タイトル	Circulating soluble LR11, a differentiation regulator for vascular cells, is increased during pregnancy and exaggerated in patients with pre-eclampsia
別タイトル	血管細胞の分化制御因子である血中可溶性LR11 は妊娠経過中に上昇し、妊娠高血圧腎症患者で増強する
作成者（著者）	石田, 洋昭
公開者	東邦大学
発行日	2020.04.23
掲載情報	東邦大学大学院医学研究科 博士論文.
資料種別	学位論文
内容記述	主査 : 森田峰人 / タイトル : Circulating soluble LR11, a differentiation regulator for vascular cells, is increased during pregnancy and exaggerated in patients with pre-eclampsia / 著者 : Hiroaki Ishida, Meizi Jiang, Hiroyuki Ebinuma, Nobuyuki Hiruta, Wolfgang J Schneider, Toshihiko Kinoshita, Hideaki Bujo / 掲載誌 : Clinica Chimica Acta / 巻号・発行年等 : 497:172-177; 2019 / 本文ファイル: 出版社
著者版フラグ	ETD
報告番号	32661乙第2925号
学位記番号	乙第2767号
学位授与年月日	2020.04.23
学位授与機関	東邦大学
DOI	info:doi/10.1016/j.cca.2019.07.001
その他資源識別子	https://www.sciencedirect.com/science/article/abs/pii/S0009898119319382?via%3Dihub
メタデータのＵＲＬ	https://mylibrary.toho-u.ac.jp/webopac/TD56395489

Clinica Chimica Acta 497 (2019) 172–177

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Circulating soluble LR11, a differentiation regulator for vascular cells, is increased during pregnancy and exaggerated in patients with pre-eclampsia

Hiroaki Ishida[a], Meizi Jiang[b], Hiroyuki Ebinuma[c], Nobuyuki Hiruta[b,d], Wolfgang J. Schneider[e], Toshihiko Kinoshita[a], Hideaki Bujo[b,*]

[a] Department of Obstetrics and Gynecology, Toho University Sakura Medical Center, Sakura, Japan
[b] Department of Clinical-Laboratory and Experimental-Research Medicine, Toho University Sakura Medical Center, Sakura, Japan
[c] Tsukuba Research Institute, Sekisui Medical Co Ltd, Ryugasaki, Japan
[d] Department of Surgical Pathology, Toho University Sakura Medical Center, Sakura, Japan
[e] Department of Medical Biochemistry, Max F. Perutz Laboratories, Medical University of Vienna, Vienna, Austria

ARTICLE INFO

Keywords:
Pre-eclampsia
Inflammation
IL-6
TNF-α
Soluble LR11

ABSTRACT

Background: Pre-eclampsia is a pregnancy-specific disease characterized by onset of hypertension and proteinuria, sometimes progressing into damaging other organs. Here, we investigated the pathological significance of the soluble fragment of LR11 (sLR11), a cell differentiation regulator, in comparison to circulating IL-6 and TNF-α, in pre-eclampsia.

Methods: The study was conducted in a cross-sectional research design with fourteen pre-eclampsia patients and fifty healthy pregnant subjects. Pre-eclampsia was defined as hypertensive disorders in pregnancy at over 20 weeks of gestation with proteinuria.

Results: Plasma levels of sLR11 as well as IL-6 in pre-eclampsia were increased compared with those in the healthy pregnant subjects at the first, the second, and the third trimester. Receiver operating characteristic analysis for the detection of pre-eclampsia among third-trimester subjects showed that the areas under the curves of sLR11 and IL-6 were equivalent. sLR11 and IL-6 correlated positively with TNF-α in healthy pregnant subjects. In the pre-eclampsia patients, there was neither a correlation between sLR11 and IL-6 nor between sLR11 and TNF-α.

Conclusions: sLR11 increases during pregnancy, with levels further exaggerated in pre-eclampsia, and may be related to the pathology of pre-eclampsia.

1. Introduction

Pre-eclampsia is a common syndrome potentially accompanied with various organ failures, and causes high risks for maternal and/or neonatal health [1,2]. The current International Society for the Study of Hypertension in Pregnancy guidelines define pre-eclampsia as de novo hypertension after the 20th week of gestation with accompanying proteinuria [3]. Although the etiology and pathophysiology of pre-eclampsia remain only incompletely elucidated, one of mechanisms underlying the development of the characteristic symptoms is believed to be based on disturbed placental function in association with inadequate spiral artery remodeling in early pregnancy with subsequent placental hypoxia or hypoxia/reperfusion injury [4,5], which causes the release of inflammatory substrate(s) into the maternal blood, and finally exaggerates endothelial cell activation to generate a hyper-inflammatory condition [2,6–8].

