Expression of Annexin A5 in maternal blood and placenta in pregnancies with preeclampsia

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Abstract

Background The placental anticoagulant protein Annexin A5 is highly expressed on the apical surfaces of syncytiotrophoblasts and plays an important role in maintaining blood fluidity in the placental circulation. We investigated the expression of Annexin A5 in maternal blood and placentas from pregnancies complicated by preeclampsia compared with uncomplicated pregnancies. Materials and Methods Placental tissue and maternal blood were collected from pregnancies complicated by preeclampsia. They were classified into two groups: early-onset group (n = 10), late-onset group (n = 10) .10 women without perinatal complications who accepted elective term cesarean section were chosen as the control group. Western blot and immunohistochemistry were used to detect the expression and localization of Annexin A5 in the placenta. Annexin A5 mRNA expression was quantified by Real-Time PCR. Annexin A5 and coagulation factor Xa in maternal blood was measured by an enzyme-linked immunosorbent assay. Prothrombin time, activated partial thromboplastin time, Thrombin time, fibrinogen were detected in each groups. Results (1) The expression of Annexin A5 in maternal blood were: 5.54±0.29 in early-onset group; 5.10±0.28 in late-onset group; 4.5±0.19 in control group. They were significantly higher in preeclampsia groups than in control group (P = 0.013). However, there was no significant difference among preeclampsia groups (P > 0.05). The mean ±SD coagulation factor Xa expression in the early-onset and late-onset group were 1229.23±45.33 and 1366.96±56.30 versus 1372.95±45.44 in the control group, which has not statistically significant(P=0.072). (2) The protein expression of Annexin A5 in placenta was not statistically significant between the early-onset, late-onset, and control groups (mean ± SD): 0.01 ± 0.02, 0.95±0.03vs.0.99 ± 0.02, p = 0.071.(3) The Annexin A5 mRNA levels in placenta were: 0.79±0.23 in early-onset group; 1.18±0.53 in late-onset group; respectively. All were significantly lower than that in control group (6.19±1.68, P =0.024), and no significant difference was found among preeclampsia groups (P > 0.05). (4) Prothrombin time, activated partial thromboplastin time, Thrombin time, fibrinogen levels were: (11.9 ± 0.23), (33.38± 0.85) s, (16.88 ± 0.45) g/L and 3.85 ± 0.23 in early-onset group; (11.65 ± 0.12), (33.15 ± 0.36) s, (16.13 ± 0.19) g/L and 4.91 ± 0.14 in late-onset group; (12.01 ± 0.09), (32.08 ± 0.41) s, (16.34 ± 0.24) g/L and 4.32 ± 0.20 in control group. The first three were no significant difference among preeclampsia groups and control group (P > 0.05), fibrinogen was statistically significant among all groups (P < 0.05). Conclusion These results suggest that Annexin A5 plays an important role in the pathogenesis and progression of preeclampsia by affecting coagulation.

Introduction

Preeclampsia(PE) is a hypertensive disorder of human pregnancy, and it affects 5%-7% of all pregnancies worldwide and remains a leading cause of fetal growth retardation, premature delivery, and maternal death[1]. PE is defined as new-onset hypertension arising after 20 weeks gestation with multiorgan system involvement resulting in complete resolution by 12 weeks after birth[2]. Among the two distinct subtypes, early-onset PE(occurs before 34 weeks) confers a higher risk of life-threatening maternal complications and fetal and perinatal death, than late-onset(occurs at 34 weeks or later), and early delivery is the only treatment[3]. This disorder develops during pregnancy, and the rapid and complete recovery after childbirth

indicates that the placenta has a pivotal role in the pathogenesis of this disease[4]. The initial event in the development of PE is generally considered to be placental ischemia/hypoxia, and this manifestation of the placenta is closely related to the imbalance of the coagulation-anticoagulation system in the body[5]. Placentas from women with PE often display various infarctions, vascular damage, and fibrin depositions [6]. Recently, Omatsu et al[7] developed a murine PE model by injecting phosphatidylserine/phosphatidylcholine(PS/PC) microvesicles in Institute of Cancer Research (ICR) pregnant mice, and diffuse fibrin deposition was observed in the placentas. These data indicate that coagulation activation should be one of the mechanisms for the development of PE.

