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Clinicopathological characteristics of miscarriages featuring placental massive perivillous fibrin deposition☆

Eun Na Kim[a,b], Joong Yeup Lee[c], Jae-Yoon Shim[b,d], Doyeong Hwang[c], Ki Chul Kim[c], So Ra Kim[b], Chong Jai Kim[a,b,*]

[a] Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea
[b] Asan Laboratory of Perinatal Science, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea
[c] Hamchoon Women's Clinic, Seoul, Republic of Korea
[d] Department of Obstetrics and Gynecology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

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ABSTRACT

Introduction: Massive perivillous fibrin deposition (MPFD) is frequently associated with detrimental pregnancy outcomes, and extensive perivillous fibrin deposition results in severe placental dysfunction and loss of maternofetal interface. Unfortunately, the fundamental pathogenesis of MPFD remains unknown, and systematic analyses of MPFD in miscarriage is lacking. We analyzed the frequency and clinicopathological characteristics of MPFD in first trimester miscarriages.

Methods: We analyzed a consecutive series of miscarriages (n = 582) gathered between March 2012 and June 2016. MPFD was classified as fibrin-type (f-MPFD) and matrix-type (m-MPFD) by immunostaining for fibrin and collagen type IV. The control group consisted of miscarriage cases (MC, n = 18) that were matched to f-MPFD with normal chromosome (f-MPFD-nc) for number of previous miscarriages and placental chromosomal status.

Results: MPFD was identified in 2.7% of miscarriages. f-MPFD was associated with recurrent abortions. Compared with miscarriages without fibrin deposition, MPFD cases had higher proportion of those with normal placental chromosome (69.2% vs. 27.4%, $P < 0.005$) and higher frequency of villous syncytiotrophoblast C4d deposition (73.3% vs. 33.9%, $P < 0.005$). All C4d(+) f-MPFD patients had more than three recurrent miscarriages, whereas C4d(−) f-MPFD patients had no history of recurrent miscarriage ($P < 0.05$). Patients with f-MPFD-nc had significantly higher HLA PRA immunopositivity rate than did MC patients ($P = 0.005$).

Discussion: MPFD was more common in miscarriages than in preterm and term pregnancies. Placental massive fibrin-type fibrinoid deposition and villous C4d immunoreactivity were associated with recurrent miscarriage.

1. Introduction

Functional integrity of the human placenta is critical for a successful pregnancy, for which timely and adequate intervillous perfusion of maternal blood is a major prerequisite [1,2]. Perivillous fibrinoid is a glassy, eosinophilic substance observed on routine hematoxylin-eosin staining, which encases the chorionic villi or fills the intervillous space [3]. Fibrinoids are commonly observed in normal-term placentas in small amounts, and their potential roles in the human placenta include supporting chorionic villous repair by filling the syncytiotrophoblast defects [3–5] and avoiding immunologic damage by preventing fetal cell antigen exposure to maternal immunocytes [6–8]. Fibrinoids can be classified into two subtypes: the fibrin type and the matrix type [9,10]; fibrin-type fibrinoid deposition is a consequence of blood coagulation, whereas matrix-type fibrinoid is secreted by extravillous trophoblasts [11,12]. Importantly, fibrinoid deposition is associated with chorionic villous damage, and an abnormal increase in perivillous fibrinoid hampers adequate intervillous circulation and may lead to serious pregnancy problems [13–16].

Massive perivillous fibrin deposition (MPFD) of the placenta, a subset of which is also known as maternal floor infarction, is a unique but detrimental condition in pregnancy. MPFD is defined as extensive deposition of intervillous fibrin/fibrinoid, and the resulting compromise in intervillous space leads to functional failure of the placenta

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* Corresponding author. Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, 88, Olympic-ro 43-gil, Songpa-gu, Seoul, 05505, Republic of Korea.
E-mail address: ckim@amc.seoul.kr (C.J. Kim).

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[17,18]. As a result, MPFD is associated with poor obstetrical outcomes such as fetal growth restriction, neonatal neurological sequelae [19], and fetal death [13,20–24]. Moreover, MPFD has been reported to recur in following pregnancies in approximately one-third of the cases [15,20,22,24,25]. The term *maternal floor infarction* is based on the gross characteristics of the placenta, but it is regarded as a misnomer in that whitish consolidation is due to excessive accumulation of fibrinoid and not related to true infarct [26]. Although MPFD has been associated with diverse clinical conditions, the fundamental pathogenesis of the disorder remains unknown.