On the basis of the systemic inflammatory condition, pre-eclampsia is indeed related not only to the characteristic symptoms hypertension and proteinuria, but also to other chronic inflammation-related diseases including insulin resistance, type 2 diabetes and obesity, known as risks for pre-eclampsia [9]. These chronic inflammatory diseases likely lead to advanced atherosclerosis, also known to be a condition of chronic inflammation in the vascular wall [10]. In such a systemic involvement, inflammatory cytokines, as well as substrates including placental cellular and subcellular debris, and the splice variant of their receptor, soluble fms-like tyrosine kinase-1 (sFlt-1), have been shown to increase in plasma of patients with pre-eclampsia (reviewed in 6), although the origin of the releasing cells remains unclear. A recent meta-analysis of

* Corresponding author at: Department of Clinical-Laboratory and Experimental-Research Medicine, Toho University Sakura Medical Center, 564-1 Shimoshizu, Sakura 285-8741, Japan.
E-mail address: hideaki.bujo@med.toho-u.ac.jp (H. Bujo).

https://doi.org/10.1016/j.cca.2019.07.001
Received 19 December 2018; Received in revised form 13 June 2019; Accepted 1 July 2019
Available online 09 July 2019
0009-8981/

independent studies has reported that Interleukin-6 (IL-6) and TNF-α were two cytokines clearly increased in the plasma of patients with pre-eclampsia [11].

We have recently identified a novel circulating molecule, the soluble form of the low-density lipoprotein receptor relative with 11 ligand- binding repeats (LR11, also called sorLA or SORL1), as a differentiation regulator in autocrine and paracrine processes for vascular cells [12,13]. Mice lacking this soluble form of LR11 (sLR11) are protected from cuff-injured arterial hyperplasia through the decreased de-differentiation into immature synthetic smooth muscle cells (SMCs) with hypersensitivities for migration and cytokine release from mature contractile SMCs [13]. In human studies, plasma sLR11 levels were increased in atherosclerotic patients with cardiovascular diseases [14–19] and also in obese patients with type 2 diabetes [17,20–22]. Notably, in these two chronic vascular inflammatory diseases, atherosclerosis and diabetes, the hyper-activated vascular cells release not only sLR11 [23], but also cytokines including IL-6 and TNF-α, both of which are the key circulating inflammatory cytokines in pre-eclampsia [11].

Based on the above background, in order to determine the significance of the circulating molecule sLR11 for cell differentiation, we have investigated its levels in comparison to those of IL-6 and TNF-α, in plasma of fifty healthy pregnant subjects and fourteen patients with pre-eclampsia.

2. Materials and methods

2.1. Subjects

Fifty normal pregnant women (16 at first trimester, 15 at second trimester and 19 at third trimester) and fourteen pre-eclampsia patients who were diagnosed and treated with conventional life-style and diet instructions at the Department of Obstetrics and Gynecology, Toho University Sakura Medical Center, Japan, between August 2016 and May 2018 were enrolled in this study (see Supplemental table). Pre-eclampsia was defined as hypertensive disorders in pregnancy at 20 weeks or later of gestation with proteinuria [3,24]. Hypertension was defined as systolic blood pressure≧140 mmHg and/or diastolic blood pressure≧90 mmHg measured on two or more occasions at least six hours apart with the patient at rest [24]. Proteinuria was defined as protein ≧300 mg/day in a 24- h-collected urine specimen [24]. Blood sampling was performed in the 6th to 8th week (first trimester), the 24th to 26th week (second trimester), and the 35th to 37th week (third trimester and pre-eclampsia) of pregnancy. There were no and ten pre-term deliveries before the 37th week for healthy pregnant women and the subjects with pre-eclampsia, respectively. Pregnant women with pre-eclampsia were hospitalized at the day or 1–2 days before the delivery by cesarean section. All pregnant women had a single fetus, no medication without life-style and diet instructions, and did not suffer from gestational diabetes. The study protocol was approved by The Ethics Committee of Toho University Sakura Medical Center (approval number 2014–029), and performed in accordance with the principles of the Declaration of Helsinki. All subjects gave informed consent for their enrollment into the study protocol.

