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Maternal vitamin D deficiency and fetal birth distress - A population-based nested case control study

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Conflicts of interest

The authors have nothing to declare.

Key words and abbreviations

Key words:

Vitamin D, asphyxia, emergency caesarean section, distress

Abbreviations:

25-OH vitamin D, 25-hydroxy vitamin D

BMI, body mass index

BNP, B-type natriuretic peptide

cAMP, cyclic adenosine monophosphate

CTG, cardiotocography

IU, international units

PTH, parathyroid hormone

SGA, small for gestational age

UV, ultraviolet

ABSTRACT

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

Design and Setting A population based nested case-control study.

Patients Banked sera of 2496 women from 12th week of gestation.

Outcome measures Vitamin D levels were analysed using a direct competitive chemiluminescence immunoassay. Vitamin D levels in early gestation in women delivered by emergency cesarean section due to suspected fetal asphyxia or not. Newborn birth distress, defined as Apgar < 7 at 5 minutes and/or umbilical cord pH $\leq$ 7.15.

Results Vitamin D levels were significantly lower in mothers delivered by emergency cesarean section due to suspected fetal asphyxia (n = 53, 43.6 $\pm$ 18 nmol/L) compared to controls (n = 120, 48.6 $\pm$ 19 nmol/L, p = 0.04). Newborn birth distress was more common in women with vitamin D deficiency (n = 95) in early pregnancy (OR 2.4 95%, confidence interval 1.1-5.7).

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

Strength and limitations of this study

- This is first study to show that women who underwent emergency cesarean section due to suspected fetal asphyxia had lower vitamin D levels in early pregnancy as compared to a control group.
- We have studied a population-based sample and have used non-restricted inclusion criteria. This make the study sample representative for the general population.
- Only one blood sample drawn from each mother just 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 not only for the bone metabolism, but also 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.[5] A recent Swedish study showed the mean nutritional intake of vitamin D was less than 200 U per day.[6] 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 UV 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.[1,7] Vitamin D deficiency is common in the Nordic countries especially among those not exposing themselves to the sun.[7] 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.[8]

Birth asphyxia is associated with cardiovascular dysfunction, including low ventricular output, lower left ventricular ejection fraction and increased troponin levels.[9,10] Congestive heart failure may even occur in severe cases of asphyxia.[11] 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.[12] It is plausible that vitamin D deficiency could make the fetal heart more vulnerable for birth distress/asphyxia. Several studies have reported increased frequency of emergency cesarean delivery in relation to low vitamin D,[13,14] but no previous study has been particularly designed

to study the relation between low vitamin D levels, measured as 25-hydroxy vitamin D, in early pregnancy and the risk for fetal/newborn birth distress.

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

Material and methods

Patients

Out of a population cohort of 2496 women, we identified all the 53 women who underwent emergency cesarean section due to suspected fetal asphyxia mainly based on fetal heart rate monitoring. Controls were selected by a computerized random selection (SPSS 20.0) comprising of ten 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 4th percentile).[15] 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. Newborn birth distress was defined as Apgar < 7 at 5 minutes 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 -80ºC until analysis of 25-OH vitamin D. 25-hydroxy vitamin D levels were measured in nmol/L. Vitamin D deficiency was defined as 25-hydroxy vitamin D < 50 nmol/L and nondeficiency as ≥ 50 nmol/L according to IOM.[16] All serum samples were analyzed at the Karolinska University Laboratory, with a

direct competitive chemiluminescence immunoassay for 25-hydroxy vitamin D from DiaSorin on a LIASON instrument (DiaSorin Inc, Stillwater, MN, USA). The method measured both 25-hydroxy 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 intraassay was 5% and interassay 7-14%.[17]

Statistics

Student's t-test or cross-tabulation with χ^2-test with a 95% confidence interval was used as appropriate. We performed a logistic regression analysis and used emergency cesarean delivery due to suspected fetal asphyxia as the dependent variable and vitamin D level, smoking habits and parity as independent variables. Statistical significance was set to $p < 0.05$. For statistical analysis the SPSS 20.0 was used. The mean 25-hydroxy vitamin D level was expected to be 50 ± 27.5 nmol/L, based on a Scandinavian study.[7]

