Journal of Reproductive Immunology 110 (2015) 74–80

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Histological characteristics of the myometrium in the postpartum hemorrhage of unknown etiology: a possible involvement of local immune reactions

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ARTICLE INFO

Article history:
Received 26 March 2015
Accepted 23 April 2015

Keywords:
Pregnancy
Postpartum hemorrhage (PPH)
Uterine atony
Myometritis
Amniotic fluid embolism

ABSTRACT

The aim of this study was to evaluate the histological characteristics of the myometrium obtained in postpartum hemorrhage (PPH) of unknown etiology secondary to uterine atony. These characteristics were selected from among registered cases of clinically suspected amniotic fluid embolism (AFE) and classified as PPH of unknown etiology because of no obvious cause of PPH at Hamamatsu University School of Medicine, a registration center for clinical AFE in Japan. Immunohistochemical studies were performed on myometrium using anti-mast cell tryptase, anti-neutrophil elastase, anti-CD68, anti-CD88, anti-CD3, and anti-ZnCP-1 antibodies. Massive infiltrations of inflammatory cells with mast cell degranulation within the myometrium secondary to complement activation were observed in PPH of unknown etiology ($n = 34$), but not in control pregnant women ($n = 15$) or after delivery in women without PPH ($n = 18$). The concomitant immunohistochemical detection of meconium in myometrium suggests that amniotic fluids or fetal materials are one of the candidates for inducing maternal local immune activation in the PPH of unknown etiology. Postpartum acute myometritis in the absence of an infective etiology may be a histological characteristic of PPH of unknown etiology.

1. Introduction

Postpartum hemorrhage (PPH) is the leading cause of maternal mortality in the world, with an incidence estimated to be up to 10% (Oyelese et al., 2007; Mousa et al., 2014; Cunningham et al., 2010). Various conditions such as abnormal placentation, trauma to the genital tract, uterine atony, and coagulation defects are known causes of PPH (Cunningham et al., 2010). Among the above, uterine atony is a major cause of PPH, estimated to be responsible for 70% (Karoshi and Keith, 2009; Oyelese and Ananth, 2010); however, the etiology remains to be clarified in cases of PPH secondary to uterine atony. Over-distended uterus and exhausted myometrium are considered to be causes of uterine atony; however, it has yet been clarified why myometrium suddenly stops contraction after delivery, because most patients presenting with uterine atony have no explainable risk factors (Rouse et al., 2006).

The authors, at a registration center for clinical amniotic fluid embolism (AFE) in Japan, and others, have proposed clinical criteria for AFE with the main clinical symptom of

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http://dx.doi.org/10.1016/j.jri.2015.04.004
0165-0378/

massive PPH not explained by other diseases (Kanayama et al., 2011; Benson, 2012). Furthermore, from the data accumulated on registering cases of clinical AFE in Japan, massive PPH frequently accompanied by coagulopathy and uterine atony has been considered pathognomonic for clinical AFE (Benson, 2007). In other words, a number of cases of 'PPH of unknown etiology' have been regarded and managed as clinical AFE according to the criteria but with no physical evidence. Although the causative mechanism of clinical AFE is still unclear, a previous study demonstrating maternal complement activation in clinical AFE suggested that a pathological maternal immune reaction might be associated with the pathogenesis of 'PPH of unknown etiology' including clinical AFE (Kanayama and Tamura, 2014).

In the present study, we hypothesized that a maternal immune reaction localized in the myometrium might deteriorate the myometrial function of contraction and cause PPH of unknown etiology secondary to uterine atony. To identify immunoreactive cells in myometrium with PPH of etiology, we carried out immunohistochemical examinations.

