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Original Article

The association of serum folate, vitamin B_{12}, homocysteine levels with pregnancy complications and newborn health in pregnant women

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Abstract: Folic acid (FA) and vitamin B_{12} (VB_{12}) are important co-enzymes in cellular DNA synthesis. Insufficient intake of FA and VB_{12} during pregnancy may lead to higher serum level of homocysteine (Hcy). This study investigated the correlation between contents of FA, VB_{12} and Hcy in pregnant women, and complication incidence or healthy status of neonatal, in order to provide evidences for preventing complication and improving birth health. Fertile women at different gestation stages were recruited to compare serum contents of FA, VB_{12} and Hcy, whose levels were compared between those with pregnant complications and those without. Serum levels of these factors were further compared between pregnant women delivering neonatal with various healthy conditions in a retrospective manner, to analyze their correlation with healthy conditions. Compared to non-pregnant group, women at middle to late gestation had significantly lower FA or VB_{12} levels plus higher Hcy content ($P<0.05$). Those complicated with threatening miscarriage, anemia, myocardial injury, pregnant hypertension had further lower FA or VB_{12} levels plus higher Hcy content. Neonatal anoxic ischemic encephalopathy or restricted development groups had lower FA or VB_{12} levels plus higher Hcy content. On the other hand, deficiency of FA or VB_{12} levels or elevated Hcy contents increased the risk of neonatal anoxic ischemic encephalopathy or restricted development. Deficiency of FA or VB_{12} levels or elevated Hcy can cause complications including hypertension, threatening miscarriage, myocardial injury, and increase the risk of restricted growth and neonatal anoxic ischemic encephalopathy.

Keywords: Folic acid, vitamin B_{12}, homocysteine, pregnant complication, restricted pregnant growth, neonatal anoxic ischemic encephalopathy

Introduction

Folic acid (FA) is one necessary water-soluble B family vitamin for maintaining normal lives. Major biological function of FA is to provide methyl group for methylation reaction and DNA synthesis inside cells, making it one critical co-enzyme for cellular DNA synthesis [1]. Similar to FA, Vitamin B_{12} (VB_{12}) is also one important co-enzyme during DNA synthesis and exerts vital roles during body metabolism [2]. With sufficient supply of FA and VB_{12}, the need for body growth/development or metabolism can be satisfied [3, 4]. During pregnancy period especially middle to late gestation stage, tremendous physiological and biochemical changes occur in maternal body, significantly increasing the need of FA and VB_{12} by developing fetus. Insufficient supply cause deficiency of FA and VB_{12} in pregnant women's body, and affects the utilization of FA and VB_{12} by fetus. Deficiency of FA and VB_{12} easily cause pregnant hypertension, erythroblastic anemia or placental abruption, or even affecting feta growth, development of nervous system and intelligence [5, 6]. Homocysteine (Hcy) is one intermediate metabolite of biosynthesis of necessary methionine. Under the synergistic effects of various biological enzymes, FA and VB_{12}, Hcy can be further transformed into other substances, thus maintaining serum Hcy at relatively constant level by excretion by urine or re-synthesis of methionine. Insufficient intake and deficiency of FA and VB_{12} at pregnant stage is one of major reasons causing high serum Hcy [7]. High content of serum Hyc can damage vascular structure and function, and affect blood perfusion of placenta, eventually affecting fetal growth/development [8].

Table 1. Comparison of FA, VB_{12} and Hcy levels in pregnant women in different stages

Group	N	FA (nmol/L)	Vit B_{12} (pmol/L)	Hcy (μmol/L)
Non-pregnant	64	12.21±1.58	357.41±42.21	9.21±1.67
Early gestation (1~12 wk)	81	11.77±1.62	345.28±38.59	9.65±1.37
Middle gestation (13~28 wk)	96	10.84±1.08[a]	328.84±37.91[a]	10.15±1.55[a]
Late gestation (28~42 wk)	128	9.23±0.97[a]	305.73±33.66[a]	11.45±1.97[a]

Note: [a]P<0.05 compared to non-pregnant group.

This study thus investigated the relationship between blood contents of FA, VB_{12} and Hcy and incidence of pregnant complication and neonatal healthy status, in order to provide evidences for preventing pregnant complication and improving birth quality of neonatal in clinics.

Materials and methods

Research objects

A total of 64 healthy fertile women (aged between 20 to 36 years old with an average age of 26.9±3.1 years old), along with 81, 96 and 128 pregnant women at early, middle and late gestation stage (aged from 20 to 39 years old with an average of 27.4±2.9 years old; gestational weeks from 6-42 with an average of 29.5±7.7 weeks) who received pre-delivery check from July 2015 to May 2016 were recruited, in parallel with 363 cases of neonatal in due course and their corresponding mothers (363 cases). Among all these pregnant women, there were 11 cases of pregnancy complicated with abortion, 30 cases of anemia, 16 cases of myocardial damage, 25 cases of gestational hypertension with some pregnant women presenting two or more combined symptoms mentioned above. Among all these 363 cases, there were 150 cases of cesarean section and 213 cases of natural birth. In all these newborn babies, Ischemia anoxic encephalopathy occurred in 8 cases with 41 cases of fetal growth restriction. In this study, pregnant complications observed included threatening miscarriage, anemia, myocardial injury, and pregnant hypertension. All research objects had no primary hypertension, coronary artery heart disease, chronic/acute nephritis or diabetes, nor did habits for smoke/alcohol, drugs taken or special diet history.