Ethics committee approval

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

Results

The background characteristics were not different between cases and controls other than an anticipated increased probability of being nulliparous and having a preterm delivery among women undergoing cesarean delivery due to suspected asphyxia (Table 1). As expected, cesarean delivery due to suspected asphyxia was related to an increased proportion of newborn birth distress and newborn SGA (Table 1). In crude analysis the mean 25-hydroxy vitamin D levels in women undergoing cesarean delivery due to suspected asphyxia was 43.6 ± 18 nmol/L, which was comparable to controls, 48.6 ±19 nmol/L (P = .1). In adjusted analysis, controlling for nulliparity and smoking, the difference in vitamin D levels was significantly lower (P = .04).

To study the effect of vitamin D levels in early pregnancy and the risk for newborn birth distress we divided the study population into two groups: those with vitamin D deficiency (25-hydroxy vitamin D < 50 nmol/L, n = 95) and those non-deficient (25-hydroxy vitamin D ≥ 50 nmol/L, n = 78) (Table 2). The rate of fetal birth distress was more than doubled in women with vitamin D deficiency as compared to non-deficient women (OR = 2.4, 95% CI 1.1- 5.7). Vitamin D deficient mothers had a significantly shorter gestational age at birth (P = .02), but no 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 to May) and 36% during summer season. In stratified analysis including only lean women (≤ 25 in BMI), there were lower vitamin D levels among those delivered by cesarean section due to suspected fetal distress in adjusted analysis (p = 0.03) and the risk of newborn birth distress was doubled among those with vitamin D deficiency (OR 2.4, 95% CI [1.0-6.2]).

Discussion

This pilot study is the first to show that women who underwent emergency cesarean section due to suspected fetal asphyxia had lower vitamin D levels in early pregnancy as compared to a control group. In addition, newborn birth distress was more common in women with vitamin D deficiency than in non-deficient women. In fact, the only study previously addressing this topic was done in southern China, where vitamin D deficiency is uncommon, and no relation to newborn birth distress was found.[18] Our observational study design disables us from investigating a causal relation between vitamin D levels and fetal/newborn birth distress. Beside the two studies showing increased cesarean delivery rate with low vitamin D levels,[13,14] a large study with blood drawn in early pregnancy showed no difference between cesarean- and vaginal delivery depending on vitamin D levels after adjustments.[19] However, in the subgroup of women with caesarean delivery due to fetal distress (n = 46), the median 25-hydroxy vitamin D level was 32.9 nmol/L, as compared to 46.6 nmol/l among the control group (n = 796). There seems to be an inverse relation between active vitamin D (1,25-dihydroxy vitamin D) and meconium stained amniotic fluid, a sign of fetal distress, in pregnancies complicated by intrahepatic cholestasis.[20]

The finding that more than 2/3 of mothers were vitamin D deficient during winter/spring season and 1/3 at summer are consistent with previous reports.[21,22] In this study we used the limits of 25-hydroxy vitamin D suggested by the IOM,[16] but the discussion of what levels should be considered deficient is ongoing. The daily recommended intake of vitamin D was recently increased to 600 IU per day in USA [5] Since late 1990's in France there has been official recommendations of 1000 U vitamin D/d from 32 weeks of gestation or 100 000 IU or 200 000 IU as a single dose at 32 weeks in order to lower complications in newborns.[23]