Histological examinations of miscarriage samples are useful in retrieving information related to causative factors, confirming intrauterine pregnancy in women that were presumed to have sustained abortions, and ruling out the possibility of gestational trophoblastic disease [27]. Increased perivillous fibrin deposition has been sporadically reported in recurrent miscarriages, suggesting its role in a certain proportion of miscarriages [28–30]. However, systematic analyses of MPFD regarding its frequency, clinicopathological characteristics, and fibrinoid subtypes have not been done in the context of miscarriage. We therefore investigated the frequency and clinicopathological significance of MPFD in first-trimester miscarriage using a large number of miscarriage placental specimens from a large-sized fertility clinic.

2. Materials and methods

2.1. Patient materials

We analyzed a consecutive series of miscarriage cases with placental biopsies (n = 562) carried out at Hamchoon Women's fertility clinic in Seoul, Korea between March 2012, and June 2016, out of which a total of 582 biopsies were available. Cases without placental fibrin deposition were selected as the control group (n = 501), and those matched for gestational age, number of previous miscarriages, and placental chromosomal status were selected as the miscarriage group (MC, n = 18). To avoid maternal contamination while assessing the chromosomal status of the placenta, we selected the samples with XY chromosomes rather than XX chromosomes. Aliquots of plasma samples that were obtained at the time of biopsy were also analyzed. Blood samples were centrifuged at 1500 × *g* for 15 min at 4 °C to obtain the serum and plasma, and the resulting supernatant was aliquoted (500 μL) and stored at −80 °C until analysis. We also obtained information on the outcomes of subsequent pregnancy from women with fibrin-type MPFD with normal chromosome, and gathered their biopsy materials as well. The results of thrombophilia test including Protein S deficiency and MTHFR mutation were obtained from clinical charts.

All women provided written informed consent, and the institutional review boards of Hamchoon Women's Clinic and Asan Medical Center approved the use of clinical data and the collection and utilization of biological samples for research purposes (approval number: 2017-1010).

2.2. Placental histologic examinations

All placentas were examined by two pathologists (ENK, CJK). The diagnoses of classic and borderline MPFD were made based on the presence of perivillous fibrinoid deposition encasing > 50% or 25%–50% of the chorionic villi, respectively, according to the criteria reported by Katzman and Genest (Fig. 1A–C) [28]. Classic MPFD and borderline MPFD were both classified as MPFD in the analysis. Increased perivillous fibrinoid was defined as the extent of perivillous fibrin deposition being less than 25% of the chorionic villi in a minimum of one slide.

Automated image analysis was used to objectively determine the extent of fibrin deposition. Representative images of hematoxylin-eosin staining were scanned using a Vectra automated imaging system, and

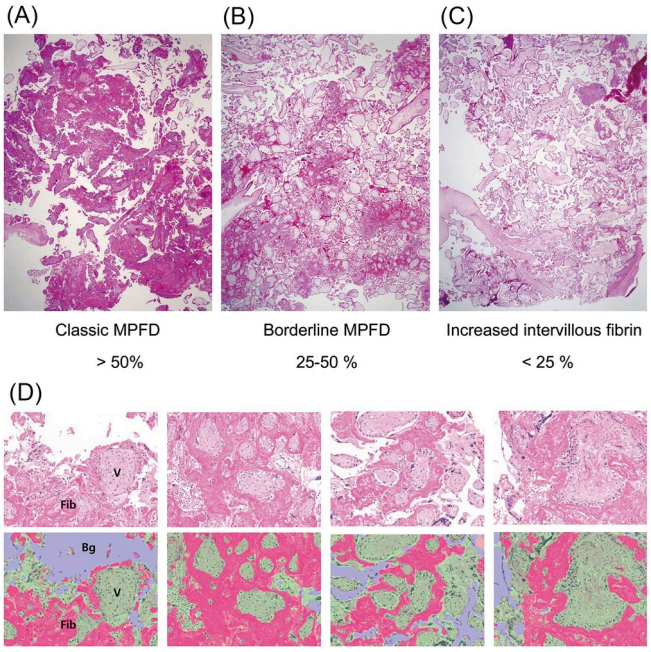

Fig. 1. A, Classic massive perivillous fibrin deposition (MPFD) showing the presence of perivillous fibrinoid deposition encasing > 50% of the chorionic villi.
B, Borderline MPFD showing the presence of perivillous fibrinoid deposition encasing 25%–50% of the chorionic villi.
C, Increased intervillous fibrin, in which the extent of perivillous fibrin deposition is < 25% of the chorionic villi.
D, Segmentation of the fibrinoid area (Fib) and chorionic villous area (V) in H&E slides. Upper, H&E; lower, segmented images. Bg: background.

inForm® Cell Analysis software (PerkinElmer, Waltham, MA, USA) was used to segment the areas with fibrin deposition and chorionic villi. In order to set up a detection algorithm for fibrinoid and chorionic villi, the imaging analysis software was trained with three MPFD cases of MPFD. By using the algorithm, the inForm® imaging analyzer automatically segmented the fibrinoid area and villous area for the rest of the MPFD cases. An independent researcher (SRK) analyzed the images (Fig. 1D) in which the fibrinoid and villi were segmented and confirmed whether more than 25% of the villi were surrounded by fibrinoid in each slide.

