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Low maternal vitamin D is associated with increased risk of congenital and peri/postnatal transmission of Cytomegalovirus in women with HIV

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Abstract

Background

CMV infection of the fetus or neonate can lead to devastating disease, and there are no effective prevention strategies to date. Vitamin D is a potent immunomodulator, supports antiviral immune responses, and plays an important role in placental immunity.

Methods

Retrospective cohort study to evaluate the impact of low maternal vitamin D on congenital and early postnatal transmission of CMV among HIV-infected, non-breastfeeding women and their HIV exposed but negative infants from an urban HIV clinic. Vitamin D panel was performed on stored maternal plasma obtained near time of delivery. Infant CMV testing at 0–6 months included urine and oral cultures, and/or serum polymerase chain reaction testing.

Results

Cohort included 340 mother-infant pairs (births 1991–2014). Among 38 infants (11%) with a CMV+ test between 0–6 months, 4.7% (14/300) had congenital CMV transmission (CMV+ test 0–3 weeks), and 7.6% (24/315) had peri/postnatal CMV (CMV+ test >3 weeks-6 months). Women with lower calcitriol (1,25-dihydroxyvitamin D), the active form of vitamin D, were more likely to have an infant with congenital (OR 12.2 [95% CI 1.61–92.2] $P = 0.02$)

University of Southern California's Office of Ethics and Compliance, there is no way to make the dataset truly de-identified. The dataset is made up solely of women with a diagnosis of HIV and their offspring. The diagnosis of HIV is an indirect identifier and considered sensitive information requiring additional protections. Additional indirect identifiers in the dataset include age, race/ethnicity, and sex. Additionally, the consent signed by the study participants did not include a provision for their data to be made publicly accessible. Data requests can be directed to department administrator, Carlota Obnillas, obnillas@usc.edu, and if the request is approved, the USC Stevens Center for Innovation will assist and create a Data Transfer Agreement.

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Competing interests: RP is the salaried Director of Pan Laboratories, Irvine, CA. There are no patents, products in development or marketed products to declare. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

and peri/postnatal (OR 9.84 [95% CI 2.63–36.8] $P = 0.0007$) infections in multivariate analyses, independent of maternal HIV viral load and CD4 count.

Conclusion

This study demonstrates an association between inadequate maternal calcitriol during pregnancy and increased congenital and early postnatal acquisition of CMV among non-breastfeeding women with HIV and their HIV negative infants.

Introduction

Human Cytomegalovirus (CMV) infection is the most common congenital infection worldwide. It is the top non-genetic cause of childhood deafness in the world and can lead to neurologic and neurodevelopmental disorders, multisystem illness, growth and development abnormalities, and death.[1,2] Approximately 50–70% of women of childbearing age in developed countries are CMV infected, with the highest prevalence among women of lower socioeconomic status.[3] Seroprevalence approaches 100% among women of child-bearing age in resource-limited countries and in those with Human Immunodeficiency Virus (HIV) infection.[2,4] Mother-to-child-transmission (MTCT) of CMV can occur prenatally (congenital infection), during birth, and postnatally through breast milk.[5] Mothers and other caregivers can also transmit CMV to their infants postnatally through infected secretions.[2] Maternal CMV infections and reactivations are often asymptomatic and unnoticed, and unlike HIV, there are currently no effective strategies widely implemented for the prevention of MTCT of CMV.[6] Rates of congenital CMV are often higher among infants of women with HIV infection, making them an ideal population for study.[2,4,7–11]

Vitamin D is obtained either from exposure to ultraviolet light or from the diet. In addition to its role in calcium homeostasis and skeletal health, Vitamin D is a well-known and potent modulator of the immune system.[12] Vitamin D supports immune system antiviral responses through the induction of autophagy and production of antimicrobial peptides like cathelicidin, and likely plays an important role in helping to protect the developing fetus from infections during pregnancy.[13–16] A multitude of cells in the body have the vitamin D receptor and many cells, including the cells of the placenta, also have the ability to convert 25-hydroxyvitamin D (25(OH)D), the main circulating form of vitamin D, to its bioactive form, 1,25-dihydroxyvitamin D (1,25(OH)D_2).[17,18] This allows for local production of 1,25(OH)D_2 and the subsequent vitamin D-dependent antimicrobial immune responses in the setting of specific conditions or stimuli.[15,18–20]

Vitamin D's important role in supporting the immune system's antiviral functions, including those at the level of the placenta, suggests its relevance to MTCT of CMV in utero. Additionally, vitamin D may contribute to the immune system's ability to limit viral shedding and therefore play a role in limiting perinatal and early postnatal CMV transmission. In order to explore these hypotheses, we conducted a retrospective study, nested within a longitudinal prospective cohort study, evaluating the impact of low maternal vitamin D on congenital and peri/postnatal acquisition of CMV among HIV-infected, non-breastfeeding women and their HIV exposed but negative infants born between 1988 through 2015 at the Maternal, Child and Adolescent/Adult Center for Infectious Diseases and Virology (MCA) at the LAC+USC Medical Center, in Los Angeles, California.