This study has been pre-approved by the ethical committee of Chengyang People's Hospital. All subjects have signed the consent forms before recruitment in this study.

Assay for serum FA, VB_{12} and Hcy

5 mL venous blood samples were collected from fasted patients in the morning. Blood samples of delivery women were collected 2-3 days before delivery, and within 7 days afterwards. Blood samples were clotted and centrifuged at 4000 rpm for 10 min to separate the upper serum, which was kept at -80°C for further use. Radioimmunology was used to test serum levels of FA and VB_{12}, whilst serum Hcy level was tested by enzymatic immunoassay (EIA).

Evaluation of neonatal healthy conditions

The diagnosis of neonatal anoxic ischemic encephalopathy was performed according to the guideline stipulated by Pediatric sub-committee of Chinese Medicine Association. Fetal growth restriction (FGR) refers to the neonatal body weight lower than two standard deviations or 10th percentiles of comparable gestation week and sex, or body weight lower than 2.5 kg after 37th gestation week [9]. Following the standard body growth parameters of 15 Chinese cities in 1987, newborns were divided into FGR and non-FGR groups based on the correlation between fetal age and percentile of delivery body weight.

Statistical analysis

SPSS18.0 software was used for data input and analysis. Measurement data were presented as mean ± standard deviation, and were compared by t-test or analysis of variance (ANOVA). Pearson analysis was performed to reveal the correlation between measurement data. A statistical significance was defined when P<0.05.

Results

Women in gestational stage had decreased levels of FA, VB_{12} and increased Hcy level

Table 1 showed lower serum FA and VB_{12} contents in early gestation women compared to

Table 2. Comparison of FA, VB_{12} and Hcy levels in pregnant women before and after delivery

Group	N	FA (nmol/L)	Vit B_{12} (pmol/L)	Hcy (μmol/L)
Non-pregnant	64	12.21±1.58	357.41±42.21	9.21±1.67
2~3 days before delivery	363	8.97±0.85[a]	301.69±35.27[a]	10.63±1.86[a]
7 days within delivery	363	10.81±1.75[a]	339.53±35.86[a]	10.21±1.11[a]

Note: [a]P<0.05 compared to non-pregnant group.

Table 3. FA, VB_{12} and Hcy levels in all research objects

Group	N	FA (nmol/L)	Vit B_{12} (pmol/L)	Hcy (μmol/L)
Threatening miscarriage	11	6.51±0.75[a,b]	179.87±34.27[a,b]	17.55±1.51[a,b]
Anemia	30	6.27±0.69[a,b]	158.15±33.64[a,b]	16.12±1.44[a,b]
Myocardial injury	16	5.49±0.58[a,b]	146.45±29.77[a,b]	19.45±1.39[a,b]
Pregnant hypertension	25	6.62±0.67[a,b]	194.74±34.82[a,b]	16.64±1.63[a,b]
Normal pregnancy	245	10.57±1.17[a]	325.61±34.49[a]	10.13±1.58[a]
Non-pregnant group	64	12.21±1.58	357.41±42.21	9.21±1.67

Note: [a]P<0.05 compared to non-pregnant group; [b]P<0.05 compared to normal pregnancy.

non-pregnant women, whilst Hcy content was marginally increased but without statistical significance (P>0.05). Compared to non-pregnant group, middle gestation and late gestation women had significantly lower FA and VB_{12} contents (P<0.05) whilst Hcy contents were remarkably elevated (P<0.05). Serum FA and VB_{12} levels in post-delivery women were elevated to some extents, whilst Hcy showed decreased levels (**Table 2**).

Lower serum FA, VB_{12} and Hcy levels in pregnant women with complications

Within 305 cases of pregnant women, there were 11 of them complicated with threatening miscarriage, 30 cases of anemia, 16 cases of myocardial injury, and 25 individuals with pregnant hypertension. Some pregnant women suffered from two or more complications. In those women having threatening, anemia, myocardial injury and hypertension, serum FA and VB_{12} contents were significantly lower than women with normal pregnancy (363 cases) and non-pregnant group (64 healthy fertile women) (P<0.05, **Table 3**).

Different serum FA, VB_{12} and Hcy levels in pregnant women with different healthy status of neonatal

In pregnant women delivering newborns with neonatal anoxic ischemic encephalopathy, serum FA and VB_{12} contents were remarkably lower than those pregnant women without neonatal anoxic ischemic encephalopathy, whilst Hcy content was significantly higher (P< 0.05 in all cases). In fetal growth restriction (FGR) group, pregnant women had significantly lower serum FA and VB_{12} contents compared to those women without FGR, whilst Hcy content was higher (P< 0.05 in all cases, **Table 4**).

