Significance of placental CD200 expression in patients with preeclampsia: Comparison between early- and late-onset patients

HIROAKI TSUBOKURA[1], MITSUAKI ISHIDA[2], AKEMI NISHIGAKI[1], TAKUYA YOKOE[1], SHINNOSUKE KOMIYA[1], YUSUKE BUTSUHARA[1], AYA YOSHIDA[1], YOJI HISAMATSU[1], YOSHIKO HASHIMOTO[1], TOMOKO TSUZUKI-NAKAO[1], HIROMI MURATA[1], KOJI TSUTA[3] and HIDETAKA OKADA[1]

[1]Department of Obstetrics and Gynecology, Kansai Medical University, Hirakata, Osaka 573-1191; [2]Department of Pathology, Osaka Medical and Pharmaceutical University, Takatsuki, Osaka 569-8686; [3]Department of Pathology and Division of Diagnostic Pathology, Kansai Medical University, Hirakata, Osaka 573-1191, Japan

Received June 29, 2022; Accepted November 10, 2022

DOI: 10.3892/mmr.2022.12905

Abstract. Preeclampsia, characterized by high blood pressure and proteinuria during pregnancy, causes serious complications in both the mother and the fetus. Although there have been several studies on the causes of preeclampsia, the detailed mechanism of this disease remains unclear. Moreover, a few reports have focused on the causes of preeclampsia in number of weeks at onset. The present study aimed to elucidate the differences between early- and late-onset preeclampsia. This study enrolled patients with preeclampsia from January 2014 to December 2020. They were classified into early- (<34 weeks) and late-onset (≥34 weeks) preeclampsia groups. The expression profiles of 770 immune-related genes were studied in the placental tissue from five patients each in the early- and late-onset groups. The expression of CD200 in the trophoblasts of the placenta of 26 and 27 patients in early- and late-onset groups, respectively, was also analyzed using immunostaining. Analysis of extracted RNA indicated that *CD200* was significantly upregulated in the early-onset group compared with late-onset group and normal control. Immunostaining for CD200 demonstrated a significantly increased expression in the early-onset group compared with the late-onset group. The present study demonstrated that upregulation of CD200, which belongs to the immunoglobulin superfamily and is recognized as a molecule that acts in immune tolerance via inhibition of classical macrophage activation, may be associated with early-onset preeclampsia, although it remains unknown whether upregulation of CD200 expression is a cause or effect of the development of early-onset preeclampsia. Early-onset preeclampsia might have a different mechanism from that of late-onset; thus, further studies are needed to clarify the mechanism of these conditions for adequate treatment.

Correspondence to: Professor Hidetaka Okada, Department of Obstetrics and Gynecology, Kansai Medical University, 2-3-1 Shinmachi, Hirakata, Osaka 573-1191, Japan
E-mail: hokada@hirakata.kmu.ac.jp

Abbreviations: DIC, disseminated intravascular coagulopathy; FFPE, formalin-fixed and paraffin-embedded; HELLP, hemolysis, elevated liver enzymes, low platelet count; HIF, hypoxia-inducible family; log2FC, log2 fold change; WHO, World Health Organization

Key words: CD200, early-onset, late-onset, placenta, preeclampsia

Introduction

Preeclampsia is a condition characterized by high blood pressure and proteinuria that develops during pregnancy (1-10). Preeclampsia affects 2.5-10% of all pregnancies (1-8) and causes serious complications for both the mother and the fetus. According to the World Health Organization (WHO) report, 14% of maternal deaths occur due to hypertensive disorders, ranking second overall (11); moreover, preeclampsia can cause serious complications, such as placental abruption, hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, and disseminated intravascular coagulopathy (DIC) (2-7,9,10). In addition, infants of mothers with preeclampsia are at twice the risk of neonatal death as those without preeclampsia and have a higher probability of admission to the neonatal intensive care unit due to preterm birth or low birth weight (12-14). Accordingly, prevention and treatment strategies for patients with preeclampsia have been one of the most important topics in obstetrics.

The pathogenesis of preeclampsia has long been shrouded in mystery but has slowly become clearer. Preeclampsia occurs only in the presence of the placenta (2,10,15), and delivery is the ultimate treatment (2-4,15), which strongly suggests a relationship between the placenta and preeclampsia. Although inadequate remodeling of the spiral artery could be one of the mechanisms for the development of preeclampsia, this condition cannot be explained by this single mechanism, and other mechanisms could be present in the development of preeclampsia (2,4,10,15). For example, an increased level of hypoxia-inducible family (HIF) 1-α in the placenta of

preeclamptic women has been reported, suggesting an association between preeclampsia and hypoxia in the placenta (16).

