

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Maternal vitamin D deficiency and fetal distress/birth asphyxia - A population-based nested case control study
AUTHORS	Lindqvist, Pelle G.; Silva, Aldo; Gustafsson, Sven; Gidlöf, Sebastian

VERSION 1 - REVIEW

REVIEWER	Bruce W Hollis, PhD Medical University of South Carolina Charleston, SC USA
REVIEW RETURNED	08-Sep-2015

GENERAL COMMENTS	The authors have performed a very important piece of work on a neglected area of medicine. I would only suggest the authors include two additional studies in their analysis,(Hossain N et al, JCEM 2014,99:2448 and Soblok A et al, Clin Endocrinol 2015,PMID 25683660).
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REVIEWER	Chrissie Yu St Mary's Hospital London UK
REVIEW RETURNED	31-Mar-2016

GENERAL COMMENTS	Sample size is very small to draw a solid conclusion. It would be good to have admission to SCBU as one of the end points as fetal distress as described in the paper is very broad. Analysing all women n= 2496 may be more appropriate as fetuses that are distressed may also be delivered by assisted vaginal route. Cord blood for vitamin D would be ideal to conclude that fetal vitamin D levels are lower in the women undergoing LSCS for fetal distress therefore may support the hypothesis by the authors.
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REVIEWER	Dr Negar Tabatabaei, PhD; Dr Shu Qin Wei, MD,PhD Department of Obstetrics and Gynecology, CHU Ste Justine, University of Montreal, Montreal, QC, Canada
REVIEW RETURNED	08-May-2016

GENERAL COMMENTS	The authors conducted a nested case control study to examine the effect of maternal vitamin D status in early pregnancy on 'newborn birth distress' which was defined as 5-min Apgar score < 7 and/or umbilical vessel pH \leq 7.15 by the authors. The authors concluded that low maternal vitamin D status in early pregnancy may be associated with emergency cesarean section due to suspected fetal asphyxia and to newborn birth distress. The conclusions are reasonable and well grounded in the data presented. There are a
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	<p>few main concerns and some moderate concerns should be addressed.</p> <p>The term fetal birth distress, fetal distress and birth asphyxia are confused. American College of Obstetricians and Gynecologists (ACOG) Committee on Obstetric Practice made a statement that the term "fetal distress" should be an antepartum or intrapartum diagnosis; while the term "birth asphyxia" should be a neonatal diagnosis (Obstet Gynecol 2005;106:1469-70). The authors mentioned 'fetal distress' in the title and main text, but there were no fetal heart rate (FHR) tracing results to support this. It might be interesting to explore if vitamin D plays a role in the fetal heart rate or vitamin D status is associated with abnormal FHR tracing. For the exposure measurement for 25-hydroxy vitamin D, the authors used a direct competitive chemiluminescence immunoassay from DiaSorin on a LIAISON instrument, this method should have an external validation of the sample reliability with an external method. There are many causes of fetal distress, such as pregnancy complications (preeclampsia, gestational diabetes), oligohydraminos, abnormal position and presentation of the fetus, multiple births, umbilical cord prolapse, nuchal cord, placental abruption, et al. The authors mentioned that they included nulliparity, smoking, and vitamin D level in the logistic regression analysis, however, the data presented as Odds Ratio (OR) only, without adjusted OR. Moreover, the other potential confounders such as maternal complications, seasons were not adjusted.</p> <p>Moderate concerns:</p> <p>The authors state "vitamin D levels" in most part of the manuscript. It is suggested to use "circulating (or serum) 25-hydroxy vitamin D concentration" instead. Since vitamin D refers to cholecalciferol and not 25(OH)D. As another alternative, "vitamin D status" but not "vitamin D levels" could be used as low, insufficient and sufficient. Vitamin D deficiency and non-deficiency are correct phrases to be used. Minor English editing is required for unclear section as stated in the review.</p> <p>Abstract: The objective is not clear. Study design and setting, 2nd sentence: "Patients Banked sera of 2496 women from 12th week of gestation" is confused. There was no 'setting' in the abstract. The authors said that this was a case-control study, but what was case, what was control were not mentioned in the abstract.</p> <p>Introduction: There are some minor edition that are suggested: U is used instead of IU in some parts of the manuscript (e.g. line 16 and 18).</p> <p>Results: Please indicate if this is season at the time pf blood collection on line 45, page 9. If season of blood collection has been considered in the analyses, it would be better to add it to the methodology section and characteristics on Table 1.</p> <p>Discussion: The authors stated IOM "limits" on line 45, page 10. The word "cut off" may fit better in this sentence.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Bruce W Hollis, PhD
 Medical University of South Carolina
 Charleston, SC USA

Comment 1

I would only suggest the authors include two additional studies in their analysis,(Hossain N et al, JCEM 2014,99:2448 and Soblok A et al, Clin Endocrinol 2015,PMID 25683660).

