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Expression of sirtuin 2 and 7 in placenta accreta spectrum

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SUMMARY

OBJECTIVE: This study aimed to investigate the expression levels of sirtuin 2 and sirtuin 7 in the placenta accreta spectrum to reveal their role in its pathogenesis.

METHODS: A total of 30 placenta accreta spectrum, 20 placenta previa, and 30 controls were experienced. The sirtuin 2 and sirtuin 7 expression levels in the placentas of these groups were determined by Western blot. sirtuin 2 and sirtuin 7 serum levels in the maternal and fetal cord blood were examined by enzyme-linked immunosorbent assay.

RESULTS: It was found that sirtuin 7 in placenta accreta spectrum was significantly lower in the placenta compared to the control and placenta previa groups ($p<0.05$). However, a significant difference was not observed between the sirtuin 2 and sirtuin 7 levels in the maternal and fetal cord serum samples of those three groups ($p>0.05$).

CONCLUSION: Sirtuin 7 may play an important role in the formation of placenta accreta spectrum. The effect of decreased expression of sirtuin 7 might be tissue-dependent in the placenta accreta spectrum and needs to be investigated further.

KEYWORDS: Epithelial-mesenchymal transition. Placenta accreta. Placenta previa. Sirtuin 2. Sirtuins.

INTRODUCTION

Placenta accreta spectrum (PAS) is the aberrant invasion of the placenta by trophoblasts into the myometrium[1]. PAS is histopathologically divided into three types according to the degree of attachment to the myometrium, namely, placenta accreta, increta, and percreta[2]. In placentation, extravillous trophoblasts (EVTs) containing interstitial and endovascular cells invade the superficial myometrium and cause remodeling of the basilar and spiral arteries[3,4]. The most common risk factors that increase the probability of PAS formation are total or partial absence of decidua and intrauterine surgical scars[5-7], presence of the placenta previa (PP), and advanced maternal age[8]. PAS has a serious effect on maternal health and increases maternal mortality and morbidity up to 0.7 and 46.9%, respectively[9,10].

The process known as epithelial-mesenchymal transition (EMT) transforms motionless epithelial cells into migratory mesenchymal cells[11]. Therefore, it is very critical for the adherence of the placenta to the myometrium during the first trimester. Although, in the second and third trimesters, EMT should not be continued[12], it was reported that if EMT is presented in the second and third trimesters, it may contribute to the formation of PAS[13].

It was observed that sirtuin 2 (SIRT2) is weakly expressed in placental disorders[14], and along with the SIRT2, sirtuin 7 (SIRT7) is known as having roles in the EMT[15]. Besides, SIRT2 has been shown to increase the abnormal proliferation and migration of cancer cells by promoting the expression of EMT-related genes[16]. Transforming growth factor-β (TGF-β) is also an important regulator of EMT, and SIRT7 is known to modulate EMT/TGF-β signaling[17]. Therefore, our study aimed to investigate the expression levels of SIRT2 and SIRT7 in the placental tissues, maternal and fetal cord of the control, PP, and PAS groups.

METHODS

Study subjects

This study was approved by and conducted with the decision of the Inonu University Clinical Research Ethics Committee (2020/51-13.05.2020), and informed consent was obtained

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from all participants. Clinical samples including placenta, fetal cord serum, and maternal serum of 80 women were used, and they were supplied by the Department of Obstetrics and Gynecology of Dicle University Faculty of Medicine. Samples were divided into three groups. The first group named the PP group (n=20) consists of patients with no previous history of cesarean section or uterine surgery but diagnosed with PP without invasion. The second group named the PAS group (n=30) has the patients who had at least one previous cesarean section along with the PP and invasion. The third group, the control group (n=30), includes healthy women with similar demographic features (Table 1). Exclusion criteria were as follows: (a) patients with PP marginalis or inferior placenta, (b) patients who underwent surgery before the 24th week of pregnancies, (c) patients who gave birth under 500 g, (d) patients under the age of 18 years, (e) patients having multiple pregnancies, (f) patients having pregnancy complications with thyroid dysfunction, hypertension, epilepsy, gestational diabetes mellitus, type 1 and type 2 diabetes mellitus, patients using any medications that may affect the cardiovascular system, and pregnant women with kidney disease were not included in the study. To make a preoperative diagnosis, abdominal, transvaginal, and Doppler ultrasonography were used. PAS or PP was defined and diagnosed according to the current American College of Obstetricians and Gynecologists[18] and Society for Maternal-Fetal Medicine guidelines as well as FIGO consensus guideline[19]. PAS was also diagnosed with the pathology results. Age of patients, gravidity, parity, pregnancy week, newborn's gender, newborn's weight, and other patient information were recorded (Table 1). Placental tissues were collected immediately after the cesarean section and stored at -80°C until Western blot analysis.