2. Materials and methods

2.1. Subjects

Cases of 'PPH with unknown etiology' were originally registered at Hamamatsu University School of Medicine from January 2011 to December 2012 as having clinically suspected AFE according to the Japan consensus criteria for the diagnosis of AFE, whereby the major symptom was PPH except for cardiac arrest or respiratory failure (Tamura et al., 2014a). They were retrospectively and carefully selected as 'PPH with unknown etiology' secondary to uterine atony according to the reports of physicians in charge. Exclusion criteria were multiple fetuses, rupture of membranes, preterm labor, chorioamnionitis, uterine or cervical laceration, placenta previa, placenta accreta, preceding DIC such as sepsis, placental abruption, and preceding sudden maternal cardiac deterioration.

Tissues after abdominal hysterectomy after the onset of PPH were collected and stored at Hamamatsu University School of Medicine. Mean time interval between deliveries and hysterectomy was 4.6 h in PPH cases. Control tissues were obtained at Hamamatsu University Hospital after receiving written informed consent. The myometrial tissues were obtained by partial resection of the anterior wall of the uterine body, 3–5 mm beneath the serosa, during ($n = 15$) or soon after ($n = 18$) the delivery of neonates by cesarean section after an uncomplicated pregnancy. 9 nulliparous and 6 multiparous pregnant women were selected for controls with mean gestational age (37.8 ± 0.7), mean parity (0.79 ± 1.05) and mean gravida (1.14 ± 1.10). Myometrial tissues from 6 nulliparous to 12 multiparous women were also obtained after delivery of neonates as controls with a mean gestational age (38.3 ± 1.5), mean parity (1.29 ± 1.11), and mean gravida (1.57 ± 0.98). All control cases were free from massive hemorrhage, hock, DIC, uterine atony, and any kind of allergic reaction. Patients' demographic data are shown in Table 1.

2.2. Immunohistochemistry

All specimens were fixed in 10% buffered formalin solution, embedded in paraffin, and cut into 3-μm-thick sections. Sections were stained with hematoxylin and eosin. For immunohistochemistry, the antigen was retrieved in a high-pressure cooker for 20 min (temperature: 95 °C) using citrate buffer (pH 6) for tryptase and CD68 and Tris/EDTA buffer (pH 9) for CD88 and CD3. Endogenous peroxidase activity was blocked by H_2O_2 for 5 min. Primary antibody was applied at a ratio of 1:10,000 for tryptase (abcam®, UK), 1:200 for elastase (DakoCytomation, Denmark), 1:200 for CD68 (Thermo, UK), 1:200 for CD3 (Novocastra™ liquid, UK), 1:4000 for CD88 (Cosmo Bio Co. Japan) with 120 ng/mL of mouse IgG for meconium-specific zinc coproporphyrin I (ZnCP-I) (Furuta et al., 2012), and incubated for 30 min. A positive reaction was visualized by 3,3-diaminobenzidine, counterstained with hematoxylin, coverslipped, and observed with an Olympus BX51 optical microscope. Halo patterns of the tryptase "golden reaction" around mast cells were considered to represent activated mast cells with degranulation (Fineschi et al., 2009; Tamura et al., 2014b).

2.3. Method for cell counts

Numbers of muscle cells and positively stained cells on a total of four digital images in each case under microscopic fields of 50 mm^2 were counted and analyzed.

2.4. Statistical analysis

All values are presented as the median ± standard error (SE). Significant differences were assessed using the Mann–Whitney *U* test. A P value of less than 0.05 was considered significant.

2.5. Approval

The Ethics Committee of Hamamatsu University School of Medicine approved all the procedures of this study (#24-130).

3. Results

3.1. Backgrounds of the subjects

Thirty-four cases were examined after being selected as 'PPH of unknown etiology', regarded as atonic uterine bleeding by excluding uterine or cervical laceration, placenta previa, placenta accreta, preceding DIC such as sepsis, placental abruption, and preceding sudden maternal cardiac deterioration.

3.2. Histological findings

Significant infiltration of inflammatory cells in myometrial stroma with a sparse structure due to edema was observed in cases of PPH of unknown etiology (Fig. 1C and F).