Preeclampsia is classified into two types: early-onset (disease onset before 34 weeks) and late-onset diseases (disease onset after 34 weeks) (1). Early-onset preeclampsia is reported to show a poorer prognosis in the neonate period and more frequent intrauterine fetal growth retardation compared with late-onset (12). Sezer *et al* reported no significant association between angiogenic factors, such as placental growth factor, vascular endothelial growth factor, and HIF-1α, in the placenta, and the number of weeks at preeclampsia onset (17). However, it has been speculated that early- and late-onset preeclampsia have different developmental mechanisms (7,12). Although several research studies have addressed the pathogenesis of preeclampsia, especially the differences between early- and late-onset preeclampsia (7,12,17), these differences have not yet been fully elucidated. CD200 belongs to the immunoglobulin superfamily, and its signaling leads to immune tolerance (18-20). Immune tolerance plays important roles in pregnancy, and the association between CD200 and preeclampsia has been addressed in a study (21). However, difference of CD200 expression between early- and late-onset preeclampsia has not yet been analyzed.

The purpose of this study was to clarify the differences between early- and late-onset preeclampsia using comprehensive gene expression and immunohistochemical analyses.

Materials and methods

Patient selection. This study enrolled patients with preeclampsia, from January 2014 to December 2020, at Kansai Medical University Hospital. Preeclampsia was defined as hypertension (systolic blood pressure of ≥140 mmHg and/or diastolic ≥90 mmHg) and proteinuria (1+ in randomly collected urine or >0.3 g in a 24-h interval) (3,5,22) We excluded patients aged <20 years at delivery, and those with chorioamnionitis, fetal anomaly, twin pregnancy, and placental abruption. Patients with preeclampsia were classified into the following two groups according to the gestational age of onset of preeclampsia: the early-onset group (gestational age <34 weeks) and the late-onset groups (gestational age ≥34 weeks) (1).

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Kansai Medical University Hospital (approval no. 2018138). Before December 2018, informed consent was obtained from patients by implementing the opt-out method, owing to the retrospective design of the study, with assurance of no risk to the participants. Information regarding this study, such as the inclusion criteria and the opportunity to opt out, was provided through the institutional website (https://www.kmu.ac.jp/hirakata/hospital/2671t800000124re-att/a1625627306468.pdf). Patients who went into delivery after January 2019 provided written informed consent for sample collection and its subsequent analysis.

RNA extraction. RNA was isolated from the archived samples. Briefly, for mRNA extraction, 5 μm-thick sections were examined from the archived formalin-fixed and paraffin-embedded (FFPE) blocks of the placenta from five patients each in the early- and late-onset groups, and two blocks from normal controls. For the placental block, the part with the attached umbilical cord was selected for each patient. RNA was extracted using a NucleoSpin total RNA FFPE kit (Macherey-Nagel GmBH & Co. KG), including an on-column DNase treatment. The quantitative evaluation of RNA was performed using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.). The quality of RNA was evaluated by measuring the ratio of 260/280 nm. We excluded samples in which the total amount of RNA was <50 ng/μl or the 260/280 ratio was <1.6 (23).

Analysis of comprehensive mRNA expression profiles. We studied the expression profiles of 770 immune-related genes using the nCounter PanCancer Immune Profiling Panel (NanoString Technologies, Inc.). The nCounter assay was performed according to the manufacturer's instructions. The RNA was hybridized with the probe sets for 16 h at 67˚C, and the samples were processed using an automated nCounter Sample Prep Station (NanoString Technologies, Inc.). Cartridges containing immobilized and aligned reporter complexes were subsequently imaged on an nCounter Digital Analyzer (NanoString Technologies, Inc.) that had been set at a data resolution of 555 fields of view. Reporter counts were collected and normalized using the nSolver analysis software (version 3.0; NanoString Technologies, Inc.). The analysis between the two groups was performed using a volcano plot after adjusting for the control group (23). Sequencing data were deposited into the DNA Data Bank of Japan Sequence Read Archive (accession nos. SAMD00549508-SAMD00549517).

Immunohistochemistry. The protein expression of CD200 was analyzed by immunohistochemistry. FFPE blocks of the placenta of the part with the attached umbilical cord were cut into 4-μm-thick sections. Subsequently, the samples were deparaffinized and rehydrated. Immunohistochemical analyses were performed using an autostainer (Discovery XT System; Roche Diagnostics), according to the manufacturer's instructions. The primary antibody used was a rabbit polyclonal antibody against CD200/OX2 (ab203887; Abcam; dilution 1:100). The staining intensity was classified into four categories as follows: 0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining. The scoring was independently done by at least two researchers blind to the clinical information.