2.2. sLR11, IL-6 and TNF-α measurements

Blood samples were collected into tubes containing EDTA as the anticoagulant, and centrifuged at 4 °C, 3000 rpm for 15 min to isolate plasma. The plasma was separated, and aliquots were then immediately refrigerated at −80 °C until processing for 1 to 6 months. All ELISAs were immediately performed at the same time using frozen samples after thawing, sLR11 was measured using a sandwich ELISA method (Sekisui Medical, Ryugasaki, Japan), using samples frozen at −80 °C as reported previously [25–27]. Briefly, 12.5 μl of each plasma was used for the measurement of sLR11 by ELISA with specific monoclonal

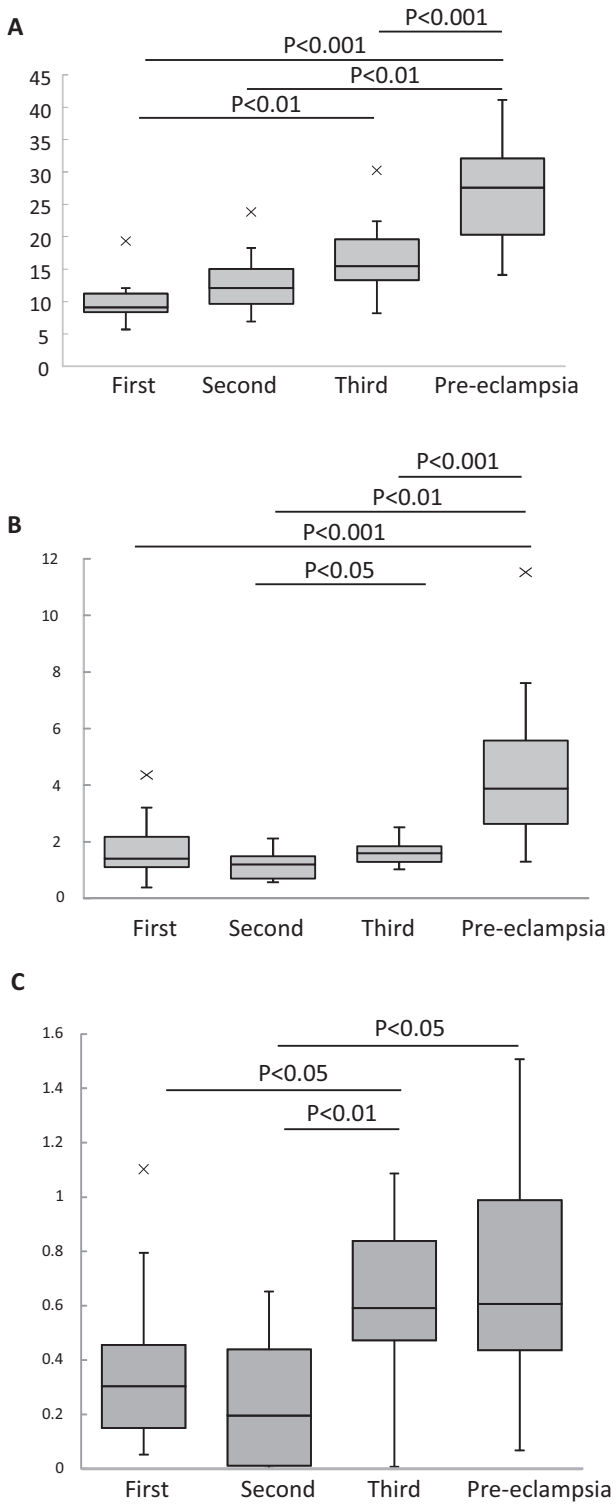

Fig. 1. Comparison of plasma sLR11 (**A**), IL-6 (**B**) and TNF-α (**C**) levels among subjects in the first trimester, the second trimester and the third trimester, and patients with pre-eclampsia, respectively. The top of each box in the box plots indicates the 75th percentile, the bottom of each box indicates the 25th percentile, the horizontal bars inside the boxes are the median values, and the whiskers extend out to the most extreme data point that is at most 1.5 times the interquartile range above the third quartile or below the first quartile. The symbols "X" above the boxes with bars indicate values from subjects or patients above this range. Values of $p < .05$ were evaluated as significant differences.

Table 1
Specificity and sensitivity of plasma sLR11,IL-6 and TNF-α for the discrimination of patients with pre-eclampsia against subjects with normal pregnancy in the third trimester.

	Cut-off value	Sensitivity	Specificity	AUC
sLR11	23.82	0.64	0.95	0.84
IL-6	2.13	0.93	0.90	0.93
TNF-α	1.13	0.21	1.00	0.52

antibodies directed against human LR11. Plasma IL-6 and TNF-α were measured using Quantikine HS ELISA (R & D systems, Tokyo), for human IL-6 (HS600B) and TNF-α (HSTA00E), respectively. The lower detection limits were 2.2 ng/mL for sLR11, 0.039 pg/mL for IL-6, and 0.022 pg/mL for TNF-α. In our studies, the coefficient of variabilities (CVs) of intra-assay were 7.6% (LR11), 5.6% (IL-6), 9.2% (TNF-α), respectively, and those of linearity were 80–120% of expected values for all measurements. Undetectable values less than the lower detection limits for TNF-α were handled as 0 pg/mL in statistical analysis.

