Evaluation of CD39, CD73, HIF-1α, and their related miRNAs expression in decidua of preeclampsia cases compared to healthy pregnant women

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Research Article

Keywords: Preeclampsia, CD39, CD73, HIF-α, miRNA

Posted Date: March 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1461130/v1

Abstract

The Preeclampsia (PE) molecular mechanisms are not fully revealed and different biological processes are involved in the pathogenesis of PE. We aimed to evaluate adenosine and hypoxia-related signaling molecules in PE patients in the current study. Decidua tissue and peripheral blood samples were taken from 25 healthy pregnant and 25 PE women at delivery time. CD39, CD73, and Hypoxia-inducible factor-alpha (HIF-α) were evaluated in mRNA and protein level using real-time PCR and western blotting techniques, respectively. Also, miR-30a, miR-206, and miR-18a expression were evaluated by real-time PCR. At last, secretion levels of IGF and TGF-β in the taken serum of blood samples were measured by ELISA. Our results revealed that Expression of CD39 is decreased in PE cases versus healthy controls at mRNA and protein levels ($p = 0.0003$ for both). CD73 and HIF-α showed an increased level of expression in PE patients at RNA and protein status ($p = 0.0157$ and $p < 0.0001$ for protein evaluation of CD73 and HIF-α, respectively). The miRNA-30a ($p = 0.0037$) and miR-206 ($p = 0.0113$) showed elevated expression in the decidua of the PE group. The concentration of secreted IGF-1 ($p = 0.0002$) and TGF-β ($p = 0.0101$) in serum samples of PE cases compared to the healthy group were decreased and increased, respectively. In conclusion, our results showed that aberrant expression of molecules that are involved in ATP catabolism and the hypoxic conditions is observed in PE cases and involved in their hypertension and inflammation could be served as PE prognosis by more confirming in comprehensive future studies.

1. Introduction

Pre-eclampsia (PE) is a usual pregnancy-related complaint characterized by hypertension with abnormal elevated serum creatinine or proteinuria, high systolic and diastolic blood pressure, damaged liver function with elevated transaminase, cerebral difficulty, thrombocytopenia, and pulmonary edema [1]. This pregnancy related severe disease affects more than 5% of all pregnancies and 10–20% of nulliparous women worldwide. There are more than 50000 deaths reports of women every year due to this complication [2, 3]. On the other hand, there is a possibility of injuries in the fetus of preeclampsia women, such as fetal growth restriction, which is one of the most important causes of neonatal mortality [4]. Despite advances in the management of this disease in recent years, the exact cause of this disease, methods of early diagnosis and prevention have not been determined. In these cases, induction of preterm labor is recommended to reduce mortality [5]. Withdrawal of the fetus and placenta from the mother's body eliminates the symptoms of the disease, but nevertheless the complications of the disease are problematic for mother and child for the rest of their lives. These complications can increase the mother's risk of cardiovascular problems and metabolic disorders [6]. In addition, infants born from pre-eclamptic pregnancies suffer from higher-than-normal systolic and diastolic blood pressure at an early age and adolescence, and are more likely to develop metabolic diseases, epilepsy, stroke, and cardiovascular disease more than normal individuals [7].

Several hypotheses have been proposed regarding the causes of preeclampsia syndrome, including the effect of genetic and immunological factors, coagulation disorders, nutritional factors, and increased production of oxygen free radicals [8–10]. The current hypothesis about the etiology of preeclampsia

focuses on an imbalance of maternal immune responses including Th17/Treg balance and its related products and incomplete invasion of trophoblasts [11–13].

Under normal physiological conditions, the concentration of extracellular ATP varies between 400 and 700 Nano moles per liter. Elevated levels of ATP have been shown to stimulate various arteries and increase blood pressure [14]. During inflammation, hypoxia or ischemia, ATP levels can increase triple. For example, these conditions are seen in diseases such as cystic fibrosis, chronic obstructive pulmonary disease and preeclampsia [15]. High levels of ATP, now known as a danger signal, cause the production of vasoactive substances, pro-inflammatory cytokines, chemokines and adhesion molecules [16]. High levels of ATP lead immature T cells to inflammatory (Th17) cells, whereas in the absence or absence of ATP, the development of regulatory T cells is supported [17]. ATP is released by hypoxic and necrosis tissue like hypoxic placenta. As a protective mechanism, ATP can be hydrolyzed to adenosine by various extracellular enzymes present in cells, including endothelial cells and trophoblast cells [18]. In order to prevent the pathological effects of ATP, cells can use extrauterine enzymes such as actonucleoside triphosphate diphosphohydrolase 1 (CD39) and alkaline phosphatase, as well as 5-actonucleotidase (CD73) to convert ATP to ADP and AMP and subsequently adenosin and phosphate. These enzymes are expressed in many tissues, including the placenta [19, 20]. Adenosine inhibits T cell proliferation, cytokine production, and cytotoxic activity. Therefore, adenosine is an important immunosuppressive molecule. Also, hypoxic conditions lead to the production of two actoenzymes, CD73 and CD39. Studies have shown that CD73 expression is regulated by HIF-1α, examination of the CD73 gene promoter shows that there is a binding site for HIF [19].

MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in regulating the progression of biological processes by interacting with cellular messenger RNAs [21]. Inflammatory responses affect miRNA expression and affect their biogenesis by altering transcription and processing of precursor transcripts or affecting the stabilization of adult miRNAs. In recent years, the number of miRNAs involved in the development and biological functions, especially of the immune system, has increased dramatically, and there has been widespread debate about their potential use as a treatment for immunological diseases. Indeed, abnormal expression of miRNAs often occurs in human diseases, including blood disorders and autoimmunity [21]. Numerous studies have been performed on the role of regulatory and stimulatory miRNAs in the immune system in preeclampsia [22]. Although the role of miRNAs involved in regulating CD39, CD73, and HIF-1α expression in cancers and autoimmune diseases, has been studied, performed studies on the role of these molecules and their regulatory miRNAs in infertility diseases especially preeclampsia has been limited. Results of these studies have shown that miR-206 regulates CD39 and IGF-1, and miR-30a regulates CD73 and IGF-1 expression, while miR-18a regulates HIF-1α and SMAD-2 expression, which is one of the downstream molecules in the TGF-β signaling pathway [23–25].

