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Title: Maternal serum 25-hydroxyvitamin D levels and infant atopic dermatitis: a prospective cohort study

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1 **Conflict of Interest**

2 All authors disclose that they have no conflict of interest.

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8 **Abstract (word count: 249)**

9 **Background:** Maternal vitamin D status during pregnancy has been linked with the risk of atopic dermatitis
10 (AD) in children, while the results were inconsistent. The objective of this study was to assess the potential
11 association.

12 **Methods:** Serum 25-hydroxyvitamin D (25(OH)D) levels were measured in pregnant women from the birth
13 cohort MKFOAD. Infant AD was diagnosed according to Williams' criteria. Multivariate logistic regression
14 model was used to examine the association of maternal serum 25(OH)D levels in the first, second and third
15 trimester of gestation with the risk of infant AD at first year of age.

16 **Results:** In total, 121 (26.5%) of 456 infants developed AD prior to 1 year of age. In general, higher maternal
17 serum 25(OH)D levels throughout pregnancy were associated with increased risks of AD in infants prior to 1
18 year of age in multivariate logistic regression models, with borderline statistical significance in the first (per ln
19 unit increase: adjusted OR = 1.93, 95% CI: 0.96, 3.88) and second (per ln unit increase: adjusted OR = 1.72,
20 95% CI: 0.93, 3.19) trimester. Multivariate logistic regression models using categorical variables of maternal
21 25(OH)D levels by tertiles showed similar results: infants born to mothers with serum 25(OH)D levels in the
22 highest tertile had higher risk of AD than those with 25(OH)D in the lowest tertile.

23 **Conclusions:** The present study found some evidence supporting that higher maternal 25(OH)D levels during
24 pregnancy increased the risk of infant AD. However, the clinical implication of the findings should be limited
25 for those with blood levels over the recommendations.

26 **Keywords:** Atopic dermatitis, birth cohort, vitamin D, 25-hydroxyvitamin D

27 **KEY MESSAGE**

28 The present study is the first longitudinal study to assess the effects of maternal serum 25(OH)D levels
29 throughout the whole pregnancy on the risk of AD in infants prior to 1 year of age, and found some evidence of
30 that higher maternal 25(OH)D levels during pregnancy was associated with higher risk of early onset infant AD.
31 Our findings suggested that vitamin D supplementation during pregnancy might be not suitable for all pregnant
32 women.

INTRODUCTION

Atopic dermatitis (AD), also known as atopic eczema, is a chronic inflammatory skin disease characterized by recurrent eczematous lesions and pruritus, and often develops in early childhood (1). The prevalence of AD in children is estimated to be about 10-20% globally (2), and is over 30% in infants prior to 1 year of age in China (3). A number of children with AD may have increasing risks of asthma or allergic rhinitis with age. However, the pathogenesis of AD is not yet fully investigated. Previous studies suggested that it might be caused by an interplay of genetic and environmental factors leading to skin barrier dysfunction, and dysregulation of innate and adaptive immunity (4). Notably, some evidence indicated that prenatal dietary exposures, such as vitamin D, might be crucial in the development of atopic diseases in childhood (5).

Vitamin D can be synthesized in the skin from ultraviolet radiation or absorbed via dietary intake (6), and then metabolized in the liver to 25-hydroxyvitamin D (25(OH)D) which is the best indicator of vitamin D status (7). In addition to its crucial role in calcium homeostasis, vitamin D also plays a regulatory role in the immune system by alternating local calcium balance and binding nuclear vitamin D receptors (8). Vitamin D receptors can be expressed across a variety of immune cells, including dendritic cells, macrophages, T cells and B cells (9). Additionally, vitamin D can stimulate the production of antimicrobial peptides in the epidermis, and regulate the expression of different inflammatory cytokines (10). Thus, multiple lines of evidence supported the hypothesis that vitamin D may be involved in the pathogenesis of AD (11).

During pregnancy, maternal 25(OH)D can be transferred to fetus via the placenta, and is the main source of fetal exposure to vitamin D (12). Several prospective cohort studies have examined the association between maternal 25(OH)D levels during pregnancy and the risk of AD, while yielding inconsistent results (13, 14). One early cohort study from the UK found that children with maternal 25(OH)D levels in late pregnancy >75 nmol/L had increased risks of atopic eczema in infants at 9 months of age (15), but a few studies found none associations (16, 17), or negative associations (18). Additionally, maternal 25(OH)D levels were measured only once in these studies, which failed to capture the effects of likely changes in 25(OH)D status over the course of pregnancy (19). Thus, the objective of this study was to examine the associations of maternal serum 25(OH)D levels in the first, second and third trimester of gestation with the risk of infant AD at first year of age.

