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Effect of the prenatal maternal environments and diets on cord blood interleukin-4 and interferon –gamma: A pilot study

Won Hee Seo,[1] Byung Min Choi,[1] Heechul Lee,[2] Young Yoo,[1] Ji Tae Choung,[1] Youngshin Han[3]

Abstract

Background & Objectives: The environment of a pregnant woman can affect not only fetal growth and development, but also diseases in childhood. Neonatal cord blood cytokines are commonly used to evaluate the immune development of neonates. The purpose of this study was to evaluate the effects of the environment and diet during pregnancy on IL-4 and IFN-γ in neonatal cord blood.

Method: A total of 111 pregnant women participated in this study from April to November 2010. Allergy history, sensitization assessed by the skin prick test, dietary intake and indoor environment were evaluated. IL-4 and IFN-γ levels were measured in the complete cord blood of neonates using real-time PCR.

Results: There were 54 pregnant women with allergic disease. Both IL-4 and IFN-γ levels in neonatal cord blood were higher in samples from allergic mothers than in non-allergic mothers (p<0.05). The indoor environment and nutrient intake were not different between allergic and non-allergic mothers, except regarding carpet use. When the cytokine levels were divided into quartiles, lower folate and vitamin B_6 intake was associated with the highest levels of IL-4 in neonatal cord blood (p<0.05), and higher folate and vitamin B_6 intake was associated with highest levels of IFN-γ in neonatal cord blood.

Conclusions: In this study, a strong association between IL-4 and IFN-γ levels in cord blood and the intake of folate and vitamin B_6 was found, which indicates that food intake during pregnancy might have a strong influence on IL-4 and IFN-γ levels in cord blood, to a greater extent than environmental factors.

Key words: Allergy, Cord blood, Diet, Folate, IL-4, IFN-γ, Vitamin B_6, Pregnancy

From:
[1] Department of Pediatrics, Korea University College of Medicine, 73, Inchon-ro, Seongbuk-gu, Seoul 12841, Korea
[2] Department of Pediatrics, Dankook University College of Medicine, Cheil General Hospital and Women's Healthcare Center,17,Seoae-ro 1-gil, Jung-gu, Seoul 04619, Korea
[3] Department of Medical Science, Sungkyunkwan University School of Medicine, 81, Irwon-ro, Gangnam-gu, Seoul 06351, Korea

Corresponding author:
Youngshin Han
Department of Medical Science, Sungkyunkwan University School of Medicine, 81, Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Email: snuhan@gmail.com

Introduction

The environment of a pregnant woman can affect not only fetal growth and development, but also disease development in childhood. Allergic disease is one of the most common disorders affected by the prenatal environment.[1] The fact that the offspring of a mother with allergies are at greater risk for the development of allergic disease than the offspring of allergic fathers suggests that allergies are not merely genetic, but are also influenced by the maternal environment during the intrauterine period through direct fetal exposure.[2,3] These factors affect the development of disease by altering cytokine production by Th1 and Th2 cells. Although the mechanism of antigen transfer from mother to fetus through the placenta or amniotic fluid and the mechanisms by which this influences the later development of allergic disease in neonates are not yet clear, the reaction to a specific antigen in the fetal circulatory system at 22 weeks of gestational age implies that a fetus is affected by allergens, suggesting that the cytokine(s) secreted from fetal T cells can be a useful indicator of the status of the immunological development of a fetus.

The prenatal environment includes the mother's food intake and residential environment during pregnancy. There have been studies on associations between indoor allergens from house dust mites and pets and the development of allergic disease in

infants through prenatal exposure.[4-6] Also, a number of studies have reported an association between prenatal diet and allergic diseases, including findings that the Mediterranean diet, which is rich in vegetables, reduces wheezing in infants, and that consumption of more vegetables and fruits during pregnancy prevents allergic diseases in infants, and that the incidence of asthma in neonates is higher in the presence of maternal vitamin and zinc deficiency.[6-9] In addition, although the exact mechanism underlying this association with the development of allergic diseases has not been elucidated, several studies have shown an association between the development of allergies and maternal intake of nutrients such as folate, vitamin B_{12}, vitamin B_6, antioxidant vitamins, and trace elements including selenium, copper, and zinc.[6,8,10,11]

Despite the importance of the maternal environment during pregnancy, however, there have been few studies on the associations between environmental and food factors with aspects of fetal immunity such as cytokine levels at birth.

Therefore, the aim of this study was to investigate the effects of environmental factors on the development of the fetal immune system evaluated by investigating the associations between the mother's food intake and indoor environment and interleukin-4 (IL-4) and interferon-γ (IFN-γ) levels in neonatal cord blood.