According to the description, this study was aimed to investigate the expression of CD39, CD73, and HIF-1α molecules and their regulatory miRNAs in the decidua of preeclampsia patients compared to healthy pregnant women.

2. Material And Methods

2.1. Study design:

25 healthy pregnant women without any previous problems and 25 preeclamptic women which are characteristic by hypertension, edema, and proteinuria were contributed in this study. Characterization of preeclampsia was done according to the International Pregnancy Hypertension Study Society (ISSHP) criteria (Table 1) [26, 27]. Patients who had historical disorders like active thromboembolic problem, fetus or mother infection like abruption, Hyper and Hypothyroidism etc. were excluded from the study. Participants have also received no immunosuppressive medication such as steroids or etc. For the control group, 25 healthy age-matched pregnant women delivery time with no sign and history or disease were contributed for current study. The ethics Code of the present study is IR.TBZMED.REC.1399.142 which is approved by the ethics committee of Tabriz University of Medical Sciences.

Table 1
Clinical characteristics of the studied population.

Characteristics	Control (Mean ± SD) N = 25	PE (Mean ± SD) N = 25	*p* value	
Maternal age (years)	27.94 ± 3.52	28.62 ± 4.11	NS	
Time of blood collection GA (week)	34.52 ± 1.23	34.1 ± 1.33	NS	
GA at delivery (week)	39.05 ± 2.15	37.7 ± 3.22	NS	
Birth weight of fetus (g)	3354 ± 381.5	2862.3 ± 832.7	NS	
BMI (kg/m2)	27.12 ± 4.77	27.92 ± 4.39	NS	
Systolic blood pressure (mmHg)	108.8 ± 8.31	152.5 ± 19.4	< 0.0001	
Diastolic blood pressure (mmHg)	72.6 ± 12.8	91.7 ± 10.2	91.7 ± 10.2	0.0086
91.7 ± 10.2				
Proteinuria	Negative	5.69 ± 4.88	-	
Fasting Blood Sugar (mg/dl)	Fasting Blood Sugar (mg/dl)	84.91 ± 12.11	86.46 ± 10.53	NS
Fasting Blood Sugar (mg/dl)				
Triglyceride(mg/dl)	Triglyceride(mg/dl)	100.85 ± 14.36	111.5 ± 21.48	NS
Triglyceride(mg/dl)				
Cholesterol(mg/dl)	169.9 ± 34.1	210.6 ± 55.73	NS	
HDL- Cholesterol(mg/dl)	55.04 ± 4.42	49.98 ± 5.64	NS	

Abbreviations: BMI: Body mass index; HDL: High density lipoprotein; LDL: Low density lipoprotein.

Characteristics	Control (Mean ± SD) N = 25	PE (Mean ± SD) N = 25	*p* value	
LDL-Cholesterol(mg/dl)	LDL-Cholesterol(mg/dl)	64.1 ± 8.14	79.92 ± 11.28	0.0012
LDL-Cholesterol(mg/dl)				
Uric acid(mg/dl)	4.31 ± 0.98	5.73 ± 1.18	0.0237	
Abbreviations: BMI: Body mass index; HDL: High density lipoprotein; LDL: Low density lipoprotein.				

2.2. Sample collection:

Decidua and blood samples of participants were collected during cesarean operation. Obtained decidua was stored for gene and protein expression analysis. Blood serums were isolated using centrifugation at 3000 rpm for 8 minutes, and then stored at -80°C for future assays.

2.3. RNA extraction & real-time polymerase chain reaction (PCR):

After slicing decidua in 10 µm sections using microtome, sliced pieces were transfered to RNX-PLUS Solution. RNA extraction was done based on the manufacturer's instructions (Sina Clon, Iran). 1.8–1.98 ratio of A260/280 absorption was observed in all extracted RNA samples. Then, Complementary DNA (cDNA) was synthesized using revert aid reverse transcriptase kit (Thermofisher, USA).

Evaluation of CD73, CD39, HIF-1α, miR-18a, miR-30a, and miR-206 genes were done using SYBR-Green master mix (Thermofisher, USA) technique in CFX Connect Real-Time PCR Detection System (Bio-Rad, USA). The real-time PCR results were measured via the $2-\Delta\Delta CT$ method. Sequences of used primers are presented in Table 2.

Table 2
Primer sequences of evaluated genes.

Gene	Primer	Sequence (5'→3')
CD39	Forward	TCGCCTCTATGGCAAGGACTAC
	Reverse	TCCAGGATGAAAGCATGGGTCC
CD73	Forward	AGTCCACTGGAGAGTTCCTGCA
	Reverse	TGAGAGGGTCATAACTGGGCAC
HIF-1α	Forward	TATGAGCCAGAAGAACTTTTAGGC
	Reverse	CACCTCTTTTGGCAAGCATCCTG
miR-206	Forward	GAATGTAAGGAAGTGTGTG
	Reverse	GAACATGTCTGCGTATCTC
miR-30a	Forward	AACATCCTCGACTGGAAG
	Reverse	GAACATGTCTGCGTATCTC
miR-18a	Forward	AGGTGCATCTAGTGCAG
	Reverse	GAACATGTCTGCGTATCTC
β-actin	Forward	CACCATTGGCAATGAGCGGTTC
	Reverse	AGGTCTTTGCGGATGTCCACGT
U6	Forward	CTCGCTTCGGCAGCACAT
	Reverse	TTTGCGTGTCATCCTTGCG

Abbreviations: CD39: Ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1); CD73: 5'-nucleotidase (5'-NT); HIF-1α: Hypoxia-inducible factor 1-alpha; miR: Micro RNA; U6: Universal primer.

