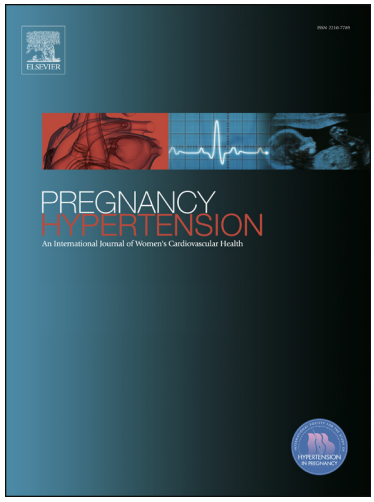

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The Effect of Low-Dose Aspirin On Serum Placental Growth Factor Levels In a High-Risk PREDO Cohort

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Abstract

Objectives

Our first aim was to study the longitudinal changes of serum placental growth factor (PlGF) concentration between 12^{+0} to 28^{+0} weeks of gestation in the prospective PREDO cohort. Our second aim was to study the effect of low-dose acetylsalicylic acid (LDA; 100mg/day), started before the 14^{th} week of gestation, on PlGF concentration.

Study design

Blood samples were collected at 12^{+0}-14^{+0}, 18^{+0}-20^{+0} and 26^{+0}-28^{+0} weeks of gestation in 101 women without and 309 with clinical risk factors for pre-eclampsia. Study cohort was divided into seven groups according to risk, treatment (no prevention/placebo/LDA) and outcome measure pre-eclampsia. Longitudinal changes in the PlGF concentration between groups were compared. To investigate the effect of LDA on serum PlGF concentration, placebo (N=62) and LDA (N=61) groups were compared. A repeated measures ANOVA was used to analyze differences in PlGF levels between the groups.

Results

The increase in serum PlGF concentration was higher in LDA than in placebo group (time×group effect, p=0.046). The increase in serum PlGF concentration during pregnancy was lower in risk-women who developed pre-eclampsia and in high-risk women who had placebo and developed pre-eclampsia compared to the other women (time×group effect, p<0.001). There were no differences in PlGF change between low-risk women, risk-women who did not develop pre-eclampsia, high-risk women in the placebo group without pre-eclampsia and high-risk women in the LDA group with and without pre-eclampsia (p=0.15).

Conclusions

Our finding suggests an association between LDA started before 14 weeks of gestation and higher increase in serum PlGF concentration.

Keywords

Pre-eclampsia, placental growth factor, low-dose acetylsalicylic acid, aspirin

Abbreviations

PlGF = placental growth factor, LDA = low-dose acetylsalicylic acid, Uta-PI = uterine artery pulsatility index, ANOVA = one-way analyses of variance, BMI = body mass index, MAP = mean arterial pressure, mmHg = millimeter of mercury

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Introduction

Pre-eclampsia is a hypertensive pregnancy disorder that affects 2-8% of pregnant women. It is a major cause of maternal and fetal morbidity and mortality [1, 2]. Blood concentration of placental growth factor (PlGF) is lower already in the early pregnancy in women who will later develop pre-eclampsia [3, 4] and therefore it is a

promising biomarker, especially for early-onset pre-eclampsia and when combined with other biomarkers and biophysical measurements [5-7]. Low-dose acetylsalicylic acid (LDA) started at 12-16 weeks of gestation reduces the risk of pre-eclampsia [8-10]. Our aim was to study maternal serum PlGF concentration in a prospective cohort of women with clinical risk factors for pre-eclampsia and of low-risk control women, and the effect of LDA (100mg/day), started in early pregnancy, on serum PlGF concentration.

Methods

The present study is a part of the multidisciplinary 'Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction' (PREDO) Project [11]. The study cohort was collected prospectively between September 2005 and December 2009 in ten participating hospital maternity clinics in Finland. The inclusion and exclusion criteria are listed in Supplementary Table 1 and the flow chart of the study is presented in Supplementary Figure 1. Originally 1082 women were recruited, 972 women that fulfilled the inclusion criteria, which had predetermined risk factors for pre-eclampsia, and 110 randomly selected women without known risk factors for pre-eclampsia as a low-risk reference group. All participants had first trimester visit at 12^{+0}-14^{+0} weeks of gestation. The bilateral waveform of uterine artery blood flow was determined and uterine artery pulsatility index (Uta-PI) was measured with Doppler ultrasound examination. Fasting blood sample was collected. During the study period, a total of 45 (4.2%) recruited women were later excluded for various reasons [10].

