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Research Article

Investigation of the Sox-9 and Cited-1 Immune Activity in Placentas of Women with Placenta Accreta

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Abstract

Aim: In this study, we investigated the immune activity of the Sox-9 and Cited-1 in women with placenta accreta.
Material and Methods: 20 healthy and 20 placenta accreta were processed for routine histological tissue processing. Placentals samples were dissected and fixed in 10% formaldehyde solution. Samples were embedded in paraffin blocks. Clinical and biochemical parameters were recorded. Placental sections were cut from paraffin blocks and stained with Sox-9 and Cited-1 immunostaining.
Results: In our study, control group showed negative Cited-1 expression in decidual cells, root villi and connective tissue areas in general. Placenta accreta group showed increased Cited-1 expression in degenerated decidual cells, fibroblastic cells and endothelium. In control group, Sox-9 expression was negative in the syncytial knots, in the vascular endothelial cells. In placenta accreta group, Sox-9 reaction was positive in the root villi, in the blood vessels, in the connective tissue.
Conclusion: It was observed that the Sox-9 reaction was increased and inflammation was induced, depending on the differences in decidual cells, in the syncytial area and in the vascular endothelium in in placentas of women with placenta accreta. It is thought that Sox-9 signaling processes are being determined and Cited-1 may be stimulants that affect cell proliferation and angiogenesis regulation and affect placental development.

Keywords: Placenta accreta, Sox-9, Cited-1, immunostaining

INTRODUCTION

Placenta is a temporary organ which regulates many activities between fetus and mother. Placenta supply nourishment to fetus, secretes hormone for the continuation of pregnancy (1). Placenta is implanted in uterus but the correct placement is important. In such cases, placement of placenta prevents delivery of baby it 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 prevent the nourishment of 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 chorionic plate and on root villi in the junction may be observed (3,4).

Placenta accreta spectrum (PAS) is a syndrome characterized by the abnormal implantation of the placenta to the uterus, where it is invasive or adherent. Its other name is morbidly adherent placenta (5). In this spectrum, trophoblast invasion occurs abnormally into the myometrium. This invasion can sometimes cross the tunica serosa. In PAS, if the placental villi are attached to the myometrium, it is defined as placenta accreta. The incidence of placenta accreta in PAS is 80% (6). The etiology of placenta accreta is unknown, but abnormal

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decidualization or absence of decidualization is the most accepted theory. Reasons such as a history of cesarean section, surgical interventions, uterine curettage, and advanced maternal age are also risk factors for placenta accreta. Another theory is excessive extra-villous trophoblastic invasion (7).

Immunohistochemistry is a molecular technique to localize the specific protein of interest in the tissue. It is commonly used for pathological diagnosis of many diseases such as cancer. In this study, we aimed to investigate expression level of Sox-9 and Cited-1 in placentas of women diagnosed with placenta accreta by immunohistochemical techniques.

MATERIAL AND METHOD

Ethical approval was taken from Dicle University Clinical Research Ethics Committee. In our study, 20 healthy women and 20 women with placenta accreta were included. Placentas were obtained from Gynecology and Obstetrics Clinics. All patients signed informed patient consent form. Biochemical and clinical parameters for each patient were recorded. Placental tissues were processed for routine paraffine wax embedding protocol.

Histological tissue processing

Placental tissues were excised and taken into 10% formaldehyde solution for 2 days. Then samples were washed in tap water for 24 hours. Samples were put into alcohols to remove the water. Tissues samples were soaked in xylol solution for 20 minutes two times. Tissues samples were taken into 58°C incubator to incubate in paraffin wax. Tissue parts were put into paraffin blocks. 5 µm thick sections were cut with rotary microtome. Sections were put into xylene to remove excess paraffine for 15 minutes two times. Sections were dipped into alcohols and washed in distilled water. Sections were stained with routine Hematoxylin and Eosin and Sox-9 and Cited-1 immunohistochemical staining (8).