2.4. Enzyme-linked immunosorbent assay (ELISA):

The secretion levels of TGF-β and IGF-1 were assessed in serum via ELISA kit in duplicates based on manufacturer's guidance (MyBioSource, San Diego, CA, USA). 450 nm wave length were selected to read the absorbance of samples in an 800™ TS absorbance reader system (BioTek, USA). Standard curve was used to evaluate concentration of these factors.

2.5. Western blotting:

The western blotting technique was used to evaluate CD73, CD39, HIF-1α, and SMAD-2 products at protein status. At first, RIPA buffer (Santa Cruz, USA) was utilized to extract decidua proteins. SDS-PAGE electrophoresis and blotting procedure were applied in order to transfer protein bands on the PVDF paper [28]. After blotting step, the PVDF paper was incubated in blocking buffer (TBS-Tween-20 with BSA 3%) for 2 hours at room temperature, which is followed by incubation of the paper in primary antibodies

(1:5000 diluted) overnight at 4°C and secondary HRP- conjugated antibody (1:3000 diluted) for 2 hours at 25°C, respectively. Enhanced Chemiluminescence (ECL) kit was used to visualize targeted proteins using western blot imaging system (SabzCo, Iran). Between the all of steps, PVDF paper was washed 5 times (3 minutes for each time) by TBS-Tween-20 buffer. All antibodies and ECL kit were purchased from Santa Cruz Biotech (Santa Cruz, USA).

2.6. Data analysis

Version 25 of SPSS software was used to analysis the study results. Normal distributions of the data were evaluated using Kolmogorov-Smirnov test and unpaired student T-test was applied to analyze different variables between PE and control groups. Variables data were showed as mean ± SD and p-value < 0.05 was considered as statistically significant. Graphical figures was illustrated using Graphpad Prism ver.9.00 (GraphPad, USA).

3. Results

3.1. Studied population clinical and demographic parameters:

A total of 50 pregnant women (25 healthy pregnant women as control group and 25 PE women as case) with matched age were contributed in this study. PE patients had positive proteinuria (5.69 ± 4.88) which is this parameter was negative for control group. Case group had elevated systolic blood pressure ($p < 0.0001$), diastolic blood pressure ($p = 0.0086$), LDL-Cholesterol ($p = 0.0012$), and uric acid ($p = 0.0237$) level compared to healthy controls. Whereas other parameters didn't showed significant differences between two groups. Demographic details of studied population were shown in Table 1.

3.2. Adenosine and hypoxia related genes and miRNAs showed different expression patterns in PE cases:

CD73, CD39, HIF-1α, and their related miRNAs including miR-30a, miR-206, and miR-18a expression were evaluated transcription level between preeclampsia and normal pregnant women decidua tissue using quantitative real-time PCR technique. CD73 ($p = 0.0231$) and CD39 ($p = 0.0003$) genes showed upper and lower expression in PE patients compared to healthy group (Fig. 1). CD73 and CD39 related regulatory miRNAs (miR-30a and miR-206, respectively) had increased expression in PE cases ($p = 0.0037$ for miR-30a and $p = 0.0113$ for miR-206) (Fig. 2). HIF-1α ($p = 0.0011$) showed up regulated gene expression in PE patients and its related miRNA (miR-18a) had down regulated expression in these patients ($p = 0.0034$) (Fig. 1&2). Details of the studied genes expression level were shown in Table 3.

Table 3
Molecular changes in preeclampsia women vs healthy pregnant women.

Real-Time PCR (Fold Change)			
Target	**Control (Mean ± SD)** **N = 25**	**PE (Mean ± SD)** **N = 25**	*p* value
CD39	1.000 ± 0.08818	0.6440 ± 0.4531	0.0003
CD73	1.000 ± 0.1102	1.285 ± 0.5975	0.0231
HIF-1α	1.000 ± 0.09359	1.438 ± 0.6247	0.0011
miR-206	1.000 ± 0.06674	1.392 ± 0.7394	0.0113
miR-30a	1.000 ± 0.07791	1.513 ± 0.8353	0.0037
miR-18a	1.000 ± 0.07929	0.7272 ± 0.4358	0.0034
ELISA (serum)			
IGF-1 (ng/ml)	817 ± 157.1	590.8 ± 235.1	0.0002
TGF-β (pg/ml)	45.96 ± 20.92	32.24 ± 14.77	0.0101
Protein Expression%			
CD39	69.5 ± 25.52	30.5 ± 18.92	0.0003
CD73	37.2 ± 14.24	62.8 ± 21.07	0.0157
HIF-1α	28.3 ± 27.13	71.3 ± 18.89	< 0.0001
SMAD-2	60 ± 6.99	40 ± 12.86	0.0406

Abbreviations: CD39: Ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1); CD73: 5'-nucleotidase (5'-NT); HIF-1α: Hypoxia-inducible factor 1-alpha; miR: Micro RNA; IGF-1: Insulin-like Growth Factor-1; TGF-β: Transforming growth factor beta; SMAD-2: Mothers against decapentaplegic homolog 2.

3.3. Western blotting analysis of CD73, CD39, and HIF-1α:

In order to evaluate post transcriptional expression and validation of CD73, CD39, and HIF-1α real-time PCR results, we aimed to investigate these markers expression at protein level using western blotting assay. Western blotting of these markers showed similar results in line with real-time PCR outcomes. A considerable increase and decrease had seen in CD73 ($p = 0.0157$) and CD39 ($p = 0.0003$) expression, respectively at protein status in PE cases in comparison with control group (Fig. 3 & Table 3). While, HIF-1α ($p < 0.0001$) showed significantly increased expression at this level in PE women (Fig. 4). SMAD-2 as a signal transducer molecule which is positively regulated by miR-18a, showed decreased ($p = 0.0406$) expression in PE patients compared to control women (Fig. 4). Details of the targeted proteins genes expression status were shown in Table 3.

