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Blood folic acid, vitamin B12, and homocysteine levels in pregnant women with fetal growth restriction

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ABSTRACT. Deficiencies in nutrients such as folic acid and vitamin B12 may play a role in fetal growth restriction (FGR). However, whether folic acid, vitamin B12, or homocysteine is associated with FGR in Chinese populations remains unclear. This study investigated the relationship between these nutrient deficiencies and FGR in pregnant Chinese women. We selected 116 mother and infant pairs, and categorized the neonates into the FGR, appropriate for gestational age, and large for gestational age groups. Birth weight, body length, head circumference, body mass index (BMI), and Rohrer's body index of the newborns were measured. Serum folic acid, vitamin B12, and homocysteine levels were measured in mothers during the first three days of their hospital stay. Results showed that the FGR group exhibited reduced folic acid and vitamin B12 levels and elevated homocysteine levels than those in the other two groups. Folic acid and vitamin B12

levels were positively correlated with birth weight, head circumference, and BMI, whereas homocysteine level was negatively correlated with these variables. The FGR ratio in the folic acid and vitamin B12 deficiency group was higher than that in the sufficiency group ($\chi^2 = 4.717$ and 4.437, $P = 0.029$ and 0.035, respectively). In addition, elevated homocysteine was associated with FGR ($\chi^2 = 5.366$, $P = 0.021$). In conclusion, we found that folic acid and vitamin B12 deficiency was associated with elevated homocysteine levels, which may increase susceptibility to FGR.

Key words: Folic acid; Vitamin B12; Fetal growth restriction; Homocysteine

INTRODUCTION

Fetal growth restriction (FGR) refers to a condition in which a fetus is unable to reach its genetic growth potential. It is defined as having a weight at two standard deviations (tenth percentile) below that of a normal fetus, or at <2.5 kg after 37 weeks of pregnancy (Seravalli and Baschat, 2015). The cause for abnormal fetal size may be either extrinsic (placenta, matrix) or intrinsic to the fetus itself (Lewis et al., 2016; Xiao et al., 2016). Studies have shown that FGR is the second leading cause of deaths among fetuses during the perinatal period, with a three-fold increase in mortality rates as compared to that of a normal neonate (Boulet et al., 2006). Moreover, long-term effects of FGR include growth retardation (Chia and Huang, 2014), developmental anomalies (Streimish et al., 2012), congenital anomalies (Clausson et al., 1999), cardiovascular diseases (Khadilkar and Parthasarathy, 2015), ischemia anoxic encephalopathy (Kovo et al., 2015), chronic lung diseases (Bose et al., 2009), gastrointestinal diseases (Liu et al., 2014), kidney diseases (Ojeda et al., 2008), and endocrine metabolic disorders (Sánchez-Vera et al., 2005). Folic acid (FA) (Walter et al., 2016) and Vitamin B12 (Vit B12) (O'Leary and Samman, 2010) are mainly obtained through diet, and are important coenzymes for DNA synthesis. The fetus requires high levels of FA and Vit B12, especially during late pregnancy. Insufficient nutrients may lead to FA and Vit B12 deficiencies in the mothers, and thus affect the fetuses as well. In addition, FA and Vit B12 deficiencies can lead to the conversion of homocysteine (Hcy) to methionine, resulting in homocystinemia (Abdollahi et al., 2008). High Hcy content can be damaging to vascular structures and functions, resulting in insufficient blood perfusion to the fetus, and abnormal fetal development. However, whether FA, Vit B12, or Hcy levels are associated with FGR in the Chinese population remains unclear. This study investigated the relationship between FA, Vit B12, Hcy content and FGR in pregnant women, and aimed to provide a reference for early diagnosis and prevention of FGR.