Statistical Analysis. In the analysis of nCounter, statistical software R version 3.5.2 (R Project, Vienna, Austria) was used to perform significance analysis of the identification of differentially expressed genes between normal and preeclampsia placental tissue samples. The counted raw RNA-seq data were normalized and analyzed between groups using the R software extension package 'DESeq' 2 available in Bioconductor (www.bioconductor.org/packages/release/bioc/html/DESeq2.html). Genes with an absolute value of log2 fold change (log2FC) >2 and the adjusted P-value <0.00001 were defined as DEGs. The volcano plot was visualized using the ggplot2 package in R (24).

Other data were presented as mean ± standard error and analyzed by unpaired and paired t-tests using Prism 8 computer

Table I. Clinicopathological characteristics of ten patients with preeclampsia who were studied the mRNA expression profiling.

Characteristics	Early-onset (n=5)	Late-onset (n=5)	P-value
Age, years	28 (26-38)	33 (29-37)	0.22
Primipara, cases (%)	3 (60%)	5 (100%)	0.44
Gestational age at onset, weeks	33.2 (24.0-33.5)	35.6 (34.2-38.1)	<0.01
Gestational age at delivery, weeks	33.5 (27.5-37.3)	35.6 (35.3-38.1)	0.13
Systolic blood pressure, mmHg	160 (143-163)	162 (141-165)	0.60
Diastolic blood pressure, mmHg	96 (89-100)	109 (70-130)	0.39
Vaginal delivery, cases (%)	3 (60%)	5 (100%)	0.44
Birth weight, g	1,512 (529-2,394)	1,806 (1,698-2,715)	0.15
Placenta weight, g	365 (220-480)	380 (310-510)	0.69

The median value is shown, and the maximum and minimum values are shown in brackets.

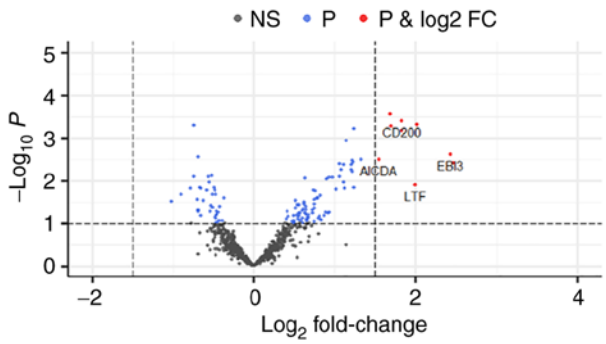

Figure 1. Comprehensive mRNA expression profiles between early- and late-onset preeclampsia patients.

software (GraphPad Software). The analysis between the two groups was performed using the Mann-Whitney test, and the analysis between the three groups was performed using the Kruskal-Wallis test (followed by the Dunn or Dunn-Bonferroni posterior test method). A probability level of <0.05 was considered statistically significant.

Results

Comprehensive mRNA expression profiles. Table I summarizes the clinicopathological features of the 10 patients with preeclampsia (5 patients each in the early- and late-onset groups) used for the comprehensive mRNA expression analysis. Gestational age at onset of preeclampsia was significantly different between the early-onset (mean 33.2 weeks) and late-onset (mean 35.6 weeks) groups (P<0.05). Age at delivery, systolic and diastolic blood pressures, birth weight, and placental weight were not significantly different between the two groups.

The volcano plot of different gene expression levels between the early- and late-onset groups is shown in Fig. 1. *CD200, AICDA, EBI3,* and *lactoferrin* were significantly upregulated in the early-onset group, as compared to the late-onset group (adjusted P<0.05).

Immunohistochemistry analysis. Table II summarizes the clinicopathological features of the 53 patients with preeclampsia (26 and 27 patients in the early- and late-onset groups, respectively) and 9 normotensive pregnant women (the control group) included in this analysis. The patients who performed the comprehensive mRNA expression analysis were also included in the immunohistochemical analysis. Gestational age at onset of preeclampsia was significantly earlier in the early-onset group than in the late-onset group (P<0.05). Gestational age at delivery was significantly earlier and birth weight was significantly lower in early-onset group than in the late-onset and control groups (P<0.05). Systolic and diastolic blood pressures were significantly higher in the early- and late-onset groups than in the control group (P<0.05). Other factors were not significantly different among the three groups.

