The effect of low-dose aspirin on serum placental growth factor levels in a high-risk PREDO cohort


ARTICLE INFO

Keywords:
Pre-eclampsia
Placental growth factor
Low-dose acetylsalicylic acid
Aspirin

ABSTRACT

Objectives: Our first aim was to study the longitudinal changes of serum placental growth factor (PlGF) concentration between 12+0 and 28+0 weeks of gestation in the prospective PREDO cohort. Our second aim was to study the effect of low-dose acetylsalicylic acid (LDA; 100 mg/day), started before the 14th 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. Risk-women were divided into two groups: to those who had medium risk for pre-eclampsia and to those who had high risk for pre-eclampsia. Finally there were 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 high-risk women who had placebo and developed pre-eclampsia and in medium-risk women who 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, medium-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.

https://doi.org/10.1016/j.preghy.2018.04.003

Received 1 December 2017; Received in revised form 16 March 2018; Accepted 6 April 2018
Available online 07 April 2018
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1. 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 (100 mg/day), started in early pregnancy, on serum PlGF concentration.

2. 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 Fig. 1a. Originally 1082 women were recruited, 972 women that fulfilled the inclusion criteria, which had predetermined risk factors for pre-eclampsia (inclusion criteria in Supplementary Table 1), 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.

Of 1082 participants 454 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. Of these 454 women 142 risk-women 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 having high-risk for pre-eclampsia. Reference groups composed of 202 women (defined as medium-risk women) with risk factors and without bilateral second-degree notch and 110 women without known risk factors for pre-eclampsia (defined as low-risk women).

During the study period, a total of 45 (9.9%) of 454 recruited women were not included to analyzes for various reasons. There were three miscarriages and one legal abortion at 20 weeks of gestation due to preterm premature rupture of membranes at 19 weeks of gestation. 25 women were lost to follow-up or discontinued for various non-
2.1. 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 and in pairwise comparisons Bonferroni correction was used.

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 PlGF 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).

3. Results

Clinical characteristics of all study groups are presented in Supplementary Table 2 and pregnancy characteristics in Supplementary Table 3. There were 40 pre-eclampsia cases (12.3% of all risk-women), ten (3.0% of all risk-women) women had early-onset pre-eclampsia and 30 (9.7% of all risk-women) women had late-onset pre-eclampsia, and 20 (6.5% of all risk-women) had severe form of the disease. There is a reduction in pre-eclampsia, especially in early-onset pre-eclampsia, in the LDA arm of the study, but the finding was not powered (Supplementary Table 5). The results has been published earlier [10].

The geometric means of PlGF concentration in each group are presented in Table 1. Change in PlGF concentration was lower in medium-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) (Fig. 1b and Table 2). The Group 5 seems to differ from other groups quite early in pregnancy. However, the interaction effect time × group of PlGF change between sample 1 and sample 2 had p-value 0.052, which we did not regard as significant. Therefore the groups 3 and 5 differed from other groups from sample 2 onwards. The change in PlGF concentration between the other groups (Group 1, 2, 4, 6 and 7) did not differ in three different time points during pregnancy (p = 0.15) (Fig. 1b and Table 2).

Clinical characteristics of three risk-women groups (medium-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 Tables 3 and 4, Fig. 2. After adjustment for BMI, chronic hypertension and MAP, the change in PlGF concentration was

### Table 1

<table>
<thead>
<tr>
<th>Weeks of Gestation/</th>
<th>12–14</th>
<th>95% CI</th>
<th>18–20</th>
<th>95% CI</th>
<th>26–28</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF concentration&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.9 23.3 26.7</td>
<td>120.5 109.3 132.9</td>
<td>322.2 287.5 361.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk women (Group 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium-risk women, no PE (Group 2)</td>
<td>24.6 23.0 26.3</td>
<td>111.5 103.4 120.2</td>
<td>299.4 269.1 333.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium-risk women, PE (Group 3)</td>
<td>23.7 19.8 28.3</td>
<td>108.5 87.5 134.4</td>
<td>133.8 91.6 195.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo, no PE (Group 4)</td>
<td>21.9 19.9 24.0</td>
<td>100.2 85.9 116.9</td>
<td>228.8 185.1 282.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo, PE (Group 5)</td>
<td>20.0 15.8 25.4</td>
<td>62.3 30.3 128.1</td>
<td>75.9 27.1 212.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDA, no PE (Group 6)</td>
<td>22.4 20.4 24.6</td>
<td>109.2 93.4 127.6</td>
<td>267.9 224.6 319.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDA, PE (Group 7)</td>
<td>28.0 22.0 35.6</td>
<td>93.7 51.6 170.1</td>
<td>220.8 125.6 388.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> geometric means.

PlGF = placental growth factor, PE = pre-eclampsia, CI = confidence interval, LDA = low-dose acetylsalicylic acid (100 mg/day).
Group 1 = Low-risk women, Group 2 = Medium-risk women, no PE, Group 3 = Medium-risk women, PE, Group 4 = High-risk women randomized to placebo group, no PE, Group 5 = High-risk women randomized to placebo group, PE, Group 6 = High-risk women randomized to LDA group, no PE, Group 7 = High-risk women randomized to LDA group, PE

The change in PlGF concentration between measurements was lower in Group 3 and Group 5 compared to other groups (interaction effect time × group, \( p < 0.001 \)). There was no difference between Groups 1, 2, 4, 6 and 7 (interaction effect time × group, \( p = 0.15 \)).