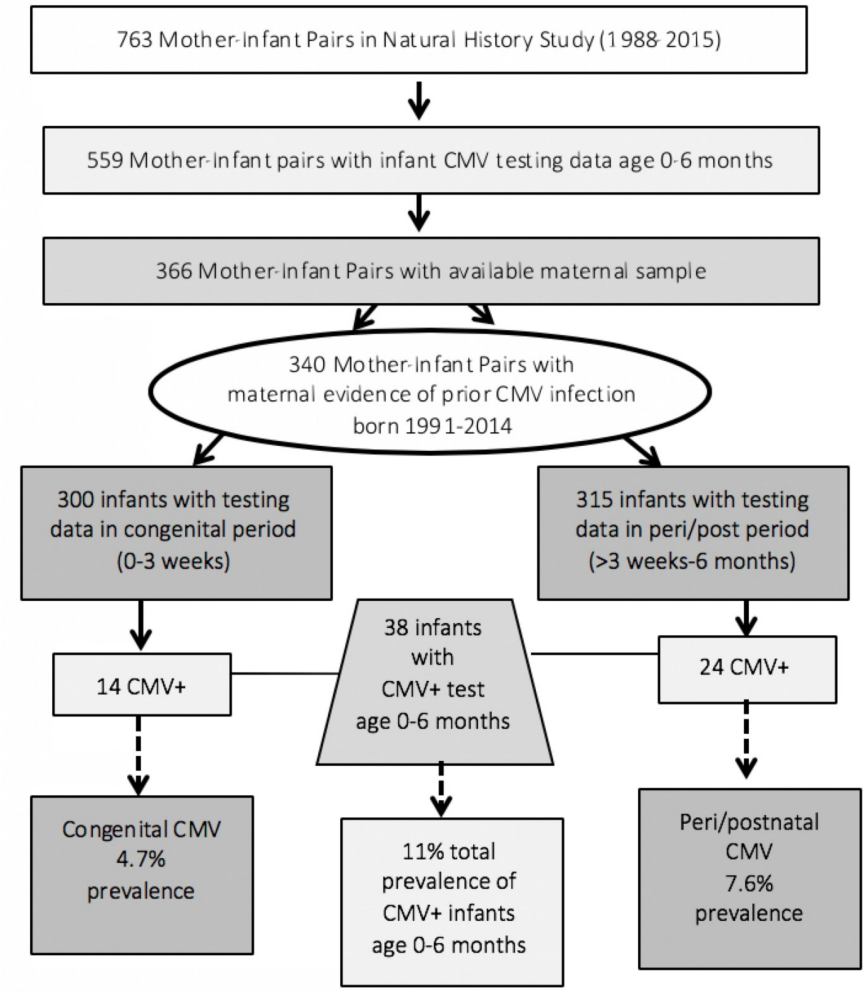

Fig 1. Study cohort based on infant CMV testing results.

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Methods

Study design and participants

MCA is a comprehensive HIV clinic, serving women and their families. It is Los Angeles County's largest referral site for HIV-infected pregnant women and their children, and cares for those who are under or uninsured. Informed consent was obtained for mothers and their newborns receiving care at MCA to participate in the University of Southern California Health Sciences Institutional Review Board–approved Natural History Study. The cohort design and participant selection for the current study are summarized in Fig 1. Mother-infant pairs were eligible for inclusion in this study if 1) they were both enrolled in MCA's Natural History Study, 2) the mother was HIV-infected with evidence of CMV infection prior to the birth of the child, 3) the infant was HIV uninfected and had CMV testing between the ages of 0 to 6 months, and 4) stored maternal plasma obtained during pregnancy was available for vitamin D analysis. Among 559 mother-infant pairs with infant CMV testing between birth and age 6 months, 366 mothers had stored plasma available for vitamin D testing. Among these women, 340 had evidence of CMV infection: 312 were CMV seropositive and 28 with missing CMV

results, had infants who were CMV IgG+ at or near the time of birth. This was considered to be a transfer of maternal antibody and thus these mothers were considered CMV+ and included in the cohort.

CMV testing in HIV-exposed infants was done as part of MCA's routine care or as part of other MCA research studies. This testing included culture of urine and oral swabs, and polymerase chain reaction (PCR) studies of blood. Cultures were performed using standard virologic methods of either shell-vial or tube cultures. PCR was performed by contracted send out laboratories (Quest Diagnostics, Focus Diagnostics) or in some cases, PCR was performed for other research studies by the MCA Laboratory. CMV tests available for analysis in this study included 1,101 urine cultures from 312 infants, 478 oral cultures from 158 infants, and 67 CMV blood PCR results from 55 infants. Congenital CMV was defined as 1 or more positive CMV tests between the ages of birth to 21 days. Peri/postnatal infection was defined as 1 or more positive results between 22 days to 6 months without a positive test during the congenital period.

Vitamin D testing

Stored maternal plasma samples were used for evaluation of vitamin D. Vitamin D is very stable and samples may be frozen indefinitely and can withstand several freeze-thaw cycles without impacting results.[21] Specimens obtained at the time closest to the infant's birth were used, with 50% obtained on the day of birth, 40% within 7 days of birth, 6.5% in the third trimester but before 7 days of birth, 3% in the second trimester, and 0.5% in the first trimester. Plasma samples were collected and stored in tubes containing either Ethylenediaminetetraacetic acid (EDTA), heparin, or Acid Citrate Dextrose (ACD). Samples were sent to Pan Laboratories, Irvine, CA for Vitamin D testing. Measurement of total 25(OH)D was performed by immunoassay (IDS ELISA, AC-57) using kits from Immuno-Diagnostic Systems (IDS), Phoenix, per package insert. The assay has a cross reactivity of 75% with 25 hydroxyvitamin D2. The assay has a sensitivity of 2.5 ng/ml and total interassay variation <8.7%.[22] Total calcitriol (1,25(OH)D_2) was measured by immunoassay (IDS ELISA, AC-62), using kits from IDS. Calcitriol (1,25(OH)D_2) was first purified by immuno-affinity purification and immunoassay was performed to quantitate calcitriol. The assay has a sensitivity of 2.5 pg/ml and total interassay variation is <15%.[23] Sufficient 25(OH)D levels were defined as ≥32 ng/mL, 80 nmol/L, insufficient as 21–31 ng/mL, 52.5–77 nmol/L, inadequate as 11–20 ng/mL, 27.5–50 nmol/L, and deficient as ≤10 ng/mL, 25 nmol/L.

Statistical analysis

Demographic and HIV related variables evaluated included maternal age, race, ethnicity, HIV viral load categorized as <400 or ≥400 copies per ml, CD4 cell count categorized as <200 or ≥200 cells/mm^3, mode of delivery, infant gender, season, and antiretroviral treatment (ART). ART was further defined as 1) non-highly active antiretroviral therapy (non-HAART) which included women not taking any ART as well as those taking a single, dual, or a combination of agents felt to be less potent based on current standards; 2) HAART with a protease inhibitor; and 3) HAART without a protease inhibitor.

