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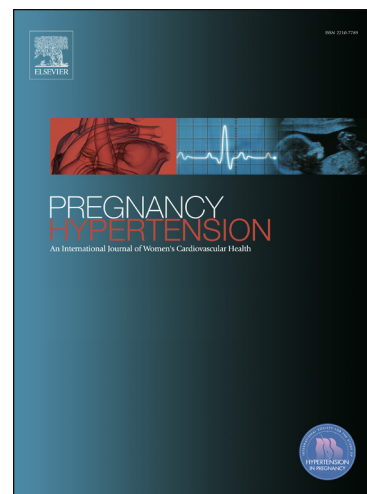
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The Effect of Low-Dose Aspirin On Serum Placental Growth Factor Levels In a High-Risk PREDO Cohort

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Abstract**Objectives**

Our first aim was to study the longitudinal changes of serum placental growth factor (PIGF) 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 14th week of gestation, on PIGF 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 PIGF concentration between groups were compared. To investigate the effect of LDA on serum PIGF concentration, placebo (N=62) and LDA (N=61) groups were compared. A repeated measures ANOVA was used to analyze differences in PIGF levels between the groups.

Results

The increase in serum PIGF concentration was higher in LDA than in placebo group (time \times group effect, $p=0.046$). The increase in serum PIGF 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 \times group effect, $p<0.001$). There were no differences in PIGF 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 PIGF concentration.

Keywords

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

Abbreviations

PIGF = 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 (PIGF) 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 PIGF 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 PIGF 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 PIGF 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 PIGF 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 PIGF concentration and changes in PIGF 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 PIGF levels between the groups. The model included the main effects of time, group and the interaction effect time \times group. Log-transformed PIGF 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 PIGF concentration in each group are presented in Table 1. Change in PIGF 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 PIGF 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 PIGF concentration and longitudinal changes in the PIGF 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 PIGF concentration was lower in the placebo group than in the LDA group (interaction effect $\text{time} \times \text{group}$, $p=0.046$), 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 ng/ml vs. LDA 174.5 ng/ml, interaction effect $\text{time} \times \text{group}$, $p=0.043$). 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 PIGF concentration (interaction effect $\text{time} \times \text{group}$, $p=0.046$ 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 PIGF 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 PIGF 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]. PIGF is a member of vascular growth factor family and secreted predominantly by the trophoblasts during pregnancy. The exact role of PIGF 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 PIGF concentration has a curvilinear relationship with gestational age. Maternal serum PIGF 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 PIGF 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 PIGF 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 PIGF 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 PIGF 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 PIGF 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 PIGF 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 PIGF. 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 PIGF 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 PIGF 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 PIGF 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.

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

Weeks of Gestation/ PIGF concentration ^a	12-14			18-20			26-28		
	ng/ml	95% CI		ng/ml	95% CI		ng/ml	95% CI	
		Lower	Upper		Lower	Upper		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

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

Table 2. The change of serum PIGF concentration from 12-14 weeks of gestation to 18-20 weeks of gestation (Δ PIGF12) and from 18-20 weeks of gestation to 26-28 weeks of gestation (Δ PIGF23)

PIGF concentration ^a / ng/ml	Δ PIGF12	95% CI		Δ PIGF23	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

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

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

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

Weeks of Gestation/ PIGF concentration ^a	12-14			18-20			26-28		
	ng/ml	95% CI		ng/ml	95% CI		ng/ml	95% CI	
		Lower	Upper		Lower	Upper		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

PIGF=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 PIGF concentration from 12-14 weeks of gestation to 18-20 weeks of gestation (Δ PIGF12) and from 18-20 weeks of gestation to 26-28 weeks of gestation (Δ PIGF23).

PIGF concentration ^a / ng/ml	Δ PIGF12	95% CI		Δ PIGF23	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

PIGF=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 PIGF concentration was lower in placebo group than in LDA group (interaction time \times 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 (PIGF).
- The effect of aspirin on PIGF levels is seen after mid-gestation.
- This finding may be related to the reduced risk of developing pre-eclampsia.