Annexin A5(ANXA5), a non-glycosylated single-chain protein discovered as a class of anticoagulant proteins of vascular tissue[8], is a member of the annexin/lipocortin family of Ca^{2+}dependent phospholipids-binding proteins, is pivotal in assuring good pregnancy outcomes by preventing local coagulation activation[9, 10]. Anticoagulant effects can be achieved by competitively binding PS sites[11]. ANX A5 exerts antithrombotic activity by binding to PS, inhibiting the activation of serine proteases important in blood coagulation. Jing J and coworkers[12] speculated that the combination of ANX A5 recombinant protein which retained ANX A5 features and PS can effectively prevent PS from participating in the activation of coagulation factor X in plasma, thereby reducing fibrin production, reducing plasma turbidity and prolonging coagulation time. It is shown that ANX A5 can intervene in the coagulation process by binding to PS, thereby exhibiting a significant anticoagulant effect.

Although several studies[13–15] have explored the adverse effects of ANXA5 in fetal growth restriction and miscarriage due to antiphospholipid syndrome, only a few reports have investigated its role in later pregnancy complications, such as preeclampsia. The present study investigate the role of ANXA5 in PE pathogenesis by evaluating its mRNA and protein expression of uncomplicated and PE-affected pregnancies and analyzing coagulation indicators.

Materials And Methods

Study group

We prospectively identified all women with PE managed and delivered at the Department of Obstetrics, Qingdao Municipal Hospital East Campus, China, during February 1, 2021and April 30, 2021. PE was defined according to ACOG[16] criteria. The control group included women with uncomplicated, normotensive singleton pregnancies who delivered healthy, appropriate-for-gestational-age babies. Exclusion criteria were stillbirth, multiple gestations, chorioamnionitis, prepregnancy hypertension, renal disease, as well as chromosomal abnormalities and fetal anatomical defects. Baseline demographic characteristics and medical history information (maternal age, gestational age, parity, maximum systolic blood pressure, maximum diastolic blood pressure) were recorded for all participants (Table 1). The study was approved by the Ethics Committee of Qingdao Municipal Hospital. Informed consent was obtained from all participants. Placental tissue and maternal blood were collected from pregnancies complicated by preeclampsia, which were classified into two groups: early-onset group (n = 10), late-onset group (n =

10) .10 women without perinatal complications who accepted elective term cesarean section were chosen as the control group.

Table 1
Comparison of general data of three groups of pregnant women

	control group(n = 15)	early-onset group(n = 15)	late-onset group(n = 15)
Maternal age(mean ± SD, years)	32.29 ± 3.41	33.54 ± 4.89	30.50 ± 6.31
Gestational age at delivery(mean ± SD, wks)	39.107 ± 0.93	32.408 ± 2.71	37.107 ± 1.25*
parity	1.57 ± 0.51	1.46 ± 0.51	1.50 ± 0.51
Maximum systolic blood pressure(mean ± SD,mmHg)	116.86 ± 7.36	162 ± 4.02	159.93 ± 4.41*
Maximum diastolic blood pressure(mean ± SD,mmHg)	70.86 ± 4.52	96.77 ± 5.36	96.50 ± 5.68*
*P < 0.05			

Tissue sampling

Patients had undergone routine blood tests on an empty stomach during routine maternity examinations. All the blood tests were performed before the preeclampsia clinical diagnosis. Blood was centrifuged and the supernatant was stored at -20°C.

Placental tissue samples were excised from randomly selected areas of central placental cotyledons within 20 min of delivery. Three fragments from each placenta were obtained and thoroughly washed in phosphate-buffered 0.9% saline (PBS) to minimize blood contamination. A 0.5 cm x 0.5 cm sample was immediately placed in formalin solution for immunohistochemical detection and the rest snap-frozen then stored at -80°C for RNA and protein analysis.

Immunohistochemistry

Immunohistochemical staining was assessed using one histological section per case (measuring 2*1 cm on average). ANX A5 expression was evaluated on formalin-fixed paraffin-embedded samples using standard immunohistochemical method. The sections were examined under a light microscope at x10 and x20 magnification (Nikon, Japan). Any staining intensity above the background of immunolabeled cells was considered positive ANXA5 expression. The mean optical density(MOD) was used to quantify the stained patches, and Image-pro plus software(Media Cybernetics, USA) was used.

RNA Extraction and cDNA Synthesis

RNA was extracted from each tissue using UNIQ-10 TRIzol® reagent (Sangon Biotech, China) according to the manufacturer's instructions. RNA concentration and purity were measured on a NanoVue ultra-trace spectrophotometer (NanoVue, China).

cDNA was synthesized with the M-MuLV RT kit (Sangon Biotech, China), following the manufacturer's instructions, using Oligo dT as amplification primers. cDNA was stored at − 20°C until use.