Immunohistochemical Analysis

Formaldehyde-fixed tissue was embedded in paraffin wax for further immunohistochemical examination. Sections were deparaffinized in absolute alcohol. Antigen retrieval process was performed twice in citrate buffer solution (pH:6.0), first for 7 minutes, and second for 5 minutes, boiled in a microwave oven at 700 W. They were allowed to cool to room temperature for 30 minutes and washed twice in distilled water for 5 minutes. Endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide for 20 minutes. Ultra V block (Cat. No:85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 10 minutes prior to the application of primary antibodies Sox-9 and Cited-1 (AFG scientific, US, 1:100) Secondary antibody (Cat. No:85- 9043, Invitrogen, Carlsbad, CA, USA) was applied for 20 minutes. Slides were then exposed to streptavidin–peroxidase for 20 minutes. Chromogen diaminobenzidine (DAB Invitrogen, Cat. No:34002 Carlsbad, CA, USA) was used. Control slides were prepared as mentioned above but omitting the primary antibodies. After counterstaining with hematoxylin and washing in tap water for 8 minutes and in distilled water for 10 minutes, the slides were mounted with Entellan (9).

Statistical Analysis

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

RESULTS

Age, gravida, parity, systolic BP, diastolic BP, hemoglobin, platelet, glucose, urea, creatinine, ALT, AST-urine protein was recorded in healthy and placenta accreta women. Data were shown in Table I.

Table 1. Patients characteristic and their blood test values were listed

Parameter	Healthy (N=20)	Accreta (N=20)
Age	20 (42-26)	21 (45-28)
Gravida	0 (5-2)	1 (8-3)
Parity	0 (3-0)	0 (7-2)
Systolic blood pressure	90 (144-110)	136 (200-148)
Diastolic blood pressure	64 (82-69)	92 (118-96)
Hemoglobin	9.8 (14.3-12)	9.45 (14.7-10.4)
Platelet	110 (358-231)	168 (412-269)
Glucose	65 (102-76)	64 (110-78)
Urea	11 (21-15)	13.5 (35.8-16)
Creatinine	0.52 (0.72-0.61)	0.51 (0.8-0.57)
ALT	7 (25-12)	8 (51-12)
AST	12 (55-18)	11 (45-22)
2h-urine protein	102 (179-142)	300 (920-541)

Histopathological staining

Figure 1 shows Cited-1 and Sox-9 immune staining. In the Cited-1 immunohistochemical staining of the control group, it was determined that the Cited-1 expression was negative in decidual cells, root villi and connective tissue areas in general, while the Cited-1 reaction was found to be positive in the sections with syncytial nodes (Figure 1a). Placenta accreta group Cited-1 immunohistochemical staining showed a positive reaction in maternal areas, especially due to the degeneration of decidual cells. It was observed that the cited-1 reaction was also positive in fibroblastic activities with collagenized degenerations where hyalinized areas were found. Positive vascular endothelium was also observed (Figure 1b). In the examination of the Sox-9 immunohistochemical staining section of the control group, it was observed that Sox-9 expression was negative especially in the syncytial regions and in the maternal area. Again, negative Sox-9 expression was found in the vascular endothelial cells in general. Positive Sox-9 expression was detected in some syncytial

nodes (Figure 1c). In the Sox-9 immunohistochemical staining of the Placenta Accreta group, it was observed that the Sox-9 reaction of the syncytial cells from the root villi in the maternal region was positive in the histopathological examination. Again, it was determined that Sox-9 was positive in the endothelial cells in the blood vessels, in the connective tissue cells in between, and in areas where leukocyte cell infiltration is intense. It was thought that Sox-9 could play an important role in trophoblastic regulation (Figure 1d).