MATERIAL AND METHODS

Study population

The present study was based on the birth cohort MKFOAD (ClinicalTrial.gov: NCT02889081), which recruited pregnant women at 12–14 weeks of gestation between June and September 2016 at the Maternal and Child Health Hospital of Minhang district in Shanghai, China (20, 21). Briefly, pregnant women who planned to attend subsequent antenatal care, deliver baby and attend routine postnatal child care at the study hospital were included, and those with hereditary dermatosis were excluded. Of 518 eligible pregnant women, 487 (94.0%) agreed to participate. Among the 487 pregnant women included in the initial cohort, 29 were excluded from the study because of migration during pregnancy (N=15), multiple pregnancy (N=3), miscarriage or stillbirth (N=9), and withdraw (N=4). Ultimately, a total of 456 infants who received routine child care visits at birth, 42nd days, 6th and 12th month after birth were included in the present study, as shown in Figure 1.

The study protocol was approved by the ethics committee of Children's Hospital of Fudan University (Approval Number: 2016-34). All participants provided written informed consents at enrollment and subsequent routine child care visits.

Maternal serum 25(OH)D levels

Fasting blood samples were collected from peripheral veins of pregnant women in the first trimester (12–14 weeks of gestation), second trimester (22–26 weeks), and third trimester (34–36 weeks). Serum samples were separated with centrifugation and stored at -80°C until for the measurement of 25(OH)D. Maternal serum 25(OH)D levels were measured using chemiluminescent microparticle immunoassay on Architect system (Architect 3L52, Abbott Diagnostics, Barcelona, Spain). All measurements of maternal serum 25(OH)D were blinded.

Assessment of infant AD

The assessment of infant AD has been described in detail in our previous study (21). Briefly, infant AD was diagnosed according to Williams' criteria by a pediatric dermatologist of the Children's Hospital of Fudan University (22). Infants who were suspected with AD were finally diagnosed at 1 year of age by three experienced pediatric dermatologists through panel discussion.

Covariates

At enrollment, all participants completed a structured questionnaire, including information on maternal age, educational level, pre-pregnancy body size, gestational week at enrollment, vitamin D supplementation before conception and during pregnancy, gravidity, self and family history of allergic disease, alcohol consumption,

smoke exposure, home decoration, and hair coloring, and pet keeping for 3 months around conception. After delivery, newborn information, such as infant sex, birth date, and birth weight, was obtained from the electronic medical records.

Statistical analyses

The distributions of participants' general characteristics were presented as mean (standard deviation, SD) for continuous variables, and as counts and percentage (%) for categorical variables. Student's t test was used for continuous variables and chi-square test was used for categorical variables to compare the differences between groups of infants with AD and without AD. Maternal 25(OH)D levels in the first, second and third trimester of gestation were natural log (ln) transformed to meet normal distributions for regression analysis. We used percentiles and geometric mean (geometric standard deviation, GSD) to describe their distributions, and used Spearman rank correlation to examine their correlations.

Multivariate logistic regression model was first used to examine the potential associations between maternal 25(OH)D (continuous variable) and the risk of infant AD. However, in model diagnostics for the linear model assumptions, the generalized additive model (GAM) indicated nonlinear relationship of maternal 25(OH)D levels in the third trimester of gestation with the risk of infant AD, as shown in Figure S1. Given that the objective of this study is inference rather than prediction, and to make our results comparable to other studies, we primarily showed the results of multivariate logistic regression model using continuous variable, and then used categorical variables of maternal 25(OH)D levels by tertiles. Model was adjusted for potential confounders that predicted the exposure or outcome with P -value < 0.1 in univariate analysis, which included maternal age (<30 , ≥ 30), maternal education (high school or below, undergraduate, graduate or above) pre-pregnancy BMI ($<18.5\text{kg/m}^2$, $18.5\text{--}24\text{ kg/m}^2$, $\geq 24\text{ kg/m}^2$), gravidity (1 , ≥ 1), maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), infant sex (boy or girl), and the season of blood draw in each trimester of gestation (summer [June–September], autumn [September–November], winter [December–February], and spring [March–May]). Considering previous reports on sex differences in the association between vitamin D and childhood AD (23), a product interaction between maternal 25(OH)D and infant sex was introduced to examine the modification effect by sex.

Sensitivity analyses

According to Holick's definition (24), maternal serum 25 (OH) D level was also studied as different degrees of

deficiency as follows: most deficiency (<10 ng/mL), deficiency (10-20 ng/mL), insufficiency (20~30 ng/mL), and sufficiency ($\geq$ 30 ng/mL) in multivariate logistic regression model. To make full use of the longitudinal repeated measures of maternal 25(OH)D levels in the first, second and third trimester of gestation, we conducted another sensitivity analyses via utilizing different combinations of 25(OH)D deficiency (<20 ng/mL) throughout pregnancy (a composite 4-level ordered set of exposure levels: (1) vitamin D deficiency throughout pregnancy, (2) vitamin D deficiency twice in the first, second or third trimester of gestation, (3) vitamin D deficiency only once in the first, second or third trimester of gestation, (4) no vitamin D deficiency throughout pregnancy). Additionally, to account for seasonal variation in both maternal 25(OH)D levels and infant AD, we performed stratified analysis by the season of blood draw and birth.

Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R 3.3.2 (R Development Core Team). Odds ratio (OR) and 95% confidence interval (CI) were reported using an alpha (α) level of 0.05. Statistical significance was set at P -value < 0.10 for interactions.