2.3. Statistical analysis

The results are presented as medians ± quartile deviations with the ranges (minimum to maximum), and analyzed using the Kruskal Wallis test. The diagnostic ability of biomarkers was evaluated using receiver operating characteristic (ROC) curves, which plot true-positive rates (sensitivity) vs. false positive rates (1 - specificity) across all possible thresholds. The cut-off values were determined as values for maximum ROC–area under the curves (AUCs), and the positive predictive value (sensitivity) and the negative predictive value (specificity) at the cut-off values were determined as a global measure of diagnostic accuracy for each biomarker. Associations between two of sLR11, IL-6 and TNF-α were examined by Pearson correlation analysis for continuous variables. The significance level was set at a p-value < .05. All statistical analyses were performed using JMP ver. 10.0.2 (SAS Institute Inc. Cary, NC).

3. Results

3.1. Circulating sLR11 levels were exaggerated in pre-eclampsia

Plasma sLR11 levels, together with IL-6 and TNF-α, in 16 normal pregnant women at the first trimester, 15 at the second trimester and 19 at the third trimester, and 14 patients with pre-eclampsia are shown in Fig. 1. The plasma levels of sLR11 were significantly increased in the third trimester, compared with those in the first trimester. (Fig. 1A). The sLR11 levels in patients with pre-eclampsia were increased when compared with the pregnant subjects in the first, second and third trimester (Fig. 1A). The plasma IL-6 levels in patients with pre-eclampsia were also significantly higher than those in any of the three trimesters (Fig. 1B). On the other hand, the TNF-α levels in patients with pre-eclampsia were significantly higher than those in the second trimester, but not different between the patients with pre-eclampsia and the normal pregnant first or third-trimester subjects (Fig. 1C). Thus, circulating sLR11 levels, together with the IL-6 levels, were increased in patients with pre-eclampsia, compared with those in the normal pregnant subjects at any trimester.

3.2. sLR11 shows a maximum ROC-AUC value equivalent to IL-6 for pre-eclampsia in the third trimester of pregnancy

We studied the effects of plasma levels of sLR11, IL-6 and TNF-α for the discrimination of patients with pre-eclampsia versus subjects with normal pregnancy. For this purpose, 33 third-trimester subjects (19 subjects with normal pregnancy in the third trimester, and 14 patients with pre-eclampsia, see Table 1) were chosen from the study subjects. Receiver operating characteristic analysis for the detection of pre-eclampsia among subjects in the third trimester showed that the maximum area under the curve (AUC) of sLR11 was 0.84 at the cut-off value 23.82 ng/ml with a sensitivity of 0.64 and a specificity of 0.95, and thus equivalent to the value of 0.93 at the cut-off value 2.13 pg/ml with a sensitivity of 0.93 and a specificity of 0.90 for IL-6, clearly superior to the value of 0.52 at the cut-off value 1.13 pg/ml with a sensitivity of 0.21 and a specificity of 1.00 for TNF-α (Fig. 2 and Table 1).

3.3. Circulating sLR11 levels are associated with TNF-α in normal pregnancy, but not in pre-eclampsia

In order to determine the pathological significance of increases in sLR11 in pregnancy and pre-eclampsia, the association between two of the three circulating biomarkers were analyzed in the fifty healthy pregnant subjects and in the fourteen patients with pre-eclampsia, respectively (Fig. 3). Pearson's correlation analysis showed that sLR11 and IL-6 positively correlated with TNF-α (panels B and C), although there was no significant correlation between sLR11 and IL-6 (panel A). Importantly, the positive correlations of sLR11 and IL-6 with TNF-α were not observed among patients with pre-eclampsia (panels E and F); instead, there was a negative correlation between IL-6 and TNF-α (panel F). Thus, the levels of sLR11, as those of IL-6, were associated with the levels of TNF-α in normal pregnancy, whereas in the pathology

