BMJ Open

Maternal vitamin D deficiency and fetal distress/birth asphyxia: a population-based nested case–control study

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To cite: Lindqvist PG, Silva AT, Gustafsson SA, *et al*. Maternal vitamin D deficiency and fetal distress/birth asphyxia: a population-based nested case–control study. *BMJ Open* 2016;**6**: e009733. doi:10.1136/bmjopen-2015-009733

▸ Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2015-009733).

Received 17 August 2015
Revised 10 August 2016
Accepted 24 August 2016

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ABSTRACT

Objective: Vitamin D deficiency causes not only skeletal problems but also muscle weakness, including heart muscle. If the fetal heart is also affected, it might be more susceptible to fetal distress and birth asphyxia. In this pilot study, we hypothesised that low maternal vitamin D levels are over-represented in pregnancies with fetal distress/birth asphyxia.

Design and setting: A population-based nested case–control study.

Patients: Banked sera of 2496 women from the 12th week of pregnancy.

Outcome measures: Vitamin D levels were analysed using a direct competitive chemiluminescence immunoassay. Vitamin D levels in early gestation in women delivered by emergency caesarean section due to suspected fetal distress were compared to those in controls. Birth asphyxia was defined as Apgar <7 at 5 min and/or umbilical cord pH≤7.15.

Results: Vitamin D levels were significantly lower in mothers delivered by emergency caesarean section due to suspected fetal distress (n=53, 43.6±18 nmol/L) compared to controls (n=120, 48.6±19 nmol/L, p=0.04). Birth asphyxia was more common in women with vitamin D deficiency (n=95) in early pregnancy (OR 2.4, 95% CI 1.1 to 5.7).

Conclusions: Low vitamin D levels in early pregnancy may be associated with emergency caesarean section due to suspected fetal distress and birth asphyxia. If our findings are supported by further studies, preferably on severe birth asphyxia, vitamin D supplementation/sun exposure in pregnancy may lower the risk of subsequent birth asphyxia.

Strengths and limitations of this study

- This is the first study to show that women who underwent an emergency caesarean section due to suspected fetal distress had lower vitamin D levels in early pregnancy as compared to those in a control group.
- We have studied a population-based sample and have used non-restricted inclusion criteria. This makes the study sample representative of the general population.
- Only one blood sample drawn from each mother gives us a snapshot of the vitamin D status in early pregnancy.
- Vitamin D non-deficient women may have a healthier lifestyle for which we could not control.

INTRODUCTION

Vitamin D is necessary for optimal skeletal function and deficiency is related to rachitis.[1]

It is, however, important for bone metabolism, as well as for optimal function of striated and smooth muscle strength including heart muscle, and is related to postnatal muscle strength.[2] Vitamin D supplementation has a positive impact on muscle strength on individuals with vitamin D deficiency.[1 3 4] The Institute of Medicine (IOM), USA recommends daily nutritional intake of vitamin D of 600 U, but others recommend higher doses.[5 6] A recent Swedish study showed that the mean nutritional intake of vitamin D was <200 U/day.[7] Although vitamin D is found in low amounts in the diet, mainly in oily fish and egg, the primary source of vitamin D for humans is skin conversion to vitamin D from solar ultraviolet radiation.[1] Pregnant women residing at high latitudes are at risk of vitamin D deficiency because of low solar intensity, especially during the winter months.[1 8] Vitamin D deficiency is common in the Nordic countries, especially among those not exposing themselves to the sun.[8] Since fetal vitamin D levels are directly related to that of their mothers there is also a high likelihood of fetal vitamin D deficiency in our population.[9]

Birth asphyxia is associated with cardiovascular dysfunction, including low ventricular output, lower left ventricular ejection fraction and increased troponin levels.[10 11] Congestive heart failure may occur in severe cases of asphyxia.[12] Intrauterine fetal distress is related to an increase in blood pressure, redistribution and a change in fetal heart rate pattern. Therefore, cardiotocography (CTG) is the main instrument of fetal surveillance.[13] It is plausible that vitamin D

deficiency could make the fetal heart more vulnerable to fetal distress/birth asphyxia. Several studies have reported increased frequency of emergency caesarean delivery in relation to low vitamin D,[14 15] but no previous study has been particularly designed to study the relation between low vitamin D levels, measured as 25-hydroxy (OH) vitamin D, in early pregnancy and the risk for fetal distress/birth asphyxia.

