Comparative Analysis of Excessive Collagen Deposition in Terminal Villi of Normal and Pre-eclamptic Placentae

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Abstract:

Background: The placenta is an essential part of the body for sustaining a pregnancy and fostering normal fetal development, it has been reported that pathological abnormalities seen in the placenta have a negative impact on fetal outcome. It has been documented that the spiral arteries of the placenta lose their elastic qualities during preeclampsia and ultimately results in inadequate placental perfusion which has been associated with a major histological alteration in the preeclamptic placenta that is fibrosis.

Objective: To compare the collagen Deposition in terminal villi of Normal and Pre-eclamptic Placentae.

Methodology: It was a cross-sectional investigation with 50 placenta samples, 25 of which were preeclamptic and 25 of which were normal. Following a caesarean section, the placentas were collected, prepared according to protocol, and examined histo-pathologically. Pre-eclampsia cases that had been registered, blood pressure that was less than 140/90 mmHg, parity between 0 and 4, a mother's age between 25 and 35, gestational age between 36 and 42 weeks, and caesarean section deliveries were the inclusion criteria. Patients with known hypertension and those who had been diagnosed with other chronic conditions were excluded from the study.

Results: The average number of terminal Villi, in Group A and Group B with excessive collagen were 4.780.98/0.9025mm^2 and 11.361.03/0.9025mm^2. Masson's Trichrome stained sections showed a quantitative difference between collagen deposition in healthy and preeclamptic samples of placentae. It highlighted that preeclampsia leads to deposition of collagen in walls of the terminal villi.

Conclusion: In this study, pre-eclamptic placentae showed a highly significant decrease in number of villi with excessive collagen in pre-eclamptic placentae as compared to normal placentae.

Keywords: Collagen Deposition, Preeclampsia, Terminal Villi, Caesarean section

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Introduction:

The placenta is an essential part of the body for sustaining a pregnancy and fostering normal fetal development, it has been reported that pathological abnormalities seen in the placenta have a negative impact on fetal outcome (1). The structure of placenta is made up of amnion, which is smooth, shiny, and translucent, covers the placenta's fetal surface. The umbilical cord is often

attached close to the center of the fetal surface, and from this location, branches of the umbilical vessels extend out beneath the amnion (2). The nutrients and waste are transported throughout pregnancy between the maternal and fetal circulations by the terminal branches of the placental villous trees. Hence, normal development of the vessels of the placenta is a key factor to sustain a pregnancy as well as to lessen the pregnancy associated complications (3).

Preeclampsia is a pregnancy condition that affects many organ systems and contributes significantly to maternal morbidity and mortality. Preeclampsia affects 5-7% of expectant mothers worldwide but, its prevalence ranges between 1.8 - 16.7% in developing countries (4, 5). Numerous epidemiologic and experimental investigations have suggested that imbalances in circulating angiogenic factors released from the placenta could be responsible for the maternal signs and symptoms of preeclampsia, despite the fact that the etiology of the aberrant placentation is still being debated (6). It has been documented that weak immune system, renin angiotensin aldosterone pathway, oxidative stress, modulation of growth factors such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and transforming growth factor β (TGF-β) play vital role in its pathophysiology (7, 8).

The spiral arteries of the placenta lose their elastic qualities during preeclampsia and ultimately results in inadequate placental perfusion which has been associated with the a major histological alteration in the preeclamptic placenta that is fibrosis. Various studies have reported that the deposition of collagen type I, II and III is responsible for this histologic change (9, 10). The mechanism of vascular fibrosis caused by numerous vascular agents, such as cardiotonic steroids has been documented in the literature (10). However, comparative analysis how much collagen deposition effects the vessels of placenta has not been reported yet hence the current study aims to compare the collagen Deposition in terminal villi of Normal and Pre-eclamptic Placentae.

Methodology:

It was a case-control study conducted at tertiary care hospital of Sukkur, Sindh, Pakistan from January to December 2022. The calculated sample size was 50, 25 cases (Placenta of preeclamptic female) and 25 controls (Placenta of healthy female). The sample was recruited by nonprobability consecutive technique. All the placentae were obtained from females who underwent cesarean section in Gynecology and Obstetrics department of Hospital with written informed consent taken from them prior to procedure. The set inclusion criteria were, Registered cases of Pre-eclampsia, Blood Pressure ≥ 140/90 mmHg, Parity 0 – 4, Age of mother (25 – 35) Years, Gestational Age (36 – 42) Weeks and, Mode of delivery (Cesarean Sections). The Known Hypertensive and Diabetic Patients, Smokers, patients with renal and Cardiovascular diseases and those who had Multiple pregnancies were excluded.