Of 972 women with risk factors for pre-eclampsia (inclusion criteria in Supplementary Table 1) 142 were randomized to a placebo controlled LDA-trial [10].

The inclusion criterion to the LDA-trial was bilateral second-degree diastolic notch in the uterine artery Doppler ultrasound, as a reflection of increased resistance to blood flow and therefore, higher risk, performed at the first visit. All women participating in the LDA-trial were included in this substudy and were defined as high-risk. Reference groups composed of 202 women with risk factors and without bilateral second-degree notch and 110 women without known risk factors for pre-eclampsia. All gave blood samples in three time points of pregnancy: at 12^{+0} to 14^{+0}, 18^{+0} to 20^{+0} and 26^{+0} to 28^{+0} weeks of gestation. Blood samples were drawn from antecubital vein and serum was separated within an hour by centrifugation and stored in -80°C until analysis. Serum PlGF concentration was measured with AutoDelfia analyzer (Wallac, PerkinElmer, Turku, Finland).

Primary outcome was pre-eclampsia, defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg occurring after 20 weeks of gestation in a woman with previously normal blood pressure combined with a urinary 24-hour protein excretion of ≥ 0.3 g or the dipstick equivalent in two consecutive measurements [12]. Pre-eclampsia superimposed on chronic hypertension were included in the primary outcome. Secondary outcomes were early-onset pre-eclampsia (diagnosed before 34^{+0} weeks of gestation) and severe pre-eclampsia (systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg and/or proteinuria ≥ 5 g/24 hours). All diagnoses were independently confirmed by a jury of two physicians and one research nurse, as earlier described [10]. Ethics Committee of the Helsinki and Uusimaa Hospital District approved the study and written informed consent was obtained from all participants.

To study the changes in serum PlGF concentration in longitudinal samples according to outcome measure pre-eclampsia, the cohort was divided in 7 subgroups

as follows: **group 1**: low-risk women (no known risk factors) (N=101), **group 2:** risk-women (=risk factors but no bilateral second-degree diastolic notch) who did not develop pre-eclampsia (N=163), **group 3**: risk-women (=risk factors but no bilateral second-degree diastolic notch) who developed pre-eclampsia (N=23), **group 4**: high-risk women (=bilateral second-degree diastolic notch; LDA-trial participants) who had placebo treatment and did not develop pre-eclampsia (N=51), **group 5**: high-risk women (=bilateral second-degree diastolic notch; LDA-trial participants) who had placebo treatment and developed pre-eclampsia (N=11), **group 6**: high-risk women (=bilateral second-degree diastolic notch; LDA-trial participants) who had LDA treatment and did not develop pre-eclampsia (N=55) and **group 7**: high-risk women (=bilateral second-degree diastolic notch; LDA-trial participants) who had LDA treatment and developed pre-eclampsia (N=6) (Supplementary Figure 1).

To investigate the effect of LDA on PlGF concentration and changes in PlGF concentration in the longitudinal samples we compared the high-risk women who were randomized to the placebo group (n=62) (groups 4 and 5) or to LDA (n=61) group (groups 6 and 7) (Supplementary Figure 2).

Statistical methods

The difference of means in normally distributed baseline variables between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. Kruskal-Wallis test was used in case the data was not normally distributed. Comparison of medians of different non-normally distributed variables was done with Mann-Whitney U test. Bonferroni corrections were used in post-hoc comparisons. Categorical variables were compared with chi-square test.

The repeated measures analysis of variance (ANOVA) was used to analyze differences in serum PlGF levels between the groups. The model included the main effects of time, group and the interaction effect time×group. Log-transformed PlGF values were used in these analyses due to the positively skewed distribution. The comparisons between LDA and placebo groups were adjusted for pre-pregnancy body mass index (BMI), chronic hypertension and first trimester mean arterial pressure (MAP). Results of PIGF values are expressed as geometric means (95% confidence intervals). In all analyses p-value < 0.05 was considered statistically significant. Statistical analyses were done with SPSS for Windows version 24 (IBM Corp., Armonk, NY).