We thank reviewer for these two suggestions and add them. The study by Hosain show improved 1 min Apgar score and the study by Sablok showed improved 5 min apgar score and fetal weight and a lower risk of SGA

The text is modified accordingly in the beginning of the discussion

“In fact, the only study previously addressing this topic was done in southern China, where vitamin D deficiency relatively uncommon and no relation to birth asphyxia were found.¹ Two randomized controlled studies of antenatal vitamin D supplementation reported lower APGAR score at 1 minute and 5 minutes, respectively.^{2,3} In addition, in the latter study reported 13% of vitamin D deficient newborn had APGAR score at 5 minutes <7, as compared to 1.1% among those who were sufficient.³

Reviewer: 2

Chrissie Yu, St Mary's Hospital London UK

Comment 1

Sample size is very small to draw a solid conclusion. It would be good to have admission to SCBU as one of the end points as fetal distress as described in the paper is very broad.

We agree with reviewer that it is a small sample size. I suppose SCBU is a neonatal intensive care unit. In the original cohort there were 597 women that were delivered at other hospitals. We were collecting data by ourselves at the units in Southern Sweden, and by written questionnaire by those delivering far away. Unfortunately, we did not include a question of admittance to a neonatal intensive care unit. Therefore, regarding this variable the data is not of reliable enough to present.

Comment 2

Analysing all women n= 2496 may be more appropriate as fetuses that are distressed may also be delivered by assisted vaginal route.

The reviewer is correct; a larger study will have more power to identify differences. We did not have the possibility to analyze all these samples due to economic reasons. Still we find our results highly interesting since despite the small sample size they display a difference between the groups.

Moreover, we included all women that were delivered by cesarean section due to suspected fetal distress so by increasing the sample size we would merely include more controls.

In accordance with the reviewer's comments we do believe that doing a follow-up study on severe birth asphyxia including umbilical cord vitamin D measurements would add more knowledge to this topic .

Comment 3

Cord blood for vitamin D would be ideal to conclude that fetal vitamin D levels are lower in the women undergoing LSCS for fetal distress therefore may support the hypothesis by the authors.

The reviewer is right. We do not have access to cord blood samples from this study. However, in a future RCT on severe asphyxia we will assess cord blood samples as well.

Reviewer: 3 Dr Negar Tabatabaei, PhD; Dr Shu Qin Wei, MD,PhD. Department of Obstetrics and Gynecology, CHU Ste Justine, University of Montreal, Montreal, QC, Canada

The authors conducted a nested case control study to examine the effect of maternal vitamin D status in early pregnancy on 'newborn birth distress' which was defined as 5-min Apgar score < 7 and/or umbilical vessel pH ≤ 7.15 by the authors. The authors concluded that low maternal vitamin D status

in early pregnancy may be associated with emergency cesarean section due to suspected fetal asphyxia and to newborn birth distress. The conclusions are reasonable and well grounded in the data presented. There are a few main concerns and some moderate concerns should be addressed.

Comment 1

The term fetal birth distress, fetal distress and birth asphyxia are confused. American College of Obstetricians and Gynecologists (ACOG) Committee on Obstetric Practice made a statement that the term "fetal distress" should be an antepartum or intrapartum diagnosis; while the term "birth asphyxia" should be a neonatal diagnosis (Obstet Gynecol 2005;106:1469-70).

The reviewer is right. We correct the paper throughout using fetal distress as an antepartum diagnosis and birth asphyxia as the newborn diagnosis. We agree with the reviewer that this change is important for the clarity of the study.