3.4. Evaluation of IGF-1 and TGF-β secretion level:

Investigation of IGF-1 and TGF-β secretion level in serum of PE and healthy pregnant women was done by enzyme-linked immunosurbent assay (ELISA). Results of ELISA showed lower levels of both serum IGF-1 ($p = 0.0002$) and TGF-β ($p = 0.0101$) in PE women compared to healthy controls (Fig. 5). Detailed information of IGF-1 and TGF-β results are presented in Table 3.

4. Discussion

In this study, the expression of all three major molecules involved in ATP catabolism and hypoxia including CD39, CD73, HIF-1a, and their regulatory miRNAs in the decidua of PE patients was investigated. These molecules can influence placenta environment by altering ATP, adenosine and cellular oxygen status which is observed in pregnancy related complications like recurrent pregnancy loss (RPL) and PE.

The PE molecular mechanisms are not fully revealed and different biological processes are involved in the pathogenesis of PE. Improper trophoblasts invasion and proliferation are two critical factors PE development and spreading. Therefore, it is necessary to examine the basis of these defects to identify reputable and effective targets for the treatment of PE [11]. High levels of ATP are one of the danger signals, which are now found in PE. Disrupted balance of adenosine and ATP with a low ratio of adenosine/ATP is found in PE, which may be resulting in the activation of endothelial cells, systemic inflammation, and hypertension [14].

CD39 is an ectonucleotidase cell surface enzyme with a high variety of cell distribution, which is most dominantly expressed in human trophoblasts and vessels and regulates the function of trophoblasts in an ATP-dependent manner [29]. CD73 is a glycosyl-phosphatidylinositol (GPI)-linked cellular surface enzyme with 70 kDa moleculare weight found in various tissues and act as an ecto-5'-nucleotidase. CD73s first function was described as a lymphocyte differentiating antigen by participating in T lymphocytes signaling as ligand which also can acts as adhesion molecule via binding to endothelium. A long variety of biological processes like precondition of ischemia, platelet function, vascular leac, tissue injury, hypoxia, and transportation of ion and fluid in epithelial are done by CD73 involvement. CD73 functions are outcome of the regulated hydrolytic activity on extracellular nucleotides [30]. Conversion of ATP and ADP to AMP and consequence changing of AMP to adenosine via CD39 and CD73 activities notify critical role of these enzymes in calibrating duration, magnitude, and particularity of purinergic signals. It is necessary to note that CD39 acts as rate-controling enzyme in ATP/CD39/CD73/adenosine cascade [31].

There are several studies on CD39 and CD73 expression and their role in pregnancy disorders especially in PE based on CD39-CD73 roles in different inflammatory and hypoxic condition and their expression pattern. Our results showed decreased and increased expression of CD39 and CD73 in decidua tissue of PE patients compared to healthy pregnant women at both mRNA and protein level. In line with our results,

Zhu et al. [32] showed that decreased expression of ZDHHC14 and CD39 by hypomethylation is related to late-occurred PE cases via the these genes regulatory effects on trophoblast cell lines. They concluded that ZDHHC14 and CD39 may consider as potential targets and markers in PE clinical diagnosis and treatment [32]. In another study was done on mouse model of PE by McRae et al. [29], they concluded that inducing gestational hypertension in mice could be done by transferring TH1-polarized lymphocytes which are isolated from maternal blood and CD39 overexpression has protective effects in this model. In supporting our outcomes for CD73 expression in PE cases, elevated expression of CD73 as key regulatory enzyme which cause increase of adenosine in placenta was observed by Iriyama and coworkers in their study [33]. Huang et al. [34] observed reduction of adenosine in placenta and attenuated preeclampsia features and hypomethylation of placental DNA in preeclampsia mouse model which is induced by autoantibody.

Hypoxia-inductive Factor-1α (HIF-1α) is a critical transcription factor that alters response of the cell to hypoxia in pathological situations [35]. HIF-1 has a basic and heterodimeric helix-loop-helix structure and is composed of two alpha and beta subunits. It found that the determination of HIF-1 activity is measured by HIF-1a. There is a close correlation between CD73 and HIF-1a activity, which is confirmed by Lu et al. [36] in a study was done on human gastric carcinoma. Adenosine receptors comprise ADORA2A in promoter section of placenta tissue, which is respond to hypoxia. HIF-1α and HIF-2α controls ADORA2A, and ADORA2B, respectively. It is expected that the chronic hypoxic state of PE patient's placenta is result of elevated expression of placental ADORA2A and ADORA2B [19]. Also, increased transcription of soluble endoglin (sEng), soluble fms-like tyrosine kinase-1 (sFlt-1), and endothelin-1 (ET-1), which plays key role in PE pathogenesis are related to elevated expression of HIF-1α in the PE patient's placenta [35].

HIF-1α known as one of main molecules involved in hypoxia condition which is observed in preeclamtic placenta. We observed significant increase in expression of HIF-α mRNA and protein in PE patients. In supporting our data, up-regulation of HIF-1α in placenta of PE patients was confirmed in several studies [35, 37–39]. Persistent enhancement of placental adenosine and bilateral up-regulation of HIF-1α promotes PE pathogenesis which is observed by Iriyama et al. [35] in a mouse model study. Zhao confirmed a significantly increased expression of HIF-1a and TLR4 in placental tissues from severe PE cases (late-onset and early-onset) compared to healthy control. In addition, he suggested that HIF-1α could promote human placental micro-vascular endothelial cells (hPMECs) apoptosis by upregulation of TLR4 expression during PE pathogenesis [37].

