EXPRESSION OF ERM PROTEINS IN PLACENTA PREVIA

Senem Alkan Akalin[1], Ece Öcal[2], Veysel Toprak[3], Engin Deveci[4,*]
[1]Department of Gynecology and Obstetrics, Private Medical Practice, Diyarbakir, Turkey - [2]Department of Perinatology, Private Medical Practice, Diyarbakir, Turkey - [3]Department of Gynecology and Obstetrics, Eyyübiye Education and Research Hospital, Şanlıurfa, Turkey - [4]Department of Histology and Embryology, Dicle University, Medical School, Diyarbakir, Turkey

ABSTRACT

Objective: *We investigated the immunohistochemical staining of ERM proteins expression in the placenta of pregnant women with placenta previa.*

Methods: *15 healthy and 15 placenta previa were processed for routine histological tissue processing. Samples were embedded in paraffin blocks. Placental sections were cut from paraffin blocks and stained with ERM immunostaining.*

Result: *Degenerative collagen fibers and edema were seen in root villi. Muscle cells were hypertrophied and degenerated. Increased fibrinoid accumulation and syncytial bridges and nodes, thinning of the basement membrane, and vascular dilatation were observed. The healthy group showed negative ezrin negative expression mainly in chorionic villi but it was positive in villous connective tissue. In the previa group, positive ezrin reactions were observed in vessels, fibroblast cells, and cytotrophoblasts. In the healthy group, negative radixin expression was observed in the syncytial structures. In the previa group, a positive reaction was observed in the basement membrane, endothelial cells of vessels, and connective tissue cells. In the healthy group, intense moesin expression was observed in vascular structures, connective and inflammatory cells. In the placenta, previa group, positive moesin expression was observed in endothelial cells, connective tissue cells, Hoffbauer cells, and muscle.*

Conclusion: *ERM proteins are thought to be an important marker to investigate the pathologies formed in placenta previa.*

Keywords: *Previa, ERM, immunostaining, placenta, villus.*

DOI: 10.19193/0393-6384_2023_4_148

Received *January 15, 2023;* ***Accepted*** *April 20, 2023*

Introduction

Placenta is a temporary organ that regulates many activities between the fetus and mother. The placenta supplies nourishment to the fetus, and secretes hormones for the continuation of pregnancy[1]. Placenta is implanted in the uterus, but the correct placement is important. In such cases, placement of the placenta prevents delivery of the baby. Its placenta is low lying. This clinical condition can also cause unusual bleeding during pregnancy or delivery[2]. These abnormal placental implantation causes abnormal placental development and prevents the nourishment of the fetus by fetal and maternal blood circulation. Structural changes such as thickening of the basement membrane of fetal capillaries, increased fibrous tissue in the villous stroma, and fibrinoid accumulation in a chorionic plate and on the root villi in the junction may be observed[3, 4]. Placenta previa is the placement of the placenta to the lower uterine segment partially or totally. Its incidence is about 1 in 200 pregnancies. Smoking, pregnancy at age ≥35, pre-aspiration, and curettage, multiparity, previous cesarean history, labor induction, or termination of pregnancy increases the risk of placenta previa[5, 6]. Due to its relationship with maternal morbidity and mortality, diagnosis of placenta previa is important. Placenta

previa can cause endometrial injury, uterine scar formation, and trophoblastic invasion. The etiology of placenta previa is not fully known, but it is caused due to abnormal vasculogenesis, and defective decidualization and leads to abnormal placental development[7].

The ERM protein family consists of 3 proteins that are in a relationship with each other, and these are ezrin, radixin, and moesin[8]. Members of the ERM protein family provide the link between the proteins found in the plasma membrane and the actin cytoskeleton. Proteins belonging to this family are concentrated in microvilli, in the membrane ruffles, during the assembly and division of cells[9].

Methods

Ethical approval was taken from Dicle University Medical Faculty Ethics Committee for Non-Interventional Clinical Studies (Date: 28.02.2023, protocol number: 74). In our study, 15 healthy and 15 placenta previa were collected from pregnant women (regardless of age) from Gynecology and Obstetrics Clinics. All patients signed the informed patient consent form. Placental tissues were processed for routine paraffin wax embedding protocol.

Histological tissue processing

Placental tissues were fixed with zinc-Formalin solution (catalog no: Z2902, Sigma-Aldrich, St. Louis, MO, US) and washed under tap water by 5 minutes. Tissues were passed through ascending alcohol series for about 24 hours.