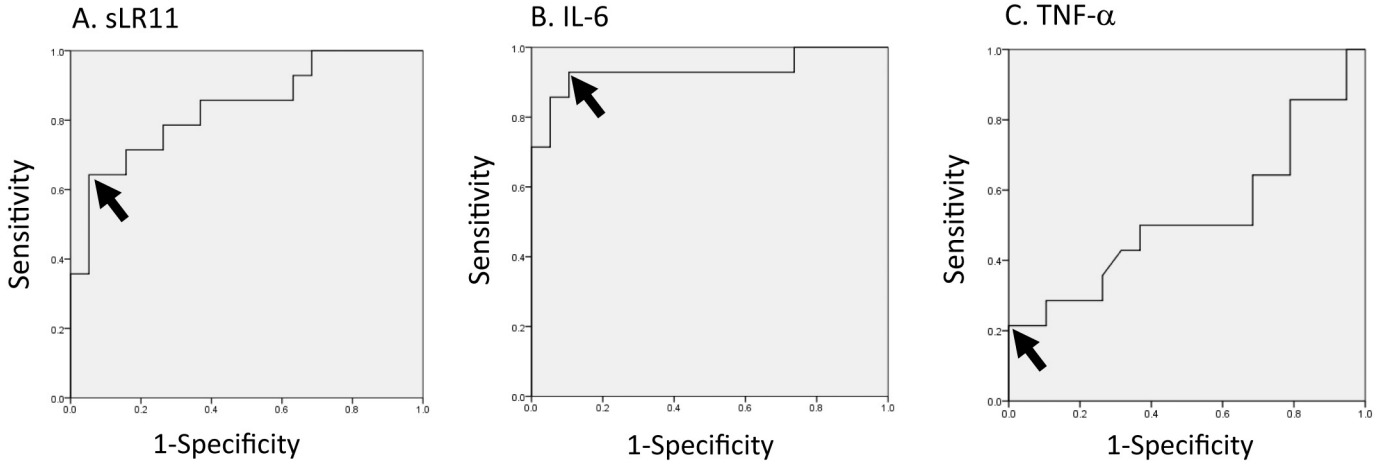

Fig. 2. Receiver operating characteristic (ROC) curves of plasma sLR11 (**A**), IL-6 (**B**) and TNF-α (**C**) concentrations for discrimination of patients with pre-eclampsia against normal pregnant subjects in the third trimester. The cut-off values are marked with arrows.

