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Clinical significance of serum sRAGE and esRAGE in women with physiologic pregnancy and preeclampsia

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<Title page>

Clinical significance of serum sRAGE and esRAGE in women with physiologic pregnancy and preeclampsia

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Thank you.

Jae Hyun Kwon

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ABSTRACT

CLINICAL SIGNIFICANCE OF SERUM sRAGE AND esRAGE IN WOMEN WITH PHYSIOLOGIC PREGNANCY AND PREECLAMPSIA

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Endogenous secretory receptor of advanced glycation end products (esRAGE) and soluble RAGE (sRAGE) have been linked to components of metabolic syndromes and pathologic pregnancy including preeclampsia, negatively. We performed this study to determine serum esRAGE and sRAGE concentrations and the esRAGE/sRAGE ratio in physiologic pregnancy and preeclampsia. Eighty-seven normal pregnant women and 28 with preeclampsia were recruited. Serum sRAGE and esRAGE levels were measured by enzyme-linked immunosorbent assay (ELISA). There were significant differences in esRAGE concentration and esRAGE/sRAGE ratio between 1st and 3rd trimester ($p = .007$ *and* $p = .003$). Serum esRAGE concentrations and esRAGE/sRAGE ratio in patients with preeclampsia significantly increased compared to controls ($p = .012$ *and* $p = .018$). Because of increased oxidative stress markers with inflammatory molecules throughout physiologic pregnancy, serum esRAGE concentration might be decreased inversely, as antioxidant defences mechanism. Elevated serum esRAGE and esRAGE/sRAGE ratio with preeclampsia might be a compensatory adaptation to neutralize oxidative stress.

Key words : sRAGE, esRAGE, preeclampsia

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I. INTRODUCTION

Advanced glycation end products (AGEs) are produced when aldose sugars respond to proteins with oxidative stress. They have reactive cross-linking molecules, produced by the non-enzymatical glycation of degrading sugars, lipids, or nucleic acids.[1-3] AGEs levels are elevated in condition of oxidative stress including diabetes, rheumatoid arthritis or renal insufficiency.[4-6] AGEs may induce the formation of oxidative stress, generation of growth factors or cytokines, chronic inflammation, and cell damage by binding to the receptor for advanced glycation end products (RAGE).[7,8] RAGE is a multiligand cell-surface receptor molecule that was found in 1992 by Schmidt and Stern.[9] RAGE contains an immunoglobin - like V-type domain and two immunoglobin - like C-type domains, a short hydrophobic transmembrane region, and a highly-charged 43-amino acid cytosoplasmic tail. Of these domains, the aminoterminal one (V-type domain) has been proven to be important for ligand formation, while the cytosolic region is critical in raising cellular effects once ligands hold the receptor.[10] Besides full-length RAGE, some truncated types of the RAGE receptor have been investigated.[11,12] First, one modified variant (N-truncated type) is free of the V-type immunoglobulin domain, however it is otherwise identical to full-length RAGE and is retained in the plasma membrane. Another variant, lacking cytosolic and transmembrane domains, is released from the cellular membrane and can be detected in blood stream as

soluble RAGE (sRAGE). Because sRAGE has been shown to successfully bind to AGEs, this soluble isoform could function as antagonist by inhibiting the action of cell-surface full-length RAGE. Recently, among total soluble RAGE variants, a novel splice isoform of RAGE mRNA coding for a C- terminally truncated secretory form, endogenous secretory RAGE (esRAGE) was identified with their cytoprotective function against AGEs and demonstrated to be actually produced by human microvascular endothelials and pericytes, and liberated extracellulary.[13] Other forms of soluble RAGE are proteolytically cleaved from the cellular surface by the action of matrix metalloproteinases and are released into the blood stream. Therefore, total circulating soluble RAGE is a sum of esRAGE and RAGE most likely shed upon fragmentation by metalloproteinases. Although recent studies have demonstrated the association between sRAGE or esRAGE levels and metabolic diseases, there is some inconsistency in results with respect to the relationship between RAGE variants and metabolic diseases.[14-17]

