Author's response to reviews

Title: Periodic Assessment of Plasma sFlt-1 and PLGF Concentrations and it's Association with Placental Morphometry in Gestational Hypertension (GH) - A Prospective Follow Study

Authors:

Kamalan Jeevaratnam (kj272@cam.ac.uk)
Vishna Devi Nadarajah (vishnadevi@gmail.com)
John Paul Judson (johnpaul_judson@imu.edu.my)
Sivalingam Nalliah (sivalingam_nalliah@imu.edu.my)
Mohd Farouk Abdullah (drmfarouk@moh.gov.my)

Version: 3 Date: 1 September 2010

Author's response to reviews: see over
Alterations in response to reviewer’s report (2nd Revision – 16th Aug 2010)

TITLE: Periodic Assessment of Plasma sFlt-1 and PLGF Concentrations and it's Association with Placental Morphometry in Gestational Hypertension (GH) - A Prospective Follow Study (MS: 2391833403680320)

AUTHORS: Kamalan Jeevaratnam, Vishna Devi Nadarajah, John Paul Judson, Sivalingam Nalliah and Mohd Farouk Abdullah

In response to editor:

Your revised manuscript has been reviewed favorably. However, the reviewers still have a few minor comments that require your attention before your manuscript can be published in BMC Pregnancy and Childbirth. Please address the following reviewer comments, and I will review your further revised manuscript in order for it to be published in a timely manner.

We are grateful for your consideration on our revised manuscript titled “Periodic Assessment of Plasma sFlt-1 and PLGF Concentrations and it's Association with Placental Morphometry in Gestational Hypertension (GH) - A Prospective Follow Study” We have once again made revisions as requested by the reviewers and hope that the manuscript will meet the publication requirement of BMC Pregnancy and Childbirth.
A. In response to REFEREE 1 (Chloe Zera):

Thank you for the very constructive comments concerning the paper. We hereby re-submit the 2nd revision in which all the points raised by both referees are dealt with.

MAJOR POINTS

1. Careful not to overstate findings in the discussion and conclusions. In particular, the clinical utility of these biomarkers remains unclear, and while you have demonstrated an association between the markers and the diagnosis of GH, this study did not address the use of sFlt-1 and PIGF for clinical diagnosis or management. I agree that the persistent elevation in sFlt-1 is interesting in terms of long-term CVD risk, but to me it's a stretch to say that there should be long-term follow-up based on elevated markers in the immediate post-partum period, as again this was not a study of clinical performance of the biomarkers but a study of associations.

We agree with this comment and have thus:


From:

Findings from these studies suggest that prolonged exposure to high levels of sFlt-1 as seen in our study population may necessitate long term follow ups in GH women.

To:

Findings from these studies suggest that it may be necessary to further evaluate the effect prolonged exposure to high levels of sFlt-1 may have and it’s subsequent clinical utility in the management of GH cases.

[2] Reworded sentences 1 and 2 in the Conclusion (Page 16)(also in line with request of referee 2)

From:

We conclude that our study, establishes periodic values for both sflt-1 and PIGF levels in a Malaysian setting and extends the idea of GH development in women assessed in this study is related to defective capillarization due to the inverse relationship between the pro-angiogenic factor, PIGF and the anti-angiogenic factor, sFlt-1. These periodic biomarker levels suggest that both sFlt-1 and PIGF, together, may also be valuable in managing GH from a surveillance point of view.
To:

Our work primarily studied biomarker values, placental morphometric parameters and their correlation in a Malaysian setting. In doing so, our findings represent an important source of information for a country with an ethnically diverse population and a maternal healthcare system that is undergoing rapid development. We therefore conclude that our study, establishes periodic values for both sFlt-1 and PlGF levels with placental morphometric correlations in a Malaysian setting and extends the idea that the development of GH in women assessed in this study is related to defective capillarization caused by an inverse relationship between the pro-angiogenic factor, PlGF and the anti-angiogenic factor, sFlt-1. These periodic biomarker levels suggest a clear need to evaluate the clinical utility of both sFlt-1 and PlGF, in managing GH from a surveillance point of view.

MINOR POINTS

1. Read carefully for grammar and punctuation.

Thank you very much for your careful attention to this matter. We have once again re-read the text and corrected for grammar and punctuation errors. This is reflected as green highlights in the newly revised version.
B. In response to REFEREE 2 (Augustine Rajakumar):

We are very grateful for these very constructive comments concerning the paper and resubmit the 2nd revision in which all the points raised by both referees are dealt with.

MINOR POINTS

1) Page 5. Sentence starting with ‘In doing so, the study establishes………..That is undergoing rapid development.’ These two sentences need to be in the discussion and not here.

Thank you for your suggestion regarding this matter. We have re-written the paragraph in the Introduction and transferred the two sentences to the conclusion section.

[a] Thus text from sentences 6, 7 and 8 in Para 3 of the Introduction (Page 5) has been removed.