Methods

Study subjects

The study subjects included 179 pregnant women ≥32 weeks of gestation. Participants were enrolled in the study from April to November 2010.

Pregnant women with a history of conditions that are known to affect the development of the fetal immune system, including congenital genetic disease, autoimmune disease, endocrine disorders such as thyroid disease, and gynecological diseases such as endometriosis, were excluded. Participants with infection during pregnancy, premature contractions, polyhydramnios, preeclampsia, and other perinatal diseases were also excluded from enrollment.

A total of 68 women (37.9%) were excluded from the study, including 42 who did not respond to the home visit or diet survey, and 26 who rejected the skin prick test. There were 111 women who completed all parts of the study, including the skin prick test and the assessment of food intake, and comprised the final study subjects.

Signed consent was obtained for participation in this study and for the use of samples, and a questionnaire about the history of allergic diseases in the pregnant subjects and their spouses, relevant family history, medical history including past diseases, age, family relationships, type of housing, duration of stay in the residence, pet ownership, history of smoking of subjects including indirect smoking, education, and income level was administered.

The study was approved by the ethics committees of Cheil General Hospital &Women's Healthcare Center (CGH-IRB-2009-04).

Diagnosis of allergy and skin prick test

Allergic diseases in pregnant women and their spouses were diagnosed by allergists. The skin prick test was performed on study subjects to determine the presence or absence of sensitization to specific allergens, including two types of house dust mites (HDM; *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), tree, grass, and weed pollens, as well as dog and cat dander.

Investigation of maternal food intake

A trained dietician evaluated the food intake status in pregnant subjects, weight gain during pregnancy, and the use of nutritional supplements or dietary restrictions by study subjects.

For the investigation of food intake, specifically, the food frequency questionnaire (FFQ) designed by the Korea Centers for Disease Control and Prevention (Korea CDC) and based on the 1998 Korean National Health and Nutrition Examination, was implemented.[12] A trained dietitian applied the semi-quantitative frequency questionnaire, which assessed the amount and frequency in the previous three months (third trimester) at 36-38 weeks of gestation. A total of 103 daily food items were included in the questionnaire. Each food that a participant reported consuming was analyzed for total energy intake (kcal) and 15 nutrients (fat, carbohydrate, protein, calcium, phosphorus, iron, zinc, vitamin A, vitamin E, vitamin B_1, vitamin B_2, niacin, vitamin B_6, folate, and vitamin C) using DS 24 (Human Nutrition Lab, Seoul National University & AI/DB Lab, Sookmyung Women's University, 1996).[12,13]

Investigation into the indoor environment

The use of a carpet, fabric sofa(s), pet ownership, frequency of home cleaning, frequency of laundry activity, frequency of window opening, use of a vacuum cleaner filter and the use of HDM-proof bedding were surveyed at home visits.

Each household of the 111 pregnant women was visited during the study period to evaluate exposure to HDM. The concentration of HDM was measured using the semi-quantitative Rapid Test for Mite Allergen® (Indoor Biotechnologies, Cardiff, UK), an ELISA-based assay and a common test for measuring allergen concentrations; this test has a sensitivity of 80.0% and a specificity of 76.5% HDM allergens.[14] Household dust samples were collected from the bedding of the mother. A dust collector filter was inserted into the collector, which was firmly attached to the end of 1.8 kW vacuum cleaner (Electrolux, Brazil). In the bedroom, the sample consisted of material from vacuuming 1 m^2 areas of the bedding for 2 minutes. In the kit, a 1% BSA PBS-T solution was poured into the dust collector and mixed by shaking for 1 minute, then left upright for four minutes . Next, five drops were transferred into the small round sample well on the test cassette. After 10 minutes, a test line developed and was compared to the intensity of the line with the colored indicator and interpreted on a scale of 0 to 3.

Measurement of cytokines in cord blood

Cord blood was collected from the neonates of study subjects, and the levels of the cytokines IL-4 and IFN-γ were measured using quantitative real-time PCR (RT-PCR). The quantitative RT-PCR method was used because it has higher sensitivity and specificity than ELISA.[15,16]

