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The role of osteopontin in the pathogenesis of placenta percreta

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ABSTRACT

Objectives: This study aims to determine how the expression of osteopontin is altered in the placenta percreta by comparing osteopontin expression in normal placentas and placenta percreta tissues.

Material and methods: Placental tissues from hysterectomy materials which were histopathologically diagnosed with placenta percreta (study group, n = 20) and placental tissues obtained from normal term pregnancies (control group, n = 20) were immunohistochemically stained with osteopontin antibody. The groups were compared with respect to the intensity of cytoplasmic staining for osteopontin.

Results: The study and control groups were similar with respect to age, gravidity, parity, gestational age at birth, number of previous cesarean deliveries and curettages and ($p > 0.05$ for all). Immediate postoperative hemoglobin was significantly lower and the need for blood transfusion was significantly higher in the study group ($p = 0.001$ for both). Placental osteopontin expression was significantly altered in the study group ($p = 0.020$). Negative staining for placental osteopontin was significantly more frequent in the placenta percreta group than the control group (9/20 vs 0/20, 45.0% vs 0%, $p = 0.037$).

Conclusions: As reduced placental osteopontin expression was determined in the placenta percreta cases compared to the normal term placenta tissues, osteopontin can be considered to have a role in morbidly adherent placentation. This study is of value as the first study to investigate the changes in osteopontin expression in placenta percreta cases.

Key words: immunohistochemistry; osteopontin; placenta percrata

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INTRODUCTION

Morbidly adherent placenta (MAP) is the terminology generally used to define the abnormal adhesion of placenta to the uterus. Depending on the depth of the placental adhesion, MAP can be described into accreta, increta, and percreta. The incidence of MAP has increased during the last 50 years in parallel with the increasing rates of cesarean section (CS), and therefore, MAP has become a significant cause of peripartum hemorrhage and thereby of maternal morbidity and mortality in recent years [1, 2].

The defective decidua basalis, due to previous CS or curettages, or over-invasive trophoblastic cells or both of these factors are thought to have a role in the pathophysiology of MAP. More specifically, it has been hypothesized that MAP occurs as the interaction between maternal endometrium and migratory trophoblasts is impaired and uteroplacental circulation is interrupted [3–5].

Osteopontin (OPN) is an extracellular matrix glycoprotein which participates in cell proliferation and invasion and, thus, contributes to the tumorigenesis in several types of malignancies [6–8]. Previously published studies have reported that the expression of osteopontin is evidently present in the placental tissues of both mice and humans. Therefore, it has been suggested that osteopontin is somehow involved in the proliferation and invasion of trophoblasts during early placentation [9–11]. Although there have been several studies showing that the OPN plays a role in implantation and in placentation, no study has

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been conducted to determine the level of OPN expression in cases with placenta percreta.

Objectives

This study aims to determine the role of OPN in the pathogenesis of placenta percreta by comparing the expression of OPN in normal placenta and placenta percreta tissues.

MATERIAL AND METHODS

This case control study was approved by the local Institutional Review Board and Ethics Committee of Kahramanmaras Sutcu Imam University Hospital where it was conducted.

The study group included placental tissues of 20 patients who underwent peripartum hysterectomy at the obstetrics department of the study center between June 2015 and December 2016 and who were histopathologically diagnosed with placenta percreta. The control group consisted of the placental tissues of 20 patients who had histopathologically normal term placentas and who underwent CS delivery due to various obstetric indications at the study center between June 2015 and December 2016. Written informed consent was obtained from all participants. Demographic and clinical characteristics of the reviewed patients were obtained from the medical records.

All of the histopathological and immunohistochemical examinations were carried out by two independent experienced histopathologists who were blinded to the demographic and clinical characteristics of the participants (A.Y. and S.K.). Placenta percreta was diagnosed when chorionic villi penetrated the uterine serosa [11].