Tissues were washed with xylene for 2x30 minutes and incubated within paraffin wax. 5 μm sections were cut with a microtome (catalog no: Leica RM2265, Wetzlar, Germany). Deparaffinized within xylene for 2X30 minutes, sections were brought to distilled water. Some of the sections were stained with immunohistochemical staining[10].

ERM immunostaining

All placental tissues were brought to distilled water. Hydrogen peroxide solution (catalog no: TA-015-HP, Thermo Fischer, Fremont, CA, USA) was dropped on sections for 20 minutes. After washing in PBS for 3X5 minutes, ultra V Block (catalog no: TA-015-UB, Thermo Fischer, Fremont, CA, USA) was applied to sections for 8 minutes. Sections were incubated with primary antibodies anti-ERM proteins (AFG Scientific, Northbrook, USA) at +4oC overnight. Sections were allowed to warm at room temperature for 30-60 minutes.

Sections were washed with biotinylated secondary antibody (catalog no:TP-015-BN, Thermo Fischer, Fremont, CA, USA) for 14 minutes. Streptavidin-peroxidase (catalog no:TS-015-HR, Thermo Fischer, Fremont, CA, USA) was dropped onto sections for 15 minutes. Clearing with PBS, DAB (catalog no: TA-001-HCX, Thermo Fischer, Fremont, CA, US) was used as the chromogen. Sections were counterstained with Gill hematoxylin (catalog no:105174, Sigma-Aldrich, St. Louis, MO, United States). and mounted with Stellan (catalog no:107961, Sigma-Aldrich, St. Louis, MO, United States). Slides were analyzed with Zeiss Imager A2 Zen 3.0 software (Germany, Carl-Zeiss-Straße, Oberkochen, Germany) and photomicrographed[11].

Statistical analysis

The data were recorded as mean (minimum-maximum). Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, US).

Results

Statistical analysis of parameters belonging to healthy and placenta previa patients were shown in Table 1. Age, gravida, parity, systolic blood pressure (BP), diastolic BP, platelet, urea, ALT, AST, and 2-h urine protein were higher in the placenta previa group than in the healthy group.

Hemoglobin, glucose, and creatinine levels were higher in a healthy group than in the placenta previa group. Graphical illustration of Table 1 is shown in Figure 1.

Parameter	*Healthy group (N=15)*	*Previa group (N=15)*
Age	26 (20-39)	28 (19-42)
Gravida	2 (1-8)	3 (1-8)
Parity	0 (0-4)	2 (0-7)
Systolic BP	115 (94-135)	148 (136-182)
Diastolic BP	69 (63-84)	96 (90-113)
Hemoglobin	12 (9.4-14.7)	10.4 (8.1-13.2)
Platelet	231 (100-364)	269 (152-408)
Glucose	76 (61-95)	72 (63-96)
Urea	14.9 (10-21)	16 (12.1-32.1)
Creatinine	0.6 (0.52-0.68)	0.57 (0.42-0.7)
ALT	11 (7-23)	12 (8-51)
AST	18 (10-49)	22 (11-45)
2h-urine protein	142 (105-184)	690 (420-6870)

Table 1: Characteristics and blood values of the patients.

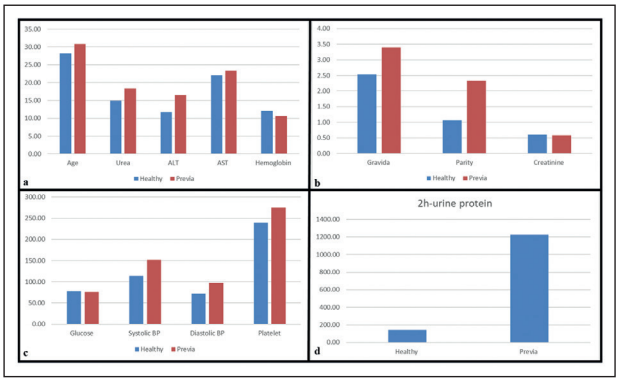

Figure 1: Graphical illustration of: a) age, urea, ALT, AST, and hemoglobin values; b) gravida, parity, creatinine values; c) glucose, systolic blood pressure (BP), diastolic BP, and platelet values; d) 2-h urine protein concentration in healthy and placenta previa patients.