Immediately after being collected, the placentas were put in 10% formalin-filled jars with patient information written on each jar. After being delivered to the lab, the placentae were kept in 10%

formalin for 24–48 hours before being cut into sections for histology. Alcohol, xylene, and paraffin infiltration were used to prepare tissue fragments for standard paraffin embedding. Paraffin blocks were made, tissue was positioned so that it covered the entire region from the chorionic plate to the placenta's basal plate, parallel tissue sections were obtained on glass slides, 4 m thick sections were cut on a rotary microtome, and the sections were left to float in a hot water bath at 42°C. Glass slides were used to remove the floating portions from the water bath. To set the sections on the slides, slides were heated to a temperature of 37°C for a period of 24 hours. Slides were correctly numbered with a lead pencil on the frosted surface.

The counting reticule and ocular micrometer were calibrated using a stage micrometer. For calibration, a stage micrometer with a scale of 10 mm divided into 100 parts was employed. In order to calibrate the counting reticule and ocular micrometer at various magnifications (4X, 10X, and 40X), their divisions were compared to those of the stage micrometer. At each magnification, the size of the counting reticule was also measured. The size of the items seen through the microscope was determined using the findings. The presence of villi was found in both groups' H and E. The quantity of collagen/connective tissue stroma in the cores of tertiary chorionic villi was examined using Masson's trichrome stain. With the use of Masson's trichrome stain, collagen was given a light green or blue color in 4 m thick paraffin sections. For all of the placentae included in this study, the average number of chorionic villi per unit area, demonstrating an excessive quantity of collagen, were counted using reticule at 10 random fields.

An independent sample t-test was used to assess the statistical significance of the differences in quantitative variables between the pre-eclamptic and control groups. For each group, the mean and standard deviation were reported. If the difference had a P-value of 0.05 or below, it was considered statistically significant. The statistical analysis was performed utilizing SPSS version 24.

Results:

Masson's Trichrome stained sections showed a quantitative difference between collagen deposition in healthy and preeclamptic samples of placentae. It highlighted that preeclampsia leads to deposition of collagen in walls of the terminal villi figure 1 (a, b) and 2 (a, b) show the histological difference between healthy (A) and preeclamptic (B) samples of placentae at 100x and 400x.