Immunohistochemistry

Sections 4 μm thick in size were cut from the normal placental tissue samples in the control group and from the invasive sections of the placentas in the study group and then stained with the immunohistochemical method. Immunohistochemical staining of the sections was made with the biotin-streptavidin peroxidase method. One section from each patient was evaluated. All the tissue sections were deparaffinised with xylol and were then passed through a series of ethanol solutions (100%, 96%, 80%) for dehydration.

The samples were then washed with PBS and left for 15 mins in 3% H2O2 for blockage of endogenous peroxidase activity, then treated with 10% bovine fetal serum to prevent non-specific connections. The sections were completely covered by drops of OPN anti-human monoclonal IgG antibody (R&D System, MN, USA, 1:100 dilution) and left for 1 hour [12]. All the samples were washed with PBS and after a secondary antibody IgG (R&D System, MN, USA) stage, a streptavidin peroxidase enzyme complex was applied. Diaminobenzidine was droppered chromogenously to bring it to a visible state and for counter staining, Mayer haematoxylin was used. For positive control purposes, breast cancer tissue slices were evaluated and for negative control, PBS was used instead of the primary antibody.

Immunohistochemical scoring

Each section was evaluated under microscope (Nikon Eclipse 80i microscope connected to a DS-Fi1 camera using NIS-Elements) by a histologist (A.Y) and a pathologist (S.K) who were blinded to the clinical and histopathological diagnoses of the patients and photographs were taken at x100 magnification.

For all the study subjects, immunohistochemical evaluation was made of 5 fields selected at random from the sections of placental tissue at x400 original magnification.The immunohistochemical staining of osteopontin was evaluated with a semi-quantitative scoring system in which cytoplasmic staining intensity was categorised as negative (0), weakly positive (1+), moderately positive (2+) or strongly positive (3+). A result of > 50% of the cells with strong staining was determined as strongly positive (3+), < 25% as weakly positive (1+) and 25–50% as moderately positive (2+) [13].

Statistical analysis

Collected data were analyzed by Statistical Package for Social Sciences version 18.0 (SPSS IBM Software, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (range: minimum-maximum) whereas categorical variables were denoted as numbers or percentages where appropriate. Data distribution was evaluated by Kolmogorov–Smirnov test while independent samples t-test and chi-square test were used for comparisons. In order to avoid the risk of Type 1 errors, post hoc pairwise comparisons were made for the chi-square test. Two-tailed p values less than 0.05 were accepted to be statistically significant.

RESULTS

Demographic and clinical characteristics of the reviewed patients are shown in Table 1. The study and control groups were similar with respect to age, gravidity, parity, gestational age at birth, number of previous CS deliveries and D&C procedures and hemoglobin levels at hospital discharge ($p > 0.05$ for all). As expected, immediate postoperative hemoglobin was significantly lower and the need for blood transfusion was significantly higher in the placenta percreta patients ($p = 0.001$ for both). The average amount of blood transfusion was 4.6 ± 2.6 units (range: 4–8 units) in the placenta percreta group whereas only one patient in the control group received two units of blood transfusion.

Table 2 shows that placental OPN expression was significantly altered in the placenta percreta group ($p = 0.020$).

Table 1. Demographic and clinical characteristics of the study and control groups

	Study group (n = 20)	Control group (n = 20)	p
Age (years)	33.2 ± 3.8	30.6 ± 5.7	0.093
Gravidity	4.5 ± 0.9	3.8 ± 1.6	0.107
Parity	2.6 ± 0.8	2.0 ± 1.3	0.057
Previous curettages	0.4 ± 0.2	0.5 ± 0.2	0.728
Previous cesarean deliveries	2.0 ± 0.9	1.4 ± 1.0	0.061
Gestational age (weeks)	36.1 ± 5.2	38.2 ± 1.5	0.073
Postoperative hemoglobin [g/dL]	8.8 ± 1.1	11.0 ± 0.8	0.001*
Need for blood transfusion	16 (80.0%)	1 (5.0%)	0.001*
Hemoglobin at hospital discharge [g/dL]	11.5 ± 1.4	11.4 ± 1.3	0.695

* $p < 0.05$ was accepted to be statistically significant

Table 2. Immunohistochemical staining for placental osteopontin in the study and control groups