Pregnancy has been shown to induce a significant elevation in RAGE protein in both myometrium and omental vasculature, which generates the aggregation of pathologic conditions by changing vascular cell function.[18] Preeclampsia is represented by systemic and uteroplacental circulatory dysfunction which, by the third trimester, demonstrates as maternal hypertension.[19] RAGE activation is connected with altering vascular cell function by promoting TNF α or controlling the vasodilator prostacyclin, and these changes are commonly observed in preeclampsia.[20] Futhermore, significant differences of total circulating sRAGE levels in physiological pregnancy and in pathological states in pregnancy was demonstrated, recently.[21] However, to date no study has been investigated whether the serum concentration of esRAGE or the proportion to total circulating sRAGE is altered according to the trimester of pregnancy and manifested in women with preeclampsia.

Therefore, we performed this pilot study to determine serum esRAGE, sRAGE concentration

and the esRAGE/sRAGE ratio in physiologic pregnancy and to investigate whether they are altered in preeclampsia.

II. MATERIALS AND METHODS

Participants

Eighty-seven healthy pregnant women and 28 pregnant women with preeclampsia who gave live singleton births from September 2009 to February 2010 at the Department of Obstetrics and Gynecology of Yonsei University Health System were recruited. The criteria for inclusion were as follows: 1) well established gestational age confirmed by ultrasonography, 2) nonsmoker, 3) normal response to glucose tolerance testing, 4) no labor, 5) no infection, 6) no family history of vascular diseases, and 7) no other medico-surgical illness. Healthy pregnant women with each trimester showed a good glycemic control and normal blood pressure throughout pregnancy. The diagnosis of preeclampsia was based on the definitions set by the American College of Obstetricians and Gynecologists.[22] The diagnostic criteria for preeclampsia includes: 1) hypertension, defined as a blood pressure of 140 mm Hg systolic or higher or 90 mm Hg diastolic or higher and 2) proteinuria, defined as urinary excretion of 0.3 g protein or higher in a 24-hour urine specimen. Severe preeclampsia was defined as the presence of hypertension and proteinuria after the 20th week of pregnancy; blood pressure elevation with a systolic blood pressure of 160 mm Hg or higher or a diastolic blood pressure of 110 mm Hg or higher; and proteinuria greater than 1000 mg per 24 hours or a reading of at least 3+ on a dipstick was considered significant. At least 2 consecutive positive measurements on urinalysis were required for diagnosis. Patients with chronic hypertension predating the pregnancy or before 20 weeks of gestation were excluded from the study. Gestational ages were confirmed by ultrasonographic examination before 20 weeks in all cases. Written informed consent was obtained from all participants and the protocol for this study was approved by the Yonsei institutional review board.

Enzyme Linked Immunosorbent Assay

Blood samples were obtained from the peripheral vein of the arm of each woman. In women with second or third trimester, sampling was done at least 24 hours before a labor onset or cesarean section to eliminate the influence of labor or pain. In women with preeclampsia, sampling was done at the time of diagnosis, promptly. Blood samples were centrifuged immediately at 4000g for 10 minutes. Serum specimens were then frozen and kept at –70℃ until the analysis. All laboratory determinations were carried out in a blinded fashion. Measurements were performed using commercially available ELISA kits (for sRAGE assay, BioVendor, Modrice, Czech Republic and esRAGE assay, B-Bridge International, Sunnyvale, California, USA). In these ELISA assays, we used the manufacturer's instructions. For sRAGE, a polyclonal antibody against receptors were used to catch them. Trapped receptors were recognized by a antihuman receptor antibody. After washing, plates were incubated with streptavidin-horseradish peroxidase, developed with appropriate substrate, and OD_{450} were settled using an ELISA plate reader. For esRAGE, samples and esRAGE antibody horseradish peroxidase conjugated are incubated in an anti-RAGE antibody coated plate. After incubation and washing, substrate is added. After incubation, the enzymatic reaction is stopped and the plates are measured at wavelength 450 nm. Measurements were conducted in duplicate and the results were averaged. The calculated inter-assay coefficients of variation(CVs) for sRAGE and esRAGE immunoassays in our laboratory were 7.9% and 5.5%, respectively. Calculated intra-assay CVs for sRAGE and esRAGE were 5.3% and 2.6%, respectively.