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 cAMP pathway and related to accelerated relaxation.[24,25] This effect was not seen in vitamin D receptor knock out mice, which implies that the effect is mediated by the vitamin D receptor which seem important for cardiac muscle function.[24,26] Using a state cardiac diagram, asphyxia is slowing the relaxation phase in the fetal heart.[27,28] Pregnancy is a condition with increased estrogen levels. Both estrogenic compounds and PTH up regulate 1,25-dihydroxy vitamin D in vascular smooth muscle cells.[29] 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 newborn birth distress 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 makes it a good representative for the general population. Furthermore, the specimens have been stored at -80°C. Antoniucci and co-workers have shown that thawing and refreezing of samples up to four times do not affect the vitamin D analysis.[30] In our study the samples from both cases and controls had been handled similarly. In the logistic regression analysis of vitamin D levels in women who underwent cesarean section due to suspected asphyxia we did not adjust for maternal BMI since it seem to be involved in a causal pathway.[31] 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.[32,33] This pilot study has some limitations and was designed to assess birth distress and suspected asphyxia and not

established fetal asphyxia. 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. However, there were indications of differences between emergency cesarean and vaginal deliveries. A problem with studying fetal distress/asphyxia is that it may represent both fetal vulnerability and suboptimal care. Further, vitamin D non-deficient women may have a healthier lifestyle, for which we could not control. Future research should aim to investigate if a similar relationship might be found in established/severe birth asphyxia. 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 youth. 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.

We found that women delivered by emergency cesarean section due to suspected fetal asphyxia had lower vitamin D levels in early pregnancy and newborn birth distress 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 birth distress/asphyxia with vitamin D supplementation/sun exposure in pregnancy.

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Funding statement

No additional data available.

Contributors

PGL contributed to the design of the study, carried out data analysis, and carried out a major part of the writing. ATS contributed to the design of the study and carried out the experimental analyses and revised and approved the final draft of the manuscript. SvG 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.

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Legends of Figures and Tables

Table 1

Mean and standard deviation or number and percentages are given. CS = cesarean section, SGA = small-for-gestational age, Birthweight deviation = Birthweight minus expected birthweight (for gestational age/expected birthweight and expressed as a percentage, fetal birth distress = 5-min Apgar score < 7 and/or umbilical vessel pH ≤ 7.15. a Logistic regression analysis including nulliparity, smoking, and vitamin D level

Table 2

Mean and standard deviation or number and percentages are given

Table 1 Characteristic 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*)[a]
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/m2)	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 (mmol/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%		
Cesarean section other reasons	0	0%	12	10%		
CS due to susp asphyxia	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	
Birthweight (gr)	2992.4	900	3550.3	619	<0.001	
Birthweight 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	
Fetal birth distress (n)	17	32.1	15	12.5	0.002	

Mean and standard deviation or number and percentages are given. CS = cesarean section, SGA = small-for-gestational age, Birthweight deviation = Birthweight minus expected birthweight (for gestational age/expected birthweight and expressed as a percentage, fetal birth distress = 5-min Apgar score < 7 and/or umbilical vessel pH ≤ 7.15. [a] Logistic regression analysis including nulliparity, smoking, and vitamin D level

Table 2 Characteristic 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/m2)	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
Cesarean section (n)	40	42.1%	25	32.1%	0.2
CS due to susp asphyxia (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
Birthweight (gr)	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 distress	23	24.2%	9	11.5%	0.03

Mean and standard deviation or number and percentages are given

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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

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Conflicts of interest

The authors have nothing to declare.

Key words and abbreviations

Key words:

Vitamin D, asphyxia, emergency caesarean section, distress

Abbreviations:

25-OH vitamin D, 25-hydroxy vitamin D

BMI, body mass index

BNP, B-type natriuretic peptide

cAMP, cyclic adenosine monophosphate

CTG, cardiotocography

IU, international units

PTH, parathyroid hormone

SGA, small for gestational age

UV, ultraviolet

ABSTRACT

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

Design and Setting A population based nested case-control study.

Patients Banked sera of 2496 women from 12th week of gestation.

Outcome measures Vitamin D levels were analysed using a direct competitive chemiluminescence immunoassay. Vitamin D levels in early gestation in women delivered by emergency cesarean section due to suspected fetal distress, or not. Birth asphyxia was defined as Apgar < 7 at 5 minutes and/or umbilical cord pH $\leq$ 7.15.