RESULTS

Participants' characteristics

Characteristics of 456 participants are presented in Table 1. A total of 121 (26.5%) infants developed AD prior to 1 year of age. The mean (SD) maternal age at enrollment was 29.9 (4.0) years, with a mean (SD) pre-pregnancy BMI of 20.8 (2.7) kg/m². The mean gestational age was 39.1 (1.3) weeks, and the mean birth weight was 3373.9 (413.1) g. Most mothers had college or higher educational level (78.0%), and reported them (78.3%) or their husbands (83.8%) without history of allergy. A few number of mothers were exposed to second-hand smoke (17.8%) and alcohol (16.2%), kept pets (14.8%), dyed hair (6.8%), and lived in newly-decorated rooms (11.6%). Of the 456 singleton live births, 218 were caesarean sections with the rate of 50.2%, and 240 (52.6%) were boys. There were significant differences in maternal history of allergy and hair coloring, paternal history of allergy, infant sex, and birth weight between groups of infants with AD and without AD.

Maternal serum 25(OH)D levels

The median level of maternal serum 25(OH)D was 16.1 ng/mL (corresponding to 40.2 nmol/L) in the first trimester, 16.3 ng/mL (corresponding to 40.7 nmol/L) in the second trimester, and 14.0 ng/mL (corresponding to 34.9 nmol/L) in the third trimester, as shown in Table 2. Maternal serum 25(OH)D levels in the first, second and

third trimester of pregnancy were moderately to highly correlated with each other (Spearman's rank correlation coefficients ($r = 0.3-0.7$, P -value <0.001 , Table S1), with the strong strongest correlation between the second and third trimester ($r = 0.7$).

Association of maternal serum 25(OH)D with infant AD

In general, higher maternal serum 25(OH)D levels throughout pregnancy were associated with increased risks of AD in infants prior to 1 year of age (Table 3 and Figure S1), especially in the second trimester (per ln unit increase: crude OR = 1.63, 95% CI: 0.93, 2.87) and the third trimester (per ln unit increase: crude OR = 1.62, 95% CI: 0.99, 2.65) during pregnancy. After adjusting for covariates, the association between maternal serum 25(OH)D level and the risk of AD in infants was strengthened in the first trimester (per ln unit increase: adjusted OR = 1.93, 95% CI: 0.96, 3.88) and in the second trimester (per ln unit increase: adjusted OR = 1.72, 95% CI: 0.93, 3.19), but was attenuated in the third trimester (per ln unit increase: adjusted OR = 1.48, 95% CI: 0.87, 2.53).

The logistic regression models using categorical variables of maternal 25(OH)D levels by tertiles showed similar results of that infants born to mothers with serum 25(OH)D levels in the highest tertile had higher risk of AD, although the associations became non-significant after adjusting for covariates (Table 3).

We found none modifying effect of infant sex on the association between maternal 25(OH)D levels during pregnancy and the risk of AD in infants (Table S2).

Sensitivity analyses

When maternal serum 25 (OH) D level was studied as different degrees of deficiency, we found that children whose mothers were vitamin D deficiency, insufficiency and sufficiency tended to have higher risks of AD in comparison with those mothers who were vitamin D most deficiency (Table S3). In another sensitivity analysis via utilizing different combinations of 25(OH)D deficiency (<20 ng/mL) throughout pregnancy, women with vitamin D deficiency twice, and only once in the first, second or third trimester of gestation and without vitamin D deficiency throughout pregnancy had higher risks of offspring AD in comparison with those with vitamin D deficiency throughout pregnancy, especially among women with vitamin D deficiency twice in the first, second or third trimester of gestation and women without vitamin D deficiency throughout pregnancy (Table S4). In analyses stratified by the season of blood draw and birth, the positive association between maternal serum 25 (OH) D levels and the risk of infant AD remained (Table S5 and Table S6)

DISCUSSION

In the present study, we found some evidence supporting that higher maternal serum 25(OH)D levels throughout pregnancy were consistently associated with increased risk of early onset infant AD, although the association became borderline statistically significant after adjustment for covariates. Additionally, we did not find any potential modifying effects of infant sex on the associations between maternal serum 25(OH)D levels during pregnancy and the risk of infant AD.