Cytoplasmic staining intensity*	Study group (n = 20)	Control group (n = 20)	p
0	9 (45.0%)	0 (0.0%)	$\chi^2 = 4{,}333$; $p = 0.037$†
1+	5 (25.0%)	7 (35.0%)	$\chi^2 = 0{,}921$; $p = 0.337$
2+	2 (10.0%)	5 (25.0%)	$\chi^2 = 1{,}558$; $p = 0.212$
3+	4 (20.0%)	8 (40.0%)	$\chi^2 = 1{,}905$; $p = 0.168$

$\chi^2 = 5{,}380$; $p = 0.020$†; *0 — negative staining, 1+ (weakly positive) — < 25% of the cells stained, 2+ (moderately positive) — 25%–50% of the cells stained; 3+ (strongly positive) — > 50% of the cells stained; †$p < 0.05$ was accepted to be statistically significant

Negative staining for placental OPN was significantly more frequent in the placenta percreta group than the control group (9/20 vs 0/20, 45.0% vs 0%, p = 0.037).

Figure 1 demonstrates the weakly positive (1+) and strongly positive (3+) staining for OPN in placental tissues in the study group. Figure 2 displays the weakly positive (1+) and strongly positive (3+) staining for OPN in placental tissues in the control group.

DISCUSSION

The results of the current study showed that trophoblastic osteopontin expression was reduced in placenta percrata compared to normal term placenta.

The invasion of uterine tissue by trophoblastic cells in the process of normal placentation shows similarities to the behaviour of malignant cells in the formation of a tumour. That is, trophoblasts invade the endometrium as tumor cells

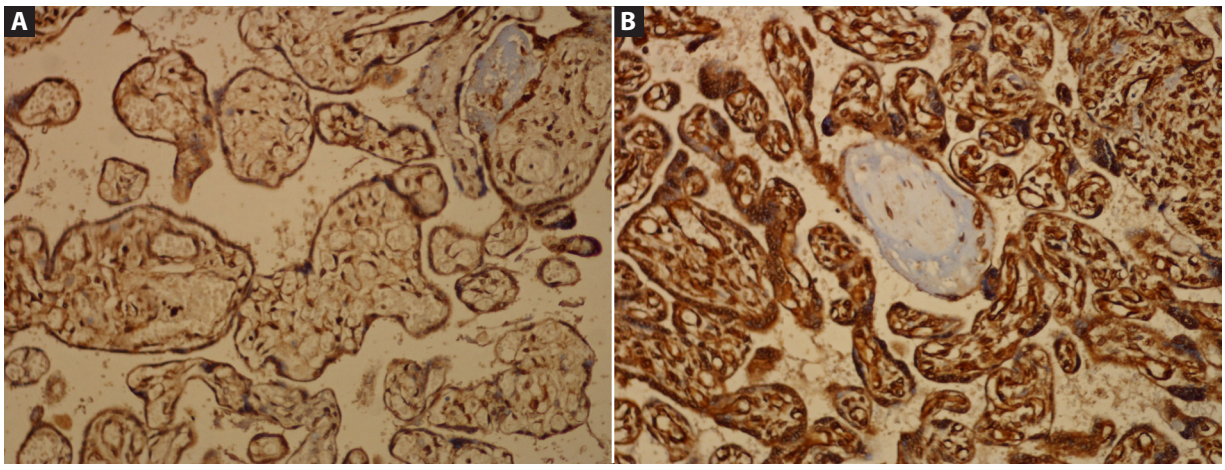

Figure 1. Weakly positive (**A**) and strongly positive (**B**) staining for osteopontin in placental tissues of the patients with placenta percreta (Mayer hematoxylin, x100 magnifications)