Western blot analysis

Placenta samples were removed from the -80°C and crushed in liquid nitrogen. Then, cold RIPA buffer containing protease-phosphatase inhibitor cocktail and nuclease (Thermo Scientific) was added to the sample. The total cellular protein concentration of lysates was determined by BCA protein assay kit (TaKaRa). Total cellular proteins (20 μg) were separated using the 10% SDS-PAGE (BIO-RAD), and the separated proteins were transferred to the PVDF transfer membrane. The membranes were incubated with anti-SIRT2 antibody (STJ25534) and anti-SIRT7 antibody (STJ25536) for 2 h at room temperature, and β-actin (Mouse IgG2b-643802-Biolegend) was used as a loading control. Appropriate HRP-conjugated secondary antibodies (Biolegend) were used to visualize the specific bands by ECL (Advansta) and the images were taken by using G: Box (Syngene).

Enzyme-linked immunosorbent assay

Maternal peripheral venous blood was obtained before the administration of anesthesia. Fetal cord blood was taken from the umbilical artery after the umbilical cord was clamped and stored at -80°C before use. Serum levels of SIRT2 and SIRT7 were examined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (SunRed bio, Shanghai, PR China). The optical density (OD) of each well was identified with a microplate reader at 450 nm.

Statistical analysis

Line intensities obtained from the Western blot analysis were evaluated by the Image J. The OD values resulted from ELISA were analyzed via the Myassays program. Statistical analysis was performed by the SPSS® 11.5 (SPSS Inc.; Chicago, IL, USA) program. After the analyses, the numerical data were given as mean±standard deviations. Normally distributed data among

Table 1. Demographic and clinical characteristics of patient groups.

Demographic and clinical characteristics	Control	PP	PAS	p-value
	Mean±std	Mean±std	Mean±std	
Age	33.3±4.8	33.4±6.0	33.9±4.9	0.883
Gravidity	4.7±1.9	3.2±2.3	5±1.8	0.009*
Parity	3.2±1.4	1.45±1.5	3.3±1.7	0.0002**
Previous cesarean section	2.7±1.1	0	2.3±1.0	0
Birth weight (g)	3,076±420	2,954±574	2,788±430	0.062
Birth week	37.2±1.2	36.5±2.4	36.3±1.2	0.106
Hemoglobin	11.7±1.4	11.3±1.6	11.5±1.2	0.689
Hematocrit	36.1±3.6	36.5±4.7	34.9±3.5	0.505
Thrombocyte	224±58	197±40	227±51	0.103

*p<0.05, **p<0.01.

the multiple groups were analyzed with the one-way ANOVA test. A value of p<0.05 was considered statistically significant.

RESULTS

In this study, we investigated the expression levels of SIRT2 and SIRT7 in control, PAS, and PP placentas. In addition, we determined the maternal and fetal cord serum levels of these three groups. The control, PP, and PAS groups have similar demographic and clinical characteristics (i.e., age, birth weight, week of birth, hemoglobin, hematocrit, and thrombocyte in all groups) (Table 1).

Our results showed that SIRT7 expression levels in placenta were lower in patients with PAS compared to the control and PP groups (*p<0.024) (Figure 1). In addition, SIRT2 expression levels were found to be decreased in PAS patients compared to the control and PP groups. However, this decrement was not statistically significant (p>0.05) (Figure 1).

SIRT2 and SIRT7 protein levels were identified by ELISA in all maternal and fetal cord sera. According to the results, there was no statistically significant difference between SIRT2 levels in both maternal serum samples of the control (n=30, mean: 1.7 ng/mL, ±std: 1.0), PP (n=20, mean: 1.5 ng/mL, ±std: 0.8), and PAS groups (n=30, mean: 2.3 ng/mL, ±std: 1.7) (p=0.0549) (Figure 2A) and fetal cord sera of the control (n=30, mean: 4.5 ng/mL, ±std: 3.13), PP (n=20, mean: 3.85 ng/mL, ±std: 2.91), and PAS groups (n=30, mean: 4.14 ng/mL, ±std: 3.09) (p=0.6932) (Figure 2B). When SIRT7 levels were compared in the maternal sera, there were no differences between control (n=30, mean: 2.2 ng/mL, ±std: 1.2), PP (n=20, mean: 2.3 ng/mL, ±std: 1.2), and PAS groups (n=30, mean: 2.5 ng/mL, ±std: 1.6) (p=0.6574) (Figure 2C). The SIRT7 levels in fetal cord serum were also measured, and a similar trend was found in three groups as in maternal sera of control (n=30, mean: 2.2 ng/mL, ±std: 1.0), PP (n=20, mean: 2.0 ng/