To our knowledge, four prospective cohort studies have examined the associations of maternal 25(OH)D levels during pregnancy with the risk of infant AD, and yielded inconsistent results (13). To some extent, the finding in our study was consistent with one early cohort study (N=440) from the UK which found that children with maternal 25(OH)D levels (median: 50.0 nmol/L) in late pregnancy >75 nmol/L had an increased risk of atopic eczema in infants at 9 months of age (15). However, another UK study (Avon Longitudinal Study of Parents and Children, ALSPC; N=5513) found none association between maternal 25(OH)D (median: 61.5 nmol/L) and atopic eczema in children at 7.5 years of age (16). The atopic disease-specific Cork BASELINE Birth Cohort Study (N=1174) also found none association between maternal serum 25(OH)D (mean: 58.6 nmol/L) at 15 weeks of gestation and atopic eczema in infants at 2 years of age (17). Contrary to our results, one study based on the Project Viva (N=1418) found that children with maternal plasma 25(OH)D (mean: 59.5 nmol/L) in the second trimester <25 nmol/L had higher risk of early childhood AD than those with levels ≥ 25 nmol/L (18). In our study and the early UK cohort study, AD was assessed in infants prior to 1 year of age, whereas AD in the ALSPC, the atopic disease-specific Cork BASELINE Birth Cohort Study and the Project Viva was assessed during childhood. The early-onset AD during infancy may be more likely affected by the epigenetic effects of *in utero* exposure on immune gene regulation across the entire period of gestation, while the later-onset AD during childhood may be attributed to more complex additional postnatal exposures, such as dietary exposure including milk, egg, or peanut (25). In addition, the diagnosis of AD in our study and the early UK cohort study was according to Williams' criteria (22), whereas in the ALSPC and the Project Viva it was obtained by maternal report through questionnaire. Thus, the inconsistent findings across different studies might be due to the different timings and definitions of outcome assessments. However, the sample size and maternal serum 25(OH)D levels in our study and the early UK study were smaller and lower than those in three other studies. Although the point estimates in our study provided some evidence for a clinically meaningful

201 association of maternal 25(OH)D with infant AD, but the 95% CI of their estimates were across null. Thus, the
202 present study just provided limited evidence of that higher maternal 25(OH)D levels were associated with higher
203 risk of infant AD, and future studies with larger sample size and wider variation in maternal 25(OH)D levels are
204 warranted to confirm our findings. Notably, unlike previous studies that 25(OH)D levels were only measured
205 once and thus failed to capture effects of likely changes in 25(OH)D status and exposure to the fetus over the
206 course of pregnancy, the present study measured maternal 25(OH)D levels in three trimesters of gestation, and
207 found consistent positive associations, which enhanced the strength of the evidence.

208 The nonlinear relationship between vitamin D status and the risk of allergic outcomes have been reported in
209 several previous studies (26-29). Savilahti *et al.* explained that the dose of vitamin D for the activation of
210 different immunological functions plausibly varied, and the balance of these functions could determine whether
211 an allergic reaction occurred, thus leading to a nonlinear association (27). However, we observed an inverted
212 U-shaped relationship between maternal 25(OH)D levels and infant AD in this study. Similar trend of
213 association was reported in a cross-sectional study for eczema in US adults (28). It is remained unexplained. In
214 the present study, the nonlinear relationship appeared only in the third trimester of gestation, the sample size was
215 smaller and maternal 25(OH)D levels were relatively lower compared with those in the first and second trimester
216 of gestation. Thus, further studies are needed to confirm such a nonlinear relationship between maternal
217 25(OH)D and infant AD.

218 Two major theories have been proposed to explain the cause of AD, the hypotheses of skin barrier
219 dysfunction and immunological dysregulation (30). The latter one focuses on the imbalance of T cells, such the
220 predominance of *Th2* cells rather than *Th1*, and thus leads to IgE mediated hypersensitivity (31). In animal
221 study, Matheu *et al.* found that excess supplementation of vitamin D in mice could induce a sustained
222 proliferation of allergic *Th2* cells, resulting in an increasing prevalence of allergy (32). In humans, two birth
223 cohort studies also found that higher cord blood 25(OH)D levels were associated with a lower number of T
224 regulatory cells, which indicated that vitamin D might repress immune tolerance (33, 34). Cord serum 25(OH)D
225 levels were significantly correlated with maternal serum 25(OH)D levels during pregnancy (35, 36). Thus,
226 vitamin D induced immunological dysregulation might be one potential mechanism of infant AD.

227 Our study has several strengths. First, this study was prospective design with high follow-up rate (93.6%),
228 which could provide potential causality between maternal 25(OH)D and the risk of infant AD. In addition,

maternal 25(OH)D were measured repeatedly throughout the whole pregnancy, which directly reflects their biological exposure of vitamin D and could assess the effects of vitamin D on infant AD by specific time period during pregnancy. Additionally, the multiple measurements of maternal 25(OH)D and consistent patterns of associations made our findings robust. Third, the diagnosis of AD was accurate with small chance of misclassification. Last, all participants were consecutively recruited from a primary health care setting, which minimized selection bias and made our findings more generalizable.

Several limitations of the present study should also be noted. First, the sample size of the present study was relatively small, and future studies with larger sample size are warranted to confirm our findings. Second, although extensive participants' characteristics have been included as potential covariates in multivariate logistic models, the possible bias from the unmeasured factors, such as maternal sun exposure, cannot be completely avoided.

In conclusion, this study found some evidence supporting that higher maternal 25(OH)D levels during pregnancy was associated with higher risk of early onset infant AD. However, the clinical implication of the findings should be interpreted with caution and limited for those with blood levels over the recommendations.