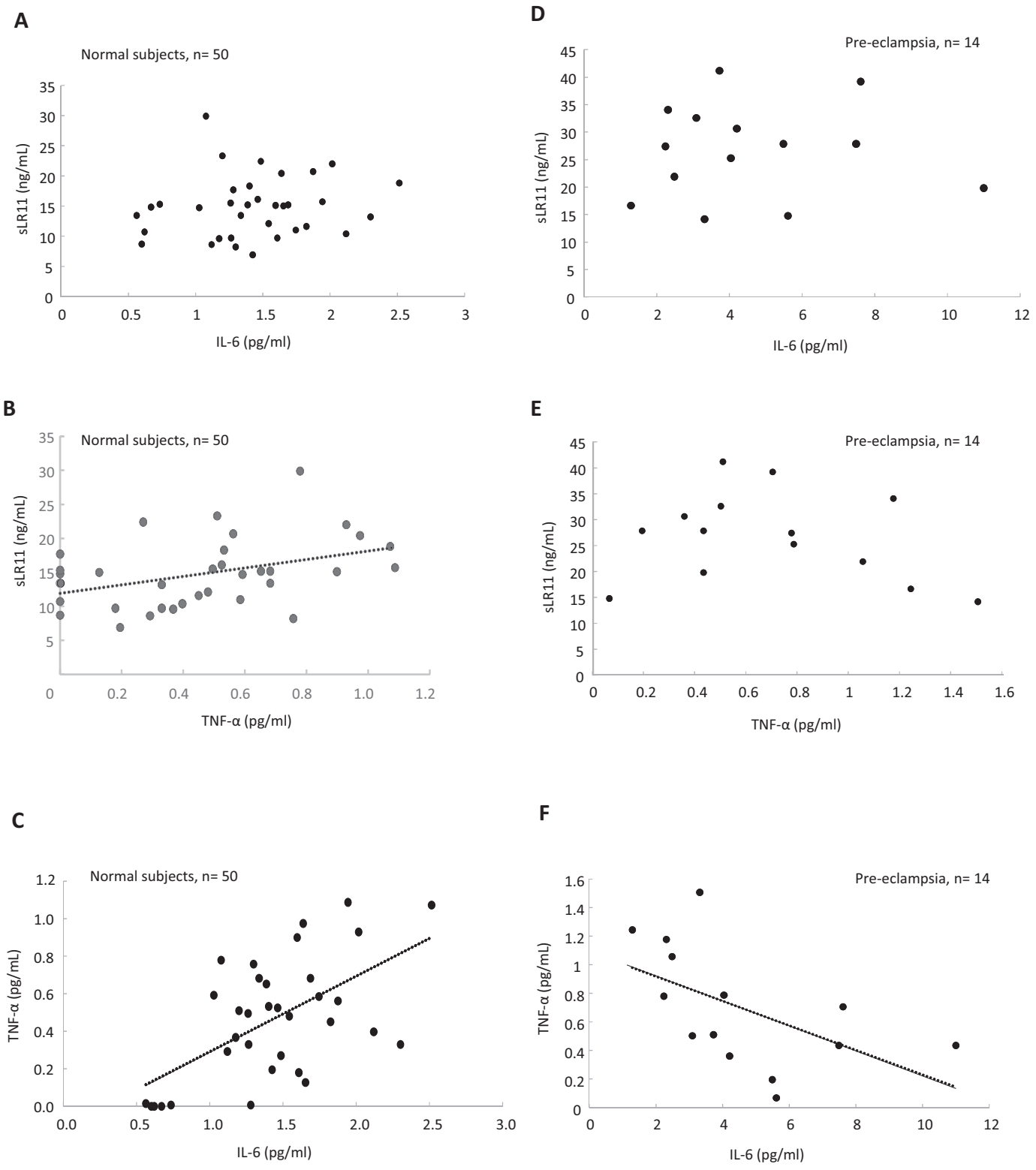

Fig. 3. Relationships between plasma sLR11 and plasma IL-6 (**A** and **D**), plasma sLR11 and plasma TNF-α (**B** and **E**), and plasma IL-6 and plasma TNF-α (**C** and **F**), in normal pregnant subjects (**A, B** and **C**) and in patients with pre-eclampsia (**D, E** and **F**). Coefficients of determination (r^2) and *p*-values in each panel are 0.013 and 0.44 (**A**), 0.185 and 0.002 (**B**), 0.088 and 0.036 (**C**), 0.0003 and 0.48 (**D**), 0.052 and 0.22 (**E**), 0.293 and 0.023 (**F**), respectively.

of pre-eclampsia, this relationship disappeared.

4. Discussion

Numerous and various kinds of functional interactions develop between the placenta and the maternal cell/tissues including immune and cardiovascular responses; pre-eclampsia possibly arises from multiple aberrations in these adaptations [2,6–8]. One potential explanation for a placental etiology of pre-eclampsia is the unsuccessful development of spiral artery remodeling in the first trimester, and the

subsequent release of inflammatory cytokines including IL-6 and TNF-α. As well, placenta-derived materials such as multinucleated syncytiotrophoblast knots and/or syncytiotrophoblast microparticles (STMPs) possibly cause systemic endothelial cell activation in the third trimester [6]. These inflammatory stimuli activate accumulation of leukocytes, the adherence of neutrophils to the endothelium, transmigration of neutrophils through the vascular endothelium, and here again release of cytokines including IL-6 and TNF-α [28,29] that mediate activation of SMCs and other atherogenic vascular cells, known as vascular chronic inflammation [10]. Accompanying additional chronic inflammatory conditions, such as type 2 diabetes, obesity, and advanced atherosclerosis itself, are known as risk factors that exaggerate the inflammatory pathology of pre-eclampsia [9]. In order to delineate the involvement of vascular cell differentiation in the complex systemic inflammatory conditions in pre-eclampsia, the current study focuses on the role of a differentiation regulator for vascular cells, sLR11. The rationale for the study lies in the observation that circulating sLR11 levels are indicative of the pathological chronic inflammatory cellular conditions in atherosclerotic patients with cardiovascular diseases [14–19] and obese patients with type 2 diabetes [17,20–22], known as chronic inflammatory diseases in the vascular wall [10,23].

LR11 was originally identified in vascular intimal de-differentiated SMCs, which display highly activated migration and cytokine release [30]. sLR11 induces the de-differentiation of SMCs from the mature contractile phenotype - observed in the medial layer - to the immature synthetic phenotype in the intimal layer in animal models and in cultured cells [13]. Indeed, in LR11-knockout mice, intimal hyperplasia is drastically inhibited after cuff injury of femoral arteries, and, in LR11-deficient cells, sensitivities of migration and cytokine release by exogeneous stimuli are clearly decreased [13]. Considering that LR11 gene expression is induced in activated endothelium in atherosclerosis [31,32], and that sLR11 induces macrophage phenotype transition [12], this de-differentiation inducer may well be involved in the inflammatory changes of vascular cells via their hyper-sensitivity against various exogeneous stimuli for migration and cytokine release.