MATERIAL AND METHODS

Materials

The following inclusion criteria were used for the study: 1) subjects have no prior histories of intrauterine distress, congenital malformations, or metabolic diseases; an Apgar score of 8-10; 2) subjects are healthy pregnant females with single pregnancy, are between 21 and 40 years of age with no essential hypertension and diabetes, no liver and kidney diseases,

no consumption of vitamin supplements two weeks before the study, no smoking and drinking history. A total of 116 pairs of maternal and neonates delivered at Fifth Affiliated Hospital Obstetrics were recruited between January 2015 and March 2016. The newborns included 56 males and 60 females. The mean gestational age was 38.6 ± 2.1 weeks, and the average birth weight was 38.6 ± 0.91 kg. The fetuses were divided into the FGR group (28 cases), appropriate for gestational age (AGA) group (62 cases), and large for gestational age (LGA) group (26 cases) according to the 15 cities newborns physique growth measurement value standards published in 1986 (Lourie et al., 1986).

This study was approved by the medical Ethics Committee of the Fifth Affiliated Hospital Obstetrics, and written informed consents were obtained from all participants prior to their enrollment into the study.

Experimental methods

Detection of serum FA, Vit B12, and Hcy

Peripheral blood (5 mL) was drawn from the mothers during the first three days of their hospital stay. The blood was incubated overnight at 4°C, and was centrifuged at 4000 rpm for 5 min. Serum samples were stored at -80°C and ELISA was used to measure FA, Vit B12, and Hcy contents.

Growth parameter (developmental coordination) detection

After birth, the umbilical cord was cut, and the newborn was dried. Body weight, body length and head circumference were measured in order to calculate the body length/head circumference ratio.

$$Body\ mass\ index\ (BMI) = weight\ (kg) / height\ (m^2) \qquad \text{(Equation 1)}$$

$$\text{Rohrer's index} = \text{birth weight (g)} \times 100 / \text{body length (cm}^3\text{)} \qquad \text{(Equation 2)}$$

Judgment criteria were as follows: Rohrer's index >2.00 (gestational age ≤ 37 weeks) or 2.20 (gestational age > 37 weeks), body length/head circumference ratio >1.36 was defined as symmetrical. Rohrer's body index <2.00 (gestational age ≤ 37 weeks) or 2.20 (gestational age > 37 weeks), or body length/head circumference ratio <1.36 were defined as a non-symmetrical.

Statistical analysis

The SPSS18.0 statistical software was used for data input and analysis. Measurement data are reported as means ± SD, and were analyzed via chi-squared tests. Enumeration data are reported as percentages, and were analyzed by ANOVA and Dunnett's *t*-test. Pearson analysis was performed to evaluate correlations among serum levels of FA, Vit B12, and Hcy. $P < 0.05$ was considered to be statistically significant.

RESULTS

Neonatal growth characteristics and maternal blood index

As shown in Table 1, birth weight, body length, head circumstance, and BMI in the FGR group were significantly lower than that of the AGA and LGA groups. In addition, FA and Vit B12 levels in the FGR group were also lower as compared with that in the other two groups. However, homocysteine level was found to be elevated than that in the AGA and LGA groups ($P < 0.05$).

Table 1. Neonatal growth characteristics and maternal blood index comparison.

Index	FGR group (N = 28)	AGA group (N = 62)	LGA group (N = 26)	F	P value
Birth weight (kg)	2.29 ± 0.29	3.28 ± 0.41[a]	4.13 ± 0.48kg[a]	142.00	<0.001
Body length (cm)	47.58 ± 2.37	48.96 ± 2.21[a]	51.11 ± 1.96[a]	17.70	<0.001
Head circumstance (cm)	30.86 ± 1.90	32.79 ± 2.00[a]	35.05 ± 2.25[a]	28.60	<0.001
BMI (kg/m^2)	9.82 ± 1.56	11.73 ± 2.11[a]	15.59 ± 2.22[a]	57.74	<0.001
FA (μg/L)	1.98 ± 2.34	15.56 ± 22.71[a]	16.23 ± 23.75[a]	4.994	0.008
Vit B_{12} (ng/L)	51.61 ± 39.23	332.51 ± 195.27[a]	298.57 ± 201.45[a]	26.39	<0.001
Hcy (μM)	17.53 ± 28.41	5.86 ± 6.17[a]	5.54 ± 6.21[a]	6.63	0.002

[a]$P < 0.05$, compared with FGR group.