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Table 1 Characteristics of the study population

Characteristics	All participants (N=456)	Infants with AD (n=121)	Infants without AD (N=335)	P-value
Continuous variables (Mean $\pm$ SD)				
Maternal age at enrollment (year)	29.9 $\pm$ 4.0	29.3 $\pm$ 4.4	29.5 $\pm$ 3.9	0.746
Pre-pregnancy BMI (kg/m ²)	20.8 $\pm$ 2.7	20.7 $\pm$ 2.6	20.8 $\pm$ 2.7	0.653
Gestational age (week)	39.1 $\pm$ 1.3	39.2 $\pm$ 1.1	39.1 $\pm$ 1.3	0.518
Birth weight (g)	3373.9 $\pm$ 413.1	3465.8 $\pm$ 445.6	3338.7 $\pm$ 395.1	0.004
Categorical variables (N (%))				
Maternal educational levels				
High school or below	100 (22.0)	22 (18.2)	78 (23.3)	0.402
Undergraduate	318 (69.9)	87 (71.9)	231 (69.2)	
Graduate or above	37 (8.1)	12 (9.9)	25 (7.5)	
Gravidity				
0	184 (40.4)	45 (37.2)	139 (41.5)	0.408
>1	272 (59.6)	76 (62.8)	196 (58.5)	
Maternal history of allergy				
No	357 (78.3)	83 (68.6)	274 (81.8)	0.003
Yes	99 (21.7)	38 (31.4)	61 (18.2)	
Paternal history of allergy				
No	382 (83.8)	94 (77.7)	288 (86.0)	0.034
Yes	74 (16.2)	27 (22.3)	47 (14.0)	
Maternal SHS exposure				
No	375 (82.2)	97 (80.2)	278 (83.0)	0.487
Yes	81 (17.8)	24 (19.8)	57 (17.0)	
Maternal alcohol consumption				
No	382 (83.8)	99 (81.8)	283 (84.5)	0.497
Yes	74 (16.2)	22 (18.2)	52 (15.5)	
Maternal vitamin D supplementation before conception				
No	393 (86.18)	105 (86.8)	288 (86.0)	0.826
Yes	63 (13.82)	16 (13.2)	47 (14.0)	
Maternal vitamin D supplementation during pregnancy				
No	222 (48.68)	52 (43.0)	170 (50.7)	0.143
Yes	234 (51.32)	69 (57.0)	165 (49.3)	
Hair coloring				
No	425 (93.2)	108 (89.3)	317 (94.6)	0.044
Yes	31 (6.8)	13 (10.7)	18 (5.4)	
Home decoration				
No	403 (88.4)	106 (87.6)	297 (88.7)	0.757
Yes	53 (11.6)	15 (12.4)	38 (11.3)	
Pet keeping				
No	391 (85.7)	103 (85.1)	288 (86.00)	0.820
Yes	65 (14.8)	18 (14.9)	47 (14.0)	
Delivery mode				

Vaginal delivery	216 (49.8)	53 (44.2)	163 (51.9)	0.149
Cesarean	218 (50.2)	67 (55.8)	151 (48.1)	
Infant gender				
Boy	240 (52.6)	79 (65.3)	161 (48.0)	0.001
Girl	216 (47.4)	42 (34.7)	174 (52.0)	

Missing data: gestational age (N=9), birth weight (N=22), maternal education (N=1), and delivery mode (N=22).

SHS: second-hand smoke.

Table 2 The distributions of maternal serum 25 (OH) D levels (ng/mL) during pregnancy.

Trimester	N	Geometric mean (GSD)	Percentiles				
			5th	25th	50th	75th	95th
First trimester	451	15.9 (1.4)	9.2	12.7	16.1	20.3	26.2
Second trimester	424	15.9 (1.5)	7.6	12.1	16.3	21.2	28.7
Third trimester	392	13.2 (1.6)	6.0	9.6	14.0	18.7	26.7

Table 3 Association of maternal serum 25 (OH) D levels with infant atopic dermatitis