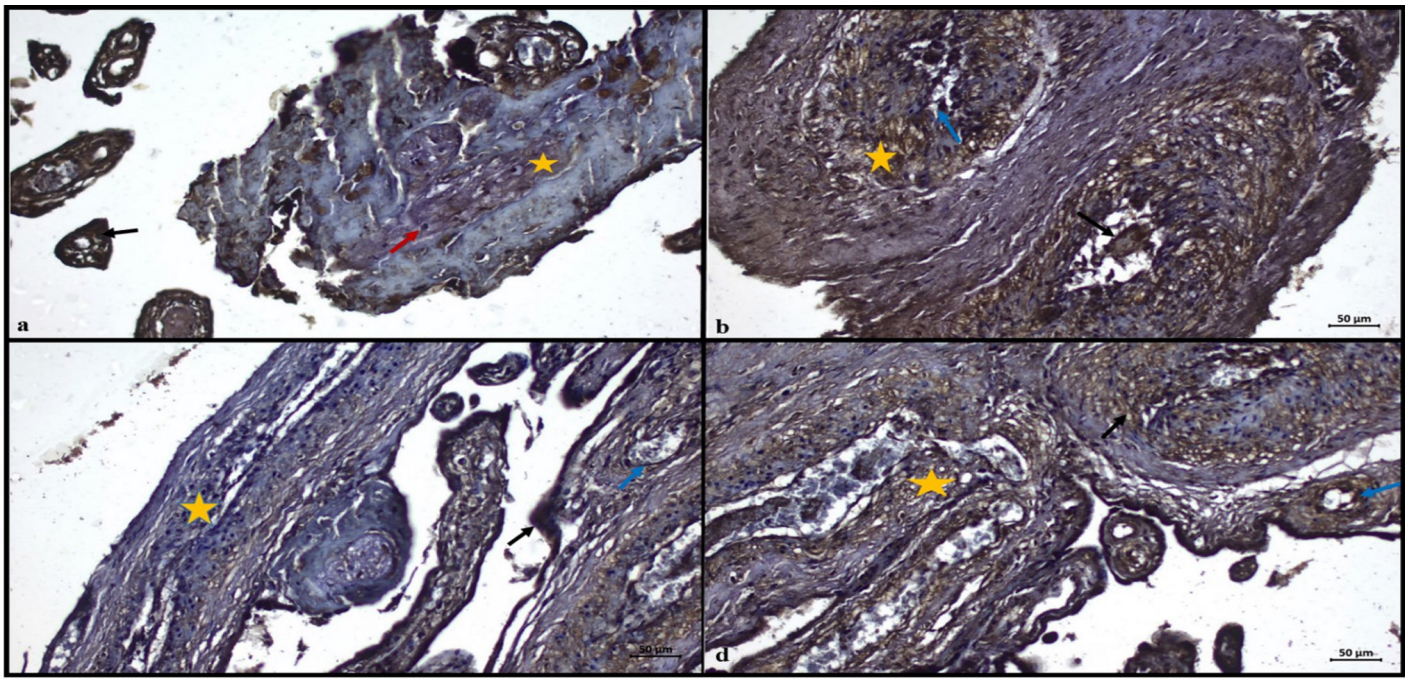

Figure 1. a) In the control group Cited-1 immunohistochemical staining, negative Cited-1 expression in decidual cells (red arrow) and connective tissue areas (yellow star), positive Cited-1 expression in syncytial nodes (black arrow); b) Placenta Acreata group Cited-1 immunohistochemical staining showed positive cited-1 reaction in degenerated decidual cells (blue arrow), connective tissue areas (yellow star), vascular endothelium (black arrow); c) Negative Sox-9 expression in maternal region (yellow star) and vessel endothelium (blue arrow) in Sox-9 immunohistochemical staining of control group, positive Sox-9 expression in syncytial nodes (black arrow); d) In the Sox-9 immunohistochemical staining of Placenta Acreata group, positive Sox-9 expression in syncytial cells (yellow star), vascular endothelium (blue arrow), and connective tissue cells in between (black arrow) from root villi