[b] The Conclusion (Page 16) has now been changed:

From:

We conclude that our study, establishes periodic values for both sflt-1 and PI GF levels in a Malaysian setting and extends the idea of GH development in women assessed in this study is related to defective capillarization due to the inverse relationship between the pro-angiogenic factor, PI GF and the anti-angiogenic factor, sFlt-1. These periodic biomarker levels suggest that both sFlt-1 and PI GF, together, may also be valuable in managing GH from a surveillance point of view.

To:

Our work primarily studied biomarker values, placental morphometric parameters and their correlation in a Malaysian setting. In doing so, our findings represent an important source of information for a country with an ethnically diverse population and a maternal healthcare system that is undergoing rapid development. We therefore conclude that our study, establishes periodic values for both sFlt-1 and PI GF levels with placental morphometric correlations in a Malaysian setting and extends the idea that the development of GH in women assessed in this study is related to defective capillarization caused by an inverse relationship between the pro-angiogenic factor, PI GF and the anti-angiogenic factor, sFlt-1. These periodic
biomarker levels suggest a clear need to evaluate the clinical utility of both sFlt-1 and PlGF, in managing GH from a surveillance point of view.

2) Page 8 ‘………villous crowding.’ Explain.

Villous crowding represents a reduction in the intervillous space due to increased villous growth and is a pathological change associated with defective capillarization. We have thus added the following sentence in Para 3 of Placental studies in the Methods section (Page 8).

For the purpose of this study, villous crowding was defined as a pathological change in which there is a reduction in intervillous space associated with defective capillarization.

3) Table 2. Authors make a reference to ‘superscript…’. But the table doesn’t show any.

We indeed made reference to superscript for Table 2. The table does not show any superscript as it was meant to reflect that none of the means were significantly different from each other. We however do agree that this may lead to confusion and have thus replaced the reference related to superscript with a new reference.

The previous reference for Table 2 which read as:

<table>
<thead>
<tr>
<th>Group</th>
<th>Villous capillarisation (VC ± SE)</th>
<th>Intervillous Space (IvS ± SE, µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central (VC-A)</td>
<td>Body (VC-B)</td>
</tr>
<tr>
<td>Normotensive (n=51)</td>
<td>0.12 ± 0.014</td>
<td>0.11 ± 0.007</td>
</tr>
<tr>
<td>GH (n=32)</td>
<td>0.15 ± 0.014</td>
<td>0.13 ± 0.015</td>
</tr>
</tbody>
</table>

Mean with similar superscript differ significantly with each other at P<0.05

The new reference for Table 2 now reads as:

<table>
<thead>
<tr>
<th>Group</th>
<th>Villous capillarisation (VC ± SE)</th>
<th>Intervillous Space (IvS ± SE, µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central (VC-A)</td>
<td>Body (VC-B)</td>
</tr>
<tr>
<td>Normotensive (n=51)</td>
<td>0.12 ± 0.014</td>
<td>0.11 ± 0.007</td>
</tr>
<tr>
<td>GH (n=32)</td>
<td>0.15 ± 0.014</td>
<td>0.13 ± 0.015</td>
</tr>
</tbody>
</table>

No significant differences were observed between means at P<0.05
4) Figure 1 and two should be combined to a single page. The ‘green’ numbers do not display well. Consider changing font color. Also if the yellow lines can be slightly darker it would aid a better visual. Mark and label the villous and capillaries.

Thank you for your valuable suggestion regarding the figures. We have thus merged figure 1 and Figure 2 as one new figure (see new Figure 1) and incorporated a new and detailed figure legend to describe both the calculation. We have also removed the green numberings and thickened the borders for better visual. The villous and capillaries are also labeled. The new manuscripts will make reference to Figure 1 only. Legend for the new Figure 1 now reads as:

**Figure 1. Morphometric calculation for villous capillarization (VC) and intervillous space (IvS) of placental villi.**

Calculation of VC was done by dividing the surface area of the capillary (blue outline) over the surface area of the villous (black outline). Calculation of IvS was done by subtracting the surface area of the photomicrograph (calculated by height \( H \) X width \( W \); green outline indicated by black arrows) with the summed up area of all villous (black outline) within a particular photomicrograph. The surface area (\( \mu m^2 \)) of the respective capillaries and villous measured was generated by the software program. This slide was viewed at 20X magnification.

5) Legend to Figure 3 and 4 refers to ‘three readings’. What do the authors mean?

The ‘three readings’ makes reference to the three intervals (ante partum, intrapartum and postpartum). We agree that the original figure legend may cause some confusion and have thus revised them. As Figure 1 and 2 have been merged (mentioned above), Figure 3 and Figure 4 will be referenced as Figure 2 and Figure 3 respectively in the new manuscript.

Legend for Figure 3 previously read as:

**Figure 3. Baseline levels of plasma PI GF in women based on period of sampling**

Baseline levels of plasma PI GF were determined using ELISA. Each value displayed is a mean ± standard error (SE) of three readings, obtained from normotensive, and GH women at antepartum, intrapartum and postpartum. Means with similar superscript differ significantly at P < 0.05.
Has now been changed to:

Figure 2. Baseline levels of plasma PlGF in women based on period of sampling

Baseline levels of plasma PlGF were determined using ELISA. Values displayed are mean ± standard error (SE) of the three intervals (antepartum, intrapartum and postpartum), obtained from normotensive and GH women. Means with similar superscript differ significantly at P < 0.05.