Table III summarizes the results of the immunohistochemical analysis. CD200 was expressed in the syncytial trophoblasts (Fig. 2). The staining intensity of CD200 was significantly stronger in the early-onset group than in the late-onset and control groups (P<0.05). There was no significant difference in the staining intensity of CD200 between the late-onset and control groups. Moreover, in the early-onset group, the staining intensity of CD200 showed a stronger tendency in patients with an earlier gestational age compared to those with a later gestational age, although the difference was not significant (P=0.09). However, this trend was not observed in the late-onset group (P=0.33) (Fig. 3).

Discussion

Preeclampsia is one of the most important conditions requiring treatment for pregnant women. Especially, early-onset preeclampsia is reported to have a poor prognosis in the neonatal period (12). In particular, early-onset preeclampsia is more likely to cause intrauterine fetal growth retardation than late-onset preeclampsia (25). In fact, significant morphological differences, including villous and vasculature features, have been reported between the placentas of normotensive pregnant women and those of pregnant women with early-onset preeclampsia, while the placentas of pregnant women with late-onset preeclampsia are not significantly different from those of normotensive pregnant women with corresponded gestational age (25,26). These facts indicate that early- and late-onset preeclampsia are not caused by the same mechanism.

Table II. Clinicopathological characteristics of 62 patients with preeclampsia, including the ones performed the immunohistochemistry analysis.

Characteristic	Control group (n=9)	Early-onset group (n=26)	Late-onset group (n=27)	P-value
Age, years	35 (24-43)	34 (24-46)	33 (23-40)	0.59
Primipara, cases (%)	9 (100%)	17 (65.3%)	17 (63.0%)	0.08
Gestational age at onset, weeks	ND	31.3 (24.0-33.6)	35.6 (34.0-40.5)	<0.01[a]
Gestational age at delivery, weeks	40.2 (37.0-41.2)	34.0 (27.0-38.0)	37.1 (34.5-40.5)	<0.01[b]
Systolic blood pressure, mmHg	116 (79-147)	163 (138-220)	156 (129-192)	<0.01[c]
Diastolic blood pressure, mmHg	64 (47-84)	102 (82-125)	98 (70-124)	<0.01[c]
Vaginal delivery, cases (%)	0 (0%)	5 (19.2%)	7 (25.9%)	0.24
Birth weight, g	2,750 (2,450-3,585)	1,514 (529-2,584)	2,355 (1,554-3,315)	<0.01[b]
Placenta weight, g	510 (350-655)	350 (220-630)	405 (260-690)	<0.01[b]

[a]Significant difference between the early onset and late-onset groups; [b]significant difference between the early-onset group vs. the late-onset and control groups; [c]significant difference between the control group vs. the early-onset and the late-onset groups. The median value is shown, and the maximum and minimum values are shown in brackets.

Table III. Results of the immunohistochemical analysis for CD200.

Staining intensity	Control group (n=9)	Early-onset group (n=26)	Late-onset group (n=27)	P-value
0	0 (0%)	0 (0%)	0 (0%)	<0.01[a]
1	8 (88.9%)	8 (30.8%)	11 (40.7%)	
2	1 (11.1%)	7 (26.9%)	14 (51.9%)	
3	0	11 (42.3%)	2 (7.4%)	

[a]Significantly higher expression of CD200 in the early-onset group compared with in the late-onset and the control groups. Staining intensity: 0, no staining; 1, low staining; 2, medium staining; 3, intense staining.

Although it has been recognized that inadequate remodeling of the spiral artery plays an important role in the development of preeclampsia, a systemic inflammatory response due to placental stress, such as hypoxia, is also considered one of the pathogenic mechanisms (27-29). Microarray studies of placental RNA have shown that gene expressions of immune and inflammatory systems in the placenta of preeclampsia patients are different from those in the normotensive placenta (30). In particular, changes in the levels of angiogenic factors, such as vascular endothelial growth factor and placental growth factor, have been strongly associated with the development of preeclampsia (31). However, the association of these factors between early- and late-onset preeclampsia has not yet been analyzed. Thus, we aimed to clarify the differences of the two subtypes of preeclampsia.

We first used the nCounter immune profiling panel to analyze differences in RNA expression of 770 genes in the placenta between early- and late-onset preeclampsia, and the results showed that four genes, including *CD200*, were significantly upregulated in early-onset preeclampsia. Among them, we focused on CD200, because this protein was found to be expressed in the placenta in a previous study (21). Following the results of the RNA expression, we performed the immunohistochemical validation study in a larger number of patients with preeclampsia. Immunohistochemical analysis also showed that staining intensity for CD200 in the trophoblasts was significantly stronger in the early-onset preeclampsia group than in the late-onset group. Accordingly, CD200 expression is considered to be significantly upregulated in patients with early-onset preeclampsia by both RNA and protein expression levels.

