (0.2–2)
UM + MM, n(%)	28 (30.4)	40 (37.7)	0.3	0.7 (0.4–1.3)
BCL2				
UU, n(%)	51 (55.4)	70 (66)		
UM, n(%)	14 (15.2)	22 (20.8)	0.7	0.9 (0.4–1.9)
MM, n(%)	27 (29.4)	14 (13.2)	0.01	2.7 (1.3–5.6)
UM + MM, n(%)	41 (44.6)	36(34)	0.13	1.6 (0.9–2.8)

U unmethylated, *M* methylated

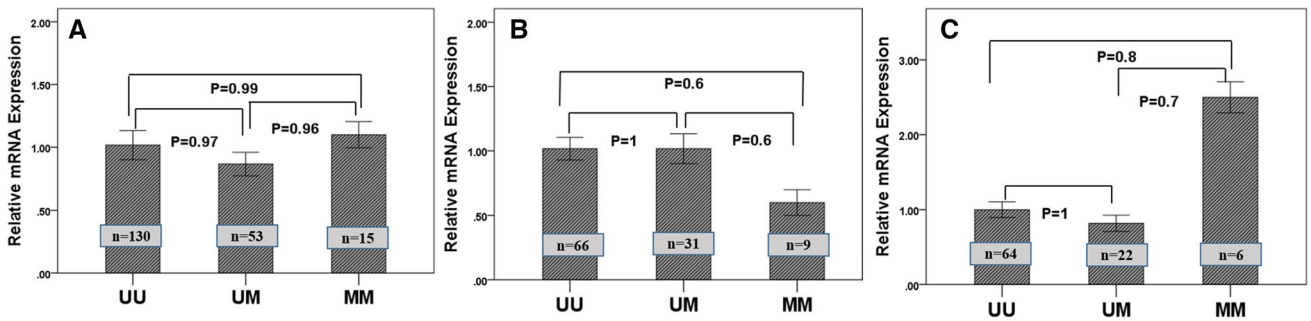


Fig. 3 The relative mRNA expression of *BAX* gene between methylated and unmethylated promoter of *BAX* gene in **a** total women, **b** control women, **c** PE women

status and mRNA expression in all women under study (Fig. 4a).

Despite the lower level of *BCL2* mRNA expression among women with MM status in the control and PE groups (Fig. 4b, c, 0.3 and 0.2-fold respectively), no significant difference was observed (P = 0.1 and P = 0.1).

Computational analysis

In-silico analysis revealed that − 938 C > A (rs2279115) SNP is located in the core recognition site of MAZ transcription factor in the promoter region of *BCL2* gene (Fig. 5). When the C allele is replaced with A allele, the MAZ binding site is missed and new binding sites are created for YY1 transcription factor (Fig. 5a, a′). Moreover, it is revealed that substitution of G allele with A allele in *BAX* − 248 G > A (rs4645878) polymorphism resulted in the loss of several binding sites of transcription factors such as E2F-1 and Sp1 in the promoter region of this gene (Fig. 5b, b′). The results of ElemeNT tool demonstrated that these SNPs could alter number, types and scores of Transcription Start Sites (TSS) and/or core promoter elements in the