Statistical Analysis

Comparisons between 2 groups were performed using Independent two sample t tests or Mann-Whitney rank-sum tests as appropriate. Multiple comparison procedures were performed using one-way or Kruskal-Wallis analysis of variance (ANOVA) followed by Bonferrony post-hoc comparisons as appropriate. Relationships between variables were

explored using Pearson's product moment. A p value <0.05 was considered statistically significant. All computations were performed using SPSS software version 18.0 (Chicago, USA).

III. RESULTS

Characteristics of Study Groups

Table 1 displays the demographic and clinical characteristics of 28 women with preeclampsia and 87 with physiologic pregnancy. Significant differences were observed in gestational age, cholesterol and creatinine level at the time of serum sampling between the two groups of pregnant women. As expected, blood pressures were significantly higher in the preeclamptic women than in the controls. By contrast, women with preeclampsia had significantly lower Apgar score, baby birth weight and higher cesarean section rate compared to healthy pregnant women.

Table 1. General characteristics

		Healthy pregnant	Preeclampsia	*p*
Number		87 (75.7%)	28 (24.3%)	
Age (years)		32.4 ± 3.5	32.3 ± 3.4	.890
Body mass index (kg/m2)		25.7 ± 3.7	27.3 ± 3.5	.076
Systolic blood pressure (mmHg)		115.2 ± 10.3	158.6 ± 16.1	<.001
Diastolic blood pressure (mmHg)		71.3 ± 9.0	98.7 ± 10.3	<.001
Gestational age at sampling (days)		174.2 ± 90.5	230.0 ± 41.2	<.001
	1st trimester	23	0	
	2nd trimester	25	7	
	3rd trimester	39	21	
Hematocrit (%)		36.8 ± 6.5	36.1 ± 3.7	.470
Platelet (1000/mm3)		235.7 ± 65.6	221.8 ± 71.1	.350
Cholesterol (mg/dL)		215.0 ± 66.8	279.2 ± 69.6	.001
Serum Creatinine (mg/dL)		0.6 ± 0.1	0.8 ± 0.2	.001
Urine Protein (Dipstick) (%)	0	87 (100.0%)	0 (0.0%)	<.001
	1+,2+	0	22 (100.0%)	
	3+,4+	0	6 (100.0 %)	
Parity (%)	0	44 (73.3%)	16 (26.7 %)	.331
	1	31 (73.8%)	11 (26.2 %)	
	2	12 (92.3%)	1 (7.7%)	

Values for mean ± standard deviation or the number and percentage of patients are shown.

Parameters were analyzed by independent two sample t-test.

Serum sRAGE, esRAGE Concentration and esRAGE/sRAGE Ratio in Physiologic Pregnancy

Serum esRAGE concentration and esRAGE/sRAGE ratio in 87 healthy pregnant women decreased during pregnancy (Figure 1A and B). There were significant differences in esRAGE concentration and esRAGE/sRAGE ratio between the 1st and 3rd trimester (156.4±70.3 pg/mL vs. 103.9±89.6 pg/mL and 0.60±0.39 vs. 0.24±0.18). Serum sRAGE concentration in healthy pregnant women varies during the pregnancy (Figure 1A). sRAGE serum concentration in the 2nd trimester increased compared to serum sRAGE levels in the 1st and 3rd trimester, but there was no significant difference.

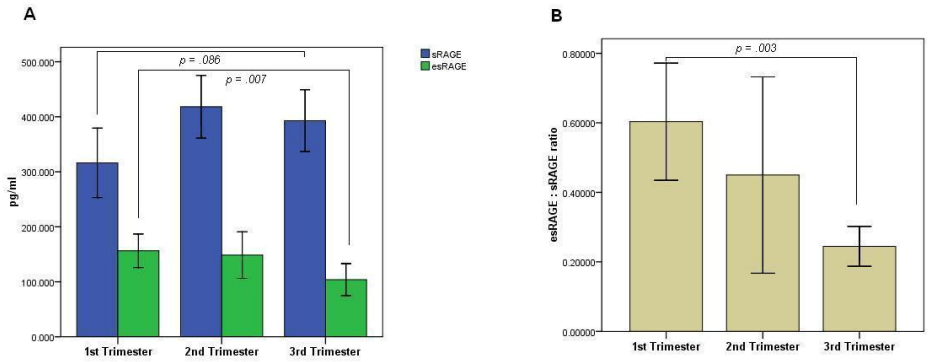

Figure 1. Serum sRAGE and esRAGE concentration (A) with esRAGEsRAGE ratio (B) in 1st, 2nd and 3rd trimester of physiologic pregnancy.