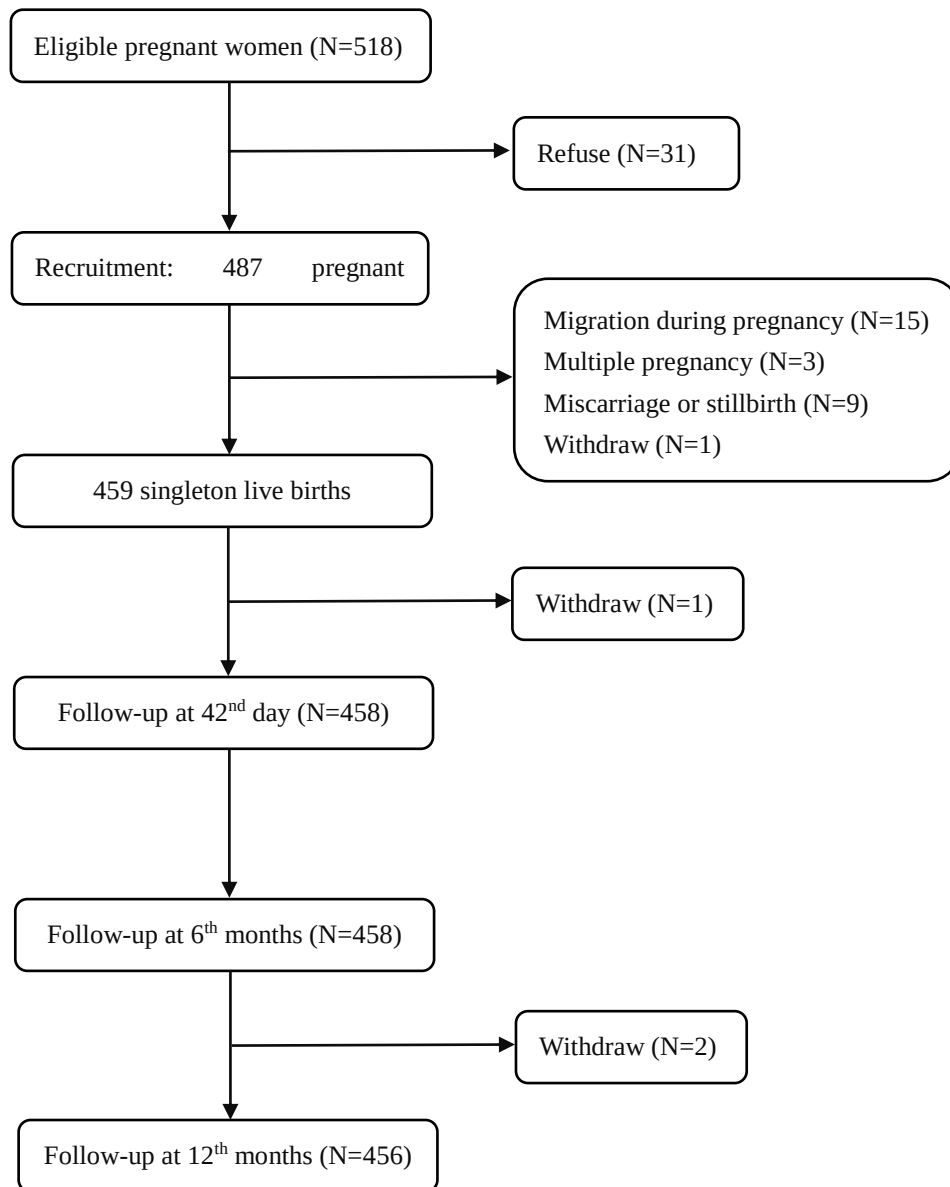
25 (OH) D levels (ng/mL)	Case (%)	Crude OR (95% CI) †	P-value	Adjusted OR (95% CI) †	P-value
Early pregnancy					
Low tertile (0~13.9)	36 (24.7)	Ref	Ref	Ref	Ref
Middle tertile (13.9~18.5)	36 (23.7)	0.95 (0.56, 1.61)	0.845	0.91 (0.52, 1.58)	0.725
High tertile (18.5~)	48 (31.4)	1.40 (0.84, 2.32)	0.197	1.53 (0.88, 2.64)	0.133
Continuous (ln-transformed)		1.64 (0.87, 3.08)	0.127	1.93 (0.96, 3.88)	0.064
Middle pregnancy					
Low tertile (0~13.6)	28 (20.3)	Ref	Ref	Ref	Ref
Middle tertile (13.6~19.0)	41 (28.5)	1.56 (0.90, 2.71)	0.112	1.53 (0.84, 2.78)	0.164
High tertile (19.0~)	43 (30.3)	1.71 (0.99, 2.95)	0.056	1.74 (0.96, 3.15)	0.070
Continuous (ln-transformed)		1.63 (0.93, 2.87)	0.089	1.72 (0.93, 3.19)	0.083
Late pregnancy					
Low tertile (0~11.2)	27 (20.9)	Ref	Ref	Ref	Ref
Middle tertile (11.2~16.6)	36 (28.1)	1.48 (0.83, 2.62)	0.182	1.48 (0.81, 2.73)	0.205
High tertile (11.2~16.6)	43 (31.9)	1.77 (1.01, 3.08)	0.046	1.65 (0.90, 3.00)	0.104
Continuous (ln-transformed)		1.62 (0.99, 2.65)	0.056	1.48 (0.87, 2.53)	0.147

† Models were adjusted for maternal age, maternal educational levels, pre-pregnancy BMI (kg/m²), gravidity, maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), infant sex (boy or girl), and the season of blood draw in each trimester of gestation.

Figure legends

Figure 1 Study population of the present study from the birth cohort MKFOAD

Figure 1 Study population of the present study from the birth cohort MKFOAD



Title: Maternal serum 25-hydroxyvitamin D levels and infant atopic dermatitis: a prospective cohort study

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Running title: Maternal 25(OH)D and infant atopic dermatitis

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Table S1 Spearman's rank correlation of maternal 25(OH)D levels among the first, second and third trimester during pregnancy

	First trimester	Second trimester	Third trimester
First trimester	1		
Second trimester	0.44*	1	
Third trimester	0.32*	0.73*	1

*Statistically significant (P -value < 0.05).