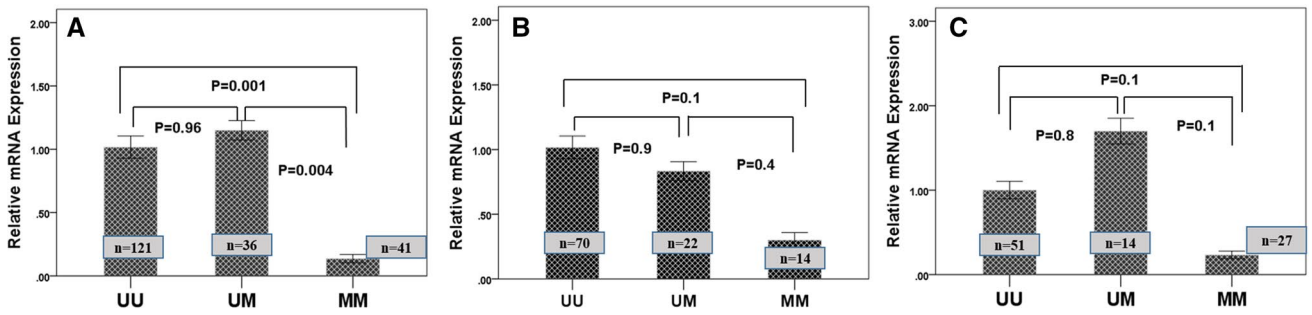
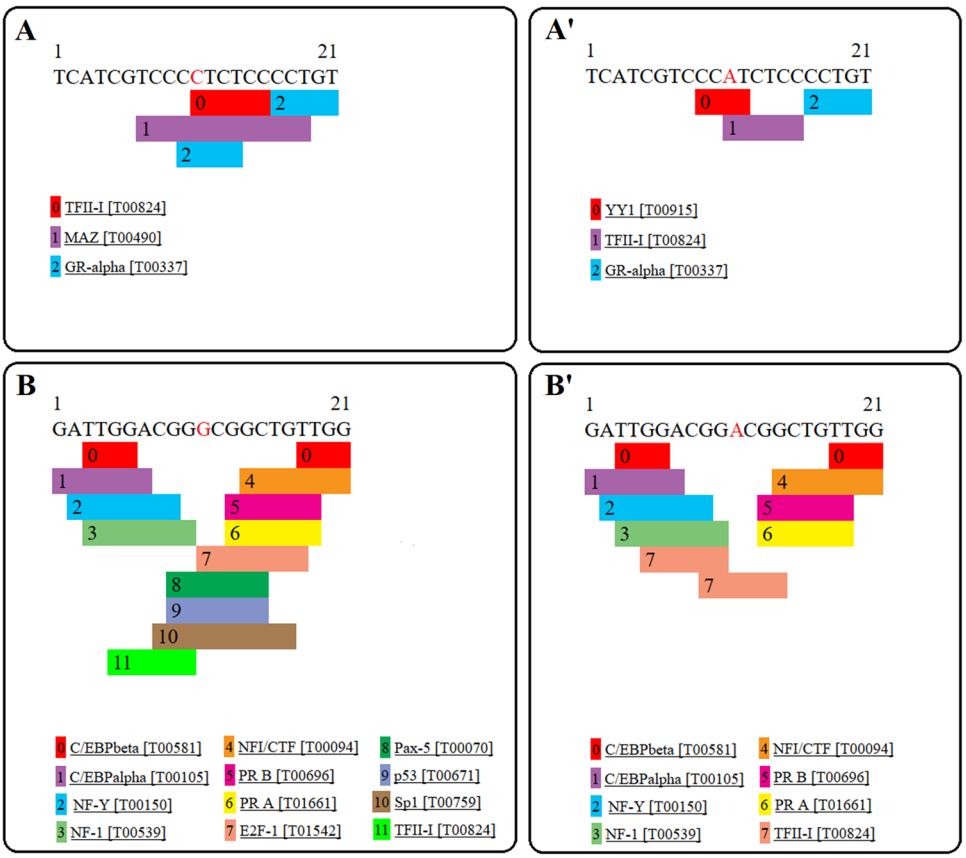


Fig. 4 The relative mRNA expression of *BCL2* gene between methylated and unmethylated promoter of *BCL2* gene in **a** total women, **b** control women, **c** PE women

Fig. 5 Potential binding sites for transcription factors of: **a, a′** the *BCL2* rs2279115 gene polymorphism, **b, b′** the *BAX* rs4645878 gene polymorphism. This analysis was shown that replacement of the wild allele with mutant allele in these polymorphisms resulted in the loss of several binding sites of transcription factors in the promoter regions of those genes