Total RNA was isolated from cord blood from the neonate by chloroform:phenol extraction and isopropanol precipitation according to the manufacturer's guidelines (Research Center Inc. (Cincinnati, OH, USA). RNeasy Mini Kits (Qiagen, Valencia, CA) were used according to the manufacturer's protocol to isolate RNA and to further purify RNA. One microgram of RNA was reverse transcribed in a 20-μl reaction containing RT Primer Mix (Qiagen, Hilden, North Rhine-Westphalia, Germany), reverse transcriptase (Qiagen), 7x gDNA Wipeout buffer (Qiagen), 5x RT buffer (Qiagen) and distilled water (Qiagen). Real time PCR was performed and analyzed by the dual-labeled fluorogenic probe method using an ABI Prism 7300 sequence detector (Applied Biosystems). Primers and probes for human GAPDH were purchased from Applied Biosystems. Amplification reactions were performed in MicroAmp optical tubes (Applied Biosystems) in a 25 μl volume containing 2× TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA), 900 nM forward primer, 900 nM reverse primer, 200 nM probe, and cDNA. Thermal cycling conditions were: 50°C for 2 min, then 95°C for 10 min for one cycle. Subsequently, 40 cycles of amplification were performed at 94°C for 15 seconds and 60°C for 1 min. Relative expression levels were calculated by the relative standard curve method as outlined in the manufacturer's technical bulletin. A standard curve was generated using the fluorescent data from the ten-fold serial dilutions of the cDNA from the sample with the highest expression. This was then used to calculate the relative amounts of target mRNA in test samples. The quantities of all targets in the test samples were normalized to the corresponding GAPDH levels in cord blood.

Statistics

SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical tests. Each environmental factor and its association with IL-4 and IFN-γ were determined by multiple regression analysis, and dependent variables were examined using the Pearson correlation. The Mann-Whitney test was used for the comparison of groups and *P* values <0.05 were considered significant.

Results

Characteristics of the subjects (Tables 1 and 2)

At the time of the survey, the mean gestational period of study subjects was 34 weeks and 4 days, and the mean age was 33.0±3.68 years (range, 23-42 years). Of the subjects, 77.7% (85 of 111) were experiencing their first pregnancy, and 63.0% (70 of 111) had an educational level beyond college.

Among the 111 subjects, there were 54 women with allergic diseases (allergic mothers). Among the allergic mothers, allergic rhinitis was the predominant allergic disease (33 of 54, 61.1%), and there were seven subjects with two or more allergic diseases. These allergic mothers had mild intermittent symptoms without maintenance allergic therapy during pregnancy. There were 42 subjects that showed sensitization to allergens, including 35 with sensitization to HDM.

Table 1. Characteristics of the participants, allergic mother and non-allergic mother

Characteristics	Allergic mother n=54	Non allergic mother n=57	*P* value
Maternal Age (yrs)	33.07±4.02	32.86±3.27	0.76
Paternal age (yrs)	35.64±4.82	34.81±3.29	0.31
Paternal allergic disease (n)	26(48.1%)	15(26.3%)	0.09
Maternal allergen sensitization (n)	29(53.7%)	13(22.8%)	0.01*
Weight at delivery (kg)	67.73±9.05	66.38±7.96	0.41
Weight gain during pregnancy (kg)	11.65±4.49	11.93±4.14	0.73
Mode of delivery (Caesarean section)	24(44.4%)	27(47.3%)	0.86
Maternaleducation (beyond college, n)	40(74.1%)	30(52.6%)	0.56
Paternaleducation (beyond college, n)	41(75.9%)	36(63.2%)	0.69
Nutritional supplements (n)	50(92.6%)	41(71.9%)	0.80
Dietaryintervention during pregnancy (n)	43(79.6%)	43(75.4%)	0.80

Abbreviation: yr, years. * means significance, *P*<0.05

Table 2. Comparison of Environmental characteristics between allergic mother and non-allergic mother

Environmental factor	Allergic mother (n=54)	Non allergic mother (n=57)	*P*
Carpet use	4(7.4%)	17(29.8%)	<0.01*
Detectable HDM at home	16(29.6%)	13(22.8%)	0.56
Exposure to tobacco smoke	58(52.2%)	22(20.2%)	0.08
Pet ownership	4(4.5%)	1(0.9%)	0.25
Use of HDM-proof bedding	12(22.2%)	8(14.0%)	0.62
Vacuum cleaning with heap filter	14(25.9%)	14(24.5%)	0.79

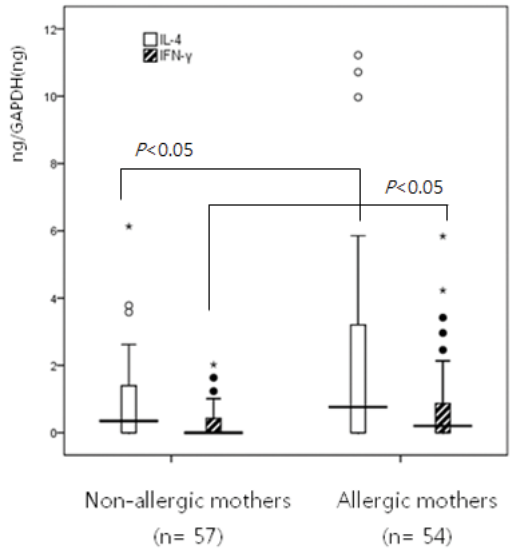
* means significance, *P*<0.05

Table 3. Comparison of nutrient intake betweengroups over the 75thpercentile and below the 25thpercentile of IL-4 and IFN-γ of allergic mother