For the purpose of this study, 25(OH)D levels were defined as sufficient if ≥32 ng/mL, 80 nmol/L. This cut point was selected based on prior research on optimal calcium homeostasis and bone health, recent clinical studies among pregnant women, and clinical applicability.[24,25] Additionally, data were divided into tertiles (rounded to the nearest 1 ng/ml) with 32 ng/mL defining the highest tertile. Sufficiency cut-points for 1,25(OH)D_2 are less well established, therefore, tertiles (rounded to the nearest 1 pg/ml) based on the frequency distribution

were used in analyses. Both 1,25(OH)D_2 and 25(OH)D were also evaluated as continuous variables. Variables associated with vitamin D levels were identified using linear regression models with either 25(OH)D or 1,25(OH)D_2 as the dependent variable and demographic and clinical characteristics as the independent variables. Generalized estimating equations (GEE) were used to account for the correlation among mothers with multiple birth outcomes. Correlates of 25(OH)D and 1,25(OH)D_2 were analyzed separately.

Univariate analyses of CMV infection were conducted using GEE logistic regression models with infant CMV status (positive/negative) as the outcome variable. Levels of 25(OH)D, 1,25 (OH)D_2 and demographic and clinical characteristics were tested for their association with congenital CMV and peri/postnatal CMV in separate models

GEE logistic regression models were used in multivariate modeling of CMV. Separate models for 25(OH)D and 1,25(OH)D_2 were analyzed for those with congenital CMV test results but only 1,25(OH)D_2 for those with peri/postnatal results as no univariate relationship between 25(OH)D and peri/postnatal CMV infection was found. Factors associated with vitamin D and/or CMV in univariate models with *P-value* <0.15 were initially included in the multivariate models. Backward elimination was used to remove variables not associated with CMV ($P>0.05$). Final multivariate models controlled for CD4 count and HIV viral load due to their presumed impact on both vitamin D levels and CMV transmission. In the congenital group, covariates analyzed included ART category, race, ethnicity, HIV viral load, CD4 count, CD4 nadir during pregnancy, season, and maternal age at collection. In the peri/postnatal group, the covariates included ART category, race, ethnicity, HIV viral load, CD4 count, season, and mode of delivery (vaginal versus caesarian birth).

Ethics statement

This study was approved by the University of Southern California's Office for the Protection of Research Subjects, Health Sciences IRB. This was a retrospective study using data from an ongoing, IRB approved Natural History Study, for which patients had signed consent. The current study posed no more than minimal risk and need for consent was waived.

Results

Among the entire cohort of 340 women/infant pairs, 38 infants had a positive CMV test between the age of birth and 6 months (11%). All positive infant test results are listed in S1 Table. Among the 300 infants with CMV testing between birth and 3 weeks, 14 or 4.7% were congenitally infected. Among the 315 infants with a CMV test between 22 days and 6 months (excluding the congenital cases), 24 infants or 7.6% were peri/postnatally infected. Among these 24 infants, 54% had a first positive test between age 4 and 10 weeks of life, 8% between weeks 11–17, and 38% between 18 and 26 weeks. Only 1 of these 24 infants was not tested in the congenital period while the other 23 had at least 1 negative test during the congenital period.

Study cohort characteristics are summarized in Table 1 and are categorized based on infant CMV transmission category. The majority of women in the cohort self-identified as Hispanic white (73%) and were between the ages of 20 and 34 years. Most (80%) had HIV viral loads <400 copies/ml, 40% had CD4 counts above 500 cells/mm^3, and 78.5% were on HAART regimens.

Maternal vitamin D levels are described in Table 1 and Fig 2A and 2B. Overall, two-thirds of the women had low vitamin D with 25(OH)D levels <32 ng/ml. Forty-two percent had insufficient 25(OH)D levels between 21–31 ng/ml, 24% had inadequate levels of 11–20 ng/ml,

Table 1. Study cohort characteristics.

Variables	Total sample (N = 340)	Congenital CMV+ (N = 14)	Peri/postnatal CMV+ (N = 24)	CMV negative (N = 302)
Maternal age (years)				
<20	30 (8.8%)	2 (14.3%)	2 (8.3%)	26 (8.6%)
20–34	240 (70.6%)	12 (85.7%)	17 (70.8%)	211 (69.9%)
≥ 35	70 (20.6%)	0	5 (20.8%)	65 (21.5%)
Race				
Hispanic white	248 (72.9%)	8 (57.1%)	22 (91.7%)	218 (72.2%)
Non-Hispanic white	11 (3.2%)	1 (7.1%)	0	10 (3.3%)
Black	77 (22.7%)	5 (35.7%)	0	72 (23.8%)
Other	4 (1.2%)	0	2 (8.3%)	2 (0.7%)
Plasma HIV-1 RNA (copies/mL)				
<400	266 (80.1%)	11 (84.6%)	21 (95.5%)	234 (78.8%)
400+	66 (19.9%)	2 (15.4%)	1 (4.5%)	63 (21.2%)
Data missing	8	1	2	5
CD4 cell count (cells/mm^3)				
<200	39 (11.6%)	3 (21.4%)	3 (12.5%)	33 (11.0%)
200–500	163 (48.4%)	4 (28.6%)	10 (41.7%)	149 (49.8%)
>500	135 (40.1%)	7 (50.0%)	11 (45.8%)	117 (39.1%)
Data missing	3	0	0	3
ART regimen				
non-HAART*	74 (22%)	2 (14.3%)	4 (16.7%)	68 (22.8%)
HAART without PI	77 (22.9%)	5 (35.7%)	2 (8.3%)	70 (23.5%)
HAART with PI	185 (55.6%)	7 (50%)	18 (75%)	160 (53.7%)
Data missing	4	0	0	4
Mode of delivery				
Cesarean section	137 (40.9%)	4 (28.6%)	5 (21.7%)	128 (42.9%)
Vaginal	198 (59.1%)	10 (71.4%)	18 (78.3%)	170 (57.1%)
Data missing	5	0	1	4
Infant gender				
Female	155 (45.6%)	8 (57.1%)	13 (54.2%)	134 (44.4%)
Male	185 (54.4%)	6 (42.9%)	11 (45.8%)	168 (55.6%)
Season				
Summer/Fall	163 (47.9%)	5 (35.7%)	10 (41.7%)	148 (49%)
Winter/Spring	177 (52.1%)	9 (64.3%)	14 (58.3%)	154 (51%)
25(OH)D levels, ng/ml				
<23	111 (32.7%)	3 (21.4%)	7 (29.2%)	101 (33.4%)
23–31	117 (34.4%)	9 (64.3%)	7 (29.2%)	101 (33.4%)
≥ 32	112 (32.9%)	2 (14.3%)	10 (41.7%)	100 (33.1%)
1,25 $(OH)D_2$ pg/ml				
median (IQR)	95.6 (69.8–125.4)	66.8 (54.9–73)	67.9 (57.9–80.5)	100 (73.5–128.9)
≤ 74	116 (34.1%)	11 (78.6%)	17 (70.8%)	78 (25.8%)
75–115	118 (34.7%)	2 (14.3%)	4 (16.7%)	112 (37.1%)
116+	106 (31.2%)	1 (7.1%)	3 (12.5%)	112 (37.1%)