Table S2 P-values of interaction item (25(OH)D*infant sex) in the associations between maternal serum 25(OH)D levels and the risk of infant AD in multivariate logistic regression models.

Maternal serum 25(OH)D levels	<i>P</i> -value of interaction
Early pregnancy	
Low tertile	Ref
Middle tertile	0.875
High tertile	0.701
Continuous (ln-transformed)	0.392
Middle pregnancy	
Low tertile	Ref
Middle tertile	0.557
High tertile	0.696
Continuous (ln-transformed)	0.889
Late pregnancy	
Low tertile	Ref
Middle tertile	0.538
High tertile	0.885
Continuous (ln-transformed)	0.227

†Models were adjusted for maternal age, maternal educational levels, pre-pregnancy BMI (kg/m²), gravidity, maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), infant sex (boy or girl), and the season of blood draw in each trimester of gestation.

Table S3 Associations of maternal vitamin D status (divided by degrees of deficiency) with infant atopic dermatitis.

Maternal vitamin D status (ng/mL)	Case (%)	Adjusted OR (95% CI)	P-value
Early pregnancy			
Most deficiency (0~10)	10 (26.3)	Ref	Ref
Deficiency (10-20)	72 (25.0)	1.05 (0.46, 2.40)	0.906
Insufficiency (20~30)	35 (29.7)	1.48 (0.60, 3.64)	0.399
Sufficiency (30~)	3 (42.9)	2.23 (0.34, 14.51)	0.400
Middle pregnancy			
Most deficiency (0~10)	11 (22.0)	Ref	Ref
Deficiency (10-20)	61 (24.4)	1.29 (0.59, 2.83)	0.531
Insufficiency (20~30)	38 (34.6)	2.23 (0.96, 5.22)	0.064
Sufficiency (30~)	2 (14.29)	0.52 (0.09, 2.91)	0.458
Late pregnancy			
Most deficiency (0~10)	18 (17.1)	Ref	Ref
Deficiency (10-20)	68 (31.6)	2.24 (1.20, 4.18)	0.012
Insufficiency (20~30)	16 (26.2)	1.59 (0.70, 3.64)	0.271
Sufficiency (30~)	4 (36.4)	1.70 (0.42, 6.95)	0.462

†Models were adjusted for maternal age, maternal educational levels, pre-pregnancy BMI (kg/m²), gravidity, maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), infant sex (boy or girl), and the season of blood draw in each trimester of gestation.

Table S4 Associations of maternal vitamin D status (via utilizing different combinations of 25(OH)D deficiency (<20 ng/mL) throughout pregnancy) with infant atopic dermatitis.

Maternal vitamin D status	Case (%)	Adjusted OR (95% CI)	P- value
Deficiency throughout pregnancy	49 (23.6)	Ref	Ref
Deficiency twice in the first, second or third trimester of gestation	30 (33.71)	1.69 (0.94, 3.03)	0.079
Deficiency only once in the first, second or third trimester of gestation	15 (29.4)	1.39 (0.67, 2.88)	0.374
No vitamin D deficiency throughout pregnancy	9 (34.6)	1.85 (0.74, 4.66)	0.192

†Models were adjusted for maternal age, maternal educational levels, pre-pregnancy BMI (kg/m²), gravidity, maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), infant sex (boy or girl).

Table S5 Associations between maternal serum 25 (OH) D levels and the risk of infant AD stratified analysis by the season of blood draw in each trimester of gestation.

Maternal serum 25 (OH) D levels	Adjusted RR (95% CI)	P-value
Early pregnancy		
Summer (N=319)		
Low tertile (0~13.9)	Ref	Ref
Middle tertile (13.9~18.5)	0.88 (0.46, 1.67)	0.689
High tertile (18.5~)	1.47 (0.76, 2.84)	0.251
Continuous	1.65 (0.74, 3.68)	0.222
Autumn (N=133)		
Low tertile (0~13.9)	Ref	Ref
Middle tertile (13.9~18.5)	0.92 (0.26, 3.25)	0.891
High tertile (18.5~)	1.90 (0.60, 6.03)	0.278
Continuous	3.55 (0.75, 16.89)	0.112
Middle pregnancy		
Autumn (N=352)		
Low tertile (0~13.9)	Ref	Ref
Middle tertile (13.9~18.5)	1.67 (0.85, 3.28)	0.140
High tertile (18.5~)	2.16 (1.11, 4.23)	0.024
Continuous	2.14 (1.06, 4.29)	0.033
Winter (N=77)		
Low tertile (0~13.9)	Ref	Ref
Middle tertile (13.9~18.5)	1.53 (0.34, 6.91)	0.583
High tertile (18.5~)	0.61 (0.11, 3.58)	0.586
Continuous	1.01 (0.21, 4.85)	0.995
Late pregnancy		
Winter (N=97)		
Low tertile (0~13.9)	Ref	Ref
Middle tertile (13.9~18.5)	1.98 (0.38, 10.35)	0.417
High tertile (18.5~)	2.55 (0.51, 12.86)	0.257
Continuous	1.56 (0.43, 5.66)	0.498
Spring (N=295)		
Low tertile (0~13.9)	Ref	Ref
Middle tertile (13.9~18.5)	1.52 (0.76, 3.06)	0.237
High tertile (18.5~)	1.59 (0.79, 3.19)	0.195
Continuous	1.65 (0.88, 3.08)	0.116