Nutrient	quartiles	IL-4			IFN-γ		
		n	Mean±SD	*p*	n	Mean±SD	*p*
Protein (g/d)	≥75th	21	62.45±4.71	0.06	18	65.18±6.44	0.11
	≤25th	23	73.55±4.75		24	73.25±4.69	
Calcium (mg/d)	≥75th	21	598.65±66.26	0.15	18	635.47±59.65	0.23
	≤25th	23	719.77±66.37		24	730.00±62.79	
Phosphorous (mg/d)	≥75th	21	1003.55±75.12	0.05	18	1057.18±85.41	0.20
	≤25th	23	1179.82±83.19		24	1170.46±82.19	
Iron (mg/d)	≥75th	21	10.60±0.99	0.06	18	10.96±1.17	0.09
	≤25th	23	12.25±.90		24	12.43±.0.92	
Zinc (mg/d)	≥75th	21	8.24±0.64	0.11	18	8.44±0.79	0.05
	≤25th	23	9.34±.0.56		24	9.28±0.59	
Vitamin A (μg/d)	≥75th	21	481.35±52.86	0.07	18	500.35±51.01	0.06
	≤25th	23	646.32±70.66		24	640.13±67.69	
Vitamin E (μg/d)	≥75th	21	7.73±0.67	0.06	18	8.31±0.77	0.12
	≤25th	23	9.66±0.97		24	9.63±0.93	
Vitamin B_1 (mg/d)	≥75th	21	1.04±0.08	0.09	18	1.100±0.09	0.06
	≤25th	23	1.23±0.07		24	1.22±0.07	
Vitamin B_2 (mg/d)	≥75th	21	1.075±0.09	0.06	18	1.15±0.09	0.27
	≤25th	23	1.305±0.09		24	1.275±0.09	
Niacin (mg/d)	≥75th	21	13.08±1.07	0.07	18	13.38±1.24	0.14
	≤25th	23	15.61±1.05		24	15.50±1.07	
Vitamin B_6 (mg/d)	≥75th	21	1.50±0.11	0.03*	18	1.60±0.17	0.05
	≤25th	23	1.81±0.11		24	1.77±0.10	
Folate (μg/d)	≥75th	21	204.40±19.11	0.01*	18	222.82±24.01	0.08
	≤25th	23	276.27±20.61		24	271.88±21.61	
Vitamin C (mg/d)	≥75th	21	98.20±11.61	0.1	18	91.88±11.36	0.04*
	≤25th	23	122.41±8.88		24	121.75±8.32	

* means significance, $P<0.05$

Figure 1. The expressions in neonatal cord blood IL-4 and IFN-γ from the non-allergic mothers and allergic mothers. The expressions IL-4 and IFN-γ in cord blood with allergic mother were significantly higher than those with non-allergic mother. (* means significance, $P<0.05$)



Measurement of IL-4 and IFN-γ in neonatal cord blood

The mean and standard deviation of IL-4 levels in the cord blood of neonates was 1.40±2.15 ng/GAPDH (ng) [range, 0-11.22 ng/GAPDH (ng)] and the IFN-γ was 0.57±1.17 ng/GAPDH (ng) [range, 0-8.23 ng/GAPDH (ng)] (mean±SD). The measured concentrations of IL-4 and IFN-γ in neonatal cord blood showed no correlation (r=0.14).

IL-4 and IFN-γ in neonatal cord blood in allergic and non-allergic mothers

The measured levels of IL-4 and IFN-γ in cord blood from the neonates of allergic mothers (n=54) were significantly higher than those in the neonates of non-allergic mothers (p=0.035 and p= 0.042, respectively; Figure 1). There were no significant differences in IL-4 and IFN-γ levels between allergic and non-allergic mothers (p=0.516).