In this study, first we show that circulating sLR11 levels gradually increased in the progression of normal pregnancy, and that the changes were obviously different from those in IL-6 and TNF-α, which did not show a gradual increase throughout pregnancy (see Fig. 1). Notably, a sharp difference in sLR11 levels was observed between patients with pre-eclampsia and those in normal pregnant subjects in any trimester, as also observed for IL-6, but not for TNF-α (see Fig. 1). Subsequent ROC analysis for the discrimination of pre-eclampsia against normal pregnancy in the third trimester revealed that the AUC value of sLR11 for the highest sensitivity-and-specificity was equivalent to that of IL-6 (see Fig. 2 and Table 1). The association analysis for the various combinations of two of the three plasma markers showed that the levels of sLR11 and IL-6 were associated with the levels of TNF-α in normal pregnancy, but the relationship disappeared in pre-eclampsia (see Fig. 3). Thus, the increased circulating sLR11 levels during pregnancy may reflect the de-differentiated phenotype with hyper-activated gene expression levels, including that of IL-6, in maternal and/or placental cell/tissues. Considered together, the clearly increased levels of sLR11 in pre-eclampsia when compared with normal pregnancy at earlier trimesters suggest the potential clinical utility of sLR11 as a sensitive marker for earlier diagnosis and increased diagnostic accuracy for pre-eclampsia.

One limitation of the present study is the restricted sample availability for extensive clinical analysis. Subjects were collected from a single institute, and therefore the subject numbers may not be fully sufficient for using complete multivariate regression analyses including other clinical and biochemical factors. The current study has been principally conducted in a cross-sectional research design, limiting the possibilities to identify causal relationships among sLR11, pregnancy period, IL-6, and TNF-α. Particularly, the changes in sLR11 levels at the timing of blood sampling during the first to third trimester need to be examined in terms of the causal relationships between the increased sLR11 and pre-eclampsia. Clearly, further studies using subjects with different characteristics (age, risk factors, and treatment conditions) will be helpful for the evaluation of pathological mechanisms involving sLR11 in pre-eclampsia. Based on the current results, a future prospective larger-scale study for the comparison of subjects with or without pre-eclampsia is indicated.

In conclusion, the present study demonstrates that circulating sLR11 gradually increases during progression of pregnancy, and sharply increases even further in pre-eclampsia. The measurement of sLR11 can contribute to a better understanding of the pathophysiology of vascular cell adaptation in pregnancy and the aberrations in pre-eclampsia.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.07.001.

Acknowledgments

This study was supported, in part, by Grants-in–aid for Scientific Research from Japanese Ministry of Education, Culture, Sports, Science and Technology to Hideaki Bujo (16H05231) and Meizi Jang (18 K07428), Japan Health and Labour Sciences Research grant US-Japan cooperative medical science program to MJ, Naito Foundation to HB, Uehara Memorial Foundation to HB, Smoking Research Foundation to HB, Takeda Scientific Foundation to MJ, and Kurozumi Medical Foundation to MJ.

References

[1] E.A. Steegers, P. von Dadelszen, J.J. Duvekot, R. Pijnenborg, Pre-eclampsia, Lancet 376 (2010) 631–644.
[2] L. Duley, The global impact of pre-eclampsia and eclampsia, Semin. Perinatol. 33 (2009) 130–133.
[3] M.A. Brown, M.D. Lindheimer, M. de Swiet, et al., The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP), Hypertens. Pregnancy. 20 (2001) ix–xiv.
[4] T.H. Hung, J.N. Skepper, G.J. Burton, In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies, Am. J. Pathol. 159 (2001) 1031–1043.
[5] T.H. Hung, J.N. Skepper, D.S. Charnock-Jones, et al., Hypoxiareoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia, Circ. Res. 90 (2002) 1274–1281.
[6] J.L. James, G.S. Whitley, J.E. Cartwright, Pre-eclampsia: fitting together the placental, immune and cardiovascular pieces, J. Pathol. 221 (2010) 363–378.
[7] B. Huppertz, Placental origins of preeclampsia: challenging the current hypothesis, Hypertension 51 (2008) 970–975.
[8] J.M. Roberts, C. Escudero, The placenta in preeclampsia, Pregnancy. Hypertens. 2 (2012) 72–83.
[9] A.L. Tranquilli, B. Landi, S.R. Giannubilo, et al., Preeclampsia: no longer solely a pregnancy disease, Pregnancy. Hypertens. 2 (2012) 350–357.
[10] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, Nature 473 (2011) 317–325.
[11] S.Y. Lau, S.J. Guild, C.J. Barrett, et al., Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: a systematic review and meta-analysis, Am. J. Reprod. Immunol. 70 (2013) 412–427.
[12] K. Ohwaki, H. Bujo, M. Jiang, H. Yamazaki, et al., A secreted soluble form of LR11, specifically expressed in intimal smooth muscle cells, accelerates formation of lipid-laden macrophages, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 1050–1056.
[13] M. Jiang, H. Bujo, K. Ohwaki, et al., Ang II-stimulated migration of vascular smooth muscle cells is dependent on LR11 in mice, J. Clin. Invest. 118 (2008) 2733–2746.
[14] A. Nohara, J. Kobayashi, M.A. Kawashiri, et al., Clinical significance of measuring soluble LR11, a circulating marker of atherosclerosis and HbA1c in familial hypercholesterolemia, Clin. Biochem. 47 (2014) 1326–1328.
[15] R. Vongpromek, H. Bujo, M. Hoekstra, et al., LR11/SorLA links triglyceride-rich lipoproteins to risk of developing cardiovascular disease in FH patients, Atherosclerosis 243 (2015) 429–437.
[16] R. Vongpromek, S. Bos, G. ten Kate, et al., Soluble LR11 associates with aortic root calcification in asymptomatic treated male patients with familial hypercholesterolemia, Atherosclerosis 265 (2017) 299–304.
[17] M. Takahashi, H. Bujo, M. Jiang, et al., Enhanced circulating soluble LR11 in patients with coronary organic stenosis, Atherosclerosis 210 (2010) 581–584.
[18] M. Ogita, K. Miyauchi, T. Dohi, et al., Increased circulating soluble LR11 in patients with acute coronary syndrome, Clin. Chim. Acta 415 (2013) 191–194.
[19] M. Ogita, K. Miyauchi, M. Jiang, et al., Circulating soluble LR11, a novel marker of smooth muscle cell proliferation, is enhanced after coronary stenting in response to vascular injury, Atherosclerosis 237 (2014) 374–378.