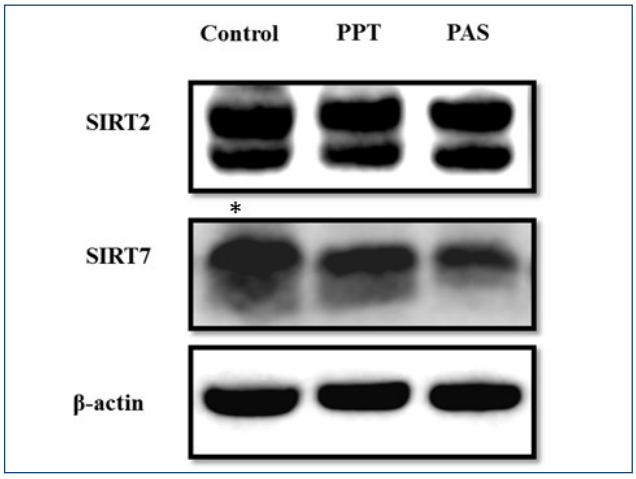

Figure 1. Expression level of sirtuin 2 and sirtuin 7 in placentas of control, placenta previa, and placenta accreta spectrum groups. sirtuin 2 expression levels were found to be not decreased statistically significant in placenta accreta spectrum patients compared to control and placenta previa groups. β-actin was used as a loading control. Sirtuin 7 expression levels were significantly lower in placental tissue of placenta accreta spectrum patients compared to the control and placenta previa groups (*p<0.024).

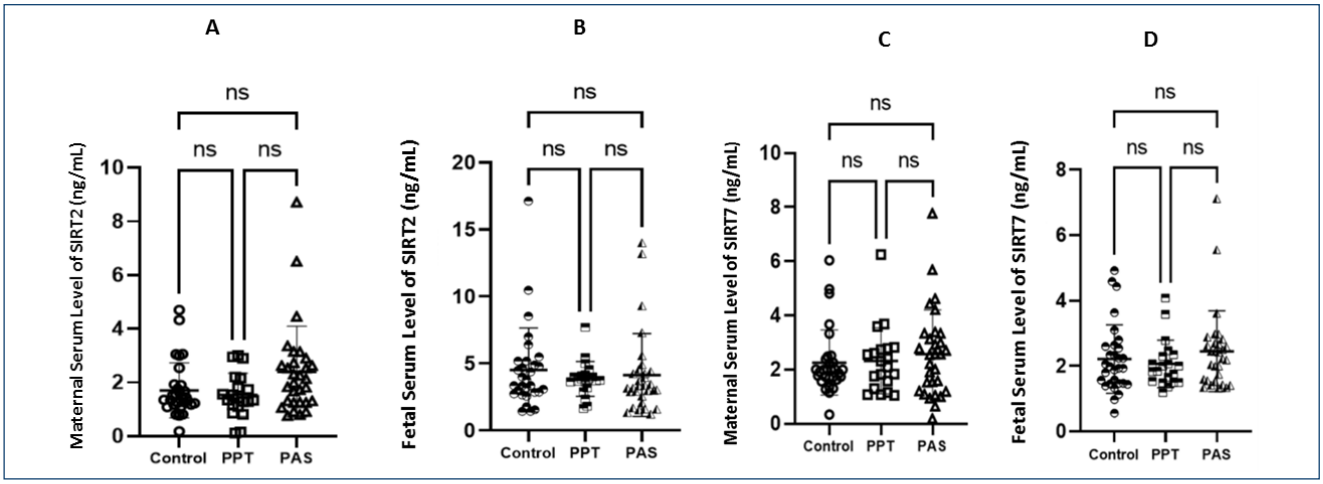

Figure 2. Maternal and fetal serum levels of sirtuin 2 and sirtuin 7 in control, placenta previa, and placenta accreta spectrum groups. (A) Comparison of sirtuin 2 levels in maternal sera from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. (B) Comparison of sirtuin 2 levels in fetal sera from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. (C) Comparison of sirtuin 7 levels in maternal sera from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. (D) Comparison of sirtuin 7 levels in fetal serum from the control (n=30), placenta previa (n=20), and placenta accreta spectrum (n=30) groups. One-way ANOVA was used for comparisons among the three groups.

mL, ±std: 0.7), and PAS (n=30, mean: 2.4 ng/mL, ±std: 1.2) (p=0.5880) (Figure 2D). Finally, no significant difference was found in the serum levels of the three groups.