The primary aim of this pilot study was to investigate 25-OH vitamin D, the main marker of vitamin D status, in women who underwent caesarean section due to suspected fetal distress compared to those who did not. Furthermore, we compared the rate of birth asphyxia in women with vitamin D deficiency and non-deficiency in early pregnancy.

MATERIALS AND METHODS

Patients

Out of a population cohort of 2496 women, we identified all the 53 women who underwent emergency caesarean section due to suspected fetal distress. The diagnosis of suspected fetal distress was carried out with the discretion of the obstetrician in charge, mainly based on fetal heart rate monitoring and/or fetal blood sampling. Controls were selected by computerised random selection (SPSS V.20.0) comprising 10 women who gave birth each month of the year (n=120).

Small-for-gestational age (SGA) was defined according to the Swedish reference algorithms (~lowest 3rd centile).[16] Preterm delivery was defined as delivery before 37 completed weeks of gestation. Gestational age was calculated by ultrasonographic measurements of femur length and biparietal diameter in all but two women, who were dated by last menstrual period. Birth asphyxia was defined as Apgar <7 at 5 min and/or umbilical cord pH≤7.15. This compound measurement was used as a secondary outcome.

Vitamin D analysis

Venous serum samples were collected at enrolment between February 1994 and June 1995 at a mean of 12 weeks of gestation, centrifuged and stored at −80°C until analysis of 25-OH vitamin D. 25-OH vitamin D levels were measured in nmol/L. Vitamin D deficiency was defined as 25-OH vitamin D <50 nmol/L and non-deficiency as ≥50 nmol/L according to IOM.[17] All serum samples were analysed at the Karolinska University Laboratory, with a direct competitive chemiluminescence immunoassay for 25-OH vitamin D from DiaSorin on a LIASON instrument (DiaSorin , Stillwater, Minnesota, USA). The method measured both 25-OH vitamin D2 and D3 with equimolar sensitivity, with a dynamic range of 10–375 nmol/L. The functional sensitivity was ≤10 nmol/L. Coefficient of variance intra-assay was 5% and interassay 7–14% and the method is accredited according to ISO15189.[18]

Statistics

Student's t-test or cross-tabulation with a χ^2 test with a 95% CI was used as appropriate. We performed a logistic regression analysis and used emergency caesarean delivery due to suspected fetal distress as the dependent variable and vitamin D level, and smoking habits and parity as independent variables. Statistical significance was set to p<0.05. For statistical analysis, SPSS V.20.0 was used. The mean 25-OH vitamin D level was expected to be 50±27.5 nmol/L, based on a Scandinavian study.[8]

RESULTS

The background characteristics were not different between cases and controls other than an expected increased probability of being nulliparous and having a preterm delivery among women undergoing caesarean delivery due to suspected fetal distress (table 1). As expected, caesarean delivery due to suspected fetal distress was related to an increased proportion of birth asphyxia and newborn SGA (table 1). In the crude analysis, the mean 25-OH vitamin D levels in women undergoing caesarean delivery due to suspected asphyxia was 43.6±18 nmol/L, which was comparable to controls, 48.6 ±19 nmol/L (p=0.1). In the adjusted analysis, controlling for nulliparity and smoking, vitamin D levels were significantly lower in cases versus controls (p=0.04).