CD200 belongs to the immunoglobulin superfamily and is expressed on diverse cell types and tissues, from B lymphocytes in the spleen to neurons in the central nervous system, and directly and continuously regulates macrophages and granulocytes through interaction with CD200R (18,19). CD200 signaling has been reported to inhibit classical macrophage activation (M1 polarization) and support an immunosuppressive M2 polarized state, resulting in immune tolerance (19,20). Immune tolerance is an important intrinsic mechanism in implantation. It has been reported that CD200 expression was significantly decreased in the villi of patients with early spontaneous abortion (32), suggesting that CD200 plays important roles in implantation. In addition, the polarization to M2 macrophages, which occurs physiologically during pregnancy, is thought to suppress the development of preeclampsia, and it has been reported that M1 macrophages were increased in the decidual tissue in patients with acute atherosis (19). Accordingly, macrophage polarization plays important roles in pregnancy and its disorders. Xu *et al* reported that CD200 expression

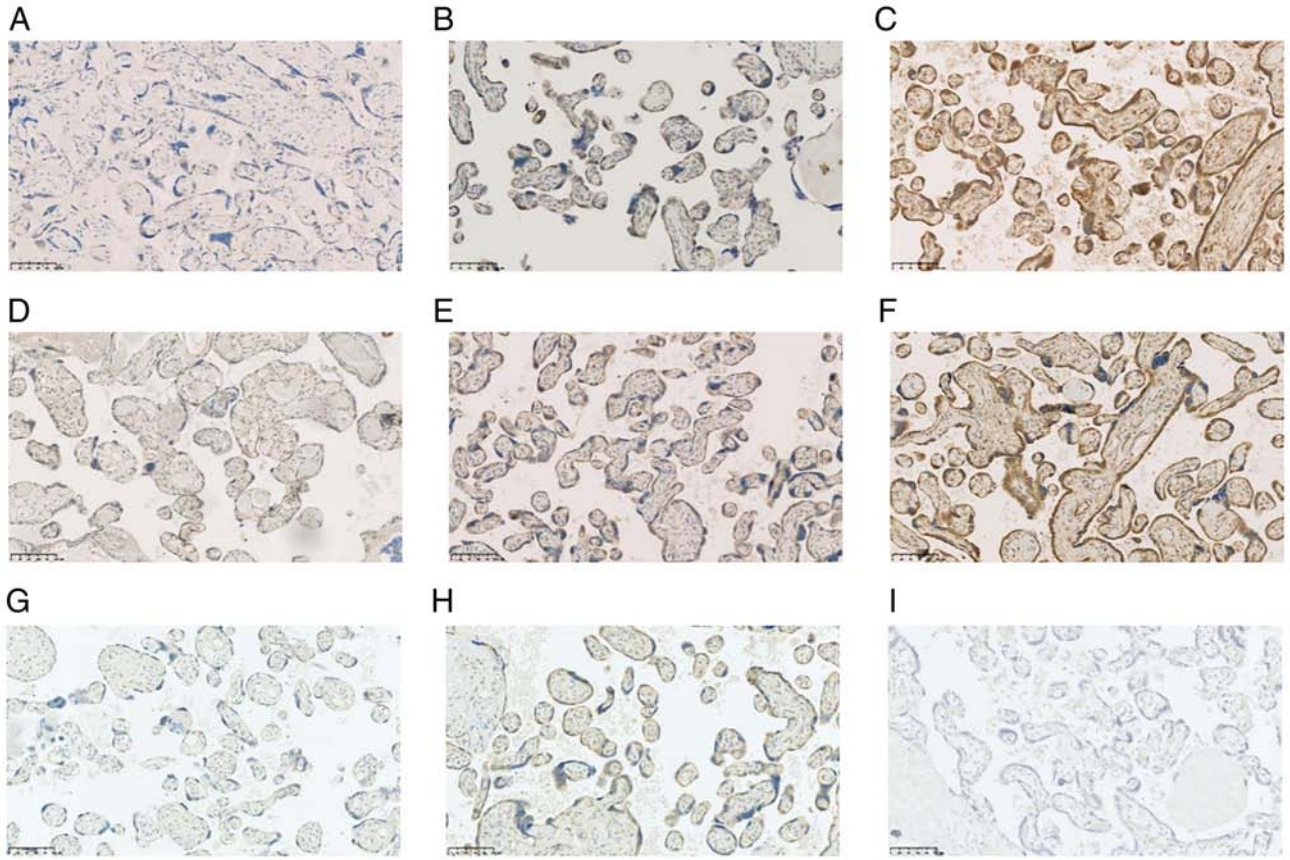

Figure 2. Representative images of the immunohistochemical expression of CD200 in the normal, early- and late-onset preeclampsia placentas. (A) Early-onset group with staining intensity is 1. (B) Early-onset group with staining intensity is 2. (C) Early-onset group with staining intensity is 3. (D) Late-onset group with staining intensity is 1. (E) Late-onset group with staining intensity is 2. (F) Late-onset group with staining intensity is 3. (G) Control group with staining intensity is 1. (H) Control group with staining intensity is 2. (I) Negative control. Scale bars, 20 μm.

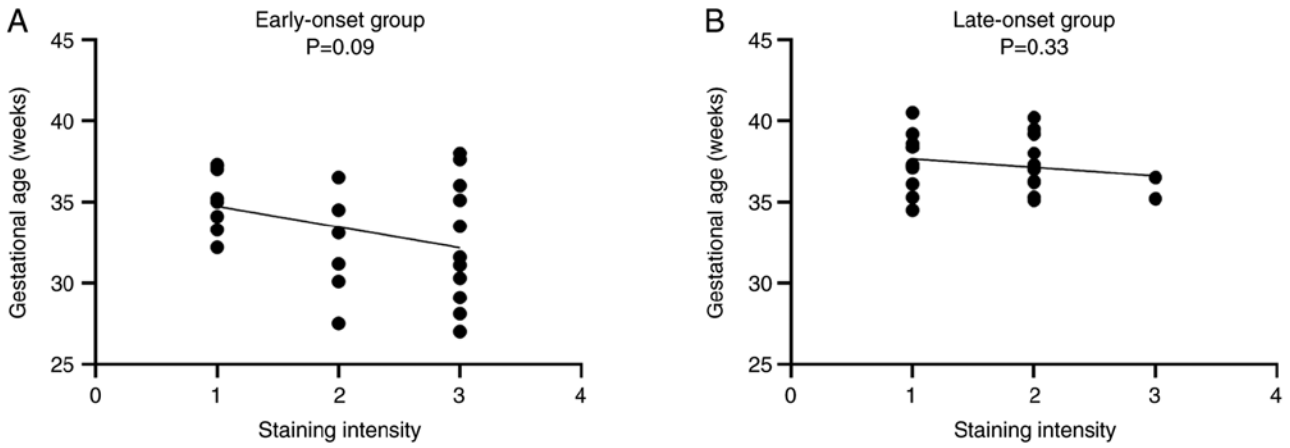