Results Vitamin D levels were significantly lower in mothers delivered by emergency cesarean section due to suspected fetal distress (n = 53, 43.6 $\pm$ 18 nmol/L) compared to controls (n = 120, 48.6 $\pm$ 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% confidence interval 1.1-5.7).

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

Strength and limitations of this study

- This is first study to show that women who underwent emergency cesarean section due to suspected fetal distress had lower vitamin D levels in early pregnancy as compared to a control group.
- We have studied a population-based sample and have used non-restricted inclusion criteria. This makes the study sample representative for the general population.
- Only one blood sample drawn from each mother just 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 not only for the bone metabolism, but also 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.[5] A recent Swedish study showed the mean nutritional intake of vitamin D was less than 200 U per day.[6] 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 UV 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.[1,7] Vitamin D deficiency is common in the Nordic countries especially among those not exposing themselves to the sun.[7] 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.[8]

Birth asphyxia is associated with cardiovascular dysfunction, including low ventricular output, lower left ventricular ejection fraction and increased troponin levels.[9,10] Congestive heart failure may occur in severe cases of asphyxia.[11] 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.[12] It is plausible that vitamin D deficiency could make the fetal heart more vulnerable for fetal distress/birth asphyxia. Several studies have reported increased frequency of emergency cesarean delivery in relation to low vitamin D,[13,14] but no previous study has been particularly designed to study

the relation between low vitamin D levels, measured as 25-hydroxy vitamin D, in early pregnancy and the risk for fetal distress/birth asphyxia.

The primary aim of this pilot study was to investigate 25-hydroxy vitamin D, the main marker of vitamin D status, in women who underwent cesarean 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.

Material and methods

Patients

Out of a population cohort of 2496 women, we identified all the 53 women who underwent emergency cesarean section due to suspected fetal distress. 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. Controls were selected by a computerized random selection (SPSS 20.0) comprising of ten 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 percentile).[15] 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 minutes 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 -80ºC until analysis of 25-OH

vitamin D. 25-hydroxy vitamin D levels were measured in nmol/L. Vitamin D deficiency was defined as 25-hydroxy vitamin D < 50 nmol/L and nondeficiency as ≥ 50 nmol/L according to IOM.[16] All serum samples were analyzed at the Karolinska University Laboratory, with a direct competitive chemiluminescence immunoassay for 25-hydroxy vitamin D from DiaSorin on a LIASON instrument (DiaSorin Inc, Stillwater, MN, USA). The method measured both 25-hydroxy 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 intraassay was 5% and interassay 7-14% and the method is accredited according to ISO15189.[17]

Statistics

Student's t-test or cross-tabulation with χ^2-test with a 95% confidence interval was used as appropriate. We performed a logistic regression analysis and used emergency cesarean delivery due to suspected fetal distress as the dependent variable and vitamin D level, smoking habits and parity as independent variables. Statistical significance was set to $p < 0.05$. For statistical analysis the SPSS 20.0 was used. The mean 25-hydroxy vitamin D level was expected to be 50 ± 27.5 nmol/L, based on a Scandinavian study.[7]

Ethics committee approval

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

Results

The background characteristics were not different between cases and controls other than an anticipated increased probability of being nulliparous and having a preterm delivery among women undergoing cesarean delivery due to suspected fetal distress (Table 1). As expected, cesarean delivery due to suspected fetal distress was related to an increased proportion of birth asphyxia and newborn SGA (Table 1). In crude analysis the mean 25-hydroxy vitamin D levels in women undergoing cesarean delivery due to suspected asphyxia was 43.6 ± 18 nmol/L, which was comparable to controls, 48.6 ±19 nmol/L (P = .1). In adjusted analysis, controlling for nulliparity and smoking, the difference in vitamin D levels was significantly lower (P = .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-hydroxy vitamin D < 50 nmol/L, n = 95) and those non-deficient (25-hydroxy 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- 5.7 and OR = 2.9, 95% CI 1.2−7.0, respectively). Vitamin D deficient mothers had a significantly shorter gestational age at birth (P = .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 to May) and 36% during summer season.