CD4, and HIV RNA levels tested at time point closest available to the vitamin D specimen test date

*non-HAART includes women on no ART, single agents, dual combinations, or any ART felt to be less active compared to current standards

Abbreviations: ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; IQR, Interquartile range; PI, protease-inhibitor; 25(OH)D, 25-hydroxyvitamin D; 1,25 $(OH)D_2$, 1,25-dihydroxyvitamin D

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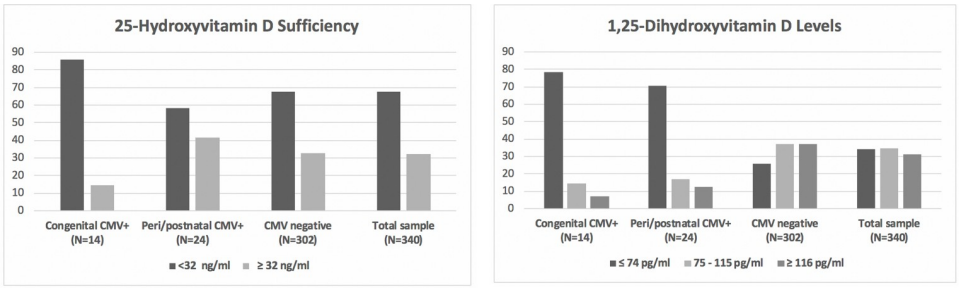

Fig 2. a. The percentage of mothers with 25(OH)D levels above and below the cut point of 32 ng/ml compared by the CMV status of their infants. **b.** The percentage of mothers with low, middle or high 1,25-dihydroxyvitamin D levels, based on tertiles, compared by CMV transmission status.

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and 2% were deficient with levels of ≤10 ng/ml (S2 Table). Maternal 25(OH)D and 1,25(OH)D_2 levels were only weakly correlated (Spearman's correlation coefficient = 0.21, $P = 0.0001$).

Table 2 summarizes the variables associated with 25(OH)D and 1,25(OH)D_2. Maternal HIV viral load was significantly associated with both 25(OH)D ($P = 0.002$) and 1,25(OH)D_2 ($P = 0.02$) with lower levels seen in women with higher viral loads. Lower 25(OH)D levels were associated with lower maternal CD4 cell counts ($P = 0.05$). Maternal ART category was significantly associated with 25(OH)D levels ($P = 0.001$) with the lowest values seen in the group on either no medications or non-HAART regimens. Season was significantly associated

Table 2. Factors univariately associated with vitamin D levels.

	25(OH)D ng/ml		1,25 (OH)D_2 pg/ml	
Variables	**Mean (95%CI)***	***P*-value**	**Mean (95% CI)***	***P*-value**
ART regimen		0.001		0.07
non-HAART	24.1 (21.6–26.6)		89.9 (80.5–99.4)	
HAART no PI	28.8 (26.6–30.9)		104.5 (95.0–113.9)	
HAART with PI	31.1 (29.3–32.9)		101.0 (95.5–106.6)	
Race		0.13		0.35
Hispanic white	28.9 (27.3–30.5)		101.3 (95.7–106.9)	
Non-Hispanic white	36.7 (28.4–44.9)		101.7 (77.8–125.6)	
Black	27.4 (24.7–30.1)		92.6 (84.5–100.8)	
Other	36.4 (28.3–44.6)		84.6 (41.7–127.6)	
Plasma HIV-1 RNA (copies/mL)		0.002		0.02
<400	30.1 (28.7–31.6)		102.5 (97.5–107.4)	
400+	24.8 (22.0–27.6)		89.1 (80.4–97.8)	
CD4 cell count (cells/mm^3)		0.05		0.07
≥ 200	29.4 (27.9–30.9)		100.1 (95.3–104.9)	
<200	25.1 (21.4–28.8)		88.0 (76.5–99.5)	
Season		0.007		0.002
Summer & Fall	30.9 (28.7–33.0)		105.9 (99.3–112.6)	
Winter & Spring	27.2 (25.6–28.8)		92.8 (87.0–98.6)	

GEE linear regression model (separate models for 25(OH)D and 1,25 (OH)D_2)

CD4, and HIV RNA levels tested at time point closest available to the vitamin D specimen test date

*Least squares mean with 95% confidence interval

Abbreviations: ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; PI, protease inhibitor; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)D_2, 1,25-dihydroxyvitamin D

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with both 25(OH)D ($P = 0.007$) and 1,25(OH)D_2 ($P = 0.002$), with lower levels among women pregnant during months with lower levels of UV light. No relationships were found between CD8 cell counts nor CD4/CD8 ratios and either 25(OH)D or 1,25(OH)D_2.

Factors associated with congenital transmission of CMV are summarized in Tables 3, 4 and 5. Lower levels of maternal bioactive vitamin D, 1,25(OH)D_2, were associated with increased congenital transmission of CMV in univariate ($P = 0.006$) and multivariate analyses ($P = 0.009$ for trend) controlling for maternal age, CD4 count, and HIV viral load categories. Women with 1,25(OH)D_2 levels $\leq$74 pg/ml were 12 times more likely to transmit CMV congenitally to their infant (OR 12.2 [95% CI 1.61–92.2] $P = 0.02$) compared to women with levels in the highest tertile of $\geq$116 pg/ml.

In univariate analyses, women with 25(OH)D levels in the middle range of 23–31 ng/dl were more likely to transmit congenital CMV (OR 4.93 [1.05–23.1] $P = 0.04$) compared to women with levels $\geq$32. Borderline significance for this association was observed in the multivariate analysis when controlling for maternal age, CD4 count, and HIV viral load categories (OR 4.88 [95% CI 0.99–24.1] $P = 0.05$). However, there was no significant difference found when comparing the lowest and highest tertile groups, nor when a cut point for vitamin D sufficiency of 32 ng/ml was used. Younger maternal age was also associated with increased risk of congenital CMV infection in univariate analysis (OR 0.90 [95% CI 0.83–0.98] $P = 0.01$) and remained significant in the multivariate model (OR 0.90 [95% CI 0.83–0.99] $P = 0.03$).