2.3. Immunohistochemistry

To assess complement activation, immunostaining for C4d was carried out. To determine the type of placental fibrinoid (fibrin type vs. matrix type), immunostaining for fibrin and collagen type IV was performed using 4-μm-thick paraffin-embedded sections [9]. For immunohistochemistry, antibodies against C4d (rabbit polyclonal, 1:200 dilution; Cell Marque Corporation, CA, USA), fibrin (mouse monoclonal, 1:10000 dilution; Biorbyt, Cambridge, UK), collagen type IV (rabbit polyclonal, 1:2000 dilution; Abcam, Cambridge, UK), and tenascin C (mouse monoclonal, 1:100, Abcam, Cambridge, UK) were used. Immunohistochemistry was carried out using the BenchMark XT automated slide preparation system (Ventana Medical Systems, Inc, AZ, USA). Placental C4d deposition was defined as linear C4d immunoreactivity along syncytiotrophoblast.

2.4. Screening for anti-HLA antibodies

For the screening of maternal HLA sensitization, serum samples obtained at the time of miscarriage were used. All samples were kept at −80 °C until use. Screening for the presence of anti-HLA class I and

anti-HLA class II antibodies was performed using the LABScreen Mixed class I and II assay (Luminex Corporation, Austin, TX, USA) and Luminex LX 200 (Luminex Corporation) according to the manufacturer's instructions. Methods for screening of maternal HLA sensitization, placental karyotyping, and assay for antithyroid and antinuclear antibodies are provided in the Supplementary Methods (Supplementary File 1).

2.5. Statistical analysis

Continuous variables are reported as medians and ranges, and categorical variables are reported as frequencies and percentages. Continuous variables were compared using the Mann–Whitney *U* test, and categorical variables were compared using the χ^2 test or the Fisher's exact test as appropriate. All statistical analyses were performed using SPSS version 18.0 (SPSS, Inc, Chicago, IL, USA).

3. Results

3.1. Frequency of MPFD in miscarriages

Among the 582 cases of miscarriage in 562 women, MPFD was identified in 17 cases from 15 women (15/562, 2.7%). Twenty women (3.6%) had repeated miscarriages during the study period. The demographics of the women with MPFD are shown in Table 1. The median gestational age of the MPFD cases (n = 15) was 6 weeks (range, 6–13). Twelve MPFD cases (80.0%) had a history of two or more prior spontaneous miscarriages.

A total of 10 cases had classic MPFD patterns, whereas the remaining five cases showed borderline MPFD patterns. Increased intervillous fibrinoid (IIF), in which the extent of the intervillous fibrinoid deposition fell short of the MPFD criteria (< 25% of the intervillous space), was found in seven cases. Compared with cases with IIF, the MPFD cases had a numerically higher proportion of cases with a history of two or more previous miscarriages (80.0% vs. 28.6%, P = 0.052).

3.2. Clinicopathological characteristics of miscarriages in women with MPFD

Clinical workup for recurrent abortions including parental chromosomal status, maternal autoantibodies, hormonal level, hysterogram, infection, and thrombophilia screening was carried out in 11 of the 15 women with MPFD. Autoantibodies (antithyroid antibody, antinuclear antibody, and antiphospholipid antibody) were positive in three cases (27.3%). A maternal tendency for thrombophilia such as protein S deficiency, methylenetetrahydrofolate reductase (MTHFR) homozygotic mutation (667C → T), and antiphospholipid antibody was present in four cases (36.4%). Among the four women with thrombophilia, three had two different types of hypercoagulable parameters simultaneously (Table 1). Only three pregnancies out of 17 MPFD cases (17.6%), one out of 7 f-MPFD-nc cases (14.3%), and 3 out of 18 MC controls (16.7%) were pregnant after *in vitro* fertilization (IVF). Also, there were no significant differences between the f-MPFD-nc group and MC control group in terms of the proportion of infertility treatment (p = 1.000). The demographics of the patients with f-MPFD with normal chromosome (f-MPFD-nc) and MC are shown in Table 2.

3.3. Clinicopathological characteristics of women with MPFD according to fibrinoid type

Evaluation of the composition of intervillous fibrinoid by immunohistochemistry for fibrin and collagen type IV revealed two

Table 1
Clinical characteristics of the women with MPFD.