Results

Clinical characteristics of all study groups are presented in Supplementary Table 2 and pregnancy characteristics in Supplementary Table 3. There were 30 pre-eclampsia cases (7.3%), 10 (2.4%) women had early-onset pre-eclampsia, and 17 (4.1%) had severe form of the disease.

The geometric means of PlGF concentration in each group are presented in Table 1. Change in PlGF concentration was lower in risk-women who developed pre-eclampsia (Group 3) and in high-risk women who had placebo treatment and developed pre-eclampsia (Group 5) compared to all other groups ($p<0.001$) (Figure 1 and Table 2). The change in PlGF concentration between the other groups did not differ in three different time points during pregnancy ($p=0.15$) (Figure 1 and Table 2).

Clinical characteristics of three risk-women groups (risk-women, placebo and LDA) are presented in Supplementary Table 4 and pregnancy characteristics in

Supplementary Table 5. Women in placebo and LDA groups had second-degree bilateral diastolic notch in the uterine artery flow and they had the highest mean Uta-PI in early pregnancy compared to the rest of the study cohort.

The adjusted geometric means of PlGF concentration and longitudinal changes in the PlGF concentration between LDA and placebo groups are presented in Table 3 and Table 4, Figure 2. After adjustment for BMI, chronic hypertension and MAP, the change in PlGF concentration was lower in the placebo group than in the LDA group (interaction effect time×group, p=0.046), particularly change between sample 2 (18^{+0}-20^{+0} weeks of gestation) and 3 (26^{+0}-28^{+0} weeks of gestation) differed in further analysis (placebo 104.9 ηg/ml vs. LDA 174.5 ηg/ml, interaction effect time×group, p=0.043). When pre-eclampsia was added as a covariant into the repeated measurements model, it did not change the result regarding the effect of LDA on serum PlGF concentration (interaction effect time×group, p=0.046 vs. p=0.047).

Conclusion and discussion

To our best knowledge, this is the first study to show the association between LDA started before 14 weeks of gestation and higher increase in serum PlGF concentration in women with high-risk for pre-eclampsia during pregnancy.

The strength of our study is a carefully characterized prospective cohort, wherein all diagnoses were confirmed from hospital records by a jury of two physicians and a study nurse. The effect of LDA on serum PlGF concentrations was studied in prospective high-risk cohort with double-blinded placebo controlled study design. However, the sample size was relatively small. Therefore, these findings need to be confirmed in a larger prospective cohort of pregnant women.

The cause of pre-eclampsia is unknown, but there is strong evidence that impaired placentation is one pathophysiological mechanism [13] and more associated with early-onset and severe pre-eclampsia [14, 15] . PlGF is a member of vascular growth factor family and secreted predominantly by the trophoblasts during pregnancy. The exact role of PlGF in pregnancy is not known, but it seems to be involved in placental growth and differentiation [16] and with an other pro-angiogenic factor, vascular endothelial growth factor (VEGF) and their anti-angiogenic counterpart, soluble fms-like tyrosine kinase-1 (sFlt-1), forms a complicated system that maintains normal endothelial structure and function [17, 18].

In screening population median serum PlGF concentration has a curvilinear relationship with gestational age. Maternal serum PlGF concentration increases in the first and second trimester, reaching a maximum level at around 30 weeks and subsequently decreases [19]. Women who develop pre-eclampsia have lower serum PlGF concentration already in the early pregnancy compared to women with normal pregnancies [20] although this difference becomes more clear with advancing pregnancy [3, 21, 22]. Low PlGF concentration is thought to reflect abnormal placental angiogenesis [23]. The timing and the site of the abnormal placentation may contribute to the pregnancy outcome [24].

In the present study the increase in PlGF concentration was lower in the risk-women with pre-eclampsia without any treatment and in high-risk women who developed pre-eclampsia in the placebo group compared to the other groups. This is in accordance with earlier studies, in which serum or plasma levels of PlGF are reduced in pregnancies complicated by pre-eclampsia [3, 4], especially in the early-onset disease [21].