MicroRNAs are small molecules with a large impact on the cellular and physiological function of the body and play an undeniable role in the pathogenesis of disorders like PE. In biogenesis of miRNA, pri-miRNA converts to single-hairpin precursor by a nuclear protein complex which has a binding site for double-strand RNA (dsRBP). After that, pri-miRNA delivers to the cytoplasm by exportin-5. Dicer is an RNase-III family cytoplasmic enzyme and modifies long dsRNA to 19–25 nucleotides named double-strand miRNA (ds-miRNA). The function of the human Dicer is consonant with dsRBP. At the end of miRNA biogenesis, produced ds-miRNA is hand-over to the Argonaute in an RNA-induced silencing complex process, and finally, one strand discard and the other was chosen to become mature miRNA [22].

We evaluated expression level of miR-30a, miR-206, and miR-18a in PE cases decidua in the current study. Our results showed decreased expression level of miR-18a in PE cases. MiR-18a regulates HIF-1α and SMAD-2 in positive and negative manner, respectively [25, 40]. In confirmation of our results, decreased levels of miR-18a in PE cases have been reported in several studies [40–42]. As result of miR-18a immune system related positive target, our group observed decreased levels of SMAD-2 and TGF-β in PE patients. SMAD-2 is a signal transduction molecule which is involved in TGF-β signaling pathway [40]. In a study was don Xu and coworkers [40], they found a reduction of miR-18a contributes to PE by down-regulating Smad-2 and reducing TGF-b Signaling. Zhu was found that promotion of apoptosis in human trophoblast cells and inhibited invasion of trophoblast occurs by targeting the ESRα gene via miR-18a suppression [41]. We reported increased expression of miR-30a and miR-206 in the decidua of preeclamptic cases. Also, our study showed decreased levels of serum IGF-1 as a common target of miR-30a and miR-206 in blood samples of PE patients. Several studies revealed decreased increased expression of miR-30a and miR-206 in PE and their regulatory effect on IGF-1 levels in these patients [43–45]. In a study which is done by Akehurst et al. [45], up and down-regulation of miR-206 and IGF-1 was seen in PE cases maternal circulations and placental tissue. They reported that mir-206 can be introduced a novel PE factor which is up-regulated during pregnancy of these patients.

One of the weaknesses of our study is the small sample population, which returns to the difficulty of sampling decidua tissue. Another weakness of this study was the lack of blood sampling at different times of the pregnancy process in PE patients, which could identify the changes in the blood variables we studied during pregnancy.

In conclusion, PE is a multifactorial pregnancy disorder with physiological, environmental, immunological, genetic, and anatomical aspects. It's difficult to know the exact etiology of PE because of such a variety of signs. We examined molecules that are involved in ATP catabolism and hypoxia as the main elements of hypertension which is the most dominant sign of PE. Our results showed that differential expressions of miR-18a, miR-30, miR-206, and their target genes could be considered as PE factors. These molecules are a few from a large scale of targets which is need to survey in future studies to find PE exact etiology.

Abbreviations

PE: Pre-eclampsia

ELISA: Enzyme-linked immunosorbent assay

HIF-1α: Hypoxia inducible factor-alpha

CD39: Actonucleoside triphosphate diphosphohydrolase 1

CD73: 5-actonucleotidase

miRNAs: MicroRNAs

SMAD-2: SMAD Family Member 2

TGF-β: Tumor growth factor beta

GPI: Glycosyl-phosphatidylinositol

sENG: Soluble endoglin

sFlt-1: Soluble fms-like tyrosine kinase-1

ET-1: Endothelin-1

hPMECs: Human placental micro-vascular endothelial cells

dsRBP: double-strand RNA binding protein

ds-miRNA: double-strand miRNA

IGF-1: Insulin-like growth factor 1

TLRs: Toll-like receptors

cDNA: Complementary DNA

PVDF: Polyvinylidene fluoride

BSA: Bovine serum albumin

ECL: Electrochemiluminescence

BMI: Body mass index

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

HDL: High-density lipoprotein

FBS: Fasting blood sugar

TG: Triglyceride

LDL: Low-density protein

Declarations

Ethics approval and consent to participate:

The ethics Code of the present study is IR.TBZMED.REC.1399.142 which is approved by the ethics committee of Tabriz University of Medical Sciences. Written informed consent was obtained from all participants after receiving an explanation of the study.

Consent for publication:

All authors read and approved the final manuscript for publication.

Availability of Data:

The data cannot be shared in public because of ethics and individual privacy restrictions but are limitedly available by contacting the corresponding author of this study, privately.

Conflict of Interest:

The authors declare that they have no conflict of interest.

Funding:

This work is financially supported by Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran [Grant NO. 65106].

Author Contributions:

M.Y., S.SF., and A.M. contributed to the conception and design of the study. Y.Y., MS.S-Z., F.P., and S.D. performed the laboratory assays. S.D. and S.T. contributed to sample collection. MS.S-Z. and L.A-M. performed the statistical analysis. Y.Y. wrote the manuscript. F.J-N., H.SK., L.K., and A.T. contributed to the acquisition of data. M.H-F. and J.AH. contributed to editing the final version of the manuscript.

Acknowledgements:

The current study is a part of the Ph.D. thesis for Yousef Yousefzadeh. Financial support of this thesis has been done by the Stem Cell Research Center of Tabriz University of Medical Sciences, Tabriz, Iran [Grant No: 65106].