ID	HLAI	HLAII	Age	GA	FT	Dx	MP	MPFD	Fib	C4d	SA No	Chromosome	Habitual abortion work up	Result of Next Pregnancy
1*	+	–	33	9	IVF	MA	No	C	F	+	5	46,XX	Polycystic ovary syndrome	Abortion (MPFD)
1*	+	–	33	8	Nat	MA	No	C	F	+	6	46,XX	Polycystic ovary syndrome	Follow-up loss
2†	+	–	31	6	Nat	MA	No	C	F	+	5	46,XX	[a] MTHFR (T/T), Protein S deficiency, Hyperlipidemia, Glucose intolerance	Abortion (MPFD)
2†	+	–	33	6	Nat	MA	No	C	F	–	6	46,XY	[a] MTHFR (T/T), Protein S deficiency, Hyperlipidemia, Glucose intolerance	Abortion (MPFD, not included in the study period)
3	+	–	30	7	Nat	MA	No	C	F	+	2	46,XY	MTHFR (T/T)	Follow-up loss
4	–	+	35	6	Nat	MA	Yes	C	F	+	5	47,XXY +8	Luteal phase defect, Chronic endometritis	Follow-up loss
5	–	+	34	8	Nat	MA	Yes	C	F	+	6	46,XX	Antinuclear antibody, Systemic lupus erythematosus	Abortion
6	+	–	29	7	Nat	MA	No	C	F	+	4	46,XX	[a] Antiphospholipid antibody, Protein S deficiency, Glucose intolerance	Follow-up loss
7	–	–	33	6	Nat	MA	Yes	C	F	+	2	47,XX +4	Not done	Follow-up loss
8	+	–	31	6	Nat	MA	No	B	F	+	3	46,XX	Previous uterine synechiae	Successful pregnancy until 21 weeks with Tx
9	+	–	38	6	IVF	MA	Yes	B	F	+	4	ND	Anti-thyroid antibody	Follow-up loss
10	–	–	34	6	Nat	BO	No	C	F	–	1	46,XX	Normal	Successful pregnancy Normal delivery with Tx
11	+	–	35	7	Nat	MA	Yes	B	F	–	1	47,XY +17	Not done	Follow-up loss
12	+	–	40	6	Nat	MA	Yes	C	M	–	3	47,XY +7	Endometriosis	Follow-up loss
13	ND	ND	33	7	Nat	RP	Yes	C	M	–	2	ND	Not done, Previous uterine synechia	Follow-up loss
14	+	–	32	13	Nat	PT	No	B	M	+	0	46,OO	Not done	Follow-up loss
15	–	–	35	6	IVF	MA	No	B	M	+	3	46,XX	[a] MTHFR (T/T), Protein S deficiency, Polycystic ovary syndrome	Follow-up loss

HLA: screening test for anti-HLA antibodies, age: maternal age (years), GA: gestational age (weeks), FT: Fertility treatment (IVF: *in vitro* fertilization, Nat: natural pregnancy), Dx: diagnosis (MA: missed abortion, BO: blighted ovum, RP: remnant placenta, PT: pregnancy termination), MP: Multiparity, MPFD: MPFD category (C: classic MPFD, B: borderline MPFD), Fib: Fibrinoid subtype (F: fibrin type, M: matrix type), C4d: C4d immunopositivity in syncytiotrophoblast, SA No: number of previous spontaneous abortions, MTHFR (T/T): homozygous MTHFR 667C→T mutation, ND: not done, Tx: immunoglobulin and heparin treatment.
*, †: Same patient.
[a] Three women had two different types of hypercoagulable parameters simultaneously.

Table 2
Clinical characteristics of the patient groups.

Demographics	f-MPFD-nc	MC	P value
Maternal age, mean (SD), y	31.86 (29–34)	32.44 (26–36)	0.607
Gestational age by CRL, mean (SD), wk	7.85 (6–13)	7.85 (6–10)	0.992
Diagnosis			0.419
Missed abortion	71.4% (5/7)	88.9% (16/18)	
Blighted ovum	14.3% (1/7)	11.1% (2/18)	
Placental chromosome			0.034
XY	14.3% (1/7)	66.7% (12/18)	
XX	71.4% (5/7)	33.3% (6/18)	
Previous spontaneous abortion ≥ 2	71.4% (5/7)	83.3% (15/18)	0.597
Previous spontaneous abortion ≥ 3	57.1% (4/7)	64.3% (9/14)	1.000
Previous spontaneous abortion ≥ 4	57.1% (4/7)	21.4% (3/14)	0.156
Pregnancy after IVF	14.3% (1/7)	16.7% (3/18)	1.000
Thrombophilia	50% (3/6)	50% (9/18)[a]	1.000
Autoantibody	33.3% (2/6)	16.7% (3/18)[b]	0.568

f-MPFD: fibrin-type massive perivillous fibrin deposition, MC: miscarriage control, CRL: crown-rump length, IVF: *in vitro* fertilization.
[a] The thrombophilia of all MC patients was Protein S deficiency.
[b] The autoantibodies of all MC patients were antithyroid antibodies.