Bilateral high resistance indices (PI and resistance index) of the uterine arteries are also associated with impairment in placentation [25, 26]. High-risk women in the placebo group who developed pre-eclampsia had higher Uta-PI than risk-women who developed pre-eclampsia (p=0.001) already at the recruitment due to the inclusion criteria (bilateral second-degree diastolic notch in Doppler ultrasound examination of uterine artery flow) of the LDA-trial. Altogether, lower serum PlGF levels and higher Uta-PI of high-risk women with pre-eclampsia in placebo group, compared with risk-women with pre-eclampsia, probably were due to more earlier or profound impairment of placentation of high-risk women in placebo group with pre-eclampsia than in risk-women with pre-eclampsia [24].

Increasing evidence suggests that LDA treatment during pregnancy reduces the risk of developing pre-eclampsia [27, 28]. However, there is still ongoing debate over the timing and dose of LDA treatment [29]. A recent prospective, double-blinded placebo-controlled study on women with high risk for pre-eclampsia showed clear evidence, that LDA 150mg per day started at 11-14 weeks of gestation reduces the risk of developing preterm pre-eclampsia resulting in delivery before 37 weeks of gestation and early-onset pre-eclampsia (delivery before 34 weeks of gestation) even more efficiently [30].

It has been suggested that LDA facilitates early placental embedding and acts as a vasodilator by increasing prostacyclin production. LDA may also have direct effect on platelets or it may decrease endothelial dysfunction by other mechanisms [29]. It may have indirect effect on PlGF concentration by dose-dependently inhibiting sFlt-1 production in cytotrophoblasts via cyclo-oxygenase-1 inhibition [31]. Recently, Panagodage et al. showed in an *in vitro* model of early-onset pre-eclampsia, that LDA modulates the production of cytokines and improves trophoblast function. By that

means LDA may increase trophoblast secretion of PlGF. LDA may also restore abnormal production of cytokines by trophoblasts, induced by pre-eclampsia serum, to levels observed in normotensive serum, and may, as well, attenuate abnormal trophoblast apoptosis and growth caused by pre-eclampsia serum [32]. This could mean that lower increase of serum PlGF levels, more evident from second trimester onward in pre-eclampsia pregnancies, reflects abnormal trophoblast function, and is partly reversible by LDA treatment. In the present study the effect of LDA on serum PlGF levels was evident from mid-gestation onward. This provides a further rationale to arguments that LDA treatment should be continued to the late third trimester.

This is the first study to show association between daily LDA started before 14 weeks of gestation and higher longitudinal increase in serum PlGF concentration in high-risk women during pregnancy. This finding may also be associated with the reduced risk of developing pre-eclampsia in high-risk women.

References

[1] L. Duley, The global impact of pre-eclampsia and eclampsia, Semin Perinatol 33(3) (2009) 130-7.

[2] L. Say, D. Chou, A. Gemmill, Ö. Tunçalp, A.B. Moller, J. Daniels, A.M. Gülmezoglu, M. Temmerman, L. Alkema, Global causes of maternal death: a WHO systematic analysis, Lancet Glob Health 2(6) (2014) 323-33.

[3] R.J. Levine, S.E. Maynard, C. Qian, K.H. Lim, L.J. England, K.F. Yu, E.F. Schisterman, R. Thadhani, B.P. Sachs, F.H. Epstein, B.M. Sibai, V.P. Sukhatme, S.A. Karumanchi, Circulating angiogenic factors and the risk of preeclampsia, N Engl J Med 350(7) (2004) 672-83.

[4] C.E. Kleinrouweler, M.M. Wiegerinck, C. Ris-Stalpers, P.M. Bossuyt, J.A. van der Post, P. von Dadelszen, B.W. Mol, E. Pajkrt, E.C. Collaboration, Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta-analysis, BJOG 119(7) (2012) 778-87.

[5] F. Crovetto, F. Figueras, S. Triunfo, F. Crispi, V. Rodriguez-Sureda, C. Dominguez, E. Llurba, E. Gratacós, First trimester screening for early and late preeclampsia based

on maternal characteristics, biophysical parameters, and angiogenic factors, Prenat Diagn 35(2) (2015) 183-91.