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Figures

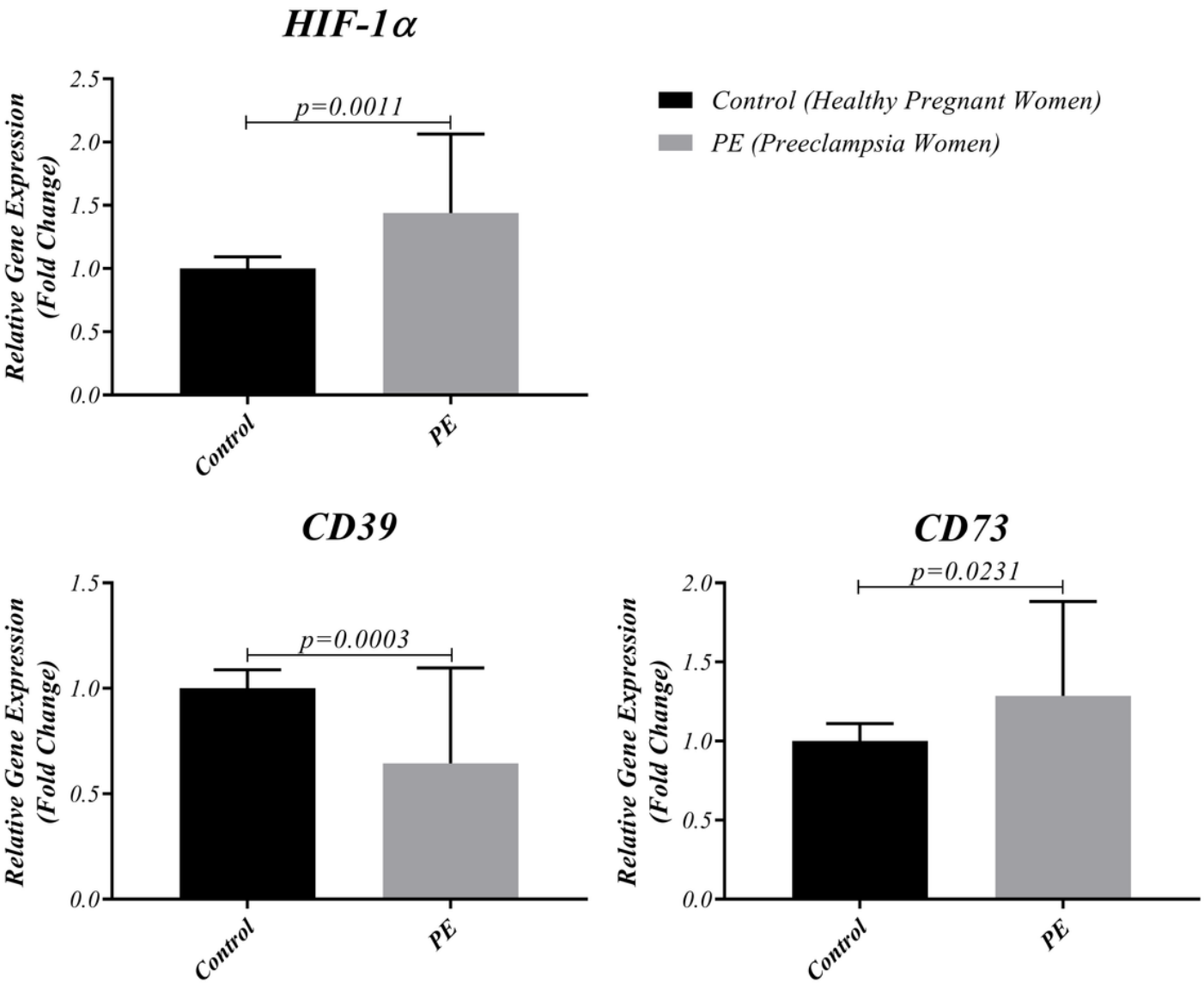

Figure 1

Expression of CD39, CD73, and HIF-1α as ATP catabolism and hypoxia related factors at mRNA level. The expression of CD39 was down-regulated and CD73 and HIF-1α was up-regulated in PE cases compared to healthy pregnant women. $p < 0.05$ was considered as statistically significant (Healthy pregnant group, n=25, PE women, n=25).