dominant patterns: the fibrin-immunopositive fibrin type and the collagen type IV-immunopositive matrix type. The perivillous deposition of fibrin-type fibrinoid was commonly associated with coagulative necrosis of the affected chorionic villi involving both the syncytiotrophoblast layer and the villous stroma (Fig. 2A–D), whereas the deposition of matrix-type fibrinoid was accompanied by a proliferation of extravillous trophoblasts (Fig. 2E–H). The staining patterns of fibrin and collagen type IV were mutually exclusive, with small amounts of matrix-type fibrinoid detected at the periphery of fibrin-type fibrinoid in some cases (Supplementary Fig. 1). Among the 15 MPFD cases, 11 (73.3%) were fibrin-type MPFD (f-MPFD), and four (26.7%) were matrix-type MPFD (m-MPFD). More than half of the f-MPFD cases (54.5%) had histories of four or more repeated miscarriages, whereas none of the patients with m-MPFD had such history. The results of tenascin C immunohistochemistry were non-specific (Supplement Fig. 2).

The placentas of women with MPFD were more likely to have normal chromosomes than those of other miscarriages without fibrin deposition (69.2% vs. 27.4%, $P < 0.005$). In contrast, within women with MPFD, the proportion of normal chromosomes was not significantly different according to fibrinoid subtype (70.0% in f-MPFD vs. 66.7% in m-MPFD, $P > 0.99$). Among cases with IIF (n = 7), all three fibrin-type cases had normal chromosomes, and all four matrix-type cases had abnormal chromosomes ($P = 0.029$).

3.4. Syncytiotrophoblast C4d immunoreactivity and HLA sensitization

The frequency of villous C4d deposition was significantly higher in MPFD cases than in other miscarriages without fibrin deposition (73.3% vs. 33.9%, $P < 0.005$; Fig. 3A). Importantly, all patients with C4d immunopositive f-MPFD had experienced more than three recurrent miscarriages (9/9), whereas no patients with C4d immunonegative f-MPFD had a history of previous recurrent miscarriage (0/2, $P < 0.05$). HLA PRA test was carried out in f-MPFD-nc cases (n = 7): all seven f-MPFD-nc cases were HLA PRA seropositive, and 85% of f-MPFD-nc cases simultaneously showed C4d immunopositivity and HLA PRA seropositivity. Patients with f-MPFD-nc showed significantly higher HLA PRA positivity rate than did MC patients ($P = 0.005$; Fig. 3B).

We analyzed the outcomes of subsequent pregnancy in five women with f-MPFD-nc, and found that of the four C4d immunopositive cases, three cases had miscarriages in the next pregnancy, and the other case became pregnant after immunoglobulin and low-molecular-weight heparin treatment. One C4d immunonegative case had successful delivery following immunoglobulin and low-molecular-weight heparin