Real-Time Polymerase Chain Reaction Assay

ANXA5 (Forward primer: 5'-CCCTCTCGGCTTTATGATGCTTAT-3'; Reverse primer: 5'-ATGGCTCTCAGTTCTTCAGGTGTC-3'; Amplicon size: 116 bp) mRNA expression was measured using a qPCR assay with SG Fast qPCR Master Mix®, with β-Actin (Forward primer: 5'-GGGAAATCGTGCGTGACATTAAG-3'; Reverse primer: 5'-TGTGTTGGCGTACAGGTCTTTG-3'; Amplicon size: 68 bp) as an internal control. cDNA (1 μl) was amplified in a PCR reaction (final volume 20μl) containing 2×SG Fast qPCR Master Mix®(Sangon Biotech, China) and 200 nM of each primer. PCR conditions were: initial denaturation at 95°C for 3 min; 40 cycles of amplification, comprised of denaturation at 95 ° for 3 sec, annealing at 53°C for 20 sec and elongation at 72°C for 20 sec. PCR experiments were conducted on a Stepone™ real-time PCR amplifier (USA). The relative expression of ANXA5 mRNA was calculated using the $2^{-\Delta\Delta CT}$ (Livak) method. Normalized ANXA5 transcription levels for each PE-affected or normal sample were then calculated using the following formula:

$$2^{-\Delta\Delta CT} = 2^{-[\Delta CT(test) - \Delta CT(control)]} = 2^{-([CT(ANXA5, test) - CT(\beta-actin, test)] - [CT(ANXA5, control) - CT(\beta-actin, control)])}$$

Western Blot Assay

Proteins were extracted with the Tissue Protein Extraction Reagent (Sangon Biotech, China), following the manufacturer's protocol, and were stored at 4°C until use.30 μg of each protein specimen were separated by 12.5% SDS polyacrylamide gel electrophoresis and were transferred to 0.45 μm nitrocellulose membranes. The membrane was closed with 5% BSA-TBST and anti-ANX A5 rabbit polyclonal antibody (36 kDa) monoclonal antibody (Sangon Biotech, China) at a dilution of 1:500 was added overnight. Rabbit anti-β-actin protein was used as an internal reference. The membrane was washed and the goat-anti-rabbit IgG-HRP secondary antibody (dilution 1:10,000) was added. It was developed by immunoblotting chemiluminescence, exposed by X-ray method, and scanned by the imaging system, and the ratio of the grayscale of the protein blot strip to the grayscale of β-actin was used as the relative expression level of ANX A5.

Enzyme-linked immunosorbent Assay

ANX A5 protein levels in maternal blood were measured by a two-site sandwich enzyme immunoassay enzyme-linked immunosorbent assay (ELISA), with the Human Annexin A5 ELISA Kit (mlbio, China).The kit assay Human ANXA5 level in the sample, use Purified Human ANXA5 antibody to coat microtiter plate wells, make solid-phase antibody, then add ANXA5 to the wells, Combined antibody which With HRP

labeled, become antibody-antigen-enzyme-antibody complex, after washing Completely, Add TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, the reaction is terminated by the addition of a sulphuric acid solution and the color change is measured at a wavelength of 450 nm with an automated ELISA reader (Rayto RT-6100, USA). The concentration of ANXA5 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Coagulation tests Assay

The changes of coagulation function including prothrombin time(PT), activated partial thromboplastin time(APTT), fibrinogen (Fib), and thrombin time(TT) were analyzed in the peripheral blood of each group of pregnant women. The coagulation function was examined using an STA Compact Max fully automated hemagglutination and corollary reagents.

Statistical analysis
All statistical analyses were performed using SPSS 26.0 software. Measurement data were expressed as mean ± standard deviation (x ± sd). The homogeneity of variance of each group was performed using Levene's test. If the variance was uniform, one-way ANOVA was used for comparison among groups, and the LSD-t method was used for further comparison between two groups. If the variances were not consistent, the Kruskal-Wallis H test was used for inter-group comparisons, and Dunnett's T3 method was used for further comparison between the two groups; $p = 0.05$ was used as the test level, and $p < 0.05$ was regarded as statistically significant.

Results

Clinical Data analysis

There were no statistically significant differences between the study and control groups in a variety of characteristics, including maternal age, parity. The control and late-onset group were not statistically significant in terms of gestational age at delivery($P > 0.05$), but were statistically significant with the early-onset group($P < 0.05$). The maximum systolic blood pressure or diastolic blood pressure in the group of pregnancies was significantly higher than controls, as expected.