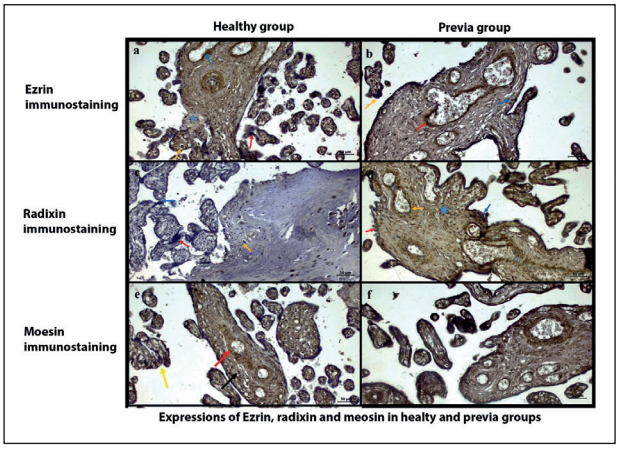

Figure 2: Ezrin immunostaining: a) Healthy group: Positive expression in vascular endothelium (blue arrow) and Hoffbauer cells (yellow arrow), negative expression in fibrinoid (blue star) and villi (red arrow), b) Previa group: Basal membrane of dilated vessels (red arrow), Hoffbauer (blue arrow) cells positive ezrin expression, negative ezrin reaction in syncytial nodes (yellow arrow); Radixin immunostaining c) Healthy group: Negative expression in syncytial nodes (red arrow) and syncytial bridges (blue arrow), positive expression in decidua cells (yellow arrow); d) Previa group: negative expression in cytotrophoblasts (red arrow) and syncytial nodes (blue arrow), positive expression in fibroblasts (blue star) and vascular endothelium (yellow arrow); Moesin immunostaining e) Healthy group: Positive expression in fibroblasts (black arrow) in the endothelium of dilated blood vessels (red arrow), negative expression in syncytial nodes (yellow arrow); f) Previa group: Edema-like formation (star), intense moesin expression in endothelial cells (orange arrow), negative expression in syncytial nodes (black arrow).

Ezrin immunostaining findings

Healthy group

Negative expression was observed in chorionic root villi, fibrinoid areas and connective tissue cells. Positive ezrin was observed in the vascular endothelium, some Hoffbauer cells, and the basement membrane. In general, moderate positivity was observed in the connective tissue inside the villi (Figure 2a). Scale Bar: 50 μm, Magnification: 20X, Ezrin immunostaining.

Previa group

Thinning of the basement membrane of dilated vessels and a positive ezrin reaction were observed. Positive expression was observed in fibroblast cells, some Hoffbauer cells and cytotrophoblasts. Expression was negative in the syncytial nodes (Figure 2b). Scale Bar: 50 μm, Magnification: 20X, Ezrin immunostaining.

Radixin immunostaining findings

Healthy group

Negative radixin expression was observed in the syncytial nodes, inter-villi syncytial bridges, and nodes in the decidual plaque region. Slight positive expression was observed in decidual cells with some maternal regions. There was no change in the intervillous area in the cross-section of the control group (Figure 2c). Scale Bar: 50 μm, Magnification: 20X, Radixin immunostaining.

Previa group

Negative expression was observed in some of the cytotrophoblast cells and syncytial nodes. A positive reaction was observed in the thinned basement membrane and in some places under the basement membrane. Along with the degenerative changes in the muscle tissue, degeneration of the endothelium and radix positivity were observed. Positive radixin expression was seen in fibroblasts (Figure 2d). Scale Bar: 50 μm, Magnification: 20X, Radixin immunostaining.

Moesin immunostaining findings

Healthy group

Degenerative changes and edema were observed in collagen fibers in some root villi. Intense moesin expression was observed in endothelial cells in dilated blood vessels, while moderate moesin expression was observed in fibroblast cells and some Hoffbauer cells in connective tissue. Negative expression was observed in the syncytial nodes. In the media layer of some blood vessels, fusiform cells

were hypertrophied and rounded, and degeneration, and positive moesin expression were observed in the muscle layer (Figure 2e). Scale Bar: 50 μm, Magnification: 20X, Moesin immunostaining.

Previa group

While degenerative changes were observed in the collagen fibers in the areas where some root villi were found, edema-like formations were observed in places. Positive moesin expression was observed in endothelial cells of dilated blood vessels, fibroblasts in connective tissue, and some Hoffbauer cells. Negative expression was observed in the syncytial nodes, while positive moesin expression was observed in the fusiform-shaped cells in the media layer of some blood vessels and in the degenerative region of the hypertrophic muscle layer (Figure 2f). Scale Bar: 50 μm, Magnification: 20X, Moesin immunostaining.