Correlations between FA, Vit B12, and Hcy

Pearson correlation analysis suggested that FA and Vit B12 levels were negatively correlated with that of Hcy ($r = -0.653$ and -0.715, respectively) (Table 2).

Table 2. Correlation relationship among FA, Vit B12, and Hcy.

	FA		Vit B12		Hcy	
	r	P	*r*	P	*r*	P
FA	-	-	0.109	0.354	-0.653	0.022
Vit B12	0.109	0.354	-	-	-0.715	0.015
Hcy	-0.653	0.022	-0.715	0.015	-	-

Correlation between FA, Vit B12, and Hcy with neonatal growth parameters

Our results showed that FA and Vit B12 levels in maternal blood were positively correlated with birth weight, head circumstance, and BMI. On the contrary, Hcy level was found to be negatively correlated with those parameters. This suggested that FA, Vit B12, and Hcy levels in the maternal blood might affect growth of the fetus (Table 3).

Table 3. Correlation relationship of FA, Vit B12, and Hcy with neonatal growth parameters.

	FA		Vit B_{12}		Hcy	
	r	P	*r*	P	*r*	P
Birth weight (kg)	0.451	0.039	0.415	0.036	-0.458	0.013
Body length (cm)	0.102	0.195	0.117	0.213	-0.425	0.032
Head circumstance (cm)	0.394	0.044	0.511	0.029	-0.401	0.034
BMI (kg/m^2)	0.489	0.021	0.427	0.016	-0.357	0.040

Impact of FA, Vit B12, and Hcy on FGR and developmental coordination

Based on previous reports, 3.0 mg/L FA and 100.3 ng/L Vit B12 were used as the limits for nutrient deficiency and sufficiency classification (Bowen et al., 2006; Khor et al., 2006). Hcy at 15.0 μM (Choi et al., 2014) was used to categorize the normal group and abnormal (elevated) groups. The ratio of FGR and inconsonant growth in each group was compared. As shown in Table 4, the FGR ratio in the FA and Vit B12 deficiency group was higher than that in the sufficiency group. Elevated Hcy was associated with higher FGR ratio ($P < 0.05$). However, levels of FA, Vit B12, and Hcy showed no obvious impact on developmental coordination.

Table 4. Impact of FA, Vit B12, and Hcy on FGR and development coordination.

Outcome	Index	Lower than cut-off value		Higher than cut-off value		χ^2	P value
FGR or not		Yes	No	Yes	No		
	FA	16	30	12	58	4.717	0.029
	Vit B_{12}	14	25	14	63	4.437	0.035
	Hcy	13	62	15	26	5.366	0.021
Coordination or not		Yes	No	Yes	No		
	FA	33	13	58	12	2.029	0.154
	Vit B_{12}	28	11	63	14	1.538	0.215
	Hcy	60	15	31	10	0.302	0.583

DISCUSSION

China is one of the countries impacted by many types of serious birth defects. Nutrient balance plays an important role in embryo development. Deficiency or excess of certain nutrients can have negative effects on embryonic development. Early diagnosis of nutrient imbalance as well as timely intervention can significantly reduce the proportion of birth defects (Gaccioli and Lager, 2016).