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

**Fig. 1b.** The geometric means of PlGF concentrations in seven study groups by weeks of gestation.

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

<table>
<thead>
<tr>
<th>PlGF concentration(^a) pg/ml</th>
<th>ΔPlGF12 Lower</th>
<th>Upper</th>
<th>ΔPlGF23 Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk women (Group 1)</td>
<td>95.6</td>
<td>86.0</td>
<td>201.6</td>
<td>178.2</td>
</tr>
<tr>
<td>Medium-risk women, no PE (Group 2)</td>
<td>86.9</td>
<td>80.4</td>
<td>187.9</td>
<td>165.6</td>
</tr>
<tr>
<td>Medium-risk women, PE (Group 3)</td>
<td>84.7</td>
<td>67.7</td>
<td>212.9</td>
<td>60.9</td>
</tr>
<tr>
<td>Placebo, no PE (Group 4)</td>
<td>78.4</td>
<td>66.0</td>
<td>188.5</td>
<td>99.1</td>
</tr>
<tr>
<td>Placebo, PE (Group 5)</td>
<td>42.3</td>
<td>14.5</td>
<td>84.1</td>
<td>-3.1</td>
</tr>
<tr>
<td>LDA, no PE (Group 6)</td>
<td>86.7</td>
<td>73.0</td>
<td>192.0</td>
<td>131.3</td>
</tr>
<tr>
<td>LDA, PE (Group 7)</td>
<td>65.7</td>
<td>29.6</td>
<td>218.1</td>
<td>134.5</td>
</tr>
</tbody>
</table>

\(^a\) calculated from geometric means.

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

<table>
<thead>
<tr>
<th>Weeks of Gestation/</th>
<th>12–14</th>
<th>95% CI</th>
<th>18–20</th>
<th>95% CI</th>
<th>26–28</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlGF concentration(^a) pg/ml</td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Placebo</td>
<td>23.5</td>
<td>20.6</td>
<td>26.8</td>
<td>94.2</td>
<td>80.0</td>
<td>110.9</td>
</tr>
<tr>
<td>LDA</td>
<td>24.3</td>
<td>21.2</td>
<td>27.9</td>
<td>113.2</td>
<td>95.7</td>
<td>134.0</td>
</tr>
<tr>
<td>Medium-risk women</td>
<td>27.2</td>
<td>25.2</td>
<td>29.4</td>
<td>123.3</td>
<td>112.2</td>
<td>135.8</td>
</tr>
</tbody>
</table>

\(^a\) 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 ± 1–14 ± 0, sample 2 at 18 ± 0–20 ± 0 and sample 3 at 26 ± 0–28 ± 0 weeks of gestation.

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

<table>
<thead>
<tr>
<th>PlGF concentration(^a) pg/ml</th>
<th>ΔPlGF12 Lower</th>
<th>Upper</th>
<th>ΔPlGF23 Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>70.7</td>
<td>59.4</td>
<td>104.9</td>
<td>81.1</td>
</tr>
<tr>
<td>LDA</td>
<td>89.0</td>
<td>74.5</td>
<td>174.5</td>
<td>136.6</td>
</tr>
<tr>
<td>Medium-risk women</td>
<td>96.1</td>
<td>87.0</td>
<td>182.2</td>
<td>157.6</td>
</tr>
</tbody>
</table>

\(^a\) calculated from geometric means.

PlGF = placental growth factor, PE = pre-eclampsia, CI = confidence interval, LDA = low-dose acetylsalicylic acid (100 mg/day).
lower in the placebo group than in the LDA group (interaction effect time × group, p = 0.046), particularly change between sample 2 (18–20 weeks of gestation) and 3 (26–28 weeks of gestation) differed in further analysis (placebo 104.9 pg/ml vs. LDA 174.5 pg/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).

4. 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 medium-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 medium-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 medium-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 medium-risk women with pre-eclampsia [24].

Increasing evidence suggests that LDA treatment during pregnancy reduces the risk of developing pre-eclampsia [27,28]. There is still ongoing debate over the timing and dose of LDA treatment [29]. However, the dose of 100 mg/day used in the present study seems to be the minimum dose at which the effect of aspirin becomes evident and the effect of aspirin for the prevention of pre-eclampsia, severe pre-eclampsia and fetal growth restriction is dose-dependent [30] A recent prospective, double-blinded placebo-controlled study on women with
high risk for pre-eclampsia showed clear evidence, that LDA 150 mg 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 [31].

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 also decrease endothelial dysfunction by other mechanisms [29]. It may have indirect effect on PI GF concentration by dose-dependently inhibiting sFlt-1 production in cytoto tropoblasts via cyclo-oxygenase-1 inhibition [32]. Recently, Panagadje 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 PI GF. 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 [33]. This could mean that lower increase of serum PI GF 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 PI GF 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 PI GF 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.

Acknowledgements

This work was supported by PerkinElmer Finland Oy (PI GF assays). We thank the doctors and study nurses for their work on the PREDO Project. We are grateful to the women who participated in the PREDO Project.

Funding

Finsk Lääkärisällkapet, Foundation of EVO research founding (A special Finnish state subsidy for science research), Academy of Finland, Signe and Ane Gyllenberg Foundation, Sigrid Juselius Foundation, University of Helsinki Research Funds, Finnish Medical Foundation, Juho Vainio Foundation, Novo Nordisk Foundation, Jane and Aatos Erkko Foundation, and Piäivikki and Sakari Sohlberg Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.preghy.2018.04.003.

References