Immunohistochemistry analysis

HE-stained placental tissue was used as a control(Fig. 1D), ANXA5 immunohistochemical expression was observed in the perivillous and extravillous trophoblast of all examined placentas as brown-staining positive particles. The staining pattern was heterogeneous with a tendency of ANXA5 positive cells towards cluster formation at the sites of syncytial knots. Immunostaining was mostly visualized as a continuous line along with the apical site of the perivillous syncytial cytoplasmic membrane or was localized in the cytoplasm of isolated trophoblastic cells (Fig. 1A-C). The brown granule expression levels were decreased in the pre-eclampsia groups compared to the healthy pregnant women group. However, no significant difference in expression was seen between the preeclampsia groups (Fig. 1a-c). The mean

± SD MOD in the early-onset and the late-onset group were 61.93 ± 5.92 and 70.75 ± 5.84 versus 88.58 ± 2.38 in the control group, a difference statistically significant (p = 0.003) (Fig. 1E). However, there was no statistical significance between the early-onset and late-onset groups(p = 0.187).

Real-time PCR analysis

PE placentas exhibited significantly lower ANX A5 mRNA levels than those obtained from uncomplicated pregnancies (Fig. 2). The mean ± SD ANXA5 mRNA expression in the early-onset and late-onset group were 0.79 ± 0.23 and 1.18 ± 0.53 versus 6.19 ± 1.68 in the control group, a difference statistically significant (p = 0.024). In contrast, the differences in ANXA5 mRNA expression levels in placental tissues of the PE groups were not statistically significant(p > 0.05).

Western blot analysis

Western Blot analysis verified that protein levels were not statistically significant between the early-onset, late-onset, and control groups (mean ± SD): 0.01 ± 0.02, 0.95 ± 0.03vs.0.99 ± 0.02, p = 0.071 (Fig. 3A, 3B).

Enzyme-linked immunosorbent assay analysis

The protein expression level of ANXA5 in peripheral blood was higher in all groups of preeclampsia than in the control group(Fig. 4A). The mean ± SD ANXA5 protein expression(ng/mL) in the early-onset and late-onset group were 5.54 ± 0.29 and 5.10 ± 0.28 versus 4.5 ± 0.19 in the control group, a difference statistically significant (p = 0.013), and the differences in placental tissues of the PE groups were not statistically significant(p > 0.05). The mean ± SD FXa expression(pg/mL) in the early-onset and late-onset group were 1229.23 ± 45.33 and 1366.96 ± 56.30 versus 1372.95 ± 45.44 in the control group, which has not statistically significant(P = 0.072)(Fig. 4B).

Coagulation parameter analysis

PT, APTT, and TT parameters have not differed significantly between healthy pregnant women and preeclampsia patients(P > 0.05). Fib was higher in the late-onset group than in the other two groups, and the differences were statistically significant (p < 0.05) (Table 2). There were no statistical differences between the early-onset group and the control group (p > 0.05).

Table 2
Changes in coagulation function parameters in three groups of pregnant women (x ± sd).

Parameter	early-onset	late-onset	control
PT(s)	11.91 ± 0.23	11.65 ± 0.12	12.01 ± 0.09
APTT(s)	33.38 ± 0.85	33.15 ± 0.36	32.08 ± 0.41
TT(s)	16.88 ± 0.45	16.13 ± 0.19	16.34 ± 0.24
Fib(g/L)	3.85 ± 0.23	4.91 ± 0.14	4.32 ± 0.20*

*P < 0.05 PT, Prothrombin time .APTT, activated partial thromboplastin time .TT, Thrombin time. Fib, fibrinogen.

Discussion

In the study, we examined whether ANXA5 mRNA or protein levels are altered in placenta tissues derived from PE-affected pregnancies compared to those with the normal outcome. We found decreased ANXA5 mRNA levels in placental tissues derived from preeclamptic women. A more detailed grouping was performed compared to past studies. The majority PE samples exhibited significantly lower ANXA5 mRNA levels compared to controls, however, the difference was little in mRNA expression between the early-onset and late-onset groups. Reduced ANXA5 mRNA and protein has been also reported in previous studies that assessed the placental expression of ANXA5 in women with PE[17, 18], and has been correlated with disease onset and the presence of FGR[17, 19]. However, Ornaghi et al[17] using the immunohistochemical Tissue Microarray (TMA) technique found ANXA5 expression to be related only to FGR but not to PE or its clinical severity. They consider that the observed decrease in ANXA5 expression is most probably a consequence of long-time exposure to the disease with its hemodynamic alterations rather than a cause of preeclampsia itself. Our result confirmed it.