Table 4. Comparison of amount of nutrients betweengroups over the 75thpercentile and below the 25thpercentile of IL-4 and IFN-γ in non- allergic mother

Nutrient	quartiles	IL-4			IFN-γ		
		n	Mean±SD	*p*	n	Mean±SD	*p*
Protein (g/d)	≥75th	7	51.57±10.85	0.21	9	74.56±7.89	0.61
	≤25th	23	66.30±5.72		26	70.00±5.62	
Calcium (mg/d)	≥75th	7	545.00±129.12	0.66	9	753.67±118.38	0.43
	≤25th	23	694.61±93.39		26	751.42±88.49	
Phosphorous (mg/d)	≥75th	7	839.29±183.12	0.24	9	1207.56±141.35	0.86
	≤25th	23	1106.00±101.06		26	1162.04±98.01	
Iron (mg/d)	≥75th	7	8.87±1.71	0.36	9	12.40±1.03	0.40
	≤25th	23	10.87±0.95		26	11.08±0.87	
Zinc (mg/d)	≥75th	7	6.69±1.41	0.18	9	9.54±0.81	0.75
	≤25th	23	8.80±0.73		26	9.21±0.72	
Vitamin A (μg/d)	≥75th	7	490.14±101.00	0.95	9	611.89±66.30	0.94
	≤25th	23	547.00±64.80		26	579.77±61.81	
Vitamin E (μg/d)	≥75th	7	9.01±5.20	0.22	9	11.567±2.4696	0.10
	≤25th	23	8.88±4.46		26	8.323±0.8644	
Vitamin B_1 (mg/d)	≥75th	7	1.08±0.11	0.40	9	1.311±0.1379	0.07
	≤25th	23	0.86±0.18		26	1.127±0.1099	
Vitamin B_2 (mg/d)	≥75th	7	1.00±0.22	0.49	9	1.400±0.1929	0.37
	≤25th	23	1.26±0.15		26	1.350±0.1480	
Niacin (mg/d)	≥75th	7	10.77±2.09	0.32	9	16.21±1.35	0.06
	≤25th	23	13.85±1.31		26	14.17±1.30	
Vitamin B_6 (mg/d)	≥75th	7	1.21±0.23	0.20	9	1.92±0.14	0.04*
	≤25th	23	1.64±0.15		26	1.65±0.14	
Folate (μg/d)	≥75th	7	178.14±37.43	0.16	9	288.78±36.62	0.03*
	≤25th	23	224.52±19.94		26	219.46±17.64	
Vitamin C (mg/d)	≥75th	7	95.00±22.46	0.32	9	154.44±27.91	0.07
	≤25th	23	113.70±12.99		26	111.62±12.37	

* means significance, $P<0.05$

Food intake and IL-4 and IFN-γ in neonatal cord blood

The mean daily calorie intake of the pregnant women was 1926.9±65.4 kcal (mean ± SD). The mean weight gain during pregnancy was 11.78±4.31 kg, and 82% of the women used nutritional supplementation. Except for phosphorus, in more than 50% of the subjects, daily nutrient intakes were less than the estimated average requirements (EAR) according to Korean recommendations. There were no differences in the intake of total energy and nutrients between allergic and non-allergic mothers.

Of all subjects, 68.4% (76 of 111) reported dietary restriction regarding some foods, and there were more allergic mothers (64.8%; 35 of 54) than non-allergic mothers with such some food restrictions; however, the difference was not statistically significant (Table 1). There was no difference in the number of restricted foods between allergic and non-allergic mothers (p=0.261).

Analysis of the quartiles of IL-4 and IFN-γ expression (Tables 3 and 4)

No associations were observed between the intake of any nutrient or nutritional supplement and IL-4 or IFN-γ levels. However, when IL-4 and IFN-γ levels were divided into quartiles and compared between groups over the 75th percentile (highest quartile group) and below the 25th percentile (lowest quartile group) in the allergic and non-allergic mothers, the highest quartile group of IL-4 was found to be significantly associated with a lower intake of folate and vitamin B_6 than those in the lowest quartile group, but only in allergic mother. Although it was not a statically significant difference, vitamin E, vitamin B_2, phosphorous and zinc were statically marginal in significance (Table 3).

Moreover, there were no differences in any environmental factors, including smoking exposure, pet ownership, paternal allergic disease, dietary restrictive intervention and nutritional supplementation between the highest quartile group and the lowest quartile group in IL-4 (p>0.05).

In non-allergic mothers, greater intake of folate and vitamin B_6 was found in the highest quartile group of IFN-γ compared to those of the lowest quartile group (Table 4).

Indoor environment during the prenatal period, and IL-4 and IFN-γ levels in neonatal cord blood

Among the indoor environmental factors, allergic mothers used less carpet than non-allergic mother. (p=0.023) Other factors, including indirect smoking, pet ownership, HDM in the residence, and the use of HDM-proof bedding showed no association with levels of IL-4 or IFN-γ in both allergic and non-allergic mothers (Tables 1 and 2).

Thirty-five subjects demonstrated sensitization to HDM in the skin prick test; however, these sensitized cases did not correlate with the detection of HDM in homes (n=29, p=0.086). There was no significant difference between mothers with an HDM positive home (1+ to 3+) and mothers with an HDM negative home with regard to levels of IL-4 or IFN-γ (p=0.323 and p=0.819, respectively).