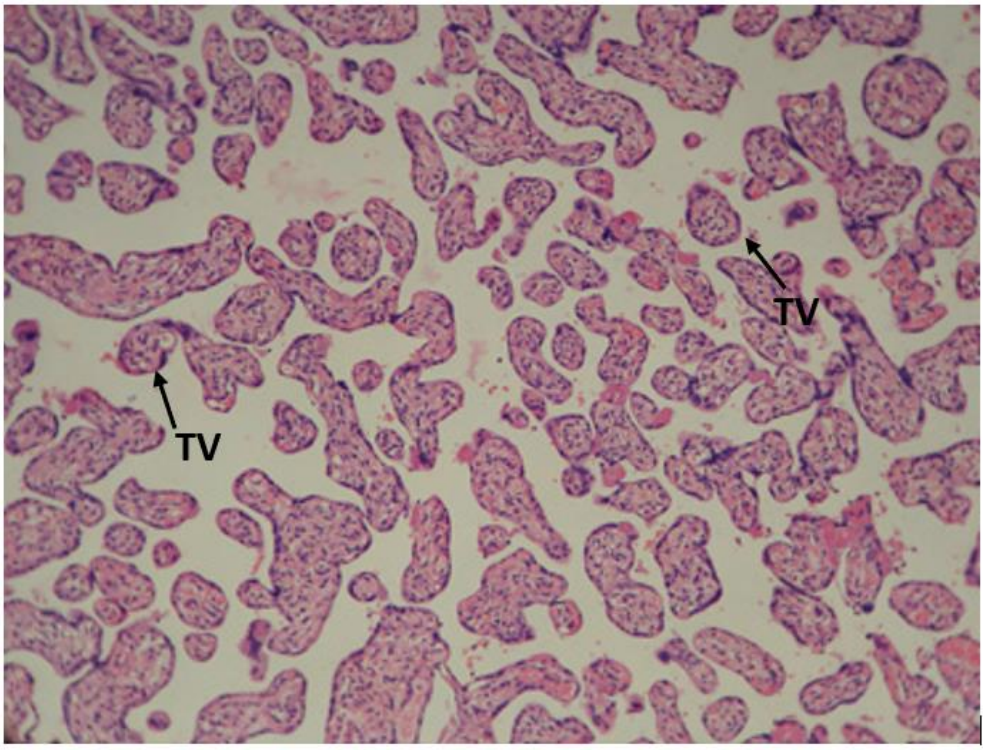

Figure 1a. A 4µm thick H&E stained paraffin section of full term normal human placenta from group-A showing terminal villi (TV). x100

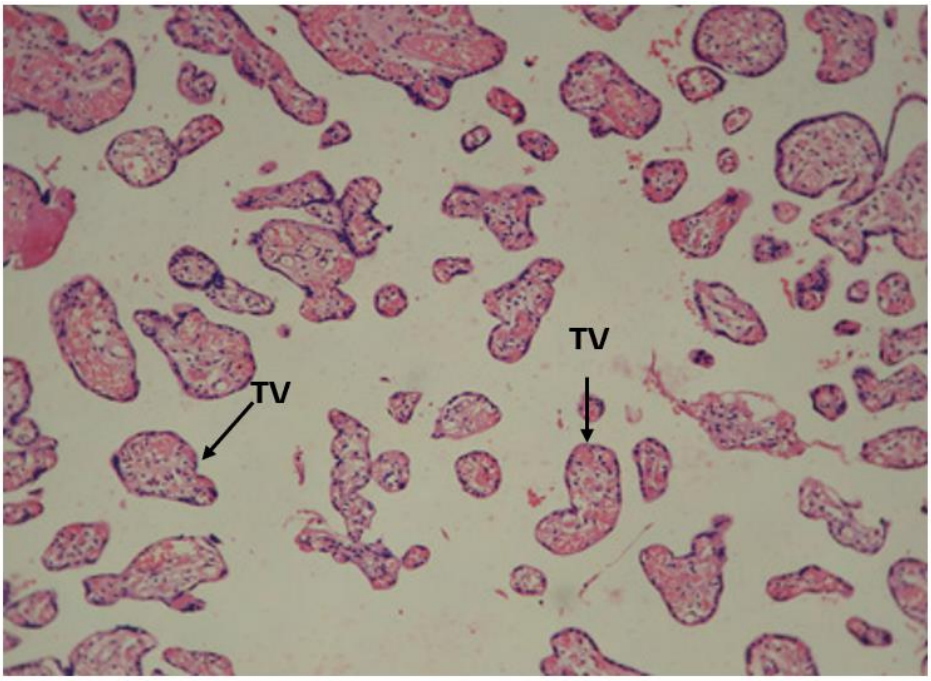

Figure 1b. A 4µm thick H&E stained paraffin section of full term pre-eclamptic human placenta from group-B showing decrease number of terminal villi (TV, black arrow). x100