Our immunohistochemistry confirmed that ANXA5 was present on the apical surface of the perivillous trophoblasts. Moreover, low staining intensity showed reduced placental ANX A5 protein levels in preeclampsia. According to the thrombomodulation of ANXA5, reduced protein expression would allow the binding of coagulation factors to phospholipid surfaces and therefore induce thrombosis. Research using streptavidin-biotin-peroxidase complex immunohistochemical methods confirmed these findings and correlated the reduced protein expression with the severity of PE. In the study, they have found that immunoreactivity of ANXA5 in preeclamptic patients was attenuated which supports diffuse arterial microthrombus progress in preeclamptic placental bed[20]. Similarly, the distribution of ANXA5 over the placental intervillous surface is significantly lower in antiphospholipid syndrome(aPLS) patients, which may be linked to placental thrombosis[21]. Considering the inherent limit of immunohistochemical placental studies, which can be performed only after delivery and are influenced by tissue heterogeneity, the results do not present consistency with western blot.

Noteworthy, there is a disassociation between ANXA5 mRNA levels, which are nearly abolished in PE, and protein expression levels. The difference of protein expression levels was not found to be statistically significant. The essential role of ANXA5 in maintaining placental integrity has been proven in animal models, specifically, anti-Annexin A5 antibodies, when injected in mice, have been proven to cause thrombosis and necrosis of the absorbed embryos[22]. Others have shown that an increased apoptosis rate has been demonstrated in cultured cytotrophoblasts isolated from placental tissues of preeclamptic and FGR pregnancies[23]. This may be attributed to the stabilization of the produced ANXA5 protein by its binding to placental tissue due to abnormally increased apoptosis. ANXA5 protein degradation would be restricted by such binding, leading protein levels to be higher than expected[19]. This accelerated apoptosis may represent either a primary pathological incident, or an etiological component leading to the development of PE. Approximately normal ANX A5 protein levels, however, were observed in PE cases examined in the present study. Consistent with this hypothesis, the low ANXA5 mRNA levels observed indicate that the placenta may not be the only source of ANXA5 protein in this type of complicated pregnancy[18]. As ANXA5 is also expressed in numerous other tissues, including platelets, the vascular endothelium, and the endothelium of the umbilical levels, the placenta may recruit the protein from the bloodstream and especially from activated platelets, which may be the actual cause of the thrombotic events observed in PE-affected placentas. Therefore, it can be speculated that ANXA5 in the intervillous circulation is derived from the systemic circulation and interferes with protein levels in placentas of PE and uncomplicated pregnancies.

Compared with past studies, our study added the measurement of ANX A5 and FXa protein levels in peripheral blood using an enzyme-linked immunoassay and the analysis of coagulation function. We found that peripheral blood from PE-affected pregnancies displayed elevated ANXA5 protein levels compared to controls. This is the opposite of the results of Xin Hong et al[24]. FXa did not change significantly in the blood of healthy and preeclamptic pregnant women. Platelet-expressed PS supports the assembly of the prothrombinase complex composed of factor Va (FVa), factor Xa (FXa), and prothrombin[25]. ANXA5 competes with FXa, and prothrombin for binding to PS, thereby preventing the formation of the prothrombinase complex and, consequently, the formation of thrombin [26]. In solution, ANXA5 is present as monomer, but once bound to PS-expressing membrane, three monomers build a trimer via protein-protein interactions and the trimers assemble into a two-dimensional lattice covering the PS expressing surface by trimer-trimer interactions[27]. The activated partial thromboplastin time (APTT) assays are useful in vitro global tests for assessing the activities of the classical extrinsic, intrinsic, and common pathways of coagulation. The APTT measures the intrinsic pathway activity, such as coagulation factors XII, XI, IX, and X, the reduction in the above coagulation factors will cause prolongation of the APTT value. It has been reported that ANXA5 showed a dose-dependent effect on APTT value. With the increase in the ANXA5 concentration, the APTT value was significantly prolonged[12]. In our study, in terms of the effects on PT value, APTT value, and TT value, neither of them caused statistically significant changes. As described in the above results, We suggest that ANXA5 affects

coagulation independent of FXa and that the pathway by which ANXA5 turns on anticoagulation needs to be confirmed by further experiments.

