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Prevalence and association with birth outcomes of low Vitamin D levels among pregnant women living with HIV

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Abstract

Objectives: To evaluate the prevalence of low vitamin D levels among well-treated pregnant women living with HIV (WLWH) on combination antiretroviral therapy in Denmark, to identify risk factors of low vitamin D levels, and to assess the association between vitamin D status and birth outcomes.

Design: Nationwide cohort study

Methods: All WLWH in Denmark giving birth from 2000-2018 with a vitamin D measurement during pregnancy were identified. Risk factors for low vitamin D (deficiency or insufficiency) were assessed using log-binomial regression models, both univariate and adjusted for maternal and HIV factors. The association between vitamin D status and birth outcomes was assessed using linear regression models for continues outcomes and log-binomial models for binary outcomes.

Results: Among 208 WLWH, the prevalence of vitamin D deficiency was 13%, insufficiency 34%, and sufficiency 53%. Being of African origin (RR 2.68, p=0.01), Asian origin (RR 3.38, p=<0.01), or having HIV RNA levels >50 copies/mL (RR 1.43, p=0.04) was associated with an increased risk of low vitamin D level. WLWH with vitamin D deficiency had an increased risk of preterm birth (RR 2.66, p=0.03) and giving birth to small for gestational age (SGA) children (RR 6.83, p=0.02) compared to WLWH with sufficient vitamin D level.

Conclusion: Low vitamin D level was prevalent among well-treated pregnant WLWH in Denmark, especially among women of African or Asian origin, and women with detectable viral loads. Vitamin D deficiency was associated with an increased risk of preterm birth and SGA.

Keywords: Vitamin D, HIV, Pregnancy, Fetal Outcomes, Nationwide Cohort Study, Women

Introduction

Low vitamin D level during pregnancy, in women without human immunodeficiency virus (HIV), has been associated with a number of adverse maternal and neonatal outcomes such as pre-eclampsia, gestational diabetes, small-for-gestational-age (SGA), low birth weight and respiratory infections in childhood [1,4].

People living with HIV might have an increased risk of vitamin D insufficiency compared to individuals living without HIV [5–7], probably due to a combination of more traditional factors such as lack of sun exposure and malabsorption of vitamin D as well as treatment with combined Anti-Retroviral Therapy (cART) [8,10].

Vitamin D is an immune regulatory hormone. *In vivo*, it has been demonstrated to affect the CD4+ Th1-Th2 balance, inhibiting Th1 and augmenting Th2 cell development [11] and Th2 enhanced cytokine expression [12]. Further vitamin D also plays a role in the control of intracellular infections [13,14].

The literature on vitamin D in pregnancy among well-treated WLWH living in developed countries is limited. Thus, the aim of this study was primarily to evaluate the prevalence of low vitamin D levels among well-treated pregnant WLWH in Denmark, and secondary to identify risk factors for low vitamin D levels and to assess the association between vitamin D status and birth outcomes.

Method

Setting

The Danish population consists of 5.7 million inhabitants with an estimated adult HIV prevalence of 0.1% [15,16]. In Denmark, WLWH are followed at eight specialized centers during pregnancy and four specialized centers for delivery. Their children are followed at four affiliated pediatric units. cART is provided free of charge to all residents in Denmark with HIV, and most WLWH (84% in this study) are well treated with undetectable viral loads [17].

Study Population

From the Danish HIV Birth Cohort (DHBC), all WLWH giving birth to one or more singleton children in Denmark in the period 2000-2018 with a vitamin D measurement during pregnancy were identified.

The Danish HIV Birth Cohort

DHBC is a nationwide database including all pregnant WLWH, followed at Danish HIV treatment clinics since year 2000 and their infants. The Danish HIV treatment clinics are

located at Copenhagen University Hospitals, Hvidovre and Rigshospitalet, Odense University Hospital, Aalborg University Hospital and Aarhus University Hospital, Skejby.

Blood Samples

Not all women had blood analyzed for vitamin D during pregnancy. Additional blood samples, drawn from the women at a routine outpatient clinic visit during their pregnancy, available at the research biobank at the Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, were analyzed for 25-hydroxyvitamin D (25(OH)D) (n=78).

All patients included in DHBC give consent to the use of their blood samples in research.

Vitamin D analyses were performed by radioimmunoassays (2000-2012) and chemiluminescence assays (2012-2018), respectively. The blood samples were tested for vitamin D-levels on a COBAS 8000 analyzer robot (Roche Diagnostics, Indianapolis (IN) USA). Inter-assay variability did not exceed 12%.