DISCUSSION

Placenta is an organ that develops within uterine wall and provide metabolic exchanges between fetus and mother (10). Placental abnormalities depend on the anatomical location of implantation. Placenta previa is a placental abnormality where placenta lie on the lower segment of the uterus, completely or partially covering cervix (11). Placenta previa is still a leading reason of 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 risk of placenta previa (12,13,14,15). Otçu et al. examined the placenta of previa patients and revealed that increased syncytial knots, intervillous hemorrhage, fibrin accumulation, and hyalinization (16). Studies on histopathology of placenta previa revealed fibrinoid necrosis, polymorphonuclear cell infiltration, abnormal vasculatures, dilated vessels. Biswas et al. recorded increased trophoblastic giant cells, hemorrhage, absence of chorionic villi in the myometrium and inflammation in placenta previa tissues (17). Silver et al. also reported increased villous infarction with fibrinoid and congested vessels in pathological examination of placenta previa (18). Jung et al. 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 (19).

Cited is a coactivator in transcription and possibly responsible for melanocytes pigmentation. It mediates events in transcription regulated by estrogen (20). Cited consists of four nuclear proteins as Cited-1, Cited-2, Cited-3, and Cited-4. Since Cited protein has no DNA binding site, its role is mainly transcriptional regulator (21). Sriraman et al. studied progesterone receptor in cultured granulosa cells and found that progesterone receptor induced many genes that regulated granulosa cells activity (22). one of the genes was Cited-1 that is affected by progesterone receptor during ovulation. Hatzirodos et al. investigated the transcriptome profile of granulosa cells in bovine ovarian follicles. author found that as follicle develops larger, transcriptional regulators was high in number (23). One of the regulators was Cited-1. In our study, control group showed negative cited-1 expression in decidual cells, root villi and connective tissue areas

in general (Figure 1a). Placenta accreta group showed increased Cited-1 expression in degenerated decidual cells, fibroblastic cells and endothelium (Figure 1b).

SRY-box transcription factor 9 or Sox-9 is a transcription factor that is required for testicular development, organogenesis of liver and pancreas, cytoskeleton and chondrocytes. Mutations in Sox-9 gene can lead to autosomal sex reversal, skeletal formation and testis development (24,25). Sekido et al. studied two genes in Sertoli cell by investigating SRY expression. They found that upregulation of Sox-9 gene in supporting cells determine their fate as Sertoli cells, which shows importance of sox-9 gene in testis (26). Zhao et al. studied endothelial to mesenchymal transition in murine endovascular progenitors. They found that endothelial to mesenchymal transition was dependent on relative expression of Sox-9 along with Notch signaling, affecting their plasticity which may be a therapy tool for fibrotic diseases (27). Xian et al. studied showed that stimulation of Sox-9 can induce cellular differentiation gene and this can be a mechanism in transformation of extra-villous trophoblast to endovascular trophoblasts during placentation (28). In control group, Sox-9 expression was negative in the syncytial knots, in the vascular endothelial cells (Figure 1c). In placenta accreta group, Sox-9 reaction was positive in the root villi, in the blood vessels, in the connective tissue (Figure 1d).

CONCLUSION

It was observed that the Sox-9 reaction was increased and inflammation was induced, depending on the differences in decidual cells, in the syncytial area and in the vascular endothelium in placentas of women with placenta accreta. It is thought that Sox-9 signaling processes are being determined and Cited-1 may be stimulants that affect cell proliferation and angiogenesis regulation and affect placental development.

Strength and Limitation

The strengths: The study strongly stated the histopathology of placenta accreta with two potential markers (Sox-9 and cited-1).

Limitations: The study only focuses on histological perspective. The result could be supported with other techniques such as TUNEL assay, western blot and flow cytometry.

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Ethical approval: *The study was carried out with the permission of Dicle University, Clinical Ethics Committee (Date. 14.10.2022 Decision No:08)*

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