We have shown that the concentration of fibrinogen in maternal plasma increases during pregnancy, and even more in preeclampsia. Our data add to previous knowledge by demonstrating that the increase in fibrinogen is significant only in late-onset, but in early-onset preeclampsia or even a decline. This study supports earlier findings that pregnancy alters the balance of the coagulation system and that this imbalance is even more pronounced when the pregnancy is complicated by preeclampsia[28, 29]. In a few earlier studies, women with severe versus mild preeclampsia have been compared concerning coagulation parameters. For example, USTüN Y and co-workers [30] found that the plasma fibrinogen level increased in women both with mild (mean gestational age 37 weeks) and severe preeclampsia (mean gestational age 35 weeks) compared to normal healthy controls. The authors did not distinguish between early-onset and late-onset disorders. Heilmann et al [31] stated that women who developed early-onset preeclampsia constitute a subgroup of patients with both more and more severe hematological abnormalities than women with late-onset preeclampsia diagnosed after 34 weeks of gestation. Kårehed K and co-workers[32] indicated that fibrinogen might be involved in the pathophysiology of early-onset preeclampsia, while they do not seem to be of importance in women with late-onset disease. The decrease in fibrinogen in association with the endothelial cells in women with early-onset preeclampsia might reflect an increased conversion of fibrinogen to fibrin. This could be a result of the systemic endothelial cell dysfunction seen in preeclampsia and may be of importance for the increased intravascular clot formation associated with the disorder[31, 33].

In conclusion, We have demonstrated altered expression of ANXA5 in the placenta as well as in peripheral blood in PE. These results suggested that it might be directly involved in impaired vascular adaptation mechanisms and defective blood flow in the metaplastic vasculature. Since the relationship between ANXA5 and the severity of preeclampsia is unclear, further studies are needed to assess its role in developmental abnormalities in PE.

