Reviewer’s report

Title: Longitudinal reference ranges for maternal plasma laeverin, and its role as a potential biomarker of preeclampsia

Version: 0 Date: 22 Mar 2016

Reviewer: Yukiyasu Sato

Reviewer's report:

The authors demonstrate the followings in the present study.

(1) Plasma laeverin levels were significantly higher in pregnant women as compared to non-pregnant women or men.

(2) Plasma laeverin levels decreased as gestation progressed and further decreased to non-pregnant levels after delivery.

(3) Half of the women who later developed preeclampsia had plasma laeverin levels below the 5th percentile at 22-24 weeks gestation.

(4) In contrast to plasma levels, laeverin concentrations in the tissue lysates were 1.6-fold higher in the preeclamptic placentas as compared to the healthy placentas.

(5) Umbilical venous samples of the neonates born to preeclamptic mothers had significantly lower laeverin levels as compared to those of the neonates born to healthy mothers.

From these findings, the authors conclude that lower serum laeverin levels at 22-24 weeks of gestation might be associated with the later development of preeclampsia.

The article contains novel and relevant information, but also contains several problems that need to be addressed. The followings are the points that I used in making my recommendations to the editor:

1) The authors should describe the criteria to diagnose preeclampsia in the Methods.

2) Among the 15 preeclamptic women whose plasma samples were analyzed for laeverin, how many women were categorized to severe type or early-onset type? Was there any difference in plasma laeverin levels between severe early-onset type and the other types?

3) To evaluate the possible usefulness of plasma laeverin measurement in the prediction of later preeclampsia, it is advisable for the authors to set the cut-off value and calculate the sensitivity, specificity, positive predictive value, and negative predictive value.
4) The authors should show the p-values in Table 1 and 2. In addition, the authors should specify the gestational ages at which the mean arterial pressure and pulsatility index of uterine artery, umbilical artery, or middle cerebral artery were measured. Were all of them measured at the time of blood sampling at 22-24 weeks of gestation?

5) The authors should also add the final antepartum data obtained from the same cohort (42 healthy pregnant women) to the box-and-whisker plots in Figure 2. In addition, the differences should be statistically evaluated (e.g., by using one-way repeated-measures ANOVA).

6) The authors describe in the abstract that the half-life of postpartum plasma laeverin was 5 days in preeclamptic women, which is not specified in the results. The authors should describe this fact in the results as well.

7) Horie et al. demonstrated that laeverin is intensely expressed on the surface of extravillous trophoblast that invades maternal decidua and vessels (Horie A, et al. Laeverin/aminopeptidase Q induces trophoblast invasion during human early placentation. Hum Reprod 2012; 27: 1267-1276). Since defective endovascular invasion of extravillous trophoblast has been considered to be a primary cause of preeclampsia, the authors should cite this article to discuss the possibility that reduced number of extravillous trophoblast colonizing inside the maternal vessels could be one of the causes of lower plasma laeverin at 22-24 weeks of gestation in the women who later developed preeclampsia.

8) The information that can be obtained from Table 3 is almost identical to Figure 1 and thus, Table 3 can be deleted.

9) Page 9, line27-44: The subsection with heading, "Plasma laeverin in women developing preeclampsia" should be moved to the back of the subsection with heading, "Plasma laeverin levels in healthy women during the second half of pregnancy."

10) Page 10, line1-2: "In the neonates of women who had preeclampsia (n=14), the mean laeverin level was significantly lower (12.02±1.00 ng/mL) compared to healthy pregnancies (p=0.001)." The authors should add "in the umbilical vein" after "the mean laeverin level".

11) Page 10, line 51: "These women most likely had defective placentation." What does the defective placentation mean? Does it mean the shallow placentation? The authors should describe more specifically.

12) Page 11, line 5: "Therefore, we hypothesize that the increased laeverin production by placenta in preeclampsia is an attempt to compensate for smaller placental size and/or defective protein function." I cannot understand why compensation for smaller placenta is associated with increased placental production of laeverin. The authors should explain the rationale more clearly.
13) Page 11, line 7: "It is known that preeclampsia leads to increased apoptosis of cytотrophoblasts or cell death, which might explain why laeverin accumulates in preeclamptic placentas." I cannot understand why increased apoptosis or cell death of cytотrophoblasts leads to placental accumulation of laeverin. The authors should clearly explain the supposed mechanism.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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