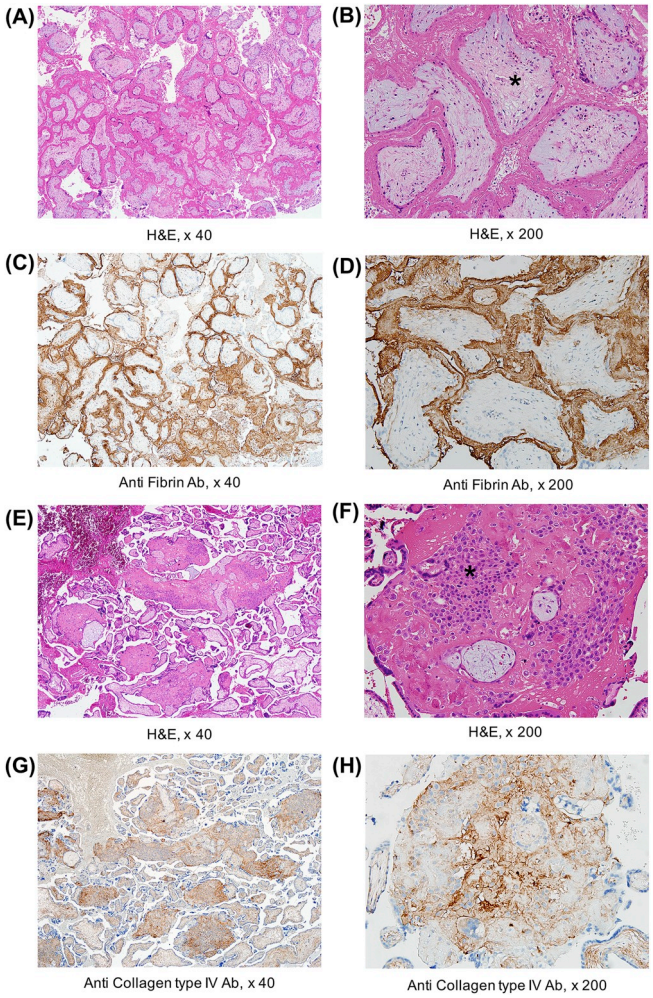

Fig. 2. A-D, Fibrin-type massive perivillous fibrin deposition (f-MPFD).
A, B, Fibrin-type fibrinoid filling the intervillous space (A, H&E, × 40; B, × 200).
C, D, Fibrin-type fibrinoid showing immunopositivity for anti-fibrin antibody (C, anti-fibrin antibody, × 40; D, × 200). Villi tightly encased by fibrin-positive fibrinoid are necrotic and sclerotic (asterisk).
E-H, Matrix-type massive perivillous fibrin deposition (m
-MPFD).
E, Matrix-type fibrinoid accumulated in the intervillous space in a nodular pattern (H&E, × 40).
F, Extravillous trophoblasts (asterisk) proliferated in matrix-type fibrinoid (H&E, x200).
G, H, Matrix-type fibrinoid showing immunopositivity for anti-collagen type IV antibody (G, anti-collagen type IV antibody, × 40; H, × 200).

treatment. Biopsy materials from subsequent miscarriages were available in two out of the three patients with repeated f-MPFD-nc. On pathologic examination, the miscarriages of both patients showed recurrent C4d-immunopositive f-MPFD; the histopathologic features were similar to those of previous miscarriages (Fig. 4).

4. Discussion

To the best of our knowledge, this is the first study on MPFD frequency in a large, consecutive cohort of miscarriages. MPFD was found in 2.7% (15/562) of consecutive miscarriages at a fertility clinic, and MPFD was more frequent in miscarriages than in preterm or term pregnancies. By dividing between the type of fibrinoid (fibrin type [f-MPFD] vs. matrix type [m-MPFD]), we observed that f-MPFD is associated with recurrent miscarriages, whereas m-MPFD is not. MPFD

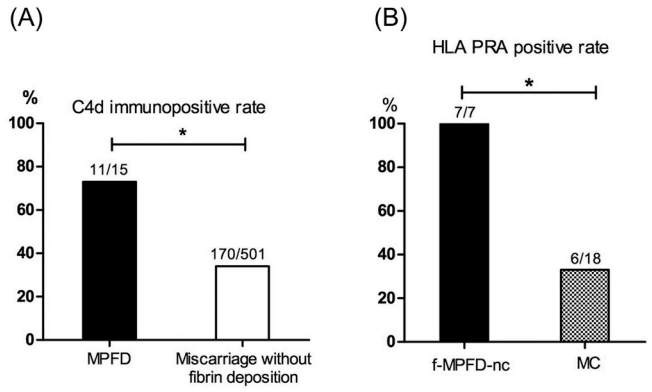

Fig. 3. A, C4d immunopositivity rate. *P < 0.005.
B, HLA PRA immunopositivity rate. *P = 0.005.

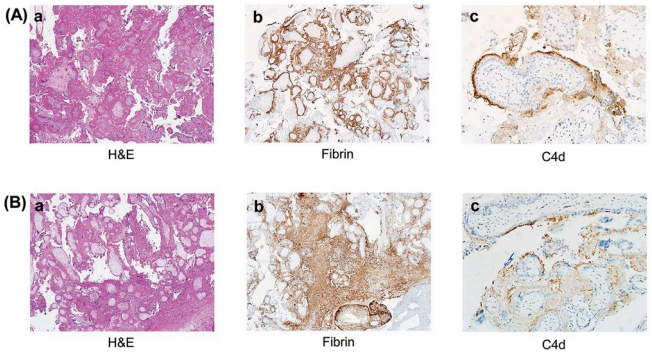

Fig. 4. f-MPFD with C4d immunoreactivity in a subsequent pregnancy (patient ID No. 1).
A, Sixth miscarriage in patient ID No. 1.
a, Placenta from a prior miscarriage showing MPFD filled with fibrin (× 40).
b, Fibrinoid showing immunopositivity for antifibrin antibody (× 40).
c, Syncytiotrophoblast showing immunopositivity for C4d (× 200).
B, Seventh miscarriage in patient ID No. 1.
a, The subsequent pregnancy in the same patient showing the same pathologic features in terms of fibrin deposition (× 40).
b, Fibrinoid showing immunoreactivity for antifibrin antibody (× 40).
c, Syncytiotrophoblast showing immunoreactivity for C4d (× 200).