Factors associated with peri/postnatal CMV infection status in both univariate and multivariate analyses are summarized in Tables 3 and 6. In univariate analyses, only 1,25(OH)D_2 was associated with peri/postnatal CMV infection ($P = 0.0002$). In addition, vaginal delivery compared to cesarean section was marginally associated with increased odds of peri/postnatal transmission. However, in multivariate analyses controlling for HIV viral load categories, CD4 count, 1,25(OH)D_2 and ART, vaginal deliveries had 3 times greater odds of peri/postnatal transmission (OR 3.03 [95% CI 1.08–8.50] $P = 0.04$). Levels of 1,25(OH)D_2 remained significant in multivariate analyses with higher maternal 1,25(OH)D_2 levels protective for peri/postpartum CMV. Women with levels $\leq$ 74 pg/ml had nearly 10 times greater odds peri/postpartum transmission compared to women with 1,25(OH)D_2 levels $\geq$ 116 pg/ml controlling for mode of delivery, CD4 and HIV viral load categories, and ART (OR 9.84 [95% CI 2.63–36.8] $P = 0.003$). There was no significant association seen between maternal 25(OH)D status and peri/postnatal CMV.

Discussion

This is the first study to evaluate the impact of maternal vitamin D status on congenital and peri/postnatal CMV transmission. In this study, lower levels of the bioactive form of vitamin D, calcitriol (1,25(OH)D_2), were associated with increased odds of both congenital and peri/postpartum CMV infections among perinatally exposed but HIV uninfected infants born to non-breastfeeding women with HIV. As seen in other cohorts, low levels of vitamin D were associated with lower maternal CD4 counts and higher HIV viral loads.[26–29] This supports the notion that vitamin D is involved in the immune functions related to HIV infection. However, the relationships found between calcitriol and CMV transmission outcomes in this study were independent of these factors.

There is growing evidence suggesting the importance of vitamin D in placental-related functions including placental development and implantation, calcium transport, immunomodulatory functions, as well as an association between low calcitriol levels and pregnancy induced hypertension. [15,30–33] During pregnancy, non-classical, extra-renal production of 1,25(OH)D_2 occurs in the placenta. In fact, healthy pregnancy is associated with a doubling or

Table 3. Factors univariately associated with congenital and perinatal/postnatal CMV transmission.

	Congenital CMV		Perinatal/Postnatal CMV	
Variable	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
25(OH)D* (ng/mL)	0.99 (0.94–1.04)	0.59	1.01 (0.99–1.04)	0.35
25(OH)D (ng/mL)		0.07		0.61
≥ 32	1.00		1.00	
23–31	4.93 (1.05–23.1)	**0.04**	0.65 (0.24–1.78)	0.40
<23	1.69 (0.28–10.3)	0.57	0.66 (0.24–1.78)	0.41
1,25(OH)D_2* (pg/mL)	0.97 (0.95–0.98)	**0.0002**	0.98 (0.97–0.99)	**0.001**
1,25(OH)D_2 (pg/mL)		**0.006**		**0.0002**
≥ 116	1.00		1.00	
75–115	2.01 (0.18–22.3)	0.57	1.32 (0.29–6.01)	0.72
≤ 74	12.6 (1.66–99.5)	0.01	7.88 (2.23–27.8)	**0.001**
ART Regimen		0.46		0.11
Non-HAART	1.00		1.00	
HAART without PI	2.29 (0.43–12.2)	0.33	0.49 (0.09–2.72)	0.41
HAART with PI	1.18 (0.24–5.82)	0.84	2.03 (0.67–6.17)	0.21
Mode of delivery				
C-section	1.00		1.00	
Vaginal	1.79 (0.55–5.80)	0.33	2.67 (0.97–7.34)	0.06
Baby gender				
Male	1.00		1.00	
Female	1.66 (0.56–4.90)	0.36	1.55 (0.67–3.58)	0.30
Maternal Age	0.90 (0.83–0.98)	**0.01**	0.99 (0.94–1.05)	0.81
Race			**	
Non-Black	1.00			
Black	2.03 (0.67–6.17)	0.21		
Ethnicity				
Non-Hispanic	1.00		1.00	
Hispanic	0.42 (0.14–1.23)	0.11	8.43 (1.12–63.5)	**0.04**
Plasma HIV-1 RNA (copies/mL)				
<400	1.00		1.00	
400+	0.80 (0.17–3.74)	0.78	0.18 (0.02–1.28)	0.09
CD4 Nadir During Pregnancy				
200+	1.00		1.00	
<200	1.72 (0.52–5.71)	0.37	1.03 (0.37–2.88)	0.95
CD4 Cell Count (copies/mL)				
200+	1.00		1.00	
<200	2.31 (0.61–8.74)	0.22	1.08 (0.30–3.90)	0.90
Season				
Summer/Fall	1.00		1.00	
Winter/Spring	1.72 (0.57–5.22)	0.34	1.31 (0.56–3.04)	0.53

* Modeled as a continuous variable

**Race was not included in the perinatal/postnatal model because no Black infants were perinatal/postnatal CMV+ GEE logistic regression model

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HAART, highly active antiretroviral therapy; OR, odds ratio; PI, protease inhibitor; 1,25(OH)D2, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D

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Table 4. Factors associated with congenital CMV in multivariate model A.