To study the effect of vitamin D levels in early pregnancy and the risk for birth asphyxia, we divided the study population into two groups: those with vitamin D deficiency (25-OH vitamin D <50 nmol/L, n=95) and those who were non-deficient (25-OH vitamin D ≥50 nmol/L, n=78) (table 2). The rate of birth asphyxia was more than doubled in women with vitamin D deficiency as compared to non-deficient women in crude and adjusted analysis (OR=2.4, 95% CI 1.1 to 5.7 and OR=2.9, 95% CI 1.2 to 7.0, respectively). Vitamin D deficient mothers had a significantly shorter gestational age at birth (p=0.02), but no significant difference in preterm birth rate (13.7% vs 5.1%, p=0.06) (table 2). The proportion of pregnant women with vitamin D deficiency (<50 nmol/L) in the whole population was 70% during winter/spring season (December–May) and 36% during summer season.

In a stratified analysis including only lean women (≤25 in body mass index (BMI)), the significance of difference of vitamin D levels among those delivered by caesarean section due to fetal distress in crude and adjusted analysis (p=0.05 and p=0.03, respectively) and the risk of birth asphyxia was more than doubled among those with vitamin D deficiency (OR=2.5, 95% CI 1.0 to 6.1 and OR=2.9, 95% CI 1.1 to 7.5, respectively).

DISCUSSION

This pilot study is the first to show that women who underwent emergency caesarean section due to suspected fetal asphyxia had lower vitamin D levels in early pregnancy as compared to a control group. In addition,

Table 1 Characteristics of study participants and control group

	CS due to suspected birth asphyxia n=53		Control group n=120		Significance of difference (p)	Adjusted significance of difference (p)*
Maternal characteristics						
Age (years)	30.4	5.7	29.2	4.6	0.2	
Height (cm)	163.2	6.4	165.2	6.7	0.07	
Body mass index (kg/m^2)	23.8	3.9	23.1	3.9	0.3	
Nulliparous	35	66.0%	50	41.7%	0.03	0.002
Smoker	15	28.3%	20	16.7%	0.08	0.1
Vitamin D level (nmol/L)	43.6	18	48.6	19	0.1	0.04
Mode of delivery						
Vaginal spontaneous	0	0%	98	81.7%		
Vaginal assisted	0	0%	10	8.3%		
Caesarean section other reasons	0	0%	12	10%		
CS due to suspected fetal distress	53	100%	0	0%		
Neonatal outcome						
Gestational age (days)	272.2	23.8	277	13.5	0.2	
Preterm delivery (n)	9	17.1%	8	6.7%	0.04	
Birth weight (g)	2992.4	900	3550.3	619	<0.001	
Birth weight deviation (%)	−9.9	18	3.0	14	<0.001	
SGA (n)	16	30.2%	0	0%	<0.001	
5 min Apgar score <7 (n)	8	15.1%	0	0%	<0.001	
Umbilical artery pH	7.20	0.09	7.22	0.08	0.3	
Umbilical vein pH	7.25	0.1	7.31	0.07	0.001	
Umbilical cord pH ≤7.15 (n)	13	24.5%	15	12.5%	0.05	
Birth asphyxia (n)	17	32.1	15	12.5	0.002	

Mean and SD or number and percentages are given. Birth weight deviation=Birth weight minus expected birth weight (for gestational age/expected birth weight and expressed as a percentage, birth asphyxia=5 min Apgar score <7 and/or umbilical vessel pH ≤7.15.
*Logistic regression analysis including nulliparity, smoking and vitamin D level.
CS, caesarean section; SGA, small-for-gestational age.