Legend for Figure 4 previously read as:

Figure 4. Baseline levels of plasma sFlt-1 in women based on period of sampling

Baseline levels of plasma sFlt-1 were determined using ELISA. Each values displayed is a mean ± SE of three readings, obtained from normotensive and GH at antepartum, intrapartum and postpartum. Means with similar superscript differ significantly at P < 0.05.

Has now been changed to:

Figure 3. Baseline levels of plasma sFlt-1 in women based on period of sampling

Baseline levels of plasma sFlt-1 were determined using ELISA. Values displayed are mean ± standard error (SE) of the three intervals (antepartum, intrapartum and postpartum), obtained from normotensive and GH women. Means with similar superscript differ significantly at P < 0.05.

6) Provide the actual ‘n’ value both in the legend and the figure.

We have now incorporated in to the figures, the ‘n’ number for all the three intervals (antepartum, intrapartum and postpartum) for both GH and normotensive women (see new
Figure 3 & 4). We have however not included the ‘n’ number for figure legend as the ‘n’ number varies for the respective intervals.

7) The manuscript reads well although the discussion portion seems lengthy.

We agree that the discussion could be improved with some reduction in words and have thus changed the following sentences in the Discussion to decrease length but increase clarity. Green highlights represent grammar or spelling changes.

[A] Page 13
From:

Recently, there has been an increase in interest on the role circulating pro and anti-angiogenic factors play in the pathophysiology of GH. However less is known on how these biomarkers vary over the duration of pregnancy and after delivery as well as if they influence placental structural development.

To:

While there has been an increase in the interest on the role circulating pro and anti-angiogenic factors play in the pathophysiology of GH, less is known on how these biomarkers vary over the duration of pregnancy and after delivery.

[B] Page 13
- From:

We observed that PlGF levels were lower in GH women in comparison to normotensive whereas sFlt-1 was higher in GH when compared to normotensive during antepartum and intrapartum, correlation analysis, confirms an inverse relationship between the two biomarkers.

To:

We observed lower PlGF but higher sFlt-1 levels in GH women in comparison to normotensive during antepartum and intrapartum, with correlation analysis confirming an inverse relationship.

[C] Page 13
- From:

More importantly, while the latter studies have proposed this for cases of preeclampsia, this study proves that both these biomarkers are also significantly altered in GH, showing a similar pattern to the more severe preeclampsia.
To:

While the latter studies proposed this for preeclampsia, this study proves these biomarkers are also significantly altered in GH.

[D]Page 14
- From:

The contrasting findings at the antepartum period between both studies could be due to variations in the study population (for example: age, parity, ethnicity, smoking status) or in the actual onset of GH versus the diagnosis of GH. Nevertheless, both studies suggest it is worthwhile to examine the levels of these biomarkers at earlier gestational ages in various populations.

To:

This contrast could be due to variations in the study population (for example: age, parity, ethnicity, smoking status) or on the actual onset of GH versus the diagnosis of GH.

[E]Page 14
- From:

PlGF levels in GH and normotensive women during the postpartum period were similar and this can be attributed to the fact that PlGF is primarily expressed by the placenta. The delivery of the placenta removes the source of PlGF synthesis, thus decreasing the levels of this biomarker in all cases.

To:

PlGF levels in GH and normotensive women during the postpartum period were similar and this can be attributed to the fact that PlGF is primarily expressed by the placenta which is no longer present at postpartum.

[F]Page 14
- From:

Findings from these studies suggest that prolonged exposure to high levels of sFlt-1 as seen in our study population may necessitate long term follow ups in GH women.
To:

Findings from these studies suggest that it may be necessary to further evaluate the effect prolonged exposure to high levels of sFlt-1 may have and it’s subsequent clinical utility in the management of GH cases.

\[G\]Page 15
- From:

For example, hypoxia occurs due to decreased blood perfusion, hypoxic experiments with placental tissue have previously demonstrated an increase in vascular endothelial growth factor expressions [17].

To:

Hypoxic experiments with placental tissue have previously demonstrated an increase in vascular endothelial growth factor expressions [17].

\[H\]Page 15
- From:

These angiogenic factors then bind to receptors to exert their effect and promote angiogenesis by increasing capillarization may explain the lack of morphometric differences between normotensive and GH as seen in our study.

To:

These angiogenic factors may thus bind to receptors to exert their effect and promote angiogenesis by increasing capillarization explaining the lack of morphometric differences between normotensive and GH as seen in our study.

\[I\]Page 15
- From:

Work done in this study examined two-dimensional evaluations of the placenta and future studies should include three-dimensional morphometric assessment in which more precise and in-depth structural and stereological features can be assessed.

To:

Furthermore as this study examined two-dimensional evaluations of the placenta, future studies should include three-dimensional morphometric assessments with precise and in-depth structural and stereological features.