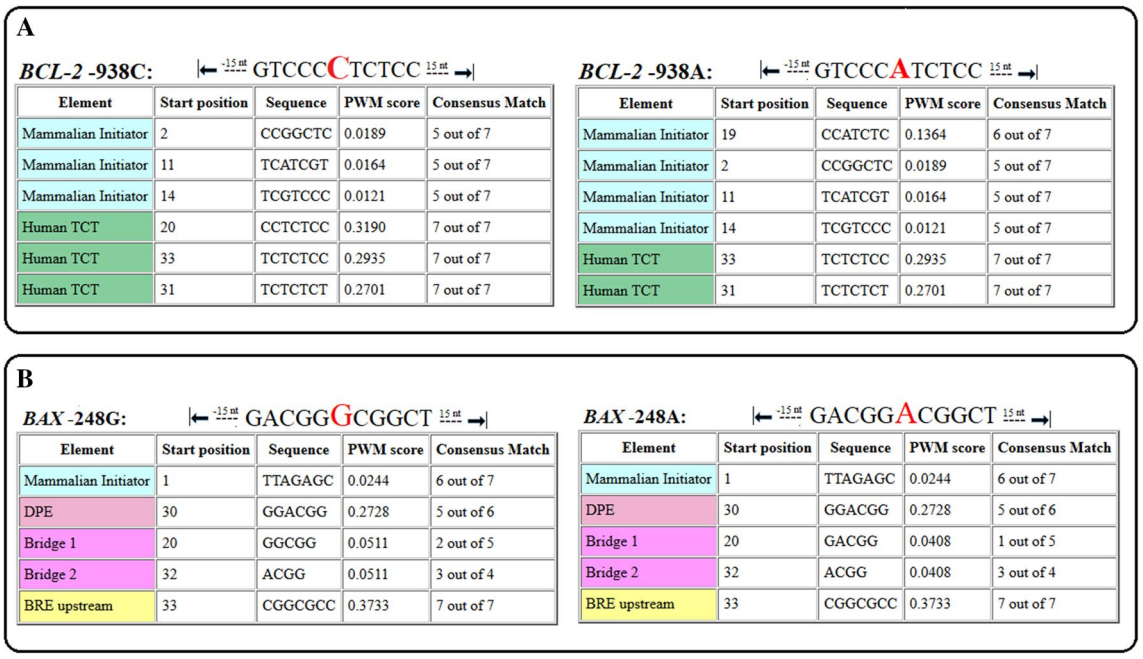
promoter regions of *BAX* and *BCL2* genes (Fig. 6a, b). Additional bioinformatics analysis by DBCAT tool showed that −938 C > A (rs2279115) and −248 G > A (rs4645878) gene polymorphisms are located in the CpG islands of the *BCL2* and *BAX* gene promoter regions (Fig. 7a, b). Furthermore, computational analysis demonstrated that these SNPs are not located in conserved regions across multiple mammalian species (Fig. 8a, b).

Discussion

Apoptosis (programmed cell death) is a critical process to maintain tissue homeostasis during life and in the placental development, it plays an important role. It influences the remodeling of spiral artery, the first trimester. Additionally, it can affect tolerance of maternal immune and differentiation of villous trophoblast and turnover of trophoblast [32]. The emergence of apoptosis can be affected by physiological, pathogenic, or cytotoxic stimuli and many proteins, such as Bcl-2 family, caspases, and inhibitors of apoptosis (IAP) families which contribute to its initiation [33, 34]. Bcl-2, a remarkable antiapoptotic protein, considerably contributes to prevention of apoptosis by blocking cytochrome C that has been released from mitochondria [35]. Bax, as another important protein of Bcl-2 family, heterodimerizes with Bcl-2 to manifest its proapoptotic functions. A series of regulated events initiate apoptosis, which is severely controlled by balance between proapoptotic and antiapoptotic proteins [33, 35, 36]. Evidences showed the altered levels of Bax and Bcl2 in the placentas of women were complicated by various disorders including preeclampsia [37, 38]. However, there is no published data on the effects of *BAX* and *BCL2* polymorphisms and methylation on PE susceptibility.

The findings of present study showed no association between placental *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms and PE risk in dominant, recessive, and allelic models. The methylation of promoter region of placental *BAX* gene was not associated with PE risk. However, the MM status of *BCL2* promoter was associated with 2.7-fold higher risk of PE. Although the relative mRNA expression of placental *BAX* gene was 1.4-fold higher in PE women and the difference was not significantly significant. The *BCL2* expression was significantly decreased in the placenta of PE group. Moreover, no association was observed between *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms and their corresponding mRNAs. The relative mRNA expression of *BAX* gene was not associated with promoter methylation of *BAX* gene neither in total study population, nor in control and PE women. The relative mRNA expression was lower in women with MM status in promoter region of *BCL2* gene; however, the difference was significant only in total studied women.