[6] R. Akolekar, A. Syngelaki, L. Poon, D. Wright, K.H. Nicolaides, Competing risks model in early screening for preeclampsia by biophysical and biochemical markers, Fetal Diagn Ther 33(1) (2013) 8-15.

[7] N. O'Gorman, D. Wright, A. Syngelaki, R. Akolekar, A. Wright, L.C. Poon, K.H. Nicolaides, Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation, Am J Obstet Gynecol 214(1) (2016) 103.e1-103.e12.

[8] S. Roberge, P. Villa, K. Nicolaides, Y. Giguère, M. Vainio, A. Bakthi, A. Ebrashy, E. Bujold, Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis, Fetal Diagn Ther 31(3) (2012) 141-6.

[9] S. Roberge, K.H. Nicolaides, S. Demers, P. Villa, E. Bujold, Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis, Ultrasound Obstet Gynecol 41(5) (2013) 491-9.

[10] P.M. Villa, E. Kajantie, K. Räikkönen, A.K. Pesonen, E. Hämäläinen, M. Vainio, P. Taipale, H. Laivuori, Aspirin in the prevention of pre-eclampsia in high-risk women: a randomised placebo-controlled PREDO Trial and a meta-analysis of randomised trials, BJOG 120(1) (2013) 64-74.

[11] P. Girchenko, E. Hämäläinen, E. Kajantie, A.K. Pesonen, P. Villa, H. Laivuori, K. Räikkönen, Cohort Profile, Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction (PREDO) study, Int J Epidemiol (2016) 1-9.

[12] ACOG Practice Bulletin No. 33: Diagnosis and Management of Preeclampsia and Eclampsia, Obstetrics & Gynecology 99(1) (2002) 159-167.

[13] I. Brosens, R. Pijnenborg, L. Vercruysse, R. Romero, The "Great Obstetrical Syndromes" are associated with disorders of deep placentation, Am J Obstet Gynecol 204(3) (2011) 193-201.

[14] A.C. Staff, S.J. Benton, P. von Dadelszen, J.M. Roberts, R.N. Taylor, R.W. Powers, D.S. Charnock-Jones, C.W. Redman, Redefining preeclampsia using placenta-derived biomarkers, Hypertension 61(5) (2013) 932-42.

[15] J.W. Meekins, R. Pijnenborg, M. Hanssens, I.R. McFadyen, A. van Asshe, A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies, Br J Obstet Gynaecol 101(8) (1994) 669-74.

[16] K. Chau, A. Hennessy, A. Makris, Placental growth factor and pre-eclampsia, J Hum Hypertens 31(12) (2017) 782-786.

[17] M. Mandalà, N. Gokina, C. Barron, G. Osol, Endothelial-derived hyperpolarization factor (EDHF) contributes to PlGF-induced dilation of mesenteric resistance arteries from pregnant rats, J Vasc Res 49(1) (2012) 43-9.

[18] C.W. Redman, A.C. Staff, Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity, Am J Obstet Gynecol 2015 Oct;213(4 Suppl) (2015) S9-S12.

[19] A. Tsiakkas, N. Duvdevani, A. Wright, D. Wright, K.H. Nicolaides, Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history, Ultrasound Obstet Gynecol 45(5) (2015) 591-8.

[20] R. Thadhani, W.P. Mutter, M. Wolf, R.J. Levine, R.N. Taylor, V.P. Sukhatme, J. Ecker, S.A. Karumanchi, First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia, J Clin Endocrinol Metab 89(2) (2004) 770-5.

[21] P.M. Villa, E. Hämäläinen, A. Mäki, K. Räikkönen, A.K. Pesonen, P. Taipale, E. Kajantie, H. Laivuori, Vasoactive agents for the prediction of early- and late-onset preeclampsia in a high-risk cohort, BMC Pregnancy Childbirth 13 (2013) 110.

[22] L.C. Chappell, S. Duckworth, P.T. Seed, M. Griffin, J. Myers, L. Mackillop, N. Simpson, J. Waugh, D. Anumba, L.C. Kenny, C.W. Redman, A.H. Shennan, Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study, Circulation 128(19) (2013) 2121-31.