birth asphyxia was more common in women with vitamin D deficiency than in non-deficient women. In fact, the only study previously addressing this topic was performed in southern China, where vitamin D deficiency is relatively uncommon and no relation to birth asphyxia was found.[19] Two randomised controlled studies of antenatal vitamin D supplementation reported lower Apgar score at 1 and 5 min, respectively.[20 21] In addition, in the latter study reported, 13% of vitamin D deficient newborn had Apgar score at 5 min <7, as compared to 1.1% among those who were sufficient.[21] Our observational study design disables us from investigating a causal relation between vitamin D levels and fetal distress/birth asphyxia. Beside the two studies showing increased caesarean delivery rate with low vitamin D levels,[14 15] a large study with blood drawn in early pregnancy showed no difference between caesarean and vaginal delivery depending on vitamin D levels after adjustments.[22] However, in the subgroup of women with caesarean delivery due to fetal distress (n=46), the median 25-OH vitamin D level was 32.9 nmol/L, as compared to 46.6 nmol/L among the control group (n=796). Vitamin D deficiency was associated with a significantly shorter gestational age at delivery, which is in line with other data.[23] Furthermore, there seems to be an inverse relation between low active vitamin D (1,25-dihydroxy vitamin D) and meconium-stained amniotic fluid, a sign of fetal distress, in pregnancies complicated by intrahepatic cholestasis.[24]

The findings that more than two-third of mothers were vitamin D deficient during winter/spring season and one-third at summer are consistent with previous reports.[25 26] In this study, we used the limits of 25-OH vitamin D suggested by the IOM,[17] but the discussion of what levels should be considered deficient is ongoing. The daily recommended intake of vitamin D is 600 IU/day in the USA[5] Since the late 1990s in France, there have been official recommendations of 1000 U vitamin D/day from 32 weeks of gestation or 100 000 or 200 000 IU as a single dose at 32 weeks in order to lower complications in newborns.[27]

Calcium homeostasis in the heart is important for the contractility and function of the heart. Animal studies show that the addition of active vitamin D to vitamin D deficient chick heart cells showed an increased Ca^{2+} influx. This was connected to the cyclic adenosine monophosphate (cAMP) pathway and related to accelerated relaxation.[28 29] This effect was not seen in vitamin D receptor knockout mice, which implies that the effect is mediated by the vitamin D receptor which seems

Table 2 Characteristics in relation to maternal vitamin D levels in the whole study group

25-OH vitamin D	<50 nmol/L Vitamin D deficiency n=95		≥ 50 nmol/L Not deficiency n=78		Significance of difference (p)
Maternal characteristics					
Age (years)	29.1	5.3	30.2	4.4	0.1
Height (cm)	163.2	5.9	166.3	7.1	0.02
Body mass index (kg/m^2)	23.7	4.1	22.9	3.6	0.2
Nulliparous (n)	42	44.2%	43	55.1%	0.2
Smoker (n)	18	18.9%	17	21.8%	0.6
Mode of delivery					
Vaginal spontaneous (n)	52	54.7%	46	59.0%	0.6
Vaginal assisted (n)	3	3.2%	7	9.0%	0.2
Caesarean section (n)	40	42.1%	25	32.1%	0.2
CS due to suspected fetal distress (n)	33	34.7%	20	25.6%	0.2
Neonatal outcome					
Gestational age (days)	273.0	19.1	278.9	14.6	0.02
Preterm delivery (n)	13	13.7%	4	5.1%	0.06
Birth weight (g)	3323.5	819	3447.5	678	0.3
SGA (n)	11	11.6%	5	6.4%	0.2
5 min Apgar score <7 (n)	6	6.3%	2	2.6%	0.3
Umbilical artery pH	7.20	0.09	7.23	0.07	0.1
Umbilical vein pH	7.28	0.09	7.30	0.08	0.4
Umbilical cord pH ≤7.15	20	21.1%	8	10.3%	0.06
Birth asphyxia	23	24.2%	9	11.5%	0.03

Mean and SD or number and percentages are given.
CS, caesarean section; OH, hydroxy; SGA, small-for-gestational age.

important for cardiac muscle function.[28 30] Using a state cardiac diagram, asphyxia is slowing the relaxation phase in the fetal heart.[31 32] Pregnancy is a condition with increased oestrogen levels. Both oestrogenic compounds and parathyroid hormone upregulate 1,25-dihydroxy vitamin D in vascular smooth muscle cells.[33] Thus, there are several vitamin D-related mechanisms that could affect the strained fetal heart during the critical time of birth. These mechanisms are possible explanations of our finding that the rate of birth asphyxia was more than doubled in women with vitamin D deficiency compared to non-deficient women.