Vitamin D

We defined vitamin D deficiency as 25(OH)D concentrations < 26 nmol/L, insufficiency as ≤ 50 nmol/L and sufficiency as > 50 nmol/L. In some analysis vitamin D was defined by two categories ≤ 50 mmol/L and >50 mmol/L.

Birth Outcomes

We defined preterm birth as gestational age <37 weeks [18], low birth weight as weight <2,500g [19] and small-for-gestational age as weight <10th percentile for gestational age by sex using the World Health Organization (WHO) fetal growth charts [20].

Statistical Models

Categorical variables were described in percentages and continuous variables were described as means (± standard deviation (SD)). Baseline characteristics were summarized and compared between women with - versus without a vitamin D measurement during pregnancy and compared between women with insufficient - versus sufficient vitamin D levels (≤50 nmol/L versus>50 nmol/L), using Pearson's χ 2 or Student unpaired *t* test as appropriate. A sensitivity analyses was performed, excluding participants who had frozen specimens obtained.

Risk factors for low vitamin D levels were assessed using log-binomial regression models, both univariate and adjusted for maternal- and HIV-related factors. We examined the following risk factors for low vitamin D levels: maternal age, maternal region of birth, CD4 cell count, HIV RNA, cART regime, smoking, season of vitamin D measurement and BMI. Multivariate models were adjusted for maternal age, maternal region of birth and HIV RNA.

Univariate models included a missing category, whereas individuals with missing explanatory values were excluded from the multivariable analyses

The association between vitamin D status and birth outcomes was assessed using linear regression models for continues outcomes and log-binomial models for binary outcomes. Multivariate models were adjusted for maternal age, maternal region of birth, HIV RNA, smoking and sex of the infant. All models were also adjusted for intragroup correlations in children born to the same mother.

Results

Study Population

By the end of December 2018, 573 infants, born to 406 mothers, were included in the DHBC. In total, 208 (37%) WLWH had information available regarding vitamin D levels during pregnancy of whom 130 (23%) had blood samples analyzed during pregnancy and 78 (13.9%) had measurements from frozen specimens obtained during pregnancy. Of the 208 women with available data on vitamin D, 13% had vitamin D deficiency, 34% had vitamin D insufficiency and 53% had sufficient vitamin D level. Of the 208 WLWH, 43% had their vitamin D levels measured from first trimester blood samples, 36% second trimester blood samples and 21% from third trimester blood samples. Table 1 shows the characteristics of the cohort. Supplementary Table 1, http://links.lww.com/QAD/C88 presents the characteristics of the 208 WLWH with available vitamin D levels.

Women with a Vitamin D Measurement in pregnancy

WLWH who had blood analyzed at a clinical visit during pregnancy had a higher mean age and BMI and were more likely to be of African origin compared to WLWH who´s frozen specimens were analyzed. There was no difference between the two groups in regard to the prevalence of vitamin D deficiency and insufficiency (supplementary Table 2, http://links.lww.com/QAD/C89). As presented in Supplementary Table 3, http://links.lww.com/QAD/C90, < 9% of WLWH had vitamin D status measured/year during pregnancy in the time period 2000-2009, versus 25% to 60% of WLWH during 2010-2018.

Risk Factors for Low Vitamin D Level

As presented in Table 2, being of African origin (RR 2.68), Asian origin (RR 3.38), and having HIV RNA levels >50 copies/mL (RR 1.43) was associated with an increased risk of low vitamin D level in the multivariable models.

Vitamin D Status and Birth Outcomes

The association between vitamin D status and birth outcomes is presented in Supplementary Table 4, http://links.lww.com/QAD/C91. WLWH with vitamin D deficiency had an increased

risk of preterm birth (RR 2.66) and SGA (RR 6.83), compared to WLWH with sufficient vitamin D levels.

Discussion

In this nationwide cohort study of WLWH in Denmark, who gave birth from 2000-2018, we found that of 208 women with available vitamin D levels included in the study, 13% had vitamin D deficiency, 34% had vitamin D insufficiency and 53% had sufficient vitamin D levels.

We found that being of African or Asian origin were associated with an increased risk of low vitamin D level. Further we found an association between having HIV RNA levels >50 copies/mL and low vitamin D level. Importantly, we found that WLWH with vitamin D deficiency had an increased risk of preterm birth and of giving birth to infants who were SGA compared to WLWH with sufficient vitamin D level. The relatively small sample size likely led to inadequate power to detect any differences in continues outcomes such as birthweight and gestational age.