	CMV		
Variables	**OR (95% CI)**	***P*-value**	***P*-value***
1,25(OH)D_2 (pg/mL)		**0.01**	**0.009**
≥ 116	1.00		
75–115	2.18 (0.20–23.6)	0.52	
≤ 74	12.2 (1.61–92.2)	**0.02**	
Plasma HIV-1 RNA (copies/mL)			
<400	1.00		
400+	0.41 (0.08–2.00)	0.27	
CD4 Cell Count (copies/mL)			
200+	1.00		
<200	2.07 (0.42–10.3)	0.37	
Maternal age	0.90 (0.83–0.99)	**0.03**	

**P*-value for trend

GEE logistic regression model

Abbreviations: CI, confidence interval; OR, odds ratio; 1,25(OH)D2, 1,25-dihydroxyvitamin D

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tripling of pre-pregnancy 1,25(OH)D_2 levels while 25(OH)D levels typically remain unchanged.[25,34] This increase occurs without impacting serum or urinary calcium levels, demonstrating an uncoupling of vitamin D metabolism from the usual calcium-parathyroid hormone axis control.[33,34] Placental trophoblasts and maternal decidua actively convert vitamin D to its active form, 1,25(OH)D_2, in an intracrine manner.[32] Decidual and placental cells also contain an abundance of the vitamin D receptor (VDR), a ligand-activated transcription factor that controls the expression of over a thousand genes, allowing for a localized response when 1,25(OH)D_2 binds. This response involves activation of the innate immune system including a dose-dependent production of the antimicrobial peptide, cathelicidin, ultimately protecting placental cells from infection and death.[18,35] It can be postulated that the

Table 5. Factors associated with congenital CMV in multivariate model B.

	CMV		
Variables	**OR (95% CI)**	***P*-value**	***P*-value***
25(OH)D (ng/mL)		0.10	0.46
≥ 32	1.00		
23–31	4.88 (0.99–24.1)	**0.05**	
<23	1.87 (0.27–12.9)	0.53	
Plasma HIV-1 RNA (copies/mL)			
<400	1.00		
400+	0.42 (0.06–3.04)	0.39	
CD4 Cell Count (copies/mL)			
200+	1.00		
<200	3.08 (0.53–18.0)	0.21	
Maternal age	0.90 (0.82–0.98)	**0.02**	

**P*-value for trend

GEE logistic regression model

Abbreviations: CI, confidence interval; OR, odds ratio; 25(OH)D, 25-hydroxyvitamin D

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Table 6. Factors associated with peri/postnatal CMV in multivariate model.

	CMV		
Variables	**OR (95% CI)**	***P*-value**	***P*-value***
1,25(OH)D_2 (pg/mL)		**0.0003**	**0.0008**
≥ 116	1.00		
75–115	1.60 (0.34–7.50)	0.55	
≤ 74	9.84 (2.63–36.8)	**0.0007**	
Plasma HIV-1 RNA (copies/mL)			
<400	1.00		
400+	2.18 (0.65–7.32)	0.21	
CD4 Cell Count (copies/mL)			
200+	1.00		
<200	1.25 (0.57–2.76)	0.58	
ART Regimen		**0.05**	
Non-HAART	1.00		
HAART no PI	0.83 (0.10–7.25)	0.87	
HAART with PI	4.21 (0.75–23.8)	0.10	
Mode of delivery			
C-section	1.00		
Vaginal	3.03 (1.08–8.50)	**0.04**	

**P*-value for trend

GEE logistic regression model

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HAART, highly active antiretroviral therapy; OR, odds ratio; PI, protease inhibitor; 1,25(OH)D2, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D

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additional 1,25(OH)D_2 available to the developing fetus is in excess of the amount required for fetal skeletal development and instead may be essential for fetal and placental immunologic and antimicrobial functions. Our findings support this notion suggesting that the increased 1,25(OH)D_2 available during pregnancy could aid immune functions important in blocking transplacental CMV transmission and possibly decrease maternal CMV shedding, thereby reducing congenital and peri/postnatal CMV infections.

A prior study of untreated HIV infected pregnant women in Tanzania demonstrated increased mother-to-child-transmission of HIV in women with 25(OH)D levels <32 ng/ml. [24] In the current study, we found only weak evidence that lower levels of 25(OH)D, the main circulating form of vitamin D, may be associated with congenital CMV transmission. There was no trend as only women with levels in the middle tertile appeared to have increased CMV transmission compared to those with the highest levels, and there was no difference when comparing those with the lowest and highest levels, nor when the cut point for vitamin D sufficiency of 32 ng/ml was used for analysis. A randomized controlled trial of vitamin D supplementation in HIV negative pregnant women by Hollis, et al, demonstrated that a serum 25(OH)D level of 40 ng/ml was needed in order to achieve the supraphysiological increase in the bioactive form of vitamin D, calcitriol (1,25(OH)D_2), during normal pregnancy.[25] It is possible a larger sample size is needed to similarly find a threshold serum value of 25(OH)D required to overcome any problems with placental dysfunction, supporting the necessary boost in calcitriol, and thereby aiding the important immunologic protective functions of the placenta.

This study had several limitations. This is a retrospective study and the cohort was created, in part, based on availability of stored samples. This may have created inadvertent bias in

cohort selection. The women in this cohort were all considered seropositive for CMV based on maternal IgG, or early infant IgG in a few cases. Therefore, it can be assumed that the transmissions during the congenital period were all non-primary infections due to reactivation of latent maternal CMV, or theoretically, reinfection with a different strain. Further study is needed to determine if vitamin D plays a role in low-seroprevalence populations as well, where higher rates of maternal primary CMV infection are more likely. The retrospective study design also limited the ability to do additional or confirmatory CMV PCR testing on infant urine, saliva or blood, due to the lack of availability of stored specimens. The results of this study were based mainly on culture results, as only 55 of the 340 infants had blood PCR test results, and there were no PCR results for urine or saliva. CMV cultures are less sensitive than PCR for detecting CMV and therefore the actual prevalence of congenital and peri/postnatal CMV infections may be underrepresented. More work is needed to demonstrate if the association between calcitriol, the active form of vitamin D, and congenital and early postnatal transmission of CMV found in this population of HIV-infected women is true in HIV uninfected women as well. Additionally, this study did not evaluate VDR expression nor the multitude of polymorphisms associated with altered VDR expression and function. It is unclear how the VDR and therefore the downstream effects of vitamin D may be impacted by CMV infection itself. In fact, a recent in vitro study demonstrated that the presence of CMV inhibited the expression of vitamin D receptors in fibroblasts.[36] Further study is needed to clarify the interplay between vitamin D and CMV infection and how this relationship may be important in protecting the developing fetus from potential infection.