Declarations

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Disclosure

The authors have no conflict of interest to declare

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Figures

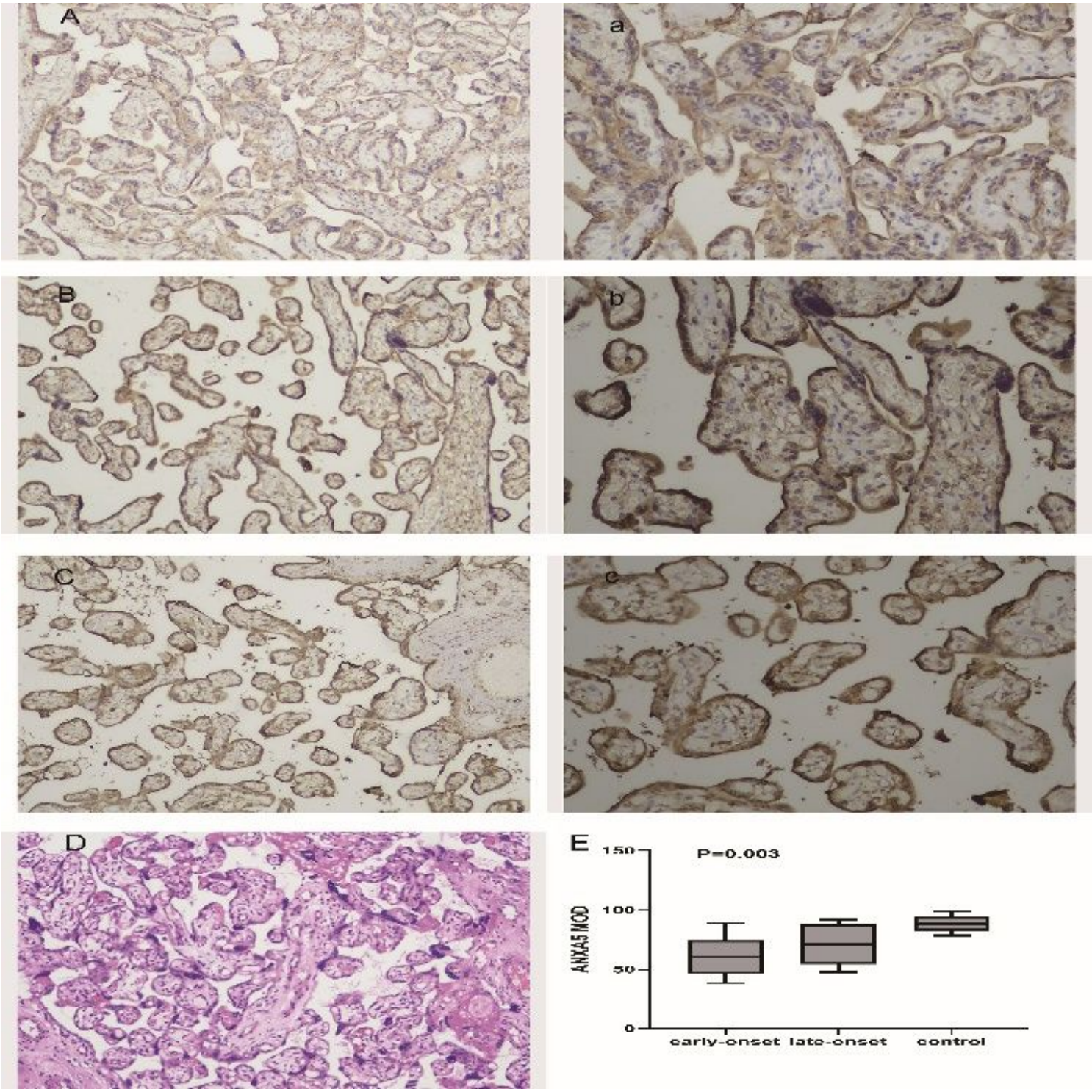

Figure 1

Positive expression of ANXA5 in placental tissue, the immunohistochemical evaluation showed that the main localization of Annexin A5 was at the apical surface of placental syncytiotrophoblast as brown-staining positive particles. (A) and (a): Immunohistochemical expression of ANXA5 in the early-onset group(×10/×20); (B) and (b): Immunohistochemical expression of ANXA5 in the late-onset group(×10/×20); (C) and (c): Immunohistochemical expression of ANXA5 in the control group(×10/×20);(D) HE staining of placental tissue;(E) Box-and-whisker plot showing the MOD in PE-affected pregnancies(early-onset and late-onset) and controls.

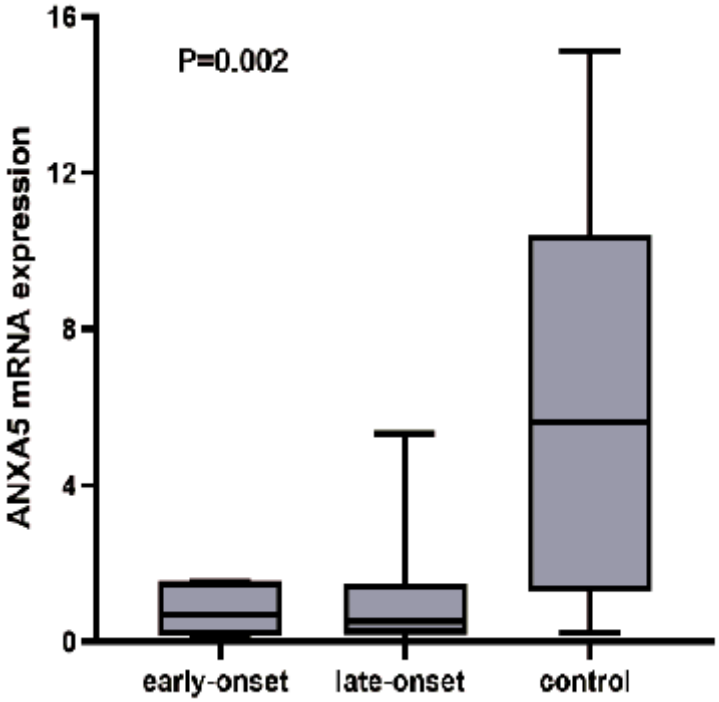

Figure 2

Box-and-whisker plot showing the normalized ANXA5 mRNA placental expression levels in PE-affected pregnancies(early-onset and late-onset) and controls. PE pregnancies show a decreased mRNA expression when compared to those with a normal outcome (p=0.002).