Serum sRAGE, esRAGE Concentration and esRAGE/sRAGE Ratio in Women with Preeclampsia Compared to Healthy Pregnant

The results of univariate and multivariate regression analysis of the characteristics associated with RAGE variants in all participants are shown in Table 2. Factors including gestational age and pregnancy with preeclampsia ($p < 0.02$) were entered into multivariate analysis. These 2 factors were the independent determinants of esRAGE concentration and esRAGE/sRAGE ratio. The serum esRAGE concentration in patients with preeclampsia significantly increased compared to healthy pregnant controls (211.9±183.1 pg/mL vs. 130.6±91.4 pg/mL, Figure 2A). Preeclamptic women had a significantly higher esRAGE/sRAGE ratio than the controls (0.60±0.48 vs. 0.40±0.45, Figure 2B). We did not find any differences in sRAGE concentration between physiologic pregnancy and preeclampsia (Figure 2A).

Table 2. Univariate and multivariate regression analysis of the factors associated with serum esRAGE and esRAGE/sRAGE ratio

	esRAGE			
	Univariate analysis		Multivariate analysis	
	coefficient	*p*	coefficient	*p*
Age(years)	-0.337	0.921		
Gestational Age (days)	-0.200	0.147	-0.501	0.023
Body mass index (kg/m^2)	-4.614	0.301		
Cholesterol (mg/dl)	0.277	0.321		
Creatinine (mg/dl)	135.866	0.170	46.038	0.648
Healthy pregnancy vs. Preeclampsia	81.249	0.031	173.544	0.012
	esRAGE/sRAGE ratio			
	Univariate analysis		Multivariate analysis	
	coefficient	*p*	coefficient	*p*
Age(years)	0.011	0.361		
Gestational Age (days)	-0.001	0.071	-0.002	0.006
Body mass index (kg/m^2)	-0.011	0.551		
Cholesterol (mg/dl)	-0.001	0.838		
Creatinine (mg/dl)	0.186	0.478		
Healthy pregnancy vs. Preeclampsia	0.201	0.047	0.253	0.018

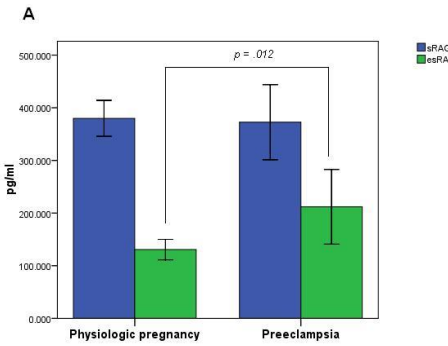

Figure 2. Serum sRAGE and esRAGE concentration (A) with esRAGEsRAGE ratio (B) in physiologic pregnancy and preeclampsia

IV. DISCUSSION

The major findings of this study are as follows. Maternal serum esRAGE concentration and esRAGE/sRAGE ratio: (1) gradually decrease with gestational age in physiologic pregnancy; and (2) are higher in patients with preeclampsia than healthy pregnant controls, significantly.