One strength of our study is the nested case–control design. The population sample is representative of women delivering and living in Malmö with good socioeconomic standard and good health resources. Another strength of the study is the non-restricted inclusion criteria of the controls that make it a good representative for the general population. Furthermore, the specimens have been stored at −80°C. Antoniucci *et al*[34] have shown that thawing and refreezing of samples up to four times do not affect the vitamin D analysis. In our study, the samples from both cases and controls have been handled similarly. In the logistic regression analysis of vitamin D levels in women who underwent caesarean section due to suspected fetal distress/birth asphyxia, we did not adjust for maternal BMI since it seems to be involved in a causal pathway.[35] However, similar results were found in stratified analysis of lean women (≤25 in BMI). We noted that women with vitamin deficiency were 3 cm shorter than non-deficient women, which is in agreement with prior observations.[36 37] This pilot study has some limitations and was designed to assess suspected fetal distress/moderate birth asphyxia and not limited to severe birth asphyxia. Since prior studies had reported on increased risk of emergency caesarean delivery in relation to low vitamin D, we used caesarean delivery due to suspected fetal distress as a main outcome and birth asphyxia as a secondary outcome. With our present knowledge, we should have designed the study to compare cases with birth asphyxia, regardless of mode of delivery, with a control group as the main outcome. The fact that there was only one blood sample drawn from each mother just gives us a snapshot of the vitamin D status in early pregnancy. We did not obtain vitamin D data in late pregnancy in these women. The limited size of the study is a limitation. However, there were indications of differences between emergency caesarean and vaginal deliveries. A problem with studying fetal distress/birth asphyxia is that it may represent fetal vulnerability and suboptimal care. Further, vitamin D non-deficient women may have a healthier lifestyle for which we could not control. 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. We speculate that our finding of shorter

maternal height among vitamin D deficient women might be due to an increased prevalence of vitamin D deficiency during childhood and adolescence. Low vitamin D levels during the longitudinal growth period might have resulted in that these individuals did not reach their full growth potential.

We found that women delivered by emergency caesarean section due to suspected fetal distress had lower vitamin D levels in early pregnancy and birth asphyxia was more common in vitamin D deficient women as compared to non-deficient women. If other groups reproduce our findings and a causal relationship can be established, we might be in a position to lower the risk of fetal distress/birth asphyxia with vitamin D supplementation/sun exposure in pregnancy.

Acknowledgements The authors would like to thank Dr Emma Öberg for linguistic review. Address all correspondence and requests for reprints to PGL, Department of Obstetrics and Gynaecology, Clintec, Karolinska University Hospital, Huddinge, Kvinnokliniken K57, SE-14186 Stockholm, Sweden. Email: Pelle.lindqvist@ki.se.

Contributors PGL contributed to the design of the study, as well as carried out data analysis and a major part of the writing. ATS contributed to the design of the study, carried out the experimental analyses and revised and approved the final draft of the manuscript. SAG supervised the experimental analyses and revised and approved the final draft of the manuscript. SG contributed to the design of the study, carried out data analysis and was responsible for major critical revisions of the manuscript.

Funding This study was supported by the Karolinska Institutet (PGL and ATS) and a postdoctoral fellowship from the Stockholm County Council to SG. The funding sources had no role in the design and conduct of the study; collection, management analysis and interpretation of the data; preparation, review or approval of the manuscript; and decision to submit the manuscript for publication.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the regional Ethics Committee, Lund University (LU 128-03).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Consent for publication of raw data was not obtained and data set could in theory pose a threat to confidentiality.

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