Comment 2

The authors mentioned 'fetal distress' in the title and main text, but there were no fetal heart rate (FHR) tracing results to support this. It might be interesting to explore if vitamin D plays a role in the fetal heart rate or vitamin D status is associated with abnormal FHR tracing.

We use threatening fetal distress to the discretion of the responsible clinician. We did not do a "second opinion" evaluation if this was correct or not. However, according to our results it may be interesting to evaluate fetal heart function depending on vitamin D levels.

We add in the paper, methods, Patients:

" The diagnosis of suspected fetal distress was done with the discretion of the obstetrician in charge, mainly based on fetal heart rate monitoring and/or fetal scalp lactate determinations."

Comment 3

For the exposure measurement for 25-hydroxy vitamin D, the authors used a direct competitive chemiluminescence immunoassay from DiaSorin on a LIAISON instrument, this method should have an external validation of the sample reliability with an external method.

Answer

Karolinska University Hospital laboratory is accredited according to ISO15189 and regarding the vitamin D analysis external validation from DEQAS is done 4 times a year.

Comment 4

There are many causes of fetal distress, such as pregnancy complications (preeclampsia, gestational diabetes), oligohydramnios, abnormal position and presentation of the fetus, multiple births, umbilical cord prolapse, nuchal cord, placental abruption, et al. The authors mentioned that they included nulliparity, smoking, and vitamin D level in the logistic regression analysis, however, the data presented as Odds Ratio (OR) only, without adjusted OR.

The reviewer is right, we have been unclear how the values are given. See changed text in the results section. A version with changes in blue text is included.

Comment 5

Moreover, the other potential confounders such as maternal complications, seasons were not adjusted.

Since season has been shown to be in the causal pathway of eclampsia, i.e., double risk in winter season, we did not include season.⁴ For the same reason, BMI was not included as a potential confounder, as stated in the manuscript. Nulliparity and smoking are prevalent and important risk factors for asphyxia. Preeclampsia (2/2) and abruptio placenta (2/2) were present in four women

each. If included in a logistic regression analysis it would become instable. Logistic regression needs a minimum number of cases in each variable to work well.

1. Zhou J, Su L, Liu M, et al. Associations between 25-hydroxyvitamin D levels and pregnancy outcomes: a prospective observational study in southern China. *Eur J Clin Nutr* 2014;68:925-30.
2. Hossain N, Kanani FH, Ramzan S, et al. Obstetric and neonatal outcomes of maternal vitamin D supplementation: results of an open-label, randomized controlled trial of antenatal vitamin D supplementation in Pakistani women. *J Clin Endocrinol Metab* 2014;99:2448-55.
3. Sablok A, Batra A, Thariani K, et al. Supplementation of vitamin D in pregnancy and its correlation with feto-maternal outcome. *Clin Endocrinol (Oxf)* 2015;83:536-41.
4. Rylander A, Lindqvist PG. Eclampsia is more prevalent during the winter season in Sweden. *Acta Obstet Gynecol Scand* 2011;90:114-7.

VERSION 2 – REVIEW

REVIEWER	Bruce W Hollis, PhD Medical Univ South Carolina Charleston, SC USA
REVIEW RETURNED	24-Jun-2016

GENERAL COMMENTS	An interesting observational study. I would suggest that the authors add and incorporate 2 additional references into the manuscript that are in line with the current findings. I would also suggest a plot of 25(OH)D vs various outcomes for clarity. Hollis and Wagner 2013 <i>Calcif Tissue Internatl</i> 92:128-39 Wagner CL et al 2016 <i>J Steroid Biochemistry Molec Biol</i> 155:245-51.
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REVIEWER	Shu Qin Wei, PhD, Negar Tabatabaei, PhD Department of Obstetrics and Gynecology, CHU Ste Justine, University of Montreal, Montreal, QC, Canada
REVIEW RETURNED	11-Jul-2016