By bonding to AGEs, RAGE can provoke acute cellular Injury via activation of signal transduction and modulation of gene expression.[23-25] Several variants may arise from alternative truncated forms of RAGE.[26] The C-truncated variant is thought to be worthy of notice due to its potential significance in RAGE-mediated diseases. This isoform has been demonstrated to be released extracellularly as esRAGE and can be found in human sera. However, Geroldi et al.[27] suggested that esRAGE may not stand for the entire pool of soluble isoforms of RAGE that is located in the serum, because it is possible that some RAGE isoforms may be derived from proteolytic cleavage from the native membranous receptor by metalloproteinases. This hypothesis was initially described as occurring in mice and lots of studies have observed that RAGE signaling is blocked by a soluble cleaved form of the RAGE receptor titled sRAGE, which binds AGEs and restrict AGE effects.[28,29] Currently, several studies have reported a relationship between RAGE variants and metabolic disorders including diabetes, hypertension and atherosclerosis.[30-32]

In this study, we demonstrated alterations in serum esRAGE concentrations throughout physiologic pregnancy. With pregnancy, oxidative stress is predicted because of an increased oxygen requirement and high energy demand.[33] Measurements of markers of oxidative stress in maternal blood and urine explain that pregnancy is a condition of oxidative stress due to the high metabolic activity.[34,35] Some reports have demonstrated the increased oxidative stress markers in physiological pregnancy along with inflammatory molecules.[36,37] Activation of RAGE on endothelium, phagocytes, and lymphocytes triggers cellular activation with

generation of proinflammatory mediators.[38] Because of increased oxidative stress markers with inflammatory molecules throughout physiologic pregnancy, serum esRAGE concentration might be decreased inversely, as antioxidant defences mechanism. Similarly, Palm et al.[39] also reported that lipid-adjusted antioxidants, α- and γ-tocopherol, decreased with advancing gestational age in response to increased oxidative stress in vivo during pregnancy.

Our data also showed that the serum esRAGE concentrations were significantly elevated in preeclamptic women compared to healthy pregnant women. Oxidative stress in the placenta and maternal vascular system has been implicated as pathophysiological features of preeclampsia.[40] Therefore, elevated serum esRAGE concentrations in preeclampsia might be explained by the compensatory adaptation to neutralize increased oxidative stress including AGE-RAGE interaction. This hypothesis is supported by paper of Gohda et. al[41] that serum esRAGE levels may reflect tissue RAGE expression and be up-regulated as a countermeasure to prevent cell damage by AGEs. Also, western blotting revealed that the levels of AGEs and RAGE in preeclamptic placenta were significantly higher than that in normal placenta, previously.[42] The observed positive association between esRAGE and preeclampsia is in agreement with earlier studies. Fasshauer et al.[43] demonstrated that that maternal serum esRAGE concentrations are significantly increased in 16 patients with preeclampsia compared to 20 healthy pregnant women. In the present study, the association between esRAGE and preeclampsia is supported by parametric statistical analysis with adequate sample size calculation. Futhermore, this is the first study dealing with the proportion of serum esRAGE to total circulating soluble RAGE in normal pregnancy and preeclampsia.

Interestingly, we could not find any significant changes of serum total sRAGE concentration throughout normal pregnancy. In addition, we could not detect any correlation of sRAGE concentration with preeclampsia. Previously, Germanova et al.[21] demonstrated that raising

serum total sRAGE concentration up to peak, which occurs in about the 24^{th}-26^{th} weeks of pregnancy, due to overproduction and up–regulation of these RAGE variants in proinflammatory circumstances. They reported that patients with gestational hypertension or preeclampsia had significantly higher sRAGE levels compared to healthy pregnant women. The reasons for these discrepancies are not clear but may be explained by differences in the backgrounds of RAGE variants. In fact, serum total soluble RAGE levels, measured by ELISA, actually detect not only native endogenous secretary RAGE isoforms, but also the amount of soluble RAGE that probably results from the cleavage of the cell-surface receptor by metalloproteinases. The inverse association between levels of sRAGE and disease risk has been demonstrated in metabolic diseases including nephropathy and atherosclerosis, previously.[44,45] However, recently, several studies have explored the association between sRAGE and pathologic pregnancy and have drawn different conclusions.[46,47] On the other hand, esRAGE has been considered to reflect homogenously the cleaved-type native soluble RAGE, without the effect of metalloproteinases. Although the number of the subjects in this study was relatively not large, our findings suggest that the endogenous action of sRAGE and esRAGE remain unknown and it is possible that these receptors have different roles in physiologic pregnancy or preeclampsia. Our data are in line with previous reports that circulating sRAGE and esRAGE levels may be under the control of different mechanisms and it is likely that circulating sRAGE and esRAGE levels are distinct markers.[48] Therefore, insignificant changes of serum sRAGE in physiologic and pathologic pregnancy in our study suggests that sRAGE acting as a potential biological marker for oxidative stress in pregnancy needs more supports of laboratory evidences. To date, no study has been investigated to compare head-to-head the value of total circulating soluble RAGE versus esRAGE, simultaneously. This is the first comparative study of sRAGE and esRAGE as markers of preeclampsia. Although it is not certain whether upregulation of serum esRAGE in