FA, a type of water-soluble vitamin B, is composed of pteridine, para-aminobenzoic acid, and glutamic acid (Kaplan and Küçüksolak, 2016). Due to the rapid growth and division of embryonic cells, the body demand for FA can increase up to 4-6 times during pregnancy. FA deficiency may lead to megaloblastic anemia, gestational hypertension, and placental abruption. It may also affect fetal growth and intellectual development. Vit B12 participates in the metabolism of various macromolecules such as carbohydrates, proteins, and lipids. Therefore, it is vital for maintenance of normal tissue and cellular functions. In addition, both Vit B12 and FA are important coenzymes in DNA synthesis. Vit B12 cannot be synthetized by the body, and deficiency in Vit B12 can significantly affect fetal growth and development (O'Leary and Samman, 2010). Scholl and Johnson (2000) found that FA deficiency was closely associated with placental abruption, FGR, fetal abnormalities, and low birth weight. Furthermore, Gomes et al. (2010) revealed that low serum FA level during pregnancy increases the incidences of low birth weight. The present study demonstrated that FA content in the FGR group was significantly lower as compared to that in the AGA and LGA groups, suggesting that it has a critical role in FGR. We also showed that FA level was positively correlated with birth weight, head circumference, and BMI. Similar to previous studies (Scholl and Johnson, 2000; Gomes et al., 2010), we found that FA deficiency increased the probability of FGR ($P < 0.05$). Similarly, Vit B12 content in the FGR group was also markedly reduced as compared to that in the AGA and LGA groups. Its level was positively correlated with birth weight, head circumference, and BMI. In addition, Vit

B12 deficiency was also correlated with elevated proportion of FGR ($P < 0.05$).

Hcy is a type of sulfur-containing amino acid produced during methionine demethylation metabolism. Its level is relatively low under normal circumstances. Methylenetetrahydrofolate reductase, cystathionine condensing enzyme, and methionine synthetase are important metabolic enzymes in methionine metabolism, while FA and Vit B12 are essential prosthetic groups. FA and/or Vit B12 deficiency is associated with hyperhomocysteinemia (Abdollahi et al., 2008). Our study indicated that FA and Vit B12 levels in the maternal blood were negatively correlated with Hcy content, confirming that FA and/or Vit B12 deficiency is associated with hyperhomocysteinemia. Eskes (2001) has also previously proposed hyperhomocysteinemia to be an independent risk factor for placental vascular disease. Hcy elevation induces production of hydrogen peroxide and superoxide free radicals. This results in oxidative damage to vascular endothelial cells, reduction in the number of villus blood vessels, and inhibition of embryo blood perfusion, which leads to adverse pregnancy outcomes (Tsen et al., 2003). It has been shown that nitric oxide release by endothelial cells is reduced by high Hcy levels. Reduction in nitric oxide leads to platelet adhesion and aggregation, thus promoting thrombosis and vascular diseases (Erol et al., 2007); this may affect blood supply to the placenta and the fetus (Zammiti et al., 2008). In addition to the vascular endothelial cells, Hcy can also affect the structure and function of blood vessels by regulating smooth muscle cell proliferation (Zou et al., 2010) and migration (Jiang et al., 2009). In this study, we demonstrated FGR is associated with high Hcy levels. Furthermore, Hcy level was negatively correlated with birth weight, body length, head circumference, and BMI, suggested that elevated Hcy negatively influences fetal growth and development. The high Hcy level in FGR suggests that it may cause abnormal development of the placenta and villous vessels via its effects on vascular endothelial cells and vascular smooth muscle cells. This can reduce placental transfer of oxygen and nutrients, ultimately leading to FGR. It is possible that immunoglobulin E may also be involved in fetal growth restrictions (Xiong et al., 2015), and future studies should be conducted to examine the exact role of Hcy in fetal growth and development.

The fetus experiences rapid growth during the gestational period, especially in the middle to late stages of pregnancy. As a result, there is an increased demand for FA and Vit B12. Nutrient deficiencies can lead to insufficient levels of FA and Vit B12, which in turn, may lead to hyperhomocysteinemia and FGR. Therefore, FA, Vit B12, and Hcy contents should be routinely monitored during middle-late stages of pregnancy. Proper FA and Vit B12 supplementation can also prevent onset of FGR.

In conclusion, out study demonstrated that FA and Vit B12 deficiency is associated with Hcy elevation, which may increase the risk of FGR.

Conflicts of interest

The authors declare no conflict of interest.

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