DISCUSSION

Placental adhesion anomally is a condition in which the placenta adheres to the uterine wall in various degrees. Although the development of PAS is a complex and multi-factor process, the molecular mechanism behind the PAS is still unknown.

As the lack of the decidua or basal layer, improper maternal revascular patterning, and excessive EVT invasion are among the postulated theories for the emergence of PAS[20], many studies have investigated the EMT markers in PAS. Although EMT is necessary for proper placental invasion and attachment to the myometrium in the first trimester, it should not continue throughout pregnancy[12]. It has been hypothesized that excessively vigorous EMT that persists during pregnancy contributes to the development of PAS[13]. N-cadherin, ZEB1, and Snail are also markers of EMT[21]. The loss of the crucial E-cadherin is the most visible symptom of EMT. The expression of E-cadherin was decreased in the chorionic villi of the invasive part of the placenta, whereas the expression of Snail and TGF-β increased in the decidual cells of the invasive region[22]. These data imply that EMT may have an important role in PAS. Sirtuins have been shown to affect epithelial plasticity by reprogramming transcription at the EMT, leading to invasion and metastasis. That is why we proposed to investigate SIRT2 and SIRT7 in PAS as they govern in EMT.

In this study, we examined SIRT2 and SIRT7 expression in the placenta, maternal serum, and fetal cord serum of PAS. Our result showed that there is a reduced expression of SIRT7 in the placental tissue of PAS patients compared to the PP and healthy groups. Likewise, it has been showed that SIRT7 is significantly downregulated in breast cancer lung metastases in humans and mice, deacetylates beta-transducin repeat containing E3 ubiquitin protein ligase (β-TrCP1), mediates SMAD family member 4 (SMAD4) degradation and SIRT7 deficiency, activates TGF-β signaling, and increases EMT[23]. It was also demonstrated that resveratrol antagonizes TGF-β signaling by activating SIRT7 deacetylase activity, inhibiting breast cancer lung metastases and increasing survival. Besides, SIRT1 can regulate SMAD4 with SIRT7 in breast cancer metastasis[23]. Interactions between the TGF-β protein family have been shown to contribute greatly to the regulation of EVT invasion[17]. As SIRT7 expression was found to be reduced in the placenta of the PAS group, but not in maternal and fetal sera in our study, and the serum level of TGF-β expression was found to be significantly higher in the placenta accreta group by another research[24], the effect of decreased expression SIRT7 and its contribution to the EMT may be the tissue depended on PAS, which needs to be further investigated.

We have also evaluated SIRT2 expression in placenta, maternal, and fetal cord of PAS patients and compared with the PP and control groups. SIRT2 is known to have dual roles in many different tumors via regulation of EMT as SIRT7. SIRT2 positively regulated EMT and upregulated the protein levels of the mesenchymal markers such as N-cadherin and vimentin and the levels of MMP2 and MMP9 in osteosarcomas[25]. However, an increase in MMP9 was observed in SIRT2-null mouse embryonic fibroblasts as was a decrease in E-cadherin, which promotes cellular migration and invasion. Even though it has also revealed an increase in the expression of MMP9 in PAS regulated by SIRT2[22], we could not detect any differences in SIRT2 levels in placental tissue, maternal, and fetal cord sera of PAS patients. Those data imply that MMP9 increase may be regulated by another way rather than SIRT2 in PAS.

CONCLUSION

Our results revealed that the expression of SIRT7 was reduced in PAS. Although this is the first study showing the relationship between the SIRT2 and SIRT7 with PAS, further studies are still needed for understanding the exact role of SIRT2 and SIRT7 in PAS.

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AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHORS' CONTRIBUTIONS

IIT: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **MSI:** Data curation, Resources, Writing – original draft. **FMF:** Data curation, Resources. **SG:** Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **DCD:** Resources, Writing – review & editing.

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