preeclampsia is a cause or consequence of this disorder, increased esRAGE and esRAGE/sRAGE ratio may be predictive markers for preeclampsia. Here, it remains to be determined to how circulating esRAGE concentration is regulated during normal and complicated pregnancy.

Some limitations should be considered in this study. First, we could not measure the levels of RAGE variants from each subject continuously throughout pregnancy before and after the development of preeclampsia. Second, it was not a population-based study. The sample size was not large enough to make definitive conclusions. Third, we did not divide the subjects by severity of preeclampsia due to the restricted number of patients.

V. CONCLUSION

In conclusion, we showed that serum esRAGE concentrations and the esRAGE/sRAGE ratio were significantly decreased throughout physiologic pregnancy, for the first time. Moreover, we demonstrated that the esRAGE and esRAGE/sRAGE ratio may be considered an important mechanism in developing preeclampsia. Further studies with a larger sample size are warranted to prove the significance of RAGE variants.

REFERENCES

1. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med.* 1988;318:315-21.

2. Kirstein M, Brett J, Radoff S, Ogawa S, Stern D, Vlassara H. Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: role in vascular disease of diabetes and aging. *Proc Natl Acad Sci U S A.* 1990;87:9010-4.

3. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascularlesions. *Arterioscler Thromb.* 1994;14:1521-8.

4. Rodriguez-Garcia J, Requena JR, Rodriguez-Segade S. Increased concentrations of serum pentosidine in rheumatoid arthritis. *Clin Chem.* 1998;44:250-5.

5. Schmidt AM, Yan SD, Wautier JL, Stern DM. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res.* 1999;84:489-97.

6. Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. *Biochim Biophys Acta.* 2000;1498:99-111.

7. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia.* 2001;44:129-46.

8. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest.* 2001;108:949-55.

9. Schmidt, AM, Vianna M, Gerlach M, et al. Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the

endothelial cell surface. *J Biol Chem.* 1992;267;14987 -97.

10. Schmidt AM, Mora R, Cap R, et al. The endothelial cell binding site for advanced glycation end products consists of a complex: an integral membrane protein and a lactoferrin-like polypeptide. *J Biol Chem.* 1994;269:9882-8.

11. Park IH, Yeon SI, Youn JH, et al. Expression of a novel secreted splice variant of the receptor for advanced glycation end products (RAGE) in human brain astrocytes and peripheral blood mononuclear cells. *Mol Immunol.* 2004;40:1203-11.

12. Schlueter C, Hauke S, Flohr AM, Rogalla P, Bullerdiek J. Tissue-specific expression patterns of the RAGE receptor and its soluble forms--a result of regulated alternative splicing? *Biochim Biophys Acta.* 2003;1630:1-6.

13. Yonekura H, Yamamoto Y, Sakurai S, et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J.* 2003;370:1097-9.

14. Falcone C, Emanuele E, D'Angelo A, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. *Arterioscler Thromb Vasc Biol.* 2005;25:1032-7.

15. Nakamura K, Yamagishi SI, Adachi H, et al. Elevation of soluble form of receptor for advanced glycation end products (sRAGE) in diabetic subjects with coronary artery disease. *Diabetes Metab Res Rev.* 2007;23:368-71.

16. Marcovecchio ML, Giannini C, Dalton RN, Widmer B, Chiarelli F, Dunger DB. Reduced endogenous secretory receptor for advanced glycation end products (esRAGE) in young people with Type 1 diabetes developing microalbuminuria. *Diabet Med.* 2009;26:815-9.

17. Koyama H, Shoji T, Yokoyama H, et al. Plasma level of endogenous secretory RAGE is associated with components of the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2005;25:2587-93.