To the best of our knowledge, there is no published report on the effects of *BAX* and *BCL2* polymorphisms on



A

***BCL-2* -938C:** -15 nt GTCCCCTCTCC 15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	2	CCGGCTC	0.0189	5 out of 7
Mammalian Initiator	11	TCATCGT	0.0164	5 out of 7
Mammalian Initiator	14	TCGTCCC	0.0121	5 out of 7
Human TCT	20	CCTCTCC	0.3190	7 out of 7
Human TCT	33	TCTCTCC	0.2935	7 out of 7
Human TCT	31	TCTCTCT	0.2701	7 out of 7

***BCL-2* -938A:** -15 nt GTCCCATCTCC 15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	19	CCATCTC	0.1364	6 out of 7
Mammalian Initiator	2	CCGGCTC	0.0189	5 out of 7
Mammalian Initiator	11	TCATCGT	0.0164	5 out of 7
Mammalian Initiator	14	TCGTCCC	0.0121	5 out of 7
Human TCT	33	TCTCTCC	0.2935	7 out of 7
Human TCT	31	TCTCTCT	0.2701	7 out of 7

B

***BAX* -248G:** -15 nt GACGGGCGGCT 15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	1	TTAGAGC	0.0244	6 out of 7
DPE	30	GGACGG	0.2728	5 out of 6
Bridge 1	20	GGCGG	0.0511	2 out of 5
Bridge 2	32	ACGG	0.0511	3 out of 4
BRE upstream	33	CGGCGCC	0.3733	7 out of 7

***BAX* -248A:** -15 nt GACGGACGGCT 15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	1	TTAGAGC	0.0244	6 out of 7
DPE	30	GGACGG	0.2728	5 out of 6
Bridge 1	20	GACGG	0.0408	1 out of 5
Bridge 2	32	ACGG	0.0408	3 out of 4
BRE upstream	33	CGGCGCC	0.3733	7 out of 7

Fig. 6 The prediction of core promoter elements; prediction of core promoter elements via ElemeNT tool for *BCL2* rs2279115 (**a**), and *BAX* rs4645878 (**b**) gene polymorphisms. ElemeNT tool revealed that these SNPs could alter the number, types, and scores of transcription start sites and/or core promoter elements in the promoter regions of *BAX* and *BCL2* genes