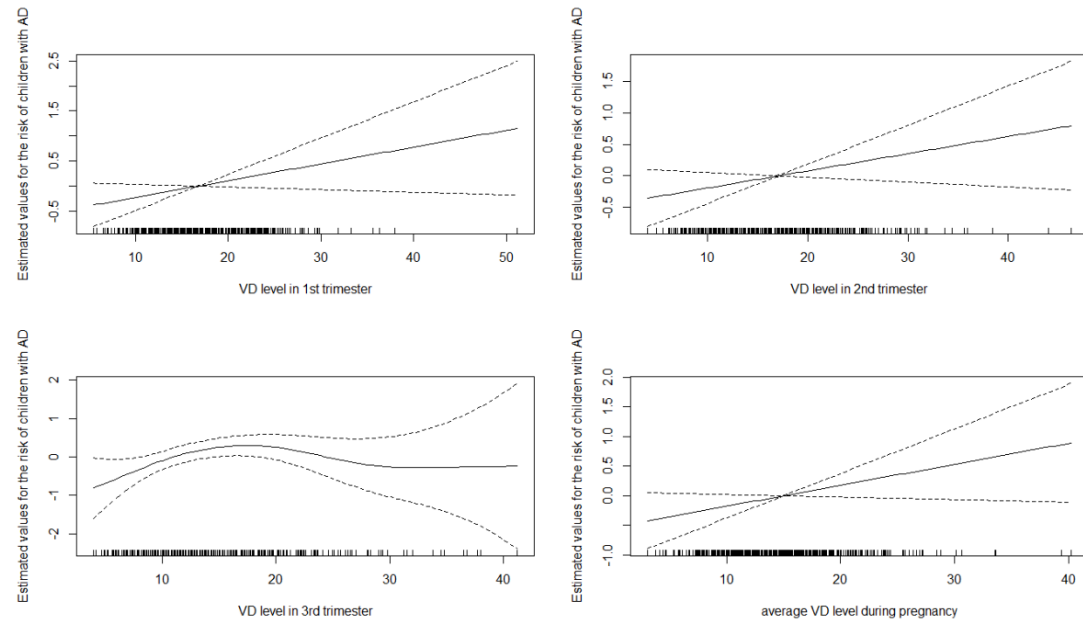
Models were adjusted for maternal age, maternal educational levels, pre-pregnancy BMI (kg/m²), gravidity, maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), and infant sex (boy or girl).

Table S6 Associations between maternal serum 25 (OH) D levels and the risk of infant AD stratified analysis by the season of birth.

Maternal serum 25 (OH) D levels	Winter (N=304)		Spring (N=142)	
	Adjusted RR (95% CI)	P-value	Adjusted RR (95% CI)	P-value
Early pregnancy				
Low tertile (0~13.9)	Ref	Ref	Ref	Ref
Middle tertile (13.9~18.5)	0.88 (0.46, 1.69)	0.699	1.11 (0.36, 3.42)	0.850
High tertile (18.5~)	1.46 (0.76, 2.80)	0.258	1.68 (0.53, 5.29)	0.376
Continuous	1.81 (0.81, 4.04)	0.148	2.56 (0.54, 12.13)	0.235
Middle pregnancy				
Low tertile (0~13.9)	Ref	Ref	Ref	Ref
Middle tertile (13.9~18.5)	1.16 (0.53, 2.50)	0.712	2.46 (0.86, 7.03)	0.094
High tertile (18.5~)	1.96 (0.93, 4.11)	0.076	0.95 (0.28, 3.24)	0.934
Continuous	1.79 (0.81, 3.94)	0.150	1.89 (0.61, 5.84)	0.272
Late pregnancy				
Low tertile (0~13.9)	Ref	Ref	Ref	Ref
Middle tertile (13.9~18.5)	1.68 (0.78, 3.60)	0.183	1.01 (0.30, 3.40)	0.989
High tertile (18.5~)	1.76 (0.81, 3.83)	0.152	1.61 (0.54, 4.81)	0.392
Continuous	1.48 (0.77, 2.87)	0.242	1.59 (0.58, 4.35)	0.366

Models were adjusted for maternal age, maternal educational levels, pre-pregnancy BMI (kg/m²), gravidity, maternal and paternal history of allergy (yes or no), hair coloring within 3 months before and after pregnancy (yes or no), and infant sex (boy or girl).

Figure S1 Adjusted generalized additive model plots of maternal serum 25(OH)D levels during pregnancy with the risk of infant AD



Note: The middle line in each panel shows the estimated effect (y-axis) of maternal serum 25(OH)D levels on the risk of infant AD plotted against the maternal serum 25(OH)D levels (x-axis), after adjusting for covariates. Upper and lower lines show 95% CI of the estimated effect.