Discussion

In this study, we measured IL-4 and IFN-γ levels in neonatal cord blood, and determined an association between these cytokine levels and the antenatal environment, especially maternal diet. There was a strong association between maternal intake of folate and vitamin B_6 and IL-4 and IFN-γ levels in neonatal cord blood, whereas there were no significant associations between these cytokines and the indoor environment. To the best of our knowledge, this is the first report to find an association between maternal intake of folate and vitamin B_6 and cord blood IL-4 and IFN-γ levels.

In the uterus, the production of IFN-γ from sensitized cells is relatively low, whereas the cytokines produced by Th2 cells, such as IL-4, IL-5, and IL-13 predominate, leading to a Th1/Th2 imbalance and Th2-biased immune response at birth.[17-21] Even in the neonatal period, a Th2 bias has also been reported without risk factors for allergic diseases.[22,23]

In this study, IFN-γ was also present at lower levels than IL-4 in the neonatal cord blood of all subjects. Both IL-4 and IFN-γ levels were higher in neonates born to allergic mothers than to non-allergic mothers (Figure 1); however, there was no difference according to maternal allergy status in the IL-4/IFN-γ ratio (p=0.516), indicating a Th1/Th2 imbalance at birth regardless of the mother's allergy history. This can be explained as a physiological mechanism to maintain both fetal viability and pregnancy through allograft tolerance promoted by Th2 cytokines, which dampen Th1 immune responses.

Cytokines in cord blood are believed to be predictive factors for the development of allergic diseases after birth, despite the Th2 cytokine bias at birth, because the Th1/Th2 imbalance is either maintained or exacerbated after birth; this can influence the development of allergic diseases. Several studies have reported that pre-existing hyper-responsive T-cell cytokine responses from birth may influence the development of allergic diseases in children.[5,24] The prenatal environment is the primary determinant of this imbalance after birth. Maternal allergic disease is an important risk factor for the development of allergic diseases in offspring. One potential explanation for the development of allergies in children of atopic mothers is Th1/Th2 regulation.[25,26] Based on the finding that IL-4 and IFN-γ levels were significantly higher in allergic mothers than in non-allergic mothers in the present study, it can be posited that the fetal immune response in neonates from allergic mothers is hypersensitive to allergens or that the fetal immune system is more active.

With respect to the association between nutrient intake and cord blood IL-4 and IFN-γ, this study showed that less intake of folate and vitamin B_6 was associated with higher IL-4 levels in allergic mothers, and more intake of folate and vitamin B_6 was associated with higher IFN-γ levels in non-allergic mothers, based on an analysis of these cytokine levels in the highest and lowest quartiles. Zinc, phosphorus, iron, vitamin E and niacin showed marginal p values. (Table 3).

Diet is suspected to impact asthma and allergy susceptibility is through epigenetic mechanisms, including DNA methylation.[27,28] Dietary methyl donors are important in the on single-carbon metabolism pathway that is essential for DNA methylation.[29] Folate and vitamins B_6, B_{12}, and B_2 are water-soluble B vitamins which are naturally occurring in many foods; they are key cofactors for single-carbon metabolism.[30] Some studies have suggested a relationship between maternal folate and vitamins B intake and allergic disease in the offspring.[31,32] Additionally, antioxidants such as zinc, vitamin C, and vitamin E are examples of dietary nutrients that have potentially immunomodulatory effects.[28] A proposed mechanism suggests that an adequate intake of vitamins (B_6, B_{12}, C, and E) as well as zinc and iron supports Th1 cytokine-mediated immune responses and the production of pro-inflammatory cytokines, and limits the anti-inflammatory Th2 cell-mediated immune response.[11] Also, folate deficiency alters the cell-mediated immune response by reducing the proportion of circulating CD8+ T cells and inhibiting the remethylation cycle, both of which alter the Th1/Th2 balance.[11,29] Moreover, a recent animal study showed that blocking folate metabolism might be helpful in suppressing allergic airway disease in mice.[33] Our results provide evidence that folate and vitamin B_6 affect fetal immunity, and it is possible that low folate levels in some pregnant women in this study had an influence on the fetuses. To the best of our knowledge, this is the first report of an association between maternal folate intake and vitamin B_6 and IL-4 and IFN-γ levels in cord blood, and might provide a mechanistic link between folate and vitamin B intake and the development of allergic disease.