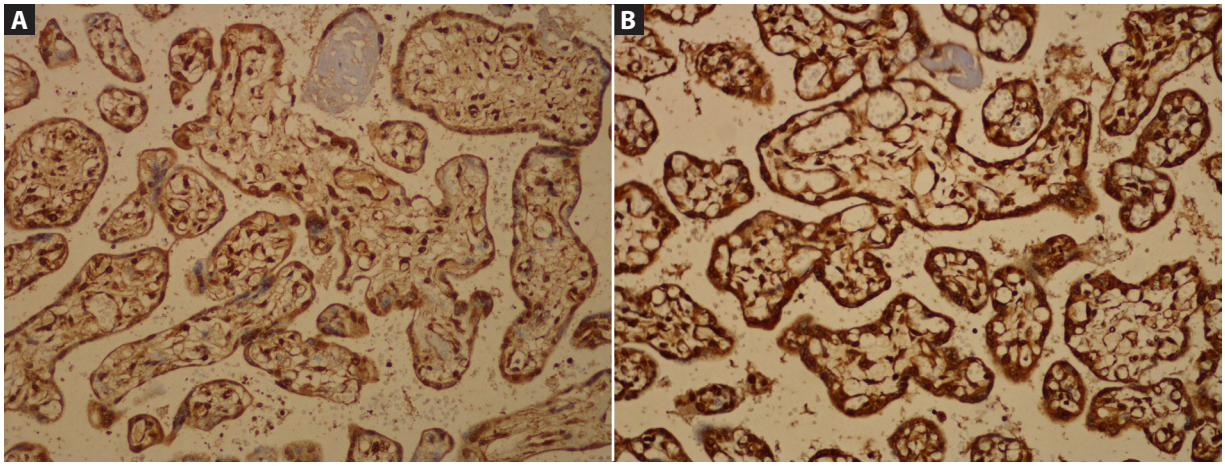

Figure 2. Weakly positive (**A**) and strongly positive (**B**) staining for osteopontin in placental tissues of the patients with normal term placenta (Mayer hematoxylin, x100 magnifications)

invade the adjacent tissues and metastasize [14]. However, the difference between normal placentation and malignant tumours is that the invasion of trophoblasts is kept under control with a complex process including cell adhesion molecules, the ligands of these adhesion molecules and the immune system [15].

Osteopontin (OPN) is a secreted glycoprotein with a role in cell-cell adhesion, cell-extra-cellular matrix (ECM) communication and cell migration. The binding of OPN to integrin, located in the membrane of uterine luminal epithelium cells, plays a role in the adhesion of the conceptus. In normal placenta, OPN is strongly present in the extravillous trophoblasts and cytotrophoblasts of the villous trophoblasts which are capable of proliferation and invasiveness [10]. An in vitro study reported that decreased OPN expression might disturb trophoblastic growth and invasion so that spontaneous abortion may occur [16]. In addition to the role in early pregnancy, the expression of OPN in the placenta has been shown in the later weeks of pregnancy [17, 18]. When these findings are interpreted together, it has been suggested that OPN acts in trophoblastic proliferation and invasion, and, thus, placentation.

To the best of our knowledge, this is the first study which aims to investigate the role of OPN in the pathogenesis of placenta percreta. Our results indicated significantly weaker staining for OPN in placenta percreta when compared to normal placenta of similar gestational age. Since OPN expression is up-regulated in several malignancies, significantly less OPN content in placenta percreta may appear as a contradictory finding [6, 8]. Defects in cellular adhesion molecule expression in cancer tissue has been shown to be associated with increased cellular invasion [19]. Supporting this information, in the placenta percreta cases of the current study, the reduced level of placental OPN expression might have decreased the decidual adhesion of trophoblasts and it can be suggested that in this way the trophoblasts could migrate more freely, forming an excessive invasion.

Supporting the findings of the current study, it has previously been shown that expression of OPN mRNA was decreased in gestational trophoblastic disease which is associated with an altered regulation of trophoblastic growth and invasion [20].

CONCLUSIONS

In conclusion, lowered expression of osteopontin seems to have a role in the pathogenesis of placenta percreta. This study is of value as the first study to investigate the changes in OPN expression in placenta percreta cases. Further research is warranted to clarify the role of osteopontin in the pathogenesis of placenta percreta.

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Disclosure

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