Discussion

Placenta is an organ that develops within the uterine wall and provides metabolic exchanges between the fetus and the mother. Placental abnormalities depend on the anatomical location of implantation[12]. Placenta previa is a placental abnormality where the placenta lies on the lower segment of the uterus, completely or partially covering the cervix[13]. Placenta previa is still a leading reason for maternal, fetal, and neonatal morbidity and mortality characterized by third-trimester bleeding. Pathophysiology of placenta previa is still not fully understood; however, many factors such as maternal age≥35, multiparity, multiple pregnancy, previous cesarean history, and smoking increases the risk of placenta previa[14].

Otçu et al.[15] examined the placenta of previa patients and revealed that increased syncytial knots, intervillous hemorrhage, fibrin accumulation, and hyalinization. Studies on histopathology of placenta previa revealed fibrinoid necrosis, polymorphonuclear cell infiltration, abnormal vasculatures, dilated vessels. Biswas et al.[16] recorded increased trophoblastic giant cells, hemorrhage, absence of chorionic villi in the myometrium and inflammation in placenta previa tissues. Silver et al.[17] also reported increased villous infarction with fibrinoid and congested vessels in pathological examination of placenta previa. Jung et al.[18] studied 93 patients with placenta previa in terms of histological perspectives. They found that maternal under perfusion, villous infarction, increased intervillous fibrin deposition in their histopathological findings. In our study, degenerative changes and edema were observed in collagen fibers in some root villi. In the media layer of some blood vessels, fusiform cells were hypertrophied and rounded, degenerated. Fibrinoid areas, increased syncytial bridges and nodes, thinning of the basement membrane of dilated vessels. Along with the degenerative changes in the muscle tissue, degeneration of the endothelium was observed (Figure 2).

The amino acid of the Ezrin (E), Radixin (R) and Moesin (M) proteins have very similar homology sequences (70-85%). Because of their functional and structural properties, these proteins are called the ERM protein family. Ezrin from the ERM protein family is highly expressed in the stomach, small intestine, kidneys, and lung, while radixin is found in the small intestine and liver, and moesin is found in the spleen and lung[19]. Ezrin was found to be highly expressed in microvilli of human placental syncytiotrophoblast cells. However, the localization of radixin differs slightly from ezrin and moesin. Unlike the other two ERM family member proteins, radixin is defined as proteins involved in adherent binding. These proteins are involved in changes in cell shape, cell adhesion, and attachment[20]. Tokunou et al.[21] found that ERM protein expression decreased in lung adenocarcinoma, stating that the expression of radixin and moesin function as tumor suppressors in lung adenocarcinoma. A similar study showed that expression of radixin and mosein were changed in prostatic adenocarcinoma, which may suggest that these proteins may be a marker in prostatic neoplasia progression[22].

In our study, in the healthy group, ezrin-negative expression was observed in chorionic root villi, but it was positive in connective tissue inside the villi. In the previa group, positive ezrin reactions were observed in vascular structures, in connective fibroblast cells, and in cytotrophoblasts (Figure 2a-b). In the healthy group, negative radixin expression was observed in the syncytial nodes, inter-villi syncytial bridges, and nodes in the decidual plaque region. In the previa group, a positive reaction was observed in the thinned basement membrane, vascular endothelial cells, and fibroblasts (Figure 2c-d). In the healthy group, intense moesin expression was observed in endothelial, fibroblast, Hoffbauer, and muscle cells. Negative expression was observed in the syncytial nodes. In the placenta, previa group, positive moesin expression was observed in

endothelial cells, connective tissue cells, Hoffbauer cells, and muscle. Negative expression was observed in the syncytial nodes (Figure 2e-f).

Conclusion

Syncytial knots and bridges formed in chorionic villi due to placenta previa development, increased cell degeneration and endothelial dysfunction, and dilatation in vessels affected ERM protein activity, resulting in disruption of angiogenesis regulation and increased apoptotic process.

ERM proteins are thought to be an important signal stimulator in the evaluation of histopathological findings in placenta previa.

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Corresponding Author:
Engin Deveci
Department of Histology and Embryology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey
Email: devecie32@hotmail.com
(Turkey)