Although our study demonstrates an association between lower calcitriol ($1,25(OH)D_2$) levels and increased CMV transmission congenitally and in the early postnatal period, we cannot comment on causation. It is unknown if the CMV and/or HIV infections deplete vitamin D as it is used in high demand in conjunction with fighting these infections, or if low maternal vitamin D leads to increased susceptibility to infection and/or increased or prolonged viral shedding. HIV and CMV infections are both known to cause significant placental damage and dysfunction and this could impact the placental production of calcitriol ($1,25(OH)D_2$), potentially contributing to the lower levels seen in this study.[37–39] Additionally, the presence of pathogens may affect local calcitriol production. In vitro, the presence of HIV and LPS impacts the production and breakdown of calcitriol by affecting CYP27B1 and CYP24A1 gene expression in monocytes.[40]

Future, prospective studies are needed in HIV positive and negative populations to further clarify these complex relationships. If found to be causal and protective in prospective studies, targeted vitamin D supplementation, with the goal of supporting the necessary rise in calcitriol during pregnancy, could represent a safe and inexpensive tool in preventing CMV transmission from mother to infant.

Supporting information

S1 Table. Number of positive CMV tests by test type for all CMV+ infants.
(DOCX)

S2 Table. 25-Hydroxyvitamin D sufficiency by infant transmission category.
(DOCX)

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References

1. Gompels UA, Larke N, Sanz-Ramos M, Bates M, Musonda K, Manno D, et al. Human Cytomegalovirus Infant Infection Adversely Affects Growth and Development in Maternally HIV-Exposed and Unexposed Infants in Zambia. CLIN INFECT DIS. 2012 Jan 13; 54(3):434–42. https://doi.org/10.1093/cid/cir837 PMID: 22247303
2. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. Clinical Microbiology Reviews. 2013 Jan; 26(1):86–102. https://doi.org/10.1128/CMR.00062-12 PMID: 23297260
3. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus Seroprevalence in the United States: The National Health and Nutrition Examination Surveys, 1988–2004. Clin Infect Dis; 2006.
4. Frederick T, Homans J, Spencer L, Kramer F, Stek A, Operskalski E, et al. The Effect of Prenatal Highly Active Antiretroviral Therapy on the Transmission of Congenital and Perinatal/Early Postnatal Cytomegalovirus Among HIV-Infected and HIV-Exposed Infants. CLIN INFECT DIS. 2012 Sep; 55(6):877–84. https://doi.org/10.1093/cid/cis535 PMID: 22675157
5. Pass RF, Anderson B. Mother-to-Child Transmission of Cytomegalovirus and Prevention of Congenital Infection. Journal of the Pediatric Infectious Diseases Society. 2014 Aug 20; 3(suppl 1):S2–S6.
6. Mehta S, Giovannucci E, Mugusi FM, Spiegelman D, Aboud S, Hertzmark E, et al. Vitamin D Status of HIV-Infected Women and Its Association with HIV Disease Progression, Anemia, and Mortality. Hernandez AV, editor. PLoS ONE. 2010 Jan 19; 5(1):e8770. https://doi.org/10.1371/journal.pone.0008770 PMID: 20098738
7. Guibert G, Warszawski J, Le Chenadec J, Blanche S, Benmebarek Y, Mandelbrot L, et al. Decreased Risk of Congenital Cytomegalovirus Infection in Children Born to HIV-1–Infected Mothers in the Era of Highly Active Antiretroviral Therapy. CLIN INFECT DIS. 2009 Jun; 48(11):1516–25. https://doi.org/10.1086/598934 PMID: 19388872
8. Mania A, Kemnitz P, Mazur-Melewska K, Figlerowicz M, Cudnoch K, Służewski W, et al. Human cytomegalovirus infection and clinical status of infants born to human immunodeficiency virus type 1 infected mothers. The Journal of Maternal-Fetal & Neonatal Medicine. 2011 Aug 25; 25(2):180–6.
9. Kovacs A, Schluchter M, Easley K, Demmler G, Shearer W, La Russa P, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. N Engl J Med. 1999 Jul 8; 341(2):77–84. https://doi.org/10.1056/NEJM199907083410203 PMID: 10395631
10. Mwaanza N, Chilukutu L, Tembo J, Kabwe M, Musonda K, Kapasa M, et al. High Rates of Congenital Cytomegalovirus Infection Linked With Maternal HIV Infection Among Neonatal Admissions at a Large Referral Center in Sub-Saharan Africa. CLIN INFECT DIS. 2014 Mar; 58(5):728–35. https://doi.org/10.1093/cid/cit766 PMID: 24265360