18. Cooke CL, Brockelsby JC, Baker PN, Davidge ST. The receptor for advanced glycation end products (RAGE) is elevated in women with preeclampsia. *Hypertens Pregnancy.* 2003;22:173–184.

19. Nakatsuka M, Takata M, Tada K, et al. A long-term transdermal nitric oxide donor improves uteroplacental circulation in women with preeclampsia. *J Ultrasound Med.* 2002;21:831-6.

20. Williams MA, Mahomed K, Farrand A, et al. Plasma tumor necrosis factor-alpha soluble receptor p55 (sTNFp55) concentrations in eclamptic, preeclamptic and normotensive pregnant Zimbabwean women. *J Reprod Immunol.* 1998;40:159–73.

21. Germanová A, Koucký M, Hájek Z, Parízek A, Zima T, Kalousová M. Soluble receptor for advanced glycation end products in physiological and pathological pregnancy. *Clin Biochem*. 2010;43:442–6.

22. American College of Obstetricians and Gynecologists Committee on Obstetric Practice. Diagnosis and management of preeclampsia and eclampsia. *Int J Gynecol Obstet.* 2002;77:67-75 [ACOG practice bulletin].

23. Rong LL, Yan SF, Wendt T, et al. RAGE modulates peripheral nerve regeneration via recruitment of both inflammatory and axonal outgrowth pathways. *FASEB J.* 2004;18:1818 – 25.

24. Bucciarelli LG, Kaneko M, Ananthakrishnan R, et al. Receptor for advanced-glycation end products: key modulator of myocardial ischemic injury. *Circulation.* 2006;113:1226-34.

25. Sakaguchi T, Yan SF, Yan SD, et al. Arterial restenosis: central role of RAGE dependent neointimal expansion. *J Clin Invest*. 2003;111:959 –72.

26. Hudson BI, Carter AM, Harja E, et al. Identification, classification, and expression of RAGE gene splice variants. *FASEB J.* 2008;22:1572-80.

27. Geroldi D, Falcone C, Emanuele E. Soluble receptor for advanced glycation end

products: from disease marker to potential therapeutic target. *Curr Med Chem.* 2006;13:1971–8.

28. Hanford LE, Enghild JJ, Valnickova Z, et al. Purification and characterization of mouse soluble receptor for advanced glycation end products (sRAGE). *J Biol Chem.* 2004;279:50019-24.

29. Yamagishi S, Matsui T. Soluble form of a receptor for advanced glycation end products (sRAGE) as a biomarker. *Front Biosci.* 2010;2:1184-95.

30. Pullerits R, Bokarewa M, Dahlberg L, Tarkowski A. Decreased levels of soluble receptor for advanced glycation end products in patients with rheumatoid arthritis indicating deficient inflammatory control. *Arthritis Res Ther.* 2005;7:817-24.

31. Geroldi D, Falcone C, Emanuele E, et al. Decreased plasma levels of soluble receptor for advanced glycation end-products in patients with essential hypertension. *Hypertens.* 2005;23:1725-9.

32. Yamamoto M, Yamaguchi T, Yamauchi M, Sugimoto T. Low serum level of the endogenous secretory receptor for advanced glycation end products (esRAGE) is a risk factor for prevalent vertebral fractures independent of bone mineral density in patients with type 2 diabetes. *Diabetes Care.* 2009;32:2263-8.

33. Fialová L, Malbohan I, Kalousová M, et al. Oxidative stress and inflammation in pregnancy. *Scand J Clin Lab Invest.* 2006;66:121–7.

34. Toescu V, Nuttall SL, Martin U, Kendall MJ, Dunne F. Oxidative stress and normal pregnancy. *Clin Endocrinol (Oxf).* 2002;57:609–13.

35. Morris JM, Gopaul NK, Endresen MJ, et al. Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclampsia. *Br J Obstet Gynaecol*. 1998;105:1195–9.

36. Arikan S, Konukoglu D, Arican C, Akcay T, Davas T. Lipid peroxidation and antioxidant

status in maternal and cord blood. *Gynecol Obstet Invest*. 2001;51:145–9.