Despite the American Academy of Pediatrics guidelines suggesting that pregnant women do not need to practice dietary restrictions in order to prevent allergic diseases, dietary interventions are still commonly performed. Most participants in this study practiced some restriction of some foods regardless of the presence of allergies. In terms of the nutrient intakes of pregnant women who participated in this study, more than 80% were taking supplements; however, except for phosphorus, intake levels were lower than the KDRI (Dietary Reference Intakes for Koreans) suggested by the Korean Nutrition Society. Dietary interventions might have resulted in lower nutrient intake than the recommended amounts;

therefore, dietary restrictions without a specific purpose in pregnant women could greatly affect the development of allergic diseases in neonates. The results of this study support the notion that dietary intervention (restriction) in pregnant women is not necessary for the prevention of allergic diseases, but rather consuming the recommended amount of nutrients is important for preventing allergic disease.

In this study, positivity to HDM antigen in the home in 35 subjects had no association with IL-4 or IFN-γ levels in neonatal cord blood and sensitized mothers. This finding may be explained that maternal allergen sensitization likely developed in early life, not during pregnancy. However, the impact of the residential environment on fetal health during pregnancy was of great interest to the subjects, considering results showing less carpet use in allergic mothers.

This study has a number of advantages concerning the investigations into food intake. First, each nutrient, rather than food units, was measured by determining the nutrients from consumed foods, because dietary intakes can vary according to socioeconomic status, culture, and lifestyle. Second, as the FFQ verified by the Korea CDC includes a list of 103 food groups and indicates the amounts of food intake through pictures, daily intake could be studied relatively accurately. Third, previous studies on the associations between dietary intake and allergic diseases have been cross-sectional in nature, whereas this study was prospective, employing surveys and interviews on dietary intake during the third trimester. Considering that it is uncommon to change dietary habits suddenly during the third trimester, the diets investigated during those three months can be assumed to be similar to the diets maintained throughout the pregnancy.

Our study has limitations; first, 63% of participants in this study had an education beyond collage and 77% were in their first pregnancy, who had great interest in fetal health, and thus were actively involved in the control of environmental factors, including dietary interventions and nutritional supplements. However, this likely had little effect on the results since there were no significant differences in the demographics of allergic and non-allergic mothers. Secondly, maternal serum levels of folate and vitamin B_6 were not measured, and thus there are some limitations to explaining the exact links between folate, vitamin B_6, IL-4 and IFN-γ levels in neonatal cord blood. Thirdly, in the measurements of indoor allergens, only HDM allergen was assessed. However, HDM is the most common and important inhaled allergen in Korea.[35] Also, the among the allergens tested in this study, HDM sensitization was most common (83%). Considering this, the investigation of other indoor allergens such as cat or dog was of little importance.

In conclusion, the maternal diet during pregnancy was found to be associated with IL-4 and IFN-γ levels in neonatal cord blood. The highest quartile of IL-4 in neonatal cord blood was associated with low intake of folate and vitamin B_6 in allergic mothers, and highest quartile of IFN-γ in neonatal cord blood was associated with more intake of folate and vitamin B_6 in non-allergic mothers. These results indicate that food intake directly affects the levels of these cytokines in neonatal cord blood, more so than environmental exposure. Therefore, further study is needed to investigate maternal diet and the development of allergic diseases after birth in an effort to prevent or control these diseases.

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002;31:1235-9.
2. Khan TK, Palmer DJ, Prescott SL. In-utero exposures and the evolving epidemiology of paediatric allergy. Curr Opin Allergy Clin Immunol. 2015;15:402-8.
3. Al-Hammadi S, Zoubeidi T, Al-Maskari F. Predictors of childhood food allergy: significance and implications. Asian Pac J Allergy Immunol. 2011;29:313-7.
4. Bahrainwala A, Hassan S, Long M, Kaplan J. Cord blood house dust mite allergen in newborns: relationship to maternal blood levels of allergen and allergen specific IgG and IgE. Ann Allergy Asthma Immunol. 2005;95:480-3.
5. Hagendorens MM, Ebo DG, Bridts CH, Van de Water L, De Clerck LS, Stevens WJ. Prenatal exposure to house dust mite allergen (Der p 1), cord blood T cell phenotype and cytokine production and atopic dermatitis during the first year of life. Pediatr Allergy Immunol. 2004;15:308-15.
6. Prescott SL, Clifton V. Asthma and pregnancy: emerging evidence of epigenetic interactions in utero. Curr Opin Allergy Clin Immunol. 2009;9:417-26.
7. Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O, et al. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. Am J Clin Nutr. 2007;85:530-7.
8. Willers SM, Devereux G, Craig LCA, McNeill G, Wijga AH, Abou El-Magd W, et al. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. Thorax. 2007;62:773-9.
9. Miller RL. Prenatal maternal diet affects asthma risk in offspring. J Clin Invest. 2008;118:3265-8.
10. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest. 2008;118:3462-9.
11. Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab. 2007;51:301-23.
12. Ahn YJ, Lee JE, Paik HY, Lee HK, Jo IH, Kimm KC. Development of a Semi-quantitative Food Frequency Questionnaire Based on Dietary Data from the Korea National Health and Nutrition Examination Survey. Nutritional Sciences. 2003;6:12.
13. Ahn Y, Kwon E, Shim JE, Park MK, Joo Y, Kimm K, et al. Validation and reproducibility of food frequency questionnaire for Korean genome epidemiologic study. Eur J Clin Nutr. 2007;61:1435-41.
14. Ha JM, Kim SW, Kim JH, Lim IS, Shin MY, Han YS, et al. Comparison of Methods for Measuring House Dust Mite Allergens. Pediatr Allergy Respir Dis. 2010;20:226-31.
15. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, Debenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2007;120:150-5.
16. Hagendorens MM, Van Bever HP, Schuerwegh AJ, De Clerck LS, Bridts CH, Stevens WJ. Determination of T-cell subpopulations and intracellular cytokine production (interleukin-2, interleukin-4, and interferon-gamma) by cord blood T-lymphocytes of neonates from atopic and non-atopic parents. Pediatr Allergy Immunol. 2000;11:12-9.
17. Prescott SL, Breckler LA, Witt CS, Smith L, Dunstan JA, Christiansen FT. Allergic women show reduced T helper type 1 alloresponses to fetal human leucocyte antigen mismatch during pregnancy. Clin Exp Immunol. 2010;159:65-72.

18. Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. Pediatr Allergy Immunol. 1996;7:109-16.
19. Devereux G, Seaton A, Barker RN. In utero priming of allergen-specific helper T cells. Clin Exp Allergy. 2001;31:1686-95.
20. Breckler LA, Hale J, Taylor A, Dunstan JA, Thornton CA, Prescott SL. Pregnancy IFN-gamma responses to foetal alloantigens are altered by maternal allergy and gravidity status. Allergy. 2008;63:1473-80.
21. Warner JO, Jones CA, Kilburn SA, Vance GH, Warner JA. Pre-natal sensitization in humans. Pediatr Allergy Immunol. 2000;11 Suppl 13:6-8.
22. Piccinni MP. T cells in normal pregnancy and recurrent pregnancy loss. Reprod Biomed Online. 2007;14 Spec No 1:95-9.
23. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol. 1993;151:4562-73.
24. Quah PL, Huang C-H, Shek LP-C, Chua KY, Lee BW, Kuo I. Hyper-responsive T-cell cytokine profile in association with development of early childhood wheeze but not eczema at 2 years. Asian Pac J Allergy Immunol. 2014;32:84-92.
25. Piccinni MP, Beloni L, Giannarini L, Livi C, Scarselli G, Romagnani S, et al. Abnormal production of T helper 2 cytokines interleukin-4 and interleukin-5 by T cells from newborns with atopic parents. Eur J Immunol. 1996;26:2293-8.
26. Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. Lancet. 1994;344:983-5.
27. Husemoen LLN, Toft U, Fenger M, Jørgensen T, Johansen N, Linneberg A. The association between atopy and factors influencing folate metabolism: is low folate status causally related to the development of atopy? Int J Epidemiol. 2006;35:954-61.
28. Amarasekera M, Prescott SL, Palmer DJ. Nutrition in early life, immune-programming and allergies: the role of epigenetics. Asian Pac J Allergy Immunol. 2013;31:175.
29. Sharma S, Litonjua A. Asthma, allergy, and responses to methyl donor supplements and nutrients. J Allergy Clin Immunol. 2014;133:1246-54.
30. Maggini S, Wintergerst ES, Beveridge S, Hornig DH. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Brit J Nutr. 2007;98:S29-S35.
31. Matsui EC, Matsui W. Higher serum folate levels are associated with a lower risk of atopy and wheeze. J Allergy Clin Immunol. 2009;123:1253-9.
32. Dunstan J, West C, McCarthy S, Metcalfe J, Meldrum S, Oddy W, et al. The relationship between maternal folate status in pregnancy, cord blood folate levels, and allergic outcomes in early childhood. Allergy. 2012; 67:50-7.
33. Pedersen B, Yang I, Stabler S, Schwartz D, Eyring K. Blocking Folate Metabolism Suppresses Allergic Airway Disease In Mice. Am J Respir Crit Care Med. 2014;189:A3391.
34. Aichbhaumik N, Zoratti EM, Strickler R, Wegienka G, Ownby DR, Havstad S, et al. Prenatal exposure to household pets influences fetal immunoglobulin E production. Clin Exp Allergy. 2008;38:1787-94.
35. Jeong KY, Park J-W, Hong C-S. House dust mite allergy in Korea: the most important inhalant allergen in current and future. Allergy Asthma Immun. 2012;4:313-25.