11. Duryea EL, Sánchez PJ, Sheffield JS, Jackson GL, Wendel GD, McElwee BS, et al. Maternal human immunodeficiency virus infection and congenital transmission of cytomegalovirus. Pediatr Infect Dis J. 2010 Oct; 29(10):915–8. https://doi.org/10.1097/INF.0b013e3181e0ce05 PMID: 20431424
12. Hewison M. An update on vitamin D and human immunity. Clin Endocrinol (Oxf). 2012 Mar; 76(3):315–25.
13. Campbell GR, Spector SA. Vitamin D Inhibits Human Immunodeficiency Virus Type 1 and Mycobacterium tuberculosis Infection in Macrophages through the Induction of Autophagy. Deretic V, editor. PLoS Pathog. 2012 May 10; 8(5):e1002689. https://doi.org/10.1371/journal.ppat.1002689 PMID: 22589721
14. Campbell GR, Spector SA. Hormonally Active Vitamin D3 (1,25-Dihydroxycholecalciferol) Triggers Autophagy in Human Macrophages That Inhibits HIV-1 Infection. Journal of Biological Chemistry. 2011 May 20; 286(21):18890–902. https://doi.org/10.1074/jbc.M110.206110 PMID: 21454634
15. Equils O, Hewison M. A Role for Vitamin D in Placental Immunology. Journal of Infectious Diseases. 2010.
16. Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. Journal of Clinical Virology. 2011 Mar 1; 50(3):194–200. https://doi.org/10.1016/j.jcv.2010.12.006 PMID: 21242105
17. Gray TK, Lester GE, Lorenc RS. Evidence for extra-renal 1 alpha-hydroxylation of 25-hydroxyvitamin D3 in pregnancy. Science. American Association for the Advancement of Science; 1979 Jun 22; 204 (4399):1311–3. https://doi.org/10.1126/science.451538 PMID: 451538
18. Liu N, Kaplan AT, Low J, Nguyen L, Liu GY, Equils O, et al. Vitamin D Induces Innate Antibacterial Responses in Human Trophoblasts via an Intracrine Pathway. Biology of Reproduction. 2009; 80:398–406. https://doi.org/10.1095/biolreprod.108.073577 PMID: 19005165
19. Holick MF. Vitamin D deficiency. N Engl J Med. Mass Medical Soc; 2007; 357(3):266–81. https://doi.org/10.1056/NEJMra070553 PMID: 17634462
20. Walker VP, Zhang X, Rastegar I, Liu PT, hollis BW, Adams JS, et al. Cord Blood Vitamin D Status Impacts Innate Immune Responses. Journal of Clinical Endocrinology & Metabolism. 2011 May 20; 96 (6):1835–43.
21. Antoniucci DM, Black DM, chemistry DSC, 2005. Serum 25-Hydroxyvitamin D Is Unaffected by Multiple Freeze-Thaw Cycles. clinchemaaccjnlsorg.
22. Yun C, Chen J, Yang C, Li Y, Piao J, Yang X. Comparison of two 25-hydroxyvitamin D immunoassays to liquid chromatography-tandem mass spectrometry in assessing samples from the Chinese population. Clin Chim Acta. 2015 Aug 25; 448:22–6. https://doi.org/10.1016/j.cca.2015.06.007 PMID: 26093339
23. Seiden-Long I, Vieth R. Evaluation of a 1,25-Dihydroxyvitamin D Enzyme Immunoassay. Clinical Chemistry. 2007 Apr 19; 53(6):1104–8. https://doi.org/10.1373/clinchem.2006.077560 PMID: 17434909
24. Mehta S, Hunter DJ, Mugusi FM, Spiegelman D, Manji KP, Giovannucci EL, et al. Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania. Journal of Infectious Diseases. 2009 Oct 1; 200(7):1022–30. https://doi.org/10.1086/605699 PMID: 19673647
25. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res. 2011 Oct; 26(10):2341–57. https://doi.org/10.1002/jbmr.463 PMID: 21706518
26. Bearden A, Abad C, Gangnon R, Sosman JM, Binkley N, Safdar N. Cross-Sectional Study of Vitamin D Levels, Immunologic and Virologic Outcomes in HIV-Infected Adults. Journal of Clinical Endocrinology & Metabolism. 2013; 98:1726–33.
27. Adeyemi OM, Agniel D, French AL, Tien PC, Weber K, Glesby MJ, et al. Vitamin D deficiency in HIV-infected and HIV-uninfected women in the United States. J Acquir Immune Defic Syndr. 2011 Jul 1; 57 (3):197–204. https://doi.org/10.1097/QAI.0b013e31821ae418 PMID: 21471818
28. Haug C, Müller F, Aukrust P, Frøland SS. Subnormal serum concentration of 1, 25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. Journal of Infectious Diseases. Oxford University Press; 1994; 169(4):889. https://doi.org/10.1093/infdis/169.4.889 PMID: 7907645
29. Teichmann J, Stephan E, Lange U, Discher T, Friese G, Lohmeyer J, et al. Osteopenia in HIV-infected Women Prior to Highly Active Antiretroviral Therapy. Journal of Infection. 2003 May 1; 46(4):221–7. https://doi.org/10.1053/jinf.2002.1109 PMID: 12799147
30. Van Winden KR, Bearden A, Kono N, Frederick T, Operskalski E, Stek A, et al. Low Bioactive Vitamin D Is Associated with Pregnancy-Induced Hypertension in a Cohort of Pregnant HIV-Infected Women Sampled Over a 23-Year Period. Amer J Perinatol. 2019 Jul 31.
31. Chan SY, Susarla R, Canovas D, Placenta EV, 2015. Vitamin D promotes human extravillous trophoblast invasion in vitro. Elsevier.

32. Ganguly A, Tamblyn JA, of SF-SJ, 2018. Vitamin D, the placenta and early pregnancy: effects on trophoblast function. Joebioscientificacom.

33. Brannon PM, Picciano MF. Vitamin D in pregnancy and lactation in humans. Annu Rev Nutr. 2011 Aug 21; 31:89–115. https://doi.org/10.1146/annurev.nutr.012809.104807 PMID: 21756132

34. Wagner CL, Taylor SN, hollis BW. Vitamin D Requirements during Pregnancy and Lactation: Lessons Learned and Unanswered Questions. Wallace TC, editor. Dietary Supplements in Health Promotion; 2015.

35. Zehnder D, Evans KN, Kilby MD, Bulmer JN, Innes BA, Stewart PM, et al. The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua. Am J Pathol. 2002 Jul; 161(1):105–14. https://doi.org/10.1016/s0002-9440(10)64162-4 PMID: 12107095

36. Rieder FJJ, Gröschel C, Kastner M-T, Kosulin K, Laengle J, Zadnikar R, et al. Human cytomegalovirus infection downregulates vitamin-D receptor in mammalian cells. Journal of Steroid Biochemistry and Molecular Biology. Elsevier Ltd; 2017 Jan 1; 165(Part B):356–62.

37. Benirschke K, Mendoza GR, Bazeley PL. Placental and fetal manifestations of cytomegalovirus infection. Virchows Arch B Cell Pathol. 1974; 16(2):121–39. https://doi.org/10.1007/bf02894070 PMID: 4373898

38. Burton GJ, O'Shea S, Rostron T, Mullen JE, Aiyer S, Skepper JN, et al. Significance of placental damage in vertical transmission of human immunodeficiency virus. J Med Virol. 1996 Nov; 50(3):237–43. https://doi.org/10.1002/(SICI)1096-9071(199611)50:3<237::AID-JMV5>3.0.CO;2-A PMID: 8923288

39. Chan G, Hemmings DG, of AYTAJ, 2002. Human cytomegalovirus-caused damage to placental trophoblasts mediated by immediate-early gene-induced tumor necrosis factor-α. Elsevier.

40. Pinzone MR, Di Rosa M, Celesia BM, Condorelli F, Malaguarnera M, Madeddu G, et al. LPS and HIV gp120 modulate monocyte/macrophage CYP27B1 and CYP24A1 expression leading to vitamin D consumption and hypovitaminosis D in HIV-infected individuals. Eur Rev Med Pharmacol Sci. 2013 Jul; 17 (14):1938–50. PMID: 23877860