37. Belo L, Santos-Silva A, Rocha S, et al. Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. *Eur J Obstet Gynecol Reprod Biol*. 2005;123:46–51.

38. Hofmann MA, Drury S, Fu C, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell.* 1999;97:889-901.

39. Palm M, Axelsson O, Wernroth L, Basu S. F(2)-isoprostanes, tocopherols and normal pregnancy. *Free Radic Res.* 2009;43:546–52.

40. Takagi Y, Nikaido T, Toki T, et al. Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. *Virchows Arch.* 2004;444:49-55.

41. Gohda T, Tanimoto M, Moon JY, et. al. Increased serum endogenous secretory receptor for advanced glycation end-product (esRAGE) levels in type 2 diabetic patients with decreased renal function. *Diabetes Res Clin Pract.* 2008;81:196-201.

42. Chekir C, Nakatsuka M, Noguchi S, et al. Accumulation of advanced glycation end products in women with preeclampsia: possible involvement of placental oxidative and nitrative stress. *Placenta*. 2006;27:225-33.

43. Fasshauer M, Seeger J, Waldeyer T, et al. Endogenous soluble receptor for advanced glycation endproducts is increased in preeclampsia. *J Hypertens*. 2008,26:1824–8.

44. Emanuele E, D'Angelo A, Tomaino C, et al. Circulating levels of soluble receptor for advanced glycation end products in Alzheimer disease and vascular dementia. *Arch Neurol.* 2005;62:1734-6.

45. Basta G, Leonardis D, Mallamaci F, et al. Circulating soluble receptor of advanced glycation end product inversely correlates with atherosclerosis in patients with chronic kidney disease. *Kidney Int.* 2010;77:225–31.

46. Pertyńska-Marczewska M, Głowacka E, Sobczak M, Cypryk K, Wilczyński J. Glycation

Endproducts, Soluble Receptor for Advanced Glycation Endproducts and Cytokines in Diabetic and Non-diabetic Pregnancies. *Am J Reprod Immunol*. 2009;61:175-82.

47. Hájek Z, Germanová A, Koucký M, et al. Detection of feto-maternal infection/inflammation by the soluble receptor for advanced glycation end products (sRAGE): results of a pilot study. *J Perinat Med*. 2008;36:399–404.

48. Katakami N, Matsuhisa M, Kaneto H, et al. Endogenous secretory RAGE but not soluble RAGE is associated with carotid atherosclerosis in type 1 diabetest patients. *Diabetes Vasc Dis Res*. 2008;5:190–7.

ABSTRACT(IN KOREAN)

정상 임신 및 전자간증에서의 sRAGE 와 esRAGE 의 임상적 유용성

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Endogenous secretory receptor of advanced glycation end products (esRAGE) 와 soluble RAGE (sRAGE) 는 대사 증후군의 한 요소이며 전자간증과도 연관되어 있다고 알려져 있다. 본 연구에서는 정상 임신과 전자간증 산모에서의 혈청 내 esRAGE 와 sRAGE 농도 및 esRAGE/sRAGE 비를 알아내고자 하였다. 87명의 정상 산모와 28명의 전자간증 산모를 대상으로 ELISA 법을 이용하여 혈청 내 sRAGE 와 esRAGE 농도를 조사하였다. 정상 산모에서 1분기와 3분기 사이에는 esRAGE 농도 및 sRAGE/sRAGE 비의 유의한 차이가 있었다. (*p = .007 and p = .003*). 전자간증 산모에서 혈청 내 esRAGE 농도와 esRAGE/sRAGE 비는 정상 산모와 비교하여 유의하게 증가하였다. (*p = .012 and p = .018*). 정상 임신에서의 증가된 oxidative stress markers with inflammatory molecules 로 인하여 혈청 내 esRAGE 농도는 antioxidant defences mechanism 으로 작용하여 역으로 감소하는 것을 추정해 볼 수 있다. 전자간증 산모에서 증가된 혈청 내 esRAGE 및 esRAGE/sRAGE 비율은 oxidative stress 에 대한 compensatory adaptation 으로 해석된다.

핵심되는 말 : sRAGE, esRAGE, 전자간증