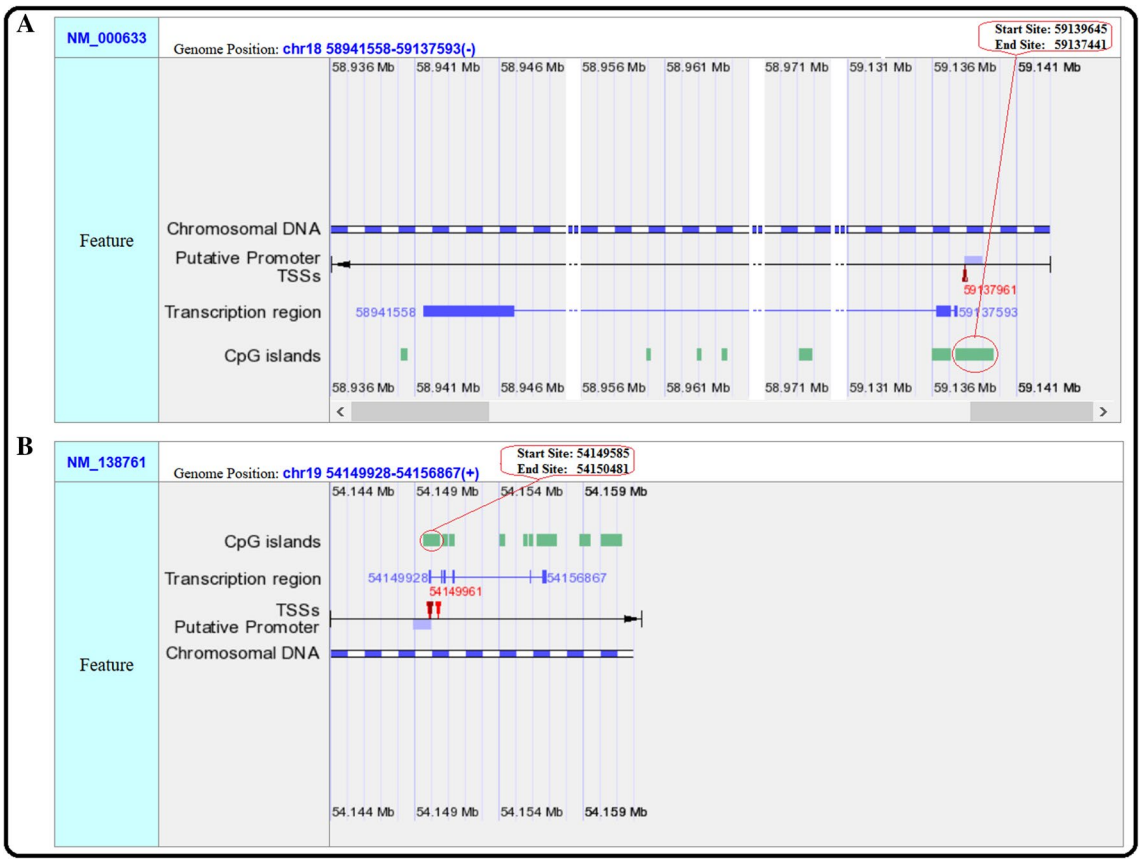


Fig. 7 The prediction of DBCAT: the results of DBCAT prediction of CpG islands of *BCL2* gene (**a**) and the results of DBCAT prediction of CpG islands of *BAX* gene (**b**). *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms were located in CpG islands of the promoters

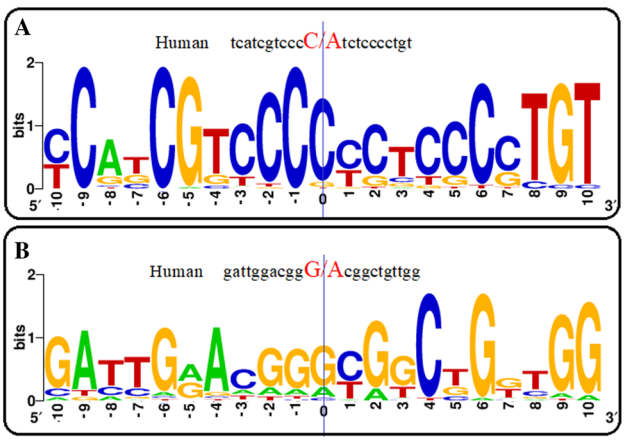


Fig. 8 Sketch of DNA sequence conservation: The WebLogo extracted results of DNA sequences conservation around *BCL2* rs2279115 (**a**) and *BAX* rs4645878 (**b**) gene polymorphisms. Human DNA sequences surrounding these loci are shown at the top. The blue vertical line indicates the variant-locus SNP position in humans and wild allele conservation in different mammalian species

PE susceptibility; however, there are several studies which assessed the relation between polymorphisms of the corresponding genes and various diseases, especially cancer [39, 40]. In another study by Dogru et al., it was shown that no significant association was observed between *BAX* rs4645878 (−248 G > A) and *BCL2* rs2279115 (−938 C > A) polymorphisms and Polycythemia vera and essential thrombocytosis [41]. In a meta-analysis by Yao et al. on 32 original articles regarding the effect of *BCL2* rs2279115 and rs1801018 polymorphisms in the promoter region on cancer risk, the association between rs2279115 was confirmed but not rs1801018 polymorphism and cancer susceptibility [39]. In Lindner et al. study, no relation was observed between *AKT1* rs1130233, BAX rs4645878, *FAS* rs223476, and *FASL* rs763110 polymorphisms as apoptosis-related genes and primary open angle glaucoma [42].

Despite the results of our study, several reports showed elevated expression of *BAX* gene in placentas of preeclamptic women; however, the difference in *BAX* expression was not observed in other studies. In addition, similar to our results, most reports showed reduced *BCL2* expression in preeclamptic placentas. In a study conducted by Mendilcioglu et al., the apoptosis rate was significantly higher in

trophoblasts of preeclamptic women. The Fas ligand expression was elevated in PE placentas; however, there was no significant increase in the expression level of *BAX, BCL2, TP53, CASP3*, and *FAS* genes between the two group [37].