Figure 3. Plotting of the staining intensity of CD200 in early- and late-onset preeclampsia. (A) Correlation between the intensity of CD200 expression in the early-onset group placenta and the gestational age of delivery is shown. There was a correlation between expression intensity of CD200 and the gestational age of delivery, but the difference was not significant (P=0.09). (B) Correlation between the intensity of CD200 expression in the late-onset group placenta and the gestational age of delivery is shown. There was no correlation between expression intensity and the gestational age of delivery (P=0.33).

was significantly downregulated in preeclampsia compared with normal placenta (21); however, they did not stratify the cases into early- and late-onset groups and might have included more patients with late-onset preeclampsia because the median gestational week was 36 weeks in their series. In addition, the period between the onset of preeclampsia and delivery might influence CD200 expression and macrophage polarization in the placenta (the data regarding this period was not available in the study by Xu *et al* (21). They also found that Th1 cytokines were upregulated and Th2 cytokines were downregulated in trophoblasts (21). In contrast, the results of the present study showed that CD200 was significantly upregulated in the early-onset group. The significance of CD200 expression in the trophoblasts might differ between

early- and late-onset preeclampsia, although it remains unclear whether CD200 expression is the mainstream of preeclampsia development. CD200 expression might be the outcome or main cause of preeclampsia development. It is possible that excessive macrophage polarization influences the development of early-onset preeclampsia. In addition, significantly lower CD200 expression was noted in oocyte donation pregnancies with preeclampsia compared to those without preeclampsia, and this was not observed in naturally conceived pregnancies (33). Similarly, in preeclampsia pregnancies, significantly lower CD200 expression was noted in oocyte donation pregnancies compared to naturally conceived pregnancies (33). Thus, CD200 might have a role in the gestation processes of oocyte donation pregnancies. Moreover, additional mechanisms other than macrophage polarization might be involved in the development of preeclampsia. Additional studies are needed to clarify this issue.