The association between low vitamin D level and African or Asian origin in pregnant women living in Northern countries has been reported in other studies [21,22]. A Swedish study found an increased OR of having vitamin D deficiency if the women were born in Asia or Africa (OR of 22.09 and 9.74 respectively) compared to women born in North-Europe. A Norwegian study found that women from South Asia, the Middle East and Sub-Saharan Africa had significantly lower vitamin D levels during their pregnancy relative to women born in Norway [21]. They found determinants of vitamin D deficiency were not taking vitamin D supplements, lack of sun exposure and low age [21,22].

Some studies have found that individuals in the US of African descent have lower vitamin D binding globulins (VDBG) which in turn leads to normal levels of bioavailable vitamin D [23]. However, this analysis is not available in our clinic.

Similar to our study, Kim *et al*. found that detectable HIV viremia (>50 copies/mL) was associated with low vitamin D level as well as a missing association between cART and low vitamin D level [10]. However, other studies have found an association between cART and low vitamin D level, especially treatment with Efavirenz or Tenofovir [9,24]. The mechanism behind has not yet been clarified [9,24] tough alterations in the kidney such as renal tubular phosphate wasting causing hypophosphatemia and secondary osteomalacia, and endocrine functions such as hyperparathyroidism has been proposed as potential factors [25,26,27]. Tenofovir has been shown to reduce free $1,25(OH)_2D_3$ levels by increasing Parathyroidhormone levels and Efavirenz induces Cytochrome P450 enzymes which may accelerate the catabolism of both 25(OH)D and $1,25(OH)_2D_3$ [26].

Our findings of an association between vitamin D deficiency and an increased risk of preterm birth and SGA is in line with the literature [28,29]. Jao *et al*. found that severe vitamin D

deficiency was associated with preterm birth [28]. It is difficult to determine whether the increased risk of preterm birth and SGA is due to low maternal vitamin D level, cART, a combination of both or whether low vitamin D level is a substitute for something else that might increase the risk.

This study has several strengths. First, it is a nationwide study including all WLWH who, since 2000, have given birth in Denmark. Second, the DHBC is a prospective cohort with consecutive ongoing enrollment. Third, the size of the population is similar to – or larger than – similar studies with a population of pregnant WLWH [29–31].

The study also has limitations. First, less than half of our population had their vitamin D status assessed during pregnancy, weakening the power of the results, which is underlined by the wide CI's of our significant results. Second, we do not know whether the women included in the present study used vitamin D supplements during pregnancy, but the Ministry of Health does advise all pregnant women to take a supplement of 10 microgram vitamin D throughout pregnancy [32]. Third, we do not have data on vitamin D binding globulin. Lastly, a longer follow-up period would give a clearer picture of the long-term effects of low vitamin D level during pregnancy on the children.

In conclusion, WLWH of African or Asian origin and WLWH with HIV RNA levels >50 copies/mL have an increased risk of having low vitamin D levels. Further WLWH with vitamin D deficiency had an increased risk of preterm birth and SGA.

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Conflict of interests

N.W. has – with no relation to the submitted work - been clinical investigator, member of advisory board or lecturer for Abbvie, Merck, BMS, Gilead and GSK and has received unrestricted grants for research or scientific meetings from Abbvie, Gilead, GSK and Novo Nordisk Foundation. E.M. reports grants from the Novo Nordisk Foundation, outside the submitted work, as well as honoraria from Gilead paid to her institution. A.B., I.J., M.S., T.K. and G.P. have no conflicts of interest.

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Table 1: Demographic Characteristics of 563 Women Living with HIV During Pregnancy in Denmark (2000-2018) With and Without Vitamin D Status

	Total number (%)[a] ($n = 563$)	Women with vitamin D [No. (%)][a] ($n = 208$)	Women without vitamin D [No. (%)][a] ($n = 355$)	P value*
Maternal age				
Mean in years ± SD	32.4 ± 5.4	32.7 ± 5.3	32.2 ± 5.4	0.37
Maternal region of birth				0.07
Denmark	125 (22)	36 (17)	89 (25)	
Africa	325 (58)	135 (65)	190 (54)	
Asia	67 (12)	23 (11)	44 (12)	
Other/unknown	46 (8)	14 (7)	32 (9)	
$CD4^+$ cell count prior to delivery				0.23
≥500 cells/μl	235 (42)	95 (46)	140 (39)	
300–499 cells/μl	173 (31)	75 (36)	97 (27)	
<300 cells/μl	68 (12)	30 (14)	38 (11)	
$CD4^+$ cell count information missing	88 (15)	8 (4)	80 (23)	
HIV RNA viral load prior to delivery				0.77
<50 copies/ml	471 (84)	181 (87)	290 (82)	
≥50 copies/ml	62 (11)	25 (12)	37 (10)	
HIV RNA viral load information missing	30 (5)	2 (1)	28 (8)	
Prepregnancy BMI				
Mean in kg/m^2 ± SD	24.6 ± 5.1	24.3 ± 5	24.6 ± 6	0.79
Prepregnancy BMI information missing	386 (69)	63 (30)	323 (91)	
Maternal smoking in pregnancy				
Yes	87 (15)	28 (13)	49 (17)	0.08
Smoking information missing	47 (9)	5 (2)	42 (12)	
Infant				
Preterm Birth (<37 weeks)				
Yes	50 (9)	20 (10)	30 (8)	0.94
Gestational age information missing	72 (13)	9 (04)	63 (18)	
Birth weight				
Mean in gram ± SD	3181 ± 613.8	3199 ± 631.5	3170 ± 616.3	0.61
Birth weight information missing	49 (9)	14 (7)	32 (10)	