Hung et al. showed that Hypoxia–reoxygenation (HR) leads to severe changes in villous apoptosis. Moreover, the mRNA expression of *BAX* and *BAK* was elevated and *BCL2* was decreased in preeclamptic placentas [43]. Similarly, higher apoptosis in cytotrophoblasts cultured under hypoxic conditions was demonstrated in the studies conducted by Hu et al. and Levy et al. They reported reduced expression of *BCL2*, and elevated expression of *BAX* in the placentas cultured under hypoxic conditions [44, 45]. Afroze et al. indicated that p38 phosphorylation, Bax/Bcl-2 ratio, caspase-9 and Cox-2 were up-regulated in PE placentas [46].

With respect to the association between *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms and mRNA expression in the placenta, there is no published report; however, there are several studies on the effects of *BAX* and *BCL2* polymorphisms on mRNA expression in tumor cells. Fernandes et al. indicated no correlation between *BCL2* mRNA-expression and *BCL2* −938C > A (rs2279115) polymorphism in patients with squamous intraepithelial neoplasia and controls [47]. Wang et al. showed lower *BAX* expression in patients with rs4645878 GA genotype in gastric cancer tissues [48]. However, Searle et al. refuted the association of rs2279115 polymorphism with *BCL2* expression in tumor tissue of breast cancer [49]. On the other hand, Hirata et al. showed lower *BCL2* expression and higher proliferative activity in rs2279115 CC genotype carriers in renal cancer tissues [50]. Pan et al. confirmed that *BCL2* rs2279115 CA and AA genotypes are associated with reduced mRNA expression of BCL-2 in both normal and cancerous esophagus tissues and indicated a genotype-phenotype correlation in this polymorphism [51]. In addition, the effect of *BCL2* rs2279115 polymorphism on *BCL2* mRNA expression has been reported by Zhang et al. in healthy subjects [52].

According to promoter methylation status of *BCL2* and *BAX* genes and their correlation with mRNA expression, no published report was observed on association between placental *BAX* and *BCL2* promoter methylation and PE susceptibility. Nevertheless, several findings have been published regarding the association between the expression of *BAX* and *BCL2* genes and promoter methylation in various cancers that their results are different and sometimes conflicting.

Similar to results of the current study in the placenta, Zhang et al. and Carvalho et al. indicated lower BCL2 expression in BCL2 promoter methylated status in rat HSC-T6 cells and prostate cancer, respectively [53, 54]. Down regulation of BCL2 expression in relation to BCL2 promoter methylation in Myelodysplastic syndrome was described by Del Rey et al. [55]. Loginov et al. showed a correlation between lower levels of expression and methylation of apoptotic genes such as BCL2 in breast cancer [56].

Recently, Nordor et al. reported the similarities in epigenetic alterations in cancerous cells and placentas in early pregnancy stage that are partially eliminated during pregnancy progression. In addition, they found that these common epigenetic alterations are associated with critical genes in placentogenesis as well as carcinogenesis [57]. In another study, Lim et al. showed a wide range of alterations in DNA methylation and mRNA expression at different gestational ages [58].

In addition, our findings regarding in silico analysis showed that BAX rs4645878 and BCL2 rs2279115 polymorphisms were located in the recognition site of various transcription factors so that the wild to mutant allele substitution might result in the loss and/ or change in these binding sites and subsequently altered the expression of *BCL*-2 and *BAX*. Moreover, *BAX* rs4645878 and *BCL2* rs2279115 polymorphisms occur in CpG islands and alteration of the sequences containing these SNPs might affect methylation status of these gene promoters.

Although the present study investigated the effects of a wide range of parameters including placental *BAX* and *BCL2* polymorphisms and methylation as well as mRNA expression on PE susceptibility with a bioinformatics aspect, it has several limitations that may affect the results, especially the relative small sample size. In addition, various exclusion criteria limited our sampling especially for the evaluation of mRNA expression. Different ethnic groups living in Southeastern Iran and various environmental conditions may be other limitations of this study. In particular, if the protein levels of BAX and BCL2 had been measured in the placenta, the results could be more valuable. Because this is the first report on the effects of placental *BAX* and *BCL2* polymorphisms and methylation on mRNA expression and PE susceptibility; further studies are needed to confirm or refute our findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The Ethics Committee of Zahedan University of Medical Science approved study protocol (IR.zaums.REC.1396.44).

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