patients had a higher frequency of normal placental karyotype and villous C4d deposition than did miscarriage patients without fibrin deposition. Most f-MPFD-nc cases were HLA PRA seropositive and C4d immunopositive, and C4d immunopositivity was associated with recurrent miscarriages in f-MPFD.

The frequency of maternal floor infarction and MPFD varied in previous studies, ranging between 0.005% and 0.5% [15,22,25,28]. In a study by Katzman and Genest [28], the prevalence of maternal floor infarction/MPFD was 0.8% (90/11,000), but their cases were mainly second- or third-trimesters. The frequency of MPFD in first-trimester miscarriages in our study was significantly higher compared with those reported in previous studies on preterm and term pregnancies. In preterm and term pregnancies, gross and microscopic evaluations of sizable placentas could be carried out. However, it was not feasible to identify the gross characteristics of MPFD in fragmented placental tissues obtained from miscarriages. Nevertheless, the MPFD cases in our study had histological and immunohistochemical characteristics pathological features that were highly similar to those of preterm and term pregnancies.

MPFD has been reported to be associated with maternal thrombophilia and autoantibodies [31–33]. In our study, thrombophilia including MTHFR mutation, protein S deficiency, and antiphospholipid antibody was identified in 36% of MPFD patients. In a report by Gogia et al., the incidence of thrombophilia ranged between 23% and 30% of that of delivered placentas with MPFD [32]; this study showed that the most common genetic thrombophilia identified in MPFD is protein S deficiency, which is consistent with the results of our study. Collectively speaking, these findings support the view that maternal thrombophilia plays a critical role in the progression of MPFD in both late and early pregnancy.

Our results suggest that the presence of different thrombophilia may be synergistically responsible for detrimental pregnancy loss. In our study, three out of four patients with thrombophilia had two different types of hypercoagulable parameters, which is a unique feature. Gris et al. reported that the rate of thrombophilia was higher in women who experienced unexpected late fetal loss than in normal pregnant women [34]. In particular, the MTHFR homozygous mutation, albeit not associated with late fetal loss, was associated with other thrombophilia diseases in our study. Additional studies are needed to determine the mechanism by which MTHFR is associated with other thrombophilia and fetal loss.

Autoantibody seems to contribute to the development of MPFD-associated placental failure, considering that autoantibodies were identified in three out of 11 MPFD patients in our study. Bendon et al. reported two cases of second-trimester maternal floor infarction in patients with autoimmune disease [16], and proposed that a possible role of autoantibodies in the pathogenesis of MPFD is interference of villous plasminogen function.

Our study underscores the importance of defining the fibrinoid subtype in miscarriages. Although extravillous trophoblast proliferation is a reliable feature for determining matrix-type fibrinoid deposition, immunostaining for fibrin and collagen type IV is a simple and valuable tool for subtyping of fibrinoid. Our data show that confirming the fibrinoid subtype in MPFD is clinically relevant, because only f-MPFD was associated with repeated miscarriages.

An interesting finding in our study was that all fibrin-type IIFs had normal placental chromosomes, whereas all matrix-type IIFs had abnormal placental chromosomes. In this regard, matrix-type fibrinoid production seems to be facilitated by extravillous trophoblasts with abnormal chromosomes.

It has been proposed that premature onset of intervillous circulation due to shallow placentation lead to miscarriage [2,35] and other major pregnancy complications such as preeclampsia and fetal growth restriction [36]. Premature onset of high-oxygen-carrying intervillous maternal circulation is detrimental, as oxygen-free radicals induce placental villous damage [37]. It is unlikely that intervillous circulation would have been established in our MPFD cases, considering the range of their gestational age; during this period, nutrients are mainly delivered to the placenta by endometrial secretion [38], and intervillous circulation is normally established at the end of the first trimester [1,39,40]. Therefore, considering that fibrin deposition is a product of blood coagulation, the presence of fibrin-type fibrinoid in MPFD cases can be considered a surrogate for premature onset of intervillous circulation and subsequent villous damage.