In stratified analysis including only lean women (≤ 25 in BMI), the significance of difference of vitamin D levels among those delivered by cesarean 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 1.0−6.1 and OR = 2.9, 95% CI 1.1−7.5, respectively).

Discussion

This pilot study is the first to show that women who underwent emergency cesarean section due to suspected fetal asphyxia had lower vitamin D levels in early pregnancy as compared to a control group. In addition, 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 done in southern China, where vitamin D deficiency relatively uncommon and no relation to birth asphyxia were found.[18] Two randomized controlled studies of antenatal vitamin D supplementation reported lower APGAR score at 1 minute and 5 minutes, repectively.[19,20] 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.[20] 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 cesarean delivery rate with low vitamin D levels,[13,14] a large study with blood drawn in early pregnancy showed no difference between cesarean- and vaginal delivery depending on vitamin D levels after adjustments.[21] However, in the subgroup of women with caesarean delivery due to fetal distress (n = 46), the median 25-hydroxy vitamin D level was 32.9 nmol/L, as compared to 46.6 nmol/l among the control group (n = 796). Furthermore, there seems to be an inverse relation between active vitamin D (1,25-dihydroxy vitamin D) and meconium stained amniotic fluid, a sign of fetal distress, in pregnancies complicated by intrahepatic cholestasis.[22]

The findings that more than 2/3 of mothers were vitamin D deficient during winter/spring season and 1/3 at summer are consistent with previous reports.[23,24] In this study we used the limits of 25-hydroxy vitamin D suggested by the IOM,[16] but the discussion of what levels should be considered deficient is ongoing. The daily recommended intake of vitamin D is 600

IU per day in USA [5] Since late 1990's in France there has been official recommendations of 1000 U vitamin D/d from 32 weeks of gestation or 100 000 IU or 200 000 IU as a single dose at 32 weeks in order to lower complications in newborns.[25]

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 cAMP pathway and related to accelerated relaxation.[26,27] This effect was not seen in vitamin D receptor knock out mice, which implies that the effect is mediated by the vitamin D receptor which seems important for cardiac muscle function.[26,28] Using a state cardiac diagram, asphyxia is slowing the relaxation phase in the fetal heart.[29,30] Pregnancy is a condition with increased estrogen levels. Both estrogenic compounds and PTH up regulate 1,25-dihydroxy vitamin D in vascular smooth muscle cells.[31] 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 makes it a good representative for the general population. Furthermore, the specimens have been stored at -80^{o}C. Antoniucci and co-workers have shown that thawing and refreezing of samples up to four times do not affect the vitamin D analysis.[32] In our study the samples from both cases and controls had been handled similarly. In the logistic regression analysis of vitamin D levels in women who underwent cesarean section due to suspected fetal distress/birth asphyxia we did not adjust for maternal BMI since

it seem to be involved in a causal pathway.[33] However, similar results were found in stratified analysis of lean women (≤ 25 in BMI). We noted that women with vitamin deficiency were three cm shorter than non-deficient women, which is in agreement with prior observations.[34,35] 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 cesarean delivery in relation to low vitamin D, we had cesarean delivery due to suspected fetal distress as main outcome and birth asphyxia as secondary outcome. With our present knowledge we should have made the opposite and modified the design accordingly. 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 cesarean and vaginal deliveries. A problem with studying fetal distress/birth asphyxia is that it may represent both fetal vulnerability and suboptimal care. Further, vitamin D non-deficient women may have a healthier lifestyle, for which we could not control. Future research should aim to investigate if a similar relationship might be found in severe birth asphyxia. 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 youth. 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.

We found that women delivered by emergency cesarean 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.

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Funding statement

No additional data available.

Contributors

PGL contributed to the design of the study, carried out data analysis, and carried out a major part of the writing. ATS contributed to the design of the study and carried out the experimental analyses and revised and approved the final draft of the manuscript. SvG 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.