[23] R.N. Taylor, J. Grimwood, R.S. Taylor, M.T. McMaster, S.J. Fisher, R.A. North, Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies, Am J Obstet Gynecol 188(1) (2003) 177-82.

[24] B. Huppertz, Placental origins of preeclampsia: challenging the current hypothesis, Hypertension 51(4) (2008) 970-5.

[25] P. Olofsson, R.N. Laurini, K. Marsál, A high uterine artery pulsatility index reflects a defective development of placental bed spiral arteries in pregnancies complicated by hypertension and fetal growth retardation, Eur J Obstet Gynecol Reprod Biol 49(3) (1993) 161-8.

[26] F. Prefumo, N.J. Sebire, B. Thilaganathan, Decreased endovascular trophoblast invasion in first trimester pregnancies with high-resistance uterine artery Doppler indices, Hum Reprod 19(1) (2004) 206-9.

[27] S. Roberge, S. Demers, E. Bujold, Antiplatelet therapy before or after 16 weeks' gestation for preventing preeclampsia, Am J Obstet Gynecol 216(6) (2017) 620-621.

[28] S. Meher, L. Duley, K. Hunter, L. Askie, Antiplatelet therapy before or after 16 weeks' gestation for preventing preeclampsia: an individual participant data meta-analysis, Am J Obstet Gynecol 216(2) (2017) 121-128.e2.

[29] S. Tong, B.W. Mol, S.P. Walker, Preventing preeclampsia with aspirin: does dose or timing matter?, Am J Obstet Gynecol 216(2) (2017) 95-97.

[30] D.L. Rolnik, D. Wright, L.C. Poon, N. O'Gorman, A. Syngelaki, C. de Paco Matallana, R. Akolekar, S. Cicero, D. Janga, M. Singh, F.S. Molina, N. Persico, J.C. Jani, W. Plasencia, G. Papaioannou, K. Tenenbaum-Gavish, H. Meiri, S. Gizurarson,

K. Maclagan, K.H. Nicolaides, Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia, N Engl J Med 377(7) (2017) 613-622.

[31] C. Li, N.S. Raikwar, M.K. Santillan, D.A. Santillan, C.P. Thomas, Aspirin inhibits expression of sFLT1 from human cytotrophoblasts induced by hypoxia, via cyclo-oxygenase 1, Placenta 36(4) (2015) 446-53.

[32] S. Panagodage, H.E. Yong, F. Da Silva Costa, A.J. Borg, B. Kalionis, S.P. Brennecke, P. Murthi, Low-Dose Acetylsalicylic Acid Treatment Modulates the Production of Cytokines and Improves Trophoblast Function in an in Vitro Model of Early-Onset Preeclampsia, Am J Pathol 186(12) (2016) 3217-3224.

Table 1. The geometric means of serum PlGF levels of study groups in three different sampling points during pregnancy.

Weeks of Gestation/	**12-14**	95% CI		**18-20**	95% CI		**26-28**	95% CI	
PlGF concentration[a]	**ηg/ml**	Lower	Upper	**ηg/ml**	Lower	Upper	**ηg/ml**	Lower	Upper
Low-risk women (Group 1)	**24.9**	23.3	26.7	**120.5**	109.3	132.9	**322.2**	287.5	361.1
Risk-women, no PE (Group 2)	**24.6**	23.0	26.3	**111.5**	103.4	120.2	**299.4**	269.1	333.1
Risk-women, PE (Group 3)	**23.7**	19.8	28.3	**108.5**	87.5	134.4	**133.8**	91.6	195.3
Placebo, no PE (Group 4)	**21.9**	19.9	24.0	**100.2**	85.9	116.9	**228.8**	185.1	282.9
Placebo, PE (Group 5)	**20.0**	15.8	25.4	**62.3**	30.3	128.1	**75.9**	27.1	212.2
LDA, no PE (Group 6)	**22.4**	20.4	24.6	**109.2**	93.4	127.6	**267.9**	224.6	319.6
LDA, PE (Group 7)	**28.0**	22.0	35.6	**93.7**	51.6	170.1	**220.8**	125.6	388.2

a geometric means

PlGF=placental growth factor, PE=pre-eclampsia, CI=confidence interval, LDA= low-dose acetylsalicylic acid (100mg/day)