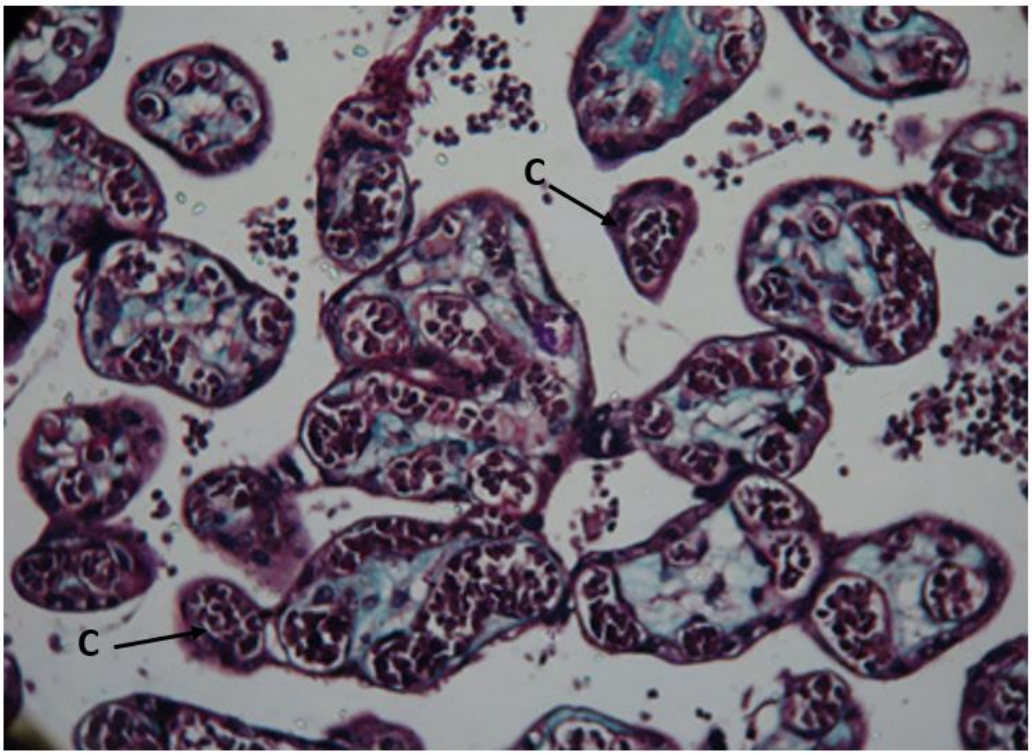

Figure 2a. A 4µm thick Masson's trichrome stained paraffin section of full term normal human placenta from group-A showing scanty terminal villous collagen (C, black arrow). x400.

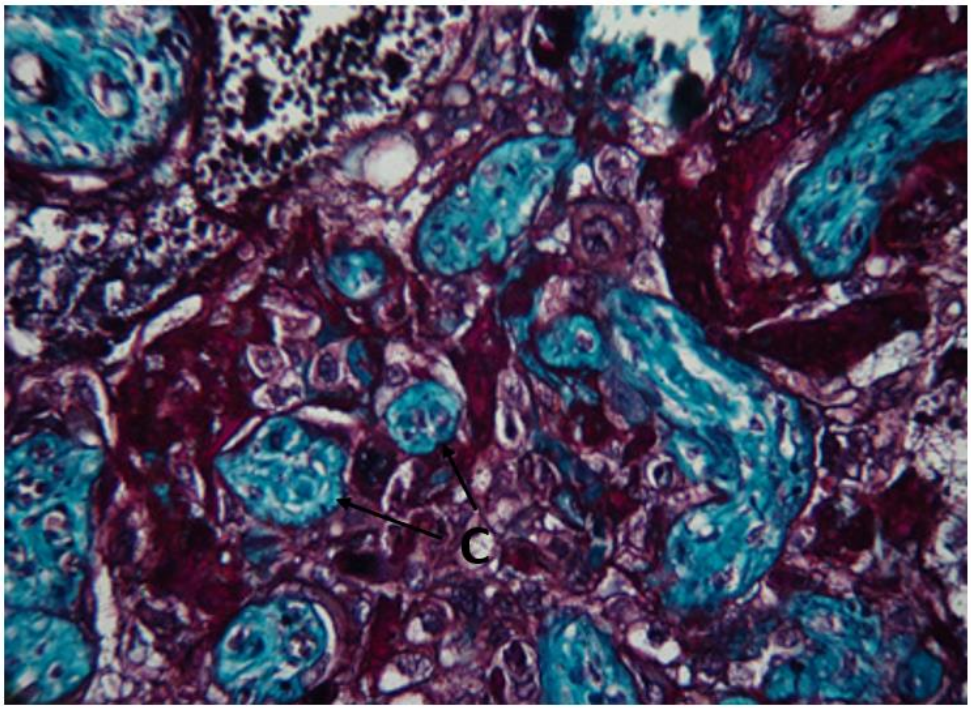

Figure 2b. A 4µm thick Masson's trichrome stained paraffin section of full term pre-eclamptic human placenta from group B showing terminal villi with excessive collagen (C, black arrow). x400

The average number of villi with excessive collagen was found using reticule in 10 random fields for each placenta in groups A and B in 4 m thick Masson's Trichrome stained paraffin sections. In the control group A and pre-eclamptic group B, the mean values for the number of villi are shown in table 1. However, the average number of terminal Villi, in Group A and Group B with excessive collagen were 4.780.98/0.9025mm^2 and 11.361.03/0.9025mm^2, respectively (table 2). Comparing group, A and group B using Masson's Trichrome stained paraffin sections revealed a highly significant rise (P 0.001) in the average number of villus with excessive collagen in group B as compared to group A.