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Legends of Figures and Tables

Table 1

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

Table 2

Mean and standard deviation or number and percentages are given

Table 1 Characteristic 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*)[a]
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/m2)	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%		
Cesarean section other reasons	0	0%	12	10%		
CS due to susp 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	
Birthweight (gr)	2992.4	900	3550.3	619	<0.001	
Birthweight 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 standard deviation or number and percentages are given. CS = cesarean section, SGA = small-for-gestational age, Birthweight deviation = Birthweight minus expected birthweight (for gestational age/expected birthweight and expressed as a percentage, Birth asphyxia = 5-min Apgar score < 7 and/or umbilical vessel pH ≤ 7.15. [a] Logistic regression analysis including nulliparity, smoking, and vitamin D level

Table 2 Characteristic 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/m2)	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
Cesarean section (n)	40	42.1%	25	32.1%	0.2
CS due to susp 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
Birthweight (gr)	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 standard deviation or number and percentages are given

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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

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The authors have nothing to declare.

Data sharing

Ddataset available from the Dryad repository

Key words and abbreviations

Key words:

Vitamin D, asphyxia, emergency caesarean section, distress

Abbreviations:

25-OH vitamin D, 25-hydroxy vitamin D

BMI, body mass index

BNP, B-type natriuretic peptide

cAMP, cyclic adenosine monophosphate

CTG, cardiotocography

IU, international units

PTH, parathyroid hormone

SGA, small for gestational age

UV, ultraviolet

ABSTRACT

Objective Vitamin D deficiency causes not only skeletal problems but also muscle weakness, including heart muscle. If also the fetal heart is affected it might be more susceptible to fetal distress and birth asphyxia. In this pilot study we hypothesized that low maternal Vitamin D levels are overrepresented 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 cesarean section due to suspected fetal distress were compared to controls. Birth asphyxia was defined as Apgar < 7 at 5 minutes and/or umbilical cord pH $\leq$ 7.15.

Results Vitamin D levels were significantly lower in mothers delivered by emergency cesarean section due to suspected fetal distress (n = 53, 43.6 $\pm$ 18 nmol/L) compared to controls (n = 120, 48.6 $\pm$ 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% confidence interval 1.1-5.7).

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

Strength and limitations of this study

- This is first study to show that women who underwent emergency cesarean section due to suspected fetal distress had lower vitamin D levels in early pregnancy as compared to a control group.
- We have studied a population-based sample and have used non-restricted inclusion criteria. This makes the study sample representative for the general population.
- Only one blood sample drawn from each mother just 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 not only for the bone metabolism, but also 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 other recommend higher doses.[5,6] A recent Swedish study showed the mean nutritional intake of vitamin D was less than 200 U per 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 UV 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 for fetal distress/birth asphyxia. Several studies have reported increased frequency of emergency cesarean 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 vitamin D, in early pregnancy and the risk for fetal distress/birth asphyxia.

The primary aim of this pilot study was to investigate 25-hydroxy vitamin D, the main marker of vitamin D status, in women who underwent cesarean 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.

Material and methods

Patients

Out of a population cohort of 2496 women, we identified all the 53 women who underwent emergency cesarean section due to suspected fetal distress. 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 blood sampling. Controls were selected by a computerized random selection (SPSS 20.0) comprising of ten 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 percentile).[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 minutes 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 -80ºC until analysis of 25-OH

vitamin D. 25-hydroxy vitamin D levels were measured in nmol/L. Vitamin D deficiency was defined as 25-hydroxy vitamin D < 50 nmol/L and nondeficiency as ≥ 50 nmol/L according to IOM.[17] All serum samples were analyzed at the Karolinska University Laboratory, with a direct competitive chemiluminescence immunoassay for 25-hydroxy vitamin D from DiaSorin on a LIASON instrument (DiaSorin Inc, Stillwater, MN, USA). The method measured both 25-hydroxy 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 intraassay was 5% and interassay 7-14% and the method is accredited according to ISO15189.[18]