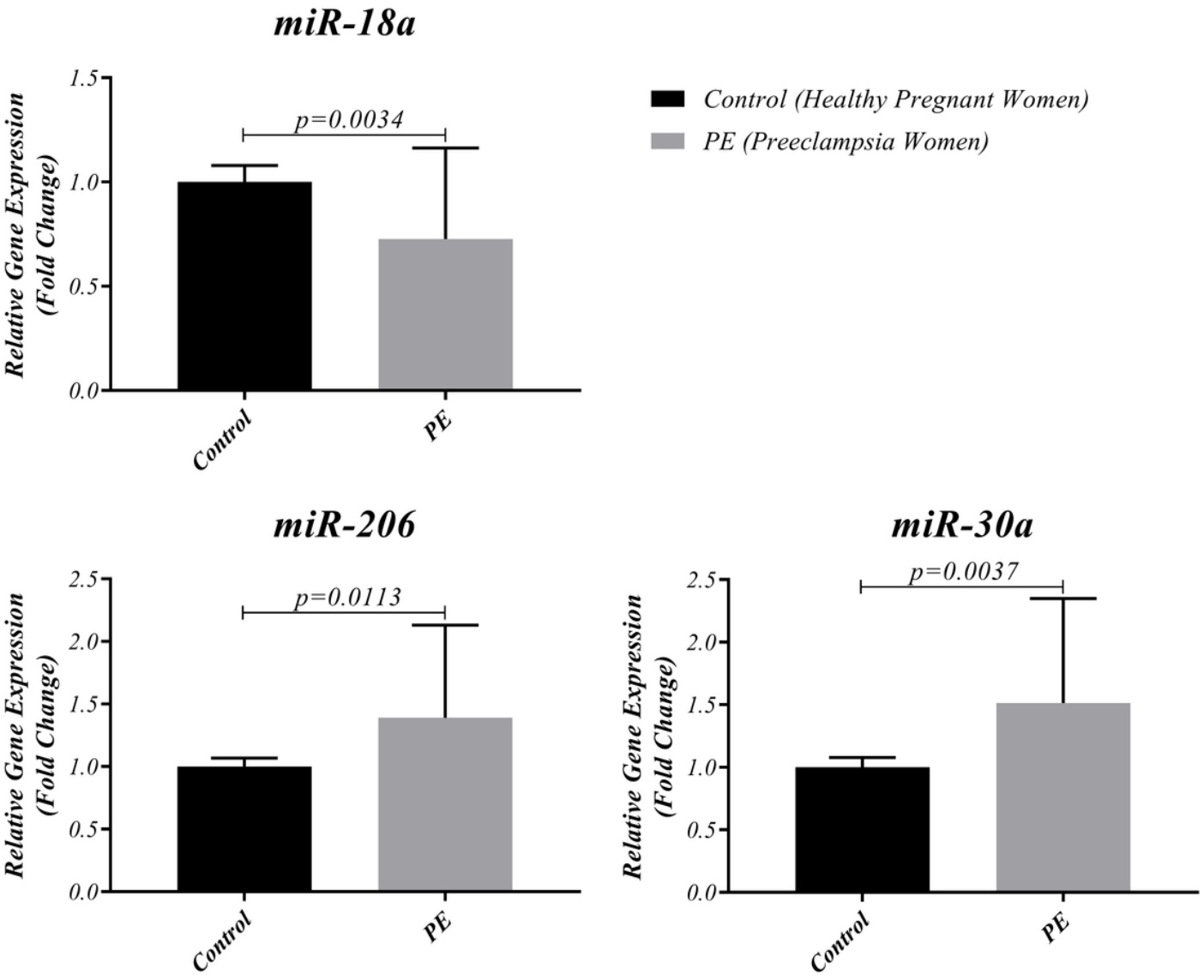

Figure 2

miR-18a, miR-30a, and miR-206 analysis in PE cases. The miR-18a had decreased expression and miR-30a and miR-206 had increased expression level in decidua of PE group versus healthy group. $p < 0.05$ was considered as statistically significant (Healthy pregnant group, n=25, PE women, n=25).

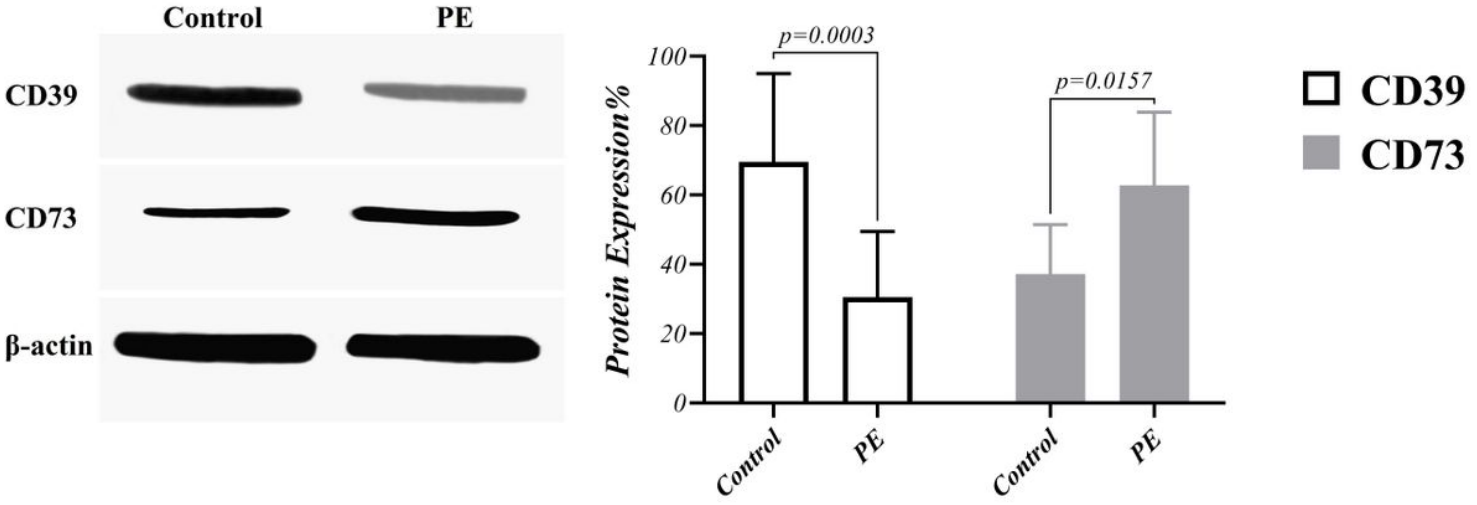

Figure 3

Protein expression analysis of CD39 and CD73. CD39 and CD73 showed decreased and increased level of protein expression in PE women compared to health group, respectively. $p < 0.05$ was considered as statistically significant (Healthy pregnant group, n=25, PE women, n=25).