Table 1. Mean Values of Number of Terminal Villi, in Group A and Group B

PARAMETER	GROUP - A		GROUP - B		
	Mean	Standard Deviation	Mean	Standard Deviation	p-value
Number of terminal villi	100.16	0.72	64.28	1.51	<0.001

Table 2. Mean Values of Average Number of Villi with Excessive Collagen/0.9025mm^2 in Group A and Group B

PARAMETER	GROUP - A		GROUP - B		
	Mean	Standard Deviation	Mean	Standard Deviation	p-value
Villi with collagen/0.9025mm^2	4.78	0.98	11.36	1.03	<0.001

Discussion:

Studies on the pathology of the placenta have been conducted; they focused mostly on the clinical outcomes of pregnancies caused by various disorders as well as gross and microscopic changes in the placenta. The involvement of collagen deposition in the pathophysiology of pre-eclampsia has been evaluated in this work. Furthermore, pre-eclampsia has been associated with significant changes in morphology, which may interfere with their function and cause an aberrant result of pregnancy (11). Pre-eclampsia patients showed placental insufficiency and parenchymal tissue fibrosis as compared to healthy samples. In the literature, this has been linked to vasospasm, apoptosis, and compensatory hyperplasia of the placental parenchyma (12). In parallel to our findings Y. Feng et., al. has documented that collagen I suppresses trophoblast

proliferation and Invasion, inducing Preeclampsia-Like Symptoms (9). Further to this, it has been demonstrated that the serum of PE patients contains vasoactive compounds that stimulate the deposition of collagen and other extracellular matrix components. Additionally, in an animal model of preeclampsia more collagen deposition in the kidney of PE animals in comparison to normal pregnant rats, in addition to the placenta. Despite these findings, it is still unknown what exact functions collagen and a fibrotic placenta play in the pathophysiology of preeclampsia. (13-15).

In our investigation, we found that group B (pre-eclamptic) had a much lower number of villi than group A (control). Placental villous hypoxia is caused by insufficient trophoblast invasion of spiral arterioles. This inhibits the placenta's ability to expand and, in turn, results in preeclampsia's decreased villi count (16, 17). Placental ischemia results from extra villous cytotrophoblast's insufficient penetration of uteroplacental spiral arteries in pre-eclampsia. There were indications of ongoing branching angiogenesis in the placental villi. These structural changes could be the result of VEGF's ongoing hypoxia-driven action. The placenta produces VEGF, which is crucial in the regulation of angiogenesis. The expression of the VEGF gene is strongly stimulated by hypoxia, which also makes the VEGF messenger RNA more stable (18, 19). Systemic vasoconstriction and hypertension are two mechanisms that could help pre-eclamptic women with more blood flowing to their uterus and placenta. As a way to bring the gestational perfusion pressure closer to normal, this may raise the perfusion pressure in the uteroplacental circulation and boost placental VEGF. These effects may then increase vasculogenic and blood flow (20, 21). The angiogenesis and vasculogenic of the uteroplacental and the fetus have been demonstrated to be regulated by a variety of hypoxia inducible genes and their protein products (22). Hence, genetic evaluation is required to further investigate and identify the mechanisms behind activation of VEGF related factors.

Conclusion:

In this study, pre-eclamptic placentae showed a highly significant decrease in number of villi with excessive collagen in pre-eclamptic placentae as compared to normal placentae.

References:

1. Nakahara A, Nair S, Ormazabal V, Elfeky O, Garvey CE, Longo S, et al. Circulating placental extracellular vesicles and their potential roles during pregnancy. 2020;20(4):439-45.

2. Sharps MC, Hayes DJ, Lee S, Zou Z, Brady CA, Almoghrabi Y, et al. A structured review of placental morphology and histopathological lesions associated with SARS-CoV-2 infection. 2020;101:13-29.

3. Plitman Mayo R, Abbas Y, Charnock-Jones DS, Burton GJ, Marom GJIF. Three-dimensional morphological analysis of placental terminal villi. 2019;9(5):20190037.