Statistics

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

Ethics committee approval

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

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 cesarean delivery due to suspected fetal distress (Table 1). As expected, cesarean 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-hydroxy vitamin D levels in women undergoing cesarean delivery due to suspected asphyxia was 43.6 ± 18 nmol/L, which was comparable to controls, 48.6 ±19 nmol/L (P = .1). In the adjusted analysis, controlling for nulliparity and smoking, vitamin D levels were significantly lower in cases versus controls (P = .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-hydroxy vitamin D < 50 nmol/L, n = 95) and those who were non-deficient (25-hydroxy 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- 5.7 and OR = 2.9, 95% CI 1.2−7.0, respectively). Vitamin D deficient mothers had a significantly shorter gestational age at birth (P = .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 to May) and 36% during summer season.

In a stratified analysis including only lean women (≤ 25 in BMI), the significance of difference of vitamin D levels among those delivered by cesarean 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 1.0–6.1 and OR = 2.9, 95% CI 1.1–7.5, respectively).

Discussion

This pilot study is the first to show that women who underwent emergency cesarean section due to suspected fetal asphyxia had lower vitamin D levels in early pregnancy as compared to a control group. In addition, 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 done in southern China, where vitamin D deficiency relatively uncommon and no relation to birth asphyxia were found.[19] Two randomized controlled studies of antenatal vitamin D supplementation reported lower Apgar score at 1 minute and 5 minutes, respectively.[20,21] 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.[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 cesarean delivery rate with low vitamin D levels,[14,15] a large study with blood drawn in early pregnancy showed no difference between cesarean- 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-hydroxy 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 2/3 of mothers were vitamin D deficient during winter/spring season and 1/3 at summer are consistent with previous reports.[25,26] In this study we used the limits of 25-hydroxy 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 per day in USA [5] Since late 1990's in France there has been official recommendations of 1000 U vitamin D/d from 32 weeks of gestation or 100 000 IU 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 cAMP pathway and related to accelerated relaxation.[28,29] This effect was not seen in vitamin D receptor knock out mice, which implies that the effect is mediated by the vitamin D receptor which seems 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 estrogen levels. Both estrogenic compounds and PTH up regulate 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 makes it a good representative for the general population. Furthermore, the specimens have been stored at -80°C. Antoniucci and co-workers have shown that thawing and refreezing of samples up to four times do not affect the vitamin D analysis.[34] 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 cesarean

section due to suspected fetal distress/birth asphyxia we did not adjust for maternal BMI since it seem 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 three 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 cesarean delivery in relation to low vitamin D, we used cesarean delivery due to suspected fetal distress as main outcome and birth asphyxia as 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 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 cesarean and vaginal deliveries. A problem with studying fetal distress/birth asphyxia is that it may represent both 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 cesarean 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.

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Funding statement

No additional data available.

Contributors

PGL contributed to the design of the study, carried out data analysis, and carried out a major part of the writing. ATS contributed to the design of the study and carried out the experimental analyses and revised and approved the final draft of the manuscript. SvG 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.

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Legends of Figures and Tables

Table 1

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

Table 2

Mean and standard deviation or number and percentages are given

Table 1 Characteristic 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*)[a]
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/m2)	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%		
Cesarean section other reasons	0	0%	12	10%		
CS due to susp 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	
Birthweight (gr)	2992.4	900	3550.3	619	<0.001	
Birthweight 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 standard deviation or number and percentages are given. CS = cesarean section, SGA = small-for-gestational age, Birthweight deviation = Birthweight minus expected birthweight (for gestational age/expected birthweight and expressed as a percentage, Birth asphyxia = 5-min Apgar score < 7 and/or umbilical vessel pH ≤ 7.15. [a] Logistic regression analysis including nulliparity, smoking, and vitamin D level

Table 2 Characteristic 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/m2)	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
Cesarean section (n)	40	42.1%	25	32.1%	0.2
CS due to susp 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
Birthweight (gr)	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 standard deviation or number and percentages are given

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.