There are some limitations in this study. First, we analyzed the immunohistochemical expression for CD200 in a relatively large number of patients (53 patients) with preeclampsia; however, RNA expression profiles analysis was performed in only 10 patients. Thus, further studies on both RNA expression and immunohistochemical analysis with a larger sample size of patients with early- and late-onset preeclampsia are needed. Second, the present study demonstrated that CD200 was significantly upregulated in early-onset preeclampsia. Although CD200 is considered to act in immune tolerance via macrophage polarization, distribution of M1 and M2 macrophages using CD68 and CD163 immunostaining in the placenta of patients with early- and late-onset preeclampsia was not analyzed in the present study. Thus, the relationship between CD200 expression and distribution of macrophages in the development of preeclampsia needs to be clarified.

In summary, the present study demonstrated that CD200 expression was significantly upregulated in patients with early-onset preeclampsia, although it remains unknown whether upregulation of CD200 is a cause or effect of development of early-onset preeclampsia. Thus, further studies are needed to clarify the mechanism of these conditions for adequate treatment.

Acknowledgements

The authors would like to thank Mr Ryosuke Yamaka who is a research assistant of Kansai Medical University (Osaka, Japan) who provided assistance in this study.

Funding

This research was partially supported by the research grant D2 from Kansai Medical University.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Sequencing data were deposited into the DNA Data Bank of Japan Sequence Read Archive (accession nos. SA MD00549508 and SAMD00549517; https://ddbj.nig.ac.jp/resource/bioproject/PRJDB14567).

Authors' contributions

HT and HO conceived and designed the study, and performed gene expression analyses. HT and MI performed immunohistochemical analyses. HT, MI, AN, TY, SK, YB, AY, YHi, YHa, TTN, HM, KT and HO acquired and analyzed data. HT, MI, and HO confirmed the authenticity of all the raw data, and drafted the manuscript, tables, and figures. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective single-institution study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the Kansai Medical University Hospital (approval no. 2018138; Hirakata, Japan). All data are completely anonymized. The Institutional Review Board waived the requirement for informed consent due to the retrospective design of the study, using medical records and archival samples with no risk of identity exposure of the patients. Moreover, the present study did not include any minors.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. von Dadelszen P, Magee LA and Roberts JM: Subclassification of preeclampsia. Hypertens Pregnancy 22: 143-148, 2003.
2. Walker JJ: Pre-eclampsia. Lancet 356: 1260-1265, 2000.
3. Wagner LK: Diagnosis and management of preeclampsia. Am Fam Physician 70: 2317-2324, 2004.
4. Report of the National high blood pressure education program working group on high blood pressure in pregnancy. Am J Obstet Gynecol 183: S1-S22, 2000.
5. Wagner SJ, Barac S and Garovic VD: Hypertensive pregnancy disorders: Current concepts. J Clin Hypertens (Greenwich) 9: 560-566, 2007.
6. Kuklina EV, Ayala C and Callaghan WM: Hypertensive disorders and severe obstetric morbidity in the United States. Obstet Gynecol 113: 1299-1306, 2009.
7. Aneman I, Pienaar D, Suvakov S, Simic TP, Garovic VD and McClements L: Mechanisms of key innate immune cells in early- and late-onset preeclampsia. Front Immunol 11: 1864, 2020.
8. Redman CW and Sargent IL: Latest advances in understanding preeclampsia. Science 308: 1592-1594, 2005.
9. Norwitz ER, Hsu CD and Repke JT: Acute complications of preeclampsia. Clin Obstet Gynecol 45: 308-329, 2002.
10. Karumanchi SA, Maynard SE, Stillman IE, Epstein FH and Sukhatme VP: Preeclampsia: A renal perspective. Kidney Int 67: 2101-2113, 2005.
11. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller AB, Daniels J, Gülmezoglu AM, Temmerman M and Alkema L: Global causes of maternal death: A WHO systematic analysis. Lancet Glob Health 2: e323-e333, 2014.
12. Lisonkova S and Joseph KS: Incidence of preeclampsia: Risk factors and outcomes associated with early-versus late-onset disease. Am J Obstet Gynecol 209: 544.e1-544.e12, 2013.
13. Basso O, Rasmussen S, Weinberg CR, Wilcox AJ, Irgens LM and Skjaerven R: Trends in fetal and infant survival following preeclampsia. JAMA 296: 1357-1362, 2006.