4. Macedo TC, Montagna E, Trevisan CM, Zaia V, de Oliveira R, Barbosa CP, et al. Prevalence of preeclampsia and eclampsia in adolescent pregnancy: A systematic review and meta-analysis of 291,247 adolescents worldwide since 1969. 2020;248:177-86.

5. Mou AD, Barman Z, Hasan M, Miah R, Hafsa JM, Das Trisha A, et al. Prevalence of preeclampsia and the associated risk factors among pregnant women in Bangladesh. 2021;11(1):21339.

6. Rana S, Burke SD, Karumanchi SAJAjoo, gynecology. Imbalances in circulating angiogenic factors in the pathophysiology of preeclampsia and related disorders. 2022;226(2):S1019-S34.

7. Johnson JD, Louis JMJAjoo, gynecology. Does race or ethnicity play a role in the origin, pathophysiology, and outcomes of preeclampsia? An expert review of the literature. 2022;226(2):S876-S85.

8. Nirupama R, Divyashree S, Janhavi P, Muthukumar S, Ravindra PJJoGO, Reproduction H. Preeclampsia: Pathophysiology and management. 2021;50(2):101975.

9. Feng Y, Chen X, Wang H, Chen X, Lan Z, Li P, et al. Collagen I induces preeclampsia-like symptoms by suppressing proliferation and invasion of trophoblasts. 2021;12:664766.

10. Bagrov A, Kolodkin N, Adair CD, Agalakova N, Trashkov A. Preeclampsia: Cardiotonic Steroids, Fibrosis and a Hint to Cancerogenesis. 2020.

11. Richards C, Sesperez K, Chhor M, Ghorbanpour S, Rennie C, Ming CLC, et al. Characterisation of cardiac health in the reduced uterine perfusion pressure model and a 3D cardiac spheroid model, of preeclampsia. 2021;12(1):1-14.

12. Edwards C, Cavanagh E, Kumar S, Clifton V, Fontanarosa DJP. The use of elastography in placental research–a literature review. 2020;99:78-88.

13. Jiang R, Teng Y, Huang Y, Gu J, Ma L, Li M, et al. Preeclampsia serum-induced collagen I expression and intracellular calcium levels in arterial smooth muscle cells are mediated by the PLC-γ1 pathway. 2014;46(9):e115-e.

14. Sudo M, Yoshita K, Ito Y, Imai N, Iino N, Narita IJPH. Histopathological features of kidney and renal prognosis in patients with preeclampsia. 2021;25:75-80.

15. Santana-Garrido Á, Reyes-Goya C, Espinosa-Martín P, Sobrevia L, Beltrán LM, Vázquez CM, et al. Oxidative and inflammatory imbalance in placenta and kidney of sFlt1-induced early-onset preeclampsia rat model. 2022;11(8):1608.

16. Khilji A, Rizvi SFA, Tarique S, Fatima S, Shah AA, Kazmi QH, et al. Morphometric Analysis of Placental Terminal Villi, Capillaries, and Intervillous Space in Normal and Pre-Eclamptic Pregnancies. 2023;17(02):826-.

17. Xu F, Yang S, Liu Y, Zheng X, Yang H, Zhang J, et al. Placental pathology and neonatal outcomes in pre-eclampsia with gestational diabetes mellitus. 2021;34(7):1149-54.

18. Witvrouwen I, Mannaerts D, Ratajczak J, Boeren E, Faes E, Van Craenenbroeck AH, et al. MicroRNAs targeting VEGF are related to vascular dysfunction in preeclampsia. 2021;41(8).

19. Nema J, Sundrani D, Joshi SJHiP. Role of vitamin D in influencing angiogenesis in preeclampsia. 2019;38(4):201-7.

20. Redman CW, Staff AC, Roberts JMJAjoo, gynecology. Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways. 2022;226(2):S907-S27.

21. Konkov DG, Piskun AO, Taran OA, Kostur GVJWL. Specialties of hystomorphometrical changes in placenta of women with early and late preeclampsia. 2020;73(1):151-5.

22. Liu Y, Ren M, Bi X, Fu Y, Jing X, Zhang H, et al. A systematic review on the application of vascular endothelial growth factors in preeclampsia. 2021;10(8):9259-66.