GENERAL COMMENTS	Major comments: 1) The authors have clarified the terms fetal distress/birth asphyxia in the manuscript. 2) It is recommended that the authors include the lack of reported fetal heart rate (FHR) in the study limitations and suggestions to be made on FHR measurement in future studies. 3) The authors have included the external validation for serum 25(OH)D measurements in the methodology. 4) The crude and adjusted statistical analyses are justified. 5) The comments are justified. Minor comments: 1) The term vitamin D level refers to cholecalciferol (vitamin D3) in the scientific literature. The term serum 25-hydroxy-vitamin D (25(OH)D) should be replaced by vitamin D levels. However, the term vitamin D deficiency and non-deficiency and vitamin D status are correctly used throughout the manuscript and refer to 25(OH)D. 2) The manuscript requires some minor editing since it includes
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	<p>some minor typos and grammatical corrections. e.g. the word "respectively" has been misspelled on line 21 of page 11 in the PDF file. The following sentence on line 19, page 13 is not correctly stated : "With our present knowledge we should have made the opposite and modified the design accordingly. " Also, line 41 on page 13 states "Low vitamin D levels at this age might have lead to that these individuals did not reach their growth potential manifested as slightly shorter height." does not seem to be correctly stated. Line 18 on page 9 states: "In adjusted analysis, controlling for nulliparity and smoking, the difference in vitamin D levels was significantly lower (P = 0.04). " Do the authors mean that vitamin D levels were significantly lower in cases vs controls (P = 0.04)?</p>
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VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Bruce W Hollis, PhD

Institution and Country, Medical Univ South Carolina, Charleston, SC USA

1. An interesting observational study. I would suggest that the authors add and incorporate 2 additional references into the manuscript that are in line with the current findings. I would also suggest a plot of 25(OH)D vs various outcomes for clarity.

Hollis and Wagner 2013 Calcif Tissue Internatl 92:128-39

Wagner CL et al 2016 J Steroid Biochemistry Molec Biol 155:245-51.

We thank the reviewer and have added the above mentioned references. First paragraph, introduction "but other recommend higher doses.^{5,6}" (Hollis and Wagner 2013). Wagner CL 2016 Discussion para 1 "Vitamin D deficiency was associated with a significantly shorter gestational age at delivery, which is in line with other data.²³"

Changes are highlighted in blue text

Reviewer: 3

Shu Qin Wei, PhD, Negar Tabatabaei, PhD

Institution and Country, Department of Obstetrics and Gynecology, CHU Ste Justine, University of Montreal, Montreal, QC, Canada

1. Major comments: The authors have clarified the terms fetal distress/birth asphyxia in the manuscript.

ok

2) It is recommended that the authors include the lack of reported fetal heart rate (FHR) in the study limitations and suggestions to be made on FHR measurement in future studies.

We have added in the limitation section of the discussion, paragraph 4:

"In addition, it was a limitation that we did not use specific CTG changes in the diagnosis of suspected fetal asphyxia. Future research should aim to investigate if a similar relationship might be found in severe birth asphyxia and including CTG changes."

3) The authors have included the external validation for serum 25(OH)D measurements in the methodology.

Ok

4) The crude and adjusted statistical analyses are justified.

Ok

5) The comments are justified.

Ok

Minor comments:

1) The term vitamin D level refers to cholecalciferol (vitamin D3) in the scientific literature. The term serum 25-hydroxy-vitamin D (25(OH)D) should be replaced by vitamin D levels. However, the term vitamin D deficiency and non-deficiency and vitamin D status are correctly used throughout the manuscript and refer to 25(OH)D.

As have been written in the methods section, the analysis measured 25-hydroxy-vitamin D3 and D2 with equimolar sensitivity. Therefore, we prefer to have the names as is.

2) The manuscript requires some minor editing since it includes some minor typos and grammatical corrections. e.g. the word "respectively" has been misspelled on line 21 of page 11 in the PDF file. The following sentence on line 19, page 13 is not correctly stated: "With our present knowledge we should have made the opposite and modified the design accordingly." Also, line 41 on page 13 states "Low vitamin D levels at this age might have lead to that these individuals did not reach their growth potential manifested as slightly shorter height." does not seem to be correctly stated.

We have scrutinized the manuscript and revised the language throughout including the examples made by the reviewer above.

3 Line 18 on page 9 states: "In adjusted analysis, controlling for nulliparity and smoking, the difference in vitamin D levels was significantly lower ($P = 0.04$). " Do the authors mean that vitamin D levels were significantly lower in cases vs controls ($P = 0.04$)?

The reviewer is right, this was not clearly stated and we have revised the text:

"In adjusted analysis, controlling for nulliparity and smoking, vitamin D levels were significantly lower in cases vs controls ($P = 0.04$)"