Table 2. The change of serum PlGF concentration from 12-14 weeks of gestation to 18-20 weeks of gestation (ΔPlGF12) and from 18-20 weeks of gestation to 26-28 weeks of gestation (ΔPlGF23)

PlGF concentration[a] / ηg/ml	**ΔPlGF12**	95% CI		**ΔPlGF23**	95% CI	
		Lower	Upper		Lower	Upper
Low-risk women (Group 1)	**95.6**	86.0	106.2	**201.6**	178.2	228.2
Risk-women, no PE (Group 2)	**86.9**	80.4	94.0	**187.9**	165.6	212.9
Risk-women, PE (Group 3)	**84.7**	67.7	106.1	**25.3**	4.1	60.9
Placebo, no PE (Group 4)	**78.4**	66.0	93.0	**128.5**	99.1	166.0
Placebo, PE (Group 5)	**42.3**	14.5	102.8	**13.6**	-3.1	84.1
LDA, no PE (Group 6)	**86.7**	73.0	103.0	**158.7**	131.3	192.0
LDA, PE (Group 7)	**65.7**	29.6	134.5	**127.1**	74.0	218.1

a calculated from geometric means

PlGF=placental growth factor, PE=pre-eclampsia, CI=confidence interval, LDA= low-dose acetylsalicylic acid (100mg/day)

The change in serum PlGF concentrations was lower in **Group 3** and **Group 5** compared to **Groups 1, 2, 4, 6 and 7** (interaction effect time×group, $p<0.001$).

Table 3. The adjusted geometric means of serum PlGF levels of the study groups in three different sampling points during pregnancy.

Weeks of Gestation/	**12-14**	95% CI		**18-20**	95% CI		**26-28**	95% CI	
PlGF concentration[a]	**ηg/ml**	Lower	Upper	**ηg/ml**	Lower	Upper	**ηg/ml**	Lower	Upper
Placebo	**23.5**	20.6	26.8	**94.2**	80.0	110.9	**199.1**	161.1	245.5
LDA	**24.3**	21.2	27.9	**113.2**	95.7	134.0	**287.7**	232.3	357.3
Risk-women	**27.2**	25.2	29.4	**123.3**	112.2	135.8	**305.5**	269.8	345.9

a geometric mean

PlGF=placental growth factor, PE=pre-eclampsia, CI=confidence interval, LDA= low-dose acetylsalicylic acid (100mg/day)

Table 4. The change of adjusted geometric mean serum PlGF concentration from 12-14 weeks of gestation to 18-20 weeks of gestation (ΔPlGF12) and from 18-20 weeks of gestation to 26-28 weeks of gestation (ΔPlGF23).

PlGF concentration[a] / ηg/ml	ΔPlGF12	95% CI		ΔPlGF23	95% CI	
		Lower	Upper		Lower	Upper
Placebo	**70.7**	59.4	84.1	**104.9**	81.1	134.6
LDA	**89.0**	74.5	106.1	**174.5**	136.6	223.3
Risk-women	**96.1**	87.0	106.5	**182.2**	157.6	210.1

a calculated from geometric means

PlGF=placental growth factor, PE=pre-eclampsia, CI=confidence interval, LDA= low-dose acetylsalicylic acid (100 mg/day)

After adjustment for BMI, chronic hypertension and MAP, the change in PlGF concentration was lower in placebo group than in LDA group (interaction time×group, p=0.046), particularly change between sample 2 and 3 differed in further analysis (p=0.043). Sample 1 was taken at 12^{+0}-14^{+0}, sample 2 at 18^{+0}-20^{+0} and sample 3 at 26^{+0}-28^{+0} weeks of gestation.

High Lights

- This study shows an association between low-dose aspirin (100mg/d) started in early pregnancy and increase in serum placental growth factor levels (PlGF).
- The effect of aspirin on PlGF levels is seen after mid-gestation.
- This finding may be related to the reduced risk of developing pre-eclampsia.