[a]Data represent no. (%) of women unless otherwise specified.
*Significant P less than 0.05.

Table 2: Risk Factors of Low Vitamin D Status (25 (OH)D≤50 nmol/L) Among 208 Pregnant Women Living with HIV in Denmark (2000-2018)

		Relative Risk [95% CI]		Relative Risk [95% CI]	
	n	Univariate	*p-value*	Multivariate*	*p-value*
Maternal age					
Less than 30 years	60	Ref		Ref	
30-39 years	131	0.85 [0.62 ; 1.15]	0.29	0.92 [0.68 ; 1.23]	0.56
40 years or older	17	0.53 [0.24 ; 1.19]	0.12	0.63 [0.30 ; 1.31]	0.22
Maternal region of birth					
Denmark	36	Ref		Ref	
Africa	135	2.78 [1.37 ; 5.63]	**<0.01**	2.68 [1.23 ; 5.83]	**0.01**
Asia	23	3.13 [1.44 ; 6.77]	**<0.01**	3.38 [1.49 ; 7.69]	**<0.01**
Other/unknown	14	1.84 [0.69 ; 4.87]	0.22	1.92 [0.69 ; 5.34]	0.21
CD4 cell count third trimester					
≥500 cells/μL	95	Ref		Ref	
300 - 499 cells/μL	75	1.00 [0.71 ; 1.40]	0.99	0.66 [0.50 ; 0.86]	**<0.01**
<300 cells/μL	30	1.33 [0.91 ; 1.92]	0.13	0.70 [0.49 ; 0.99]	**0.04**
Missing	8				
HIV RNA third trimester					
<50 copies/mL	181	Ref		Ref	
≥50 copies/mL	25	1.65 [1.20 ; 2.26]	**<0.01**	1.43 [1.01 ; 2.03]	**0.04**
Missing	2				
ART treatment initiated					
Prior to pregnancy	142	Ref		ref	
During pregnancy	65	1.27 [0.95 ; 1.69]	0.11	1.20 [0.90 ; 1.60]	0.2
Missing	1				
Treatment category					
NRTI+PI	152	Ref		Ref	
NRTI + NNRTi	22	0.62 [0.32 ; 1.18]	0.14	0.70 [0.39 ; 1.26]	0.24
NRTI + InST	12	1.01 [0.57 ; 1.80]	0.96	1.09 [0.67 ; 1.79]	0.72
Other	22	1.06 [0.68 ; 1.64]	0.79	1.30 [1.00 ; 1.68]	**0.05**
Pre-pregnancy BMI					
<18.5	12	1.33 [0.75 ; 2.35]	0.33	1.03 [0.59 ; 1.80]	0.92
18.5-25	73	Ref		Ref	
>25	60	1.27 [0.88 ; 1.83]	0.2	1.19 [0.84 ; 1.67]	0.33
Missing	63				
Maternal smoking in pregnancy					

No	175	Ref		Ref	
Yes	28	0.99 [0.63 ; 1.57]	0.97	0.94 [0.60 ; 1.48]	0.8
Missing	5				
Season for Vitamin D					
Spring	49	Ref		Ref	
Summer	42	0.85 [0.52 ; 1.39]	0.52	0.68 [0.42 ; 1.10]	0.12
Autumn	58	1.15 [0.79 ; 1.67]	0.46	0.90 [0.64 ; 1.25]	0.53
Winter	57	1.17 [0.79 ; 1.73]	0.43	1.00 [0.71 ; 1.42]	1.00

*Adjusted for maternal age, maternal country of birth, and HIV RNA at delivery