[20] W. Jin, M. Jiang, X. Han, et al., Circulating soluble form of LR11, a regulator of smooth muscle cell migration, is a novel marker for intima-media thickness of carotid arteries in type 2 diabetes, Clin. Chim. Acta 457 (2016) 137–141.
[21] M. Takahashi, H. Bujo, T. Shiba, M. Jiang, T. Maeno, K. Shirai, Enhanced circulating soluble LR11 in patients with diabetic retinopathy, Am J. Ophthalmol. 154 (2012) 187–192.
[22] K.A. Berk, R. Vongpromek, M. Jiang, et al., Levels of the soluble LDL receptor-relative LR11 decrease in overweight individuals with type 2 diabetes upon diet-induced weight loss, Atherosclerosis 254 (2016) 67–72.
[23] H. Xu, G.T. Barnes, Q. Yang, et al., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (2003) 1821–1830.
[24] M.A. Brown, L.A. Magee, L.C. Kenny, et al., International Society for the Study of Hypertension in Pregnancy (ISSHP). hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice, Hypertension 72 (2018) 24–43.
[25] M. Matsuo, H. Ebinuma, I. Fukamachi, et al., Development of an immunoassay for the quantification of soluble LR11, a circulating marker of atherosclerosis, Clin. Chem. 55 (2009) 1801–1808.
[26] K. Fujimura, H. Ebinuma, I. Fukamachi, et al., Circulating LR11 is a novel soluble receptor marker for early-stage clinical conditions in patients with non-Hodgkin's lymphoma, Clin. Chim. Acta 430 (2014) 48–54.
[27] A.J. Whittle, M. Jiang, V. Peirce, et al., Soluble LR11/SorLA represses thermogenesis in adipose tissue and correlates with BMI in humans, Nat. Commun. 20 (2015) 8951.
[28] C. Leik, S. Walsh, Neutrophils infiltrate resistance-sized vessels of subcutaneous fat in women with pre-eclampsia, Hypertension 44 (2004) 72–77.
[29] K. Tsukimori, A. Tsushima, K. Fukushima, et al., Neutrophil-derived reactive oxygen species can modulate neutrophil adhesion to endothelial cells in pre-eclampsia, Am. J. Hypertens. 21 (2008) 587–591.
[30] H. Yamazaki, H. Bujo, J. Kusunoki, et al., Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member, J. Biol. Chem. 271 (1996) 24761–24768.
[31] H.J. Ting, J.P. Stice, U.Y. Schaff, et al., Triglyceride-rich lipoproteins prime aortic endothelium for an enhanced inflammatory response to tumor necrosis factor-alpha, Circ. Res. 100 (2007) 381–390.
[32] T.M. Bajari, W. Winnicki, E.T. Gensberger, et al., Known players, new interplay in atherogenesis: chronic shear stress and carbamylated-LDL induce and modulate expression of atherogenic LR11 in human coronary artery endothelium, Thromb. Haemost. 111 (2014) 323–332.