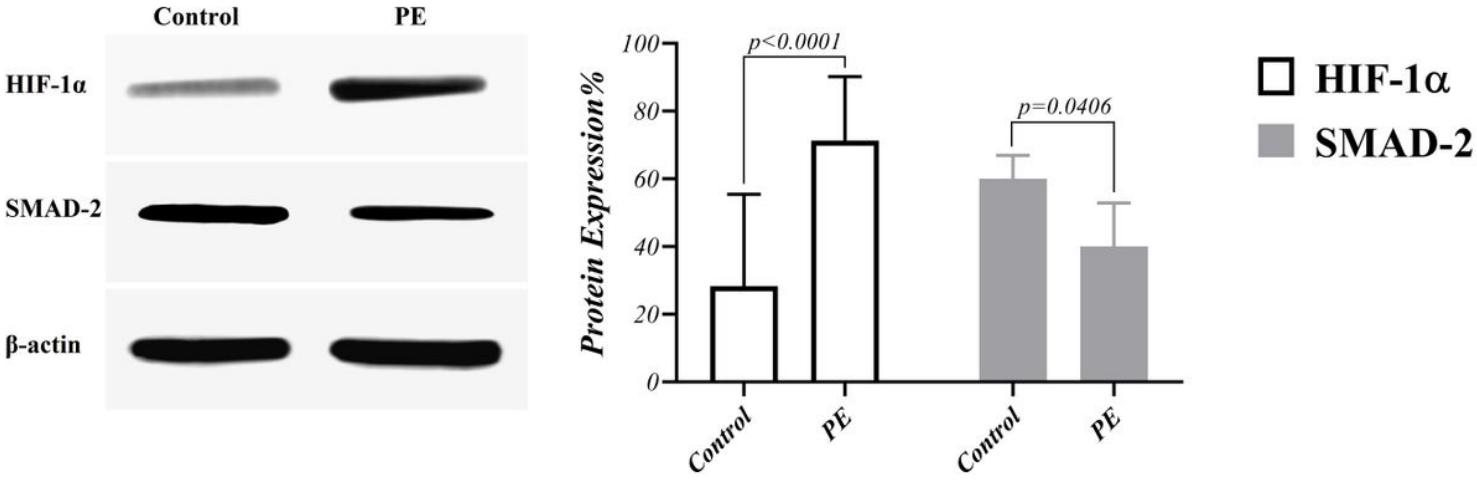

Figure 4

Protein expression analysis of Hif-1α and SMAD-2. Hif-1α and SMAD-2 showed increased and decreased expression level at protein status in PE cases compared to health pregnant women, respectively. $p < 0.05$ was considered as statistically significant (Healthy pregnant group, n=25, PE women, n=25).

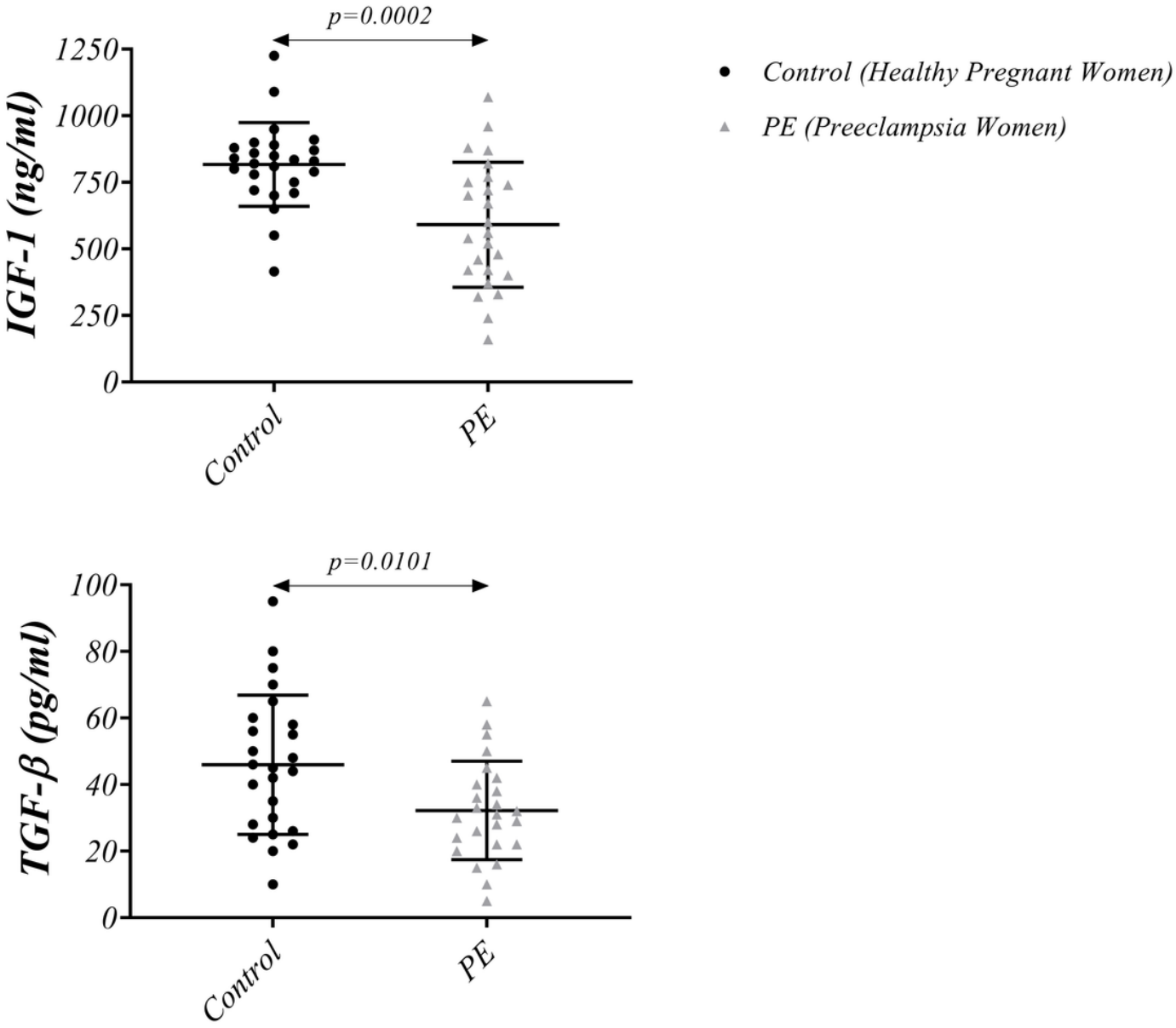

Figure 5

Serum concentration of IGF-1 and TGF-β in PE women. IGF-1 had higher and TGF-β had lower concentration in serum of PE women compared to healthy pregnant women. $p < 0.05$ was considered as statistically significant (Healthy pregnant group, n=25, PE women, n=25).