14. Backes CH, Markham K, Moorehead P, Cordero L, Nankervis CA and Giannone PJ: Maternal preeclampsia and neonatal outcomes. J Pregnancy 2011: 214365, 2011.
15. Burton GJ, Redman CW, Roberts JM and Moffett A: Pre-eclampsia: Pathophysiology and clinical implications. BMJ 366: l2381, 2019.
16. Rolfo A, Many A, Racano A, Tal R, Tagliaferro A, Ietta F, Wang J, Post M and Caniggia I: Abnormalities in oxygen sensing define early and late onset preeclampsia as distinct pathologies. PLoS One 5: e13288, 2010.
17. Sezer SD, Küçük M, Döger FK, Yüksel H, Odabaşı AR, Türkmen MK, Cakmak BÇ, Ömürlü İK and Kınaş MG: VEGF, PIGF and HIF-1α in placentas of early- and late-onset pre-eclamptic patients. Gynecol Endocrinol 29: 797-800, 2013.
18. Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, *et al*: Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290: 1768-1771, 2000.
19. Zhang S, Cherwinski H, Sedgwick JD and Phillips JH: Molecular mechanisms of CD200 inhibition of mast cell activation. J Immunol 173: 6786-6793, 2004.
20. Li Y, Zhang D, Xu L, Dong L, Zheng J, Lin Y, Huang J, Zhang Y, Tao Y, Zang X, *et al*: Cell-cell contact with proinflammatory macrophages enhances the immunotherapeutic effect of mesenchymal stem cells in two abortion models. Cell Mol Immunol 16: 908-920, 2019.
21. Xu J, Gu Y, Sun J, Zhu H, Lewis DF and Wang Y: Reduced CD200 expression is associated with altered Th1/Th2 cytokine production in placental trophoblasts from preeclampsia. Am J Reprod Immunol 79: 1 10.1111/aji.12763, 2018.
22. ACOG Committee on Obstetric Practice: ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American college of obstetricians and gynecologists. Int J Gynaecol Obstet 77: 67-75, 2002.
23. Ryota H, Ishida M, Satoi S, Yanagimoto H, Yamamoto T, Kosaka H, Hirooka S, Yamaki S, Kotsuka M, Matsui Y, *et al*: Clinicopathological and immunological features of follicular pancreatitis-a distinct disease entity characterised by Th17 activation. Histopathology 74: 709-717, 2019.
24. Anders S and Huber W: Differential expression analysis for sequence count data. Genome Biol 11: R106, 2010.
25. Egbor M, Ansari T, Morris N, Green CJ and Sibbons PD: Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. BJOG 113: 580-589, 2006.
26. van der Merwe JL, Hall DR, Wright C, Schubert P and Grové D: Are early and late preeclampsia distinct subclasses of the disease-what does the placenta reveal? Hypertens Pregnancy 29: 457-467, 2010.
27. Staff AC: The two-stage placental model of preeclampsia: An update. J Reprod Immunol 134-135: 1-10, 2019.
28. Miller D, Motomura K, Galaz J, Gershater M, Lee ED, Romero R and Gomez-Lopez N.: Cellular immune responses in the pathophysiology of preeclampsia. J Leukoc Biol 111: 237-260, 2022.
29. Dekker GA and Sibai BM: Etiology and pathogenesis of preeclampsia: Current concepts. Am J Obstet Gynecol 179: 1359-1375, 1998.
30. Enquobahrie DA, Meller M, Rice K, Psaty BM, Siscovick DS and Williams MA: Differential placental gene expression in preeclampsia. Am J Obstet Gynecol 199: 566.e1-11, 2008.
31. Shibata E, Rajakumar A, Powers RW, Larkin RW, Gilmour C, Bodnar LM, Crombleholme WR, Ness RB, Roberts JM and Hubel CA: Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: Relationship to circulating placental growth factor. J Clin Endocrinol Metab 90: 4895-4903, 2005.
32. Wang LQ, Yan CF, Zhao Y, Chu J and Yu XW: Reduced CD200 and CD200R1 expression in human chorionic villi contributes to early spontaneous abortion. Acta Obstet Gynecol Scand 93: 1248-1254, 2014.
33. van 't Hof LJ, Dijkstra KL, van der Keur C, Eikmans M, Baelde HJ, Bos M, and van der Hoorn MLP: Decreased expression of ligands of placental immune checkpoint inhibitors in uncomplicated and preeclamptic oocyte donation pregnancies. J Reprod Immunol 142: 103194, 2020.