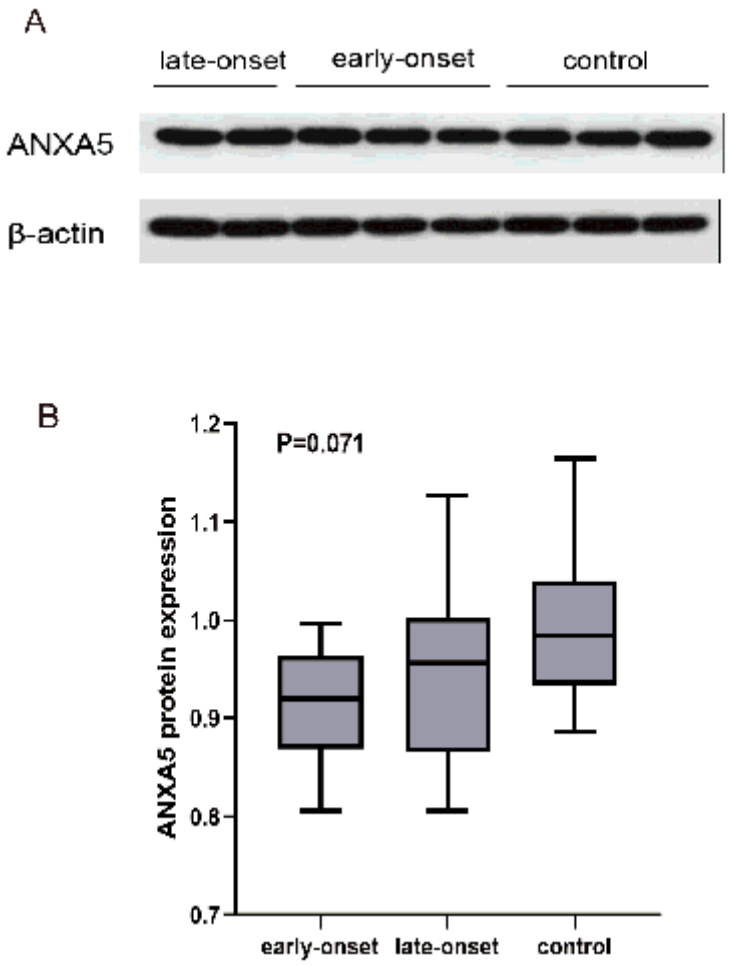

Figure 3

(A) Representative examples of ANXA5 and β-Actin Western Blot analysis in pregnancies complicated by early-onset and late-onset preeclampsia (PE) and normal pregnancies. (B) Box and whisker plots depicting normalized ANXA5 protein expression between PE patients and controls (p = 0.071).

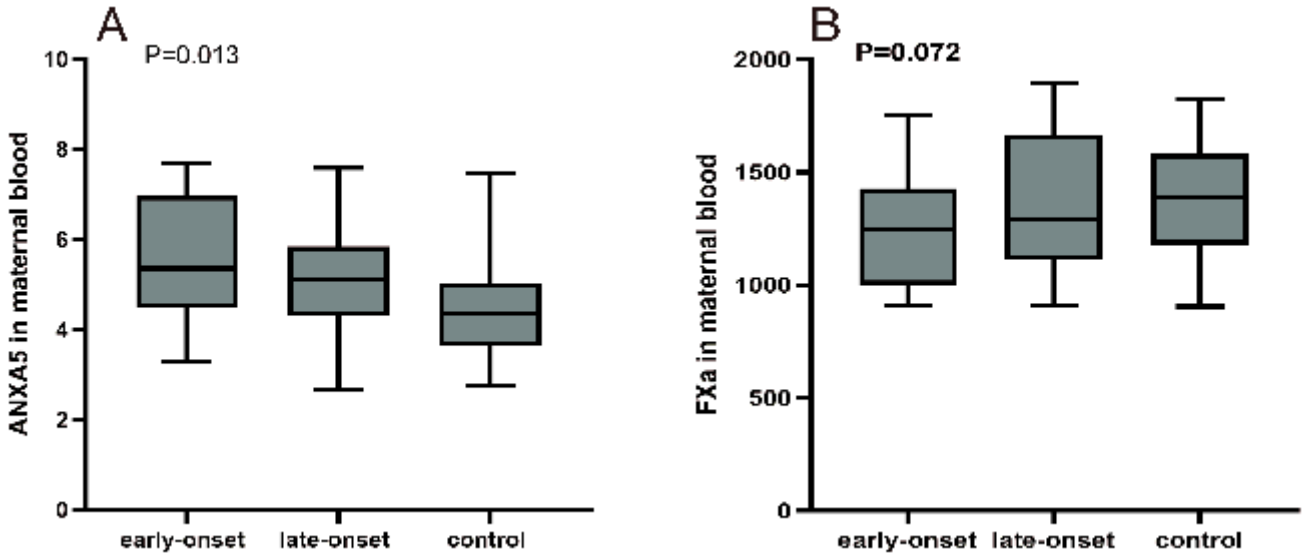

Figure 4

(A)Box-and-whisker plot showing the ANXA5 protein expression levels in maternal blood in PE-affected pregnancies(early-onset and late-onset) and controls. PE pregnancies show an increased expression when compared to those with a normal outcome (p=0.013). (B) Box-and-whisker plot showing the coagulation factor FXa expression levels in maternal blood in PE-affected pregnancies(early-onset and late-onset) and controls (p=0.072).