Fibrin-type fibrinoid deposition and villous C4d immunoreactivity seem to be putative risk factors for recurrent miscarriage. Villous C4d deposition is associated with various placental injuries such as inflammation and ischemia [41]. Accordingly, we frequently observed coagulation necrosis in C4d-immunopositive chorionic villi surrounded by fibrin-type fibrinoids. Although our present study did not include elective abortion cases, we have previously reported that the C4d-immunopositive rate in normal first-trimester pregnancy is 5% in elective abortions [42].

It is possible that the villous necrosis observed in this study is associated with immune-mediated placental injury due to anti-fetal rejection (i.e., maternal HLA sensitization to the fetus). C4d immunopositivity was more frequently observed in f-MPFD (81.8%) than in miscarriage control cases (33.3%) and elective abortions (5%) [42]. In addition, maternal HLA sensitization was more frequent in f-MPFD with normal chromosomes than in miscarriage control cases, and 85%

of f-MPFD cases with normal chromosomes showed simultaneous C4d immunopositivity and HLA PRA positivity. Similarly, Romero et al. showed evidence of anti-fetal rejection such as HLA PRA positivity, C4d deposition in the endothelial lining of the umbilical vein, and elevated CXCL10 in the maternal plasma of MPFD placentas delivered at 17 to 29 gestational weeks [43].

We speculate that anti-fetal immune response may be enhanced due to the exposure of fetal antigen to maternal blood from abnormal premature intervillous circulation. Anti-fetal rejection is considered a cause of various obstetric diseases such as preeclampsia [44], fetal growth restriction [45], intrauterine fetal death [46], and late preterm labor [47]. The findings on MPFD, C4d, and maternal HLA seropositivity in our study suggest that anti-fetal rejection plays a role in the development of MPFD in first-trimester miscarriages.

The f-MPFD-nc group showed a female predominance. The placenta had long been considered an asexual organ; however, as the placenta is the baby's organ, it can be divided into female and male placenta according to their sex chromosome. Recent evidence shows that numerous RNA expression and methylation patterns are dependent on the placental sex [48]. Further research is needed to examine the relationship between the occurrence of MPFD, maternal anti-fetal rejection, and fetal sex. The male predominance in MC control may be due to selection bias that occurred while excluding XX chromosome samples in order to avoid maternal contamination.

The limitations of this study are as follows. The small number of MPFD cases did not allow for a more robust statistical analysis of the relationship between MPFD and other clinicopathological characteristics. Also, clinical workup regarding maternal autoantibodies and thrombotic tendency was not carried out in some cases, thus hindering comparisons based on these parameters. Our cases were obtained from a single fertility clinic; therefore, there may have been a selection bias that limits the generalizability of our results. Nevertheless, the patient population in this fertility clinic comprises both sporadic miscarriage patients and normal pregnant women. Moreover, only a small portion of pregnancies included in this study were pregnancies following IVF, and the remaining pregnancies were natural pregnancies. Furthermore, we gathered a large number of consecutively admitted subjects for this study. Therefore, our study findings may have some application in general population.

Whether fibrinoid is deposited before or after embryonic death remains controversial. Secondary perivillous fibrinoid deposition can develop after embryonic death, especially between 8 and 12 weeks of gestation [49]. However, when considering the case of fetal death in utero, postmortem passive fetal vascular involution is diffuse, which is distinct from fetal vascular thrombo-occlusive disease. Accordingly, we can infer that reactive perivillous fibrin deposition after embryonic death may be diffuse, similar to the postmortem changes [50]. Nevertheless, when we examined an abortion placental specimen through scan view (Supplement Figure 3), we observed that massive perivillous fibrinoid deposition were localized to certain areas, leaving uninvolved viable villi. In addition, only the villi that were entirely encased and strangulated by fibrinoid became degenerated and sclerotic. This supports the idea that MPFD may occur before, not after, embryonic death. Moreover, it should be considered that the median gestational age of our MPFD cases was 6 weeks (range, 6–13), which is less than the gestational ages at which perivillous fibrinoid deposition normally occurs due to embryonic death (8–12 weeks) [49]. Also, gestational age of 6 weeks is far before the period when maternal blood is perfused to the intervillous space. Collectively speaking, we suggest that fibrinoid deposition occurs before embryonic death rather than after embryonic death.

In conclusion, we report that MPFD was more common in miscarriages than in preterm and term pregnancies. Fibrin/collagen type IV immunostaining was a simple and valuable tool for subtyping fibrinoid in MPFD, and fibrin-type fibrinoid deposition and villous C4d immunoreactivity were associated with recurrent miscarriages. Therefore, placental C4d immunostaining in MPFD may be a useful diagnostic tool for subtyping miscarriages and predicting outcomes of subsequent pregnancies.

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Conflicts of interest

The authors report no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.07.006.

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