Closely associations between serum FA, VB_{12} and Hcy levels and neonatal healthy status

Using 6.8 nmol/L of serum FA [10], and 74 pmol/L of VB_{12} in maternal blood [11] as the boundary lines, pregnant women were divided into normal and deficient groups. Using 15.0 μmol/L of Hcy as the boundary line [12], pregnant women were divided into normal and abnormally elevated groups. Results showed significantly higher risk of neonatal anoxic ischemic encephalopathy or FGR in those with FA or VB_{12} deficiency group (P<0.05 compared to normal group). Abnormally elevated Hcy group also had higher incidences of neonatal anoxic ischemic encephalopathy or FGR (P<0.05 compared to normal group, **Table 5**). Pearson correlation analysis showed positive relationship between FA or VB_{12} levels and body weight of neonatal (P<0.05 in both), whilst Hcy level was negatively correlated with body weight (P<0.05, **Table 6**). In order to identify the independent factors affecting neonatal healthy status, age, BMI, increased weight, pregnancy complications, serum level of FA, VB_{12} and Hcy were included as variables for logistic regression analysis. As shown in **Table 7**, age, pregnancy complications and serum level of FA, VB_{12} and Hcy were found to be independent factor for neonatal healthy status.

Discussion

The balance of nutrients plays a critical role in fetal development. The deficiency or over-sup-

Table 4. Comparison of serum FA, VB_{12} and Hcy levels

Healthy status		N	FA (nmol/L)	Vit B_{12} (pmol/L)	Hcy (μmol/L)
Neonatal anoxic ischemic encephalopathy	Yes	8	5.81±0.77[a]	179.21±25.79[a]	16.21±1.66[a]
	No	355	10.31±1.24	339.23±38.57	10.95±1.49
Fetal growth restriction (FGR)	Yes	41	6.22±0.83[b]	201.54±26.61[b]	17.02±1.87[b]
	No	322	10.21±1.19	316.74±36.65	10.81±1.50

Note: [a]P<0.05 compared to non- neonatal anoxic ischemic encephalopathy group; [b]P<0.05 compared to non-FGR group.

Table 5. Effects of maternal FA, VB_{12} and Hcy levels on neonatal healthy status

Endpoint	Index	Anoxic ischemic encephalopathy		χ^2	*P* value	FGR		χ^2	*P* value
		Yes	No			Yes	No		
FA (nmol/L)	<6.8	4	42	7.139	0.008	11	35	8.371	0.004
	≥6.8	4	313			30	287		
Vit B_{12} (pmol/L)	<74.0	3	32	7.287	0.007	9	26	6.525	0.011
	≥74.0	5	323			32	296		
Hcy (μmol/L)	≤15.0	5	318	5.850	0.016	32	291	4.447	0.035
	>15.0	3	37			9	31		

Table 6. Relationship between maternal serum FA, VB_{12} and Hcy levels and neonatal weight

	FA		Vit B_{12}		Hcy	
	r	*P*	*r*	*P*	*r*	*P*
Birth body weight (kg)	0.572	0.036	0.528	0.041	-0.603	0.033

Table 7. Logistic regression analysis of the independent factors for the neonatal healthy status

	β	Wald	*P*	OR	95% CI
Age	0.081	5.834	0.018	1.183	1.017~1.348
BMI	-0.439	1.786	0.342	0.735	0.417~1.263
Increased weight	-1.037	0.842	0.625	0.583	0.371~1.104
Pregnancy complications	0.894	6.172	0.011	2.125	1.463~2.534
FA	-0.427	5.038	0.042	0.671	0.423~0.778
Vit B_{12}	-0.345	5.371	0.036	0.611	0.403~0.811
Hcy	0.546	5.465	0.028	1.567	1.241~1.926

ply of certain components may cause adverse effects on fetal development. Therefore, the supply or correction of over-supply of certain nutrients before and during pregnancy is of critical importance for decreasing pregnant complication or adverse endpoints, decreasing birth defects, improving health status, and benefiting the birth quality [9].

FA is one water soluble B family vitamin, and is one important co-enzyme during cellular DNA synthesis, thus playing one critical role in body metabolism [13]. At middle to late stage of gestation, the remarkably increased fetal body causes effects on maternal digestive tracts, thus probably compromising FA intake and absorption of pregnant women. Moreover, due to the elevation of estrogen level in maternal body and abrupt development of fetus, both requirement and consumption of FA are largely elevated, reaching 4~6 folds of that during non-pregnant period. FA deficiency during pregnancy remarkably elevates the risk of birth defects including fetal neural tube malformation, cheilopalatognathus, FGR, and lower birth weight. FA deficiency can also cause adverse effects on pregnant women, as it frequently leads to natural miscarriage, pregnant anemia, hypertension and placental abruption.

Similar to FA, VB_{12} is one important co-enzyme in cellular DNA synthesis, and plays an important role in body metabolism [2]. VB_{12} plays a role in metabolism of various substances including carbohydrate, protein and lipid, thus

maintaining normal function of tissues and cells, plus development of nervous system. Human body cannot synthesize VB_{12} by themselves, and require the food supply, whose deficiency can affect fetal growth and development [2]. Hcy is one sulfide-containing amino acid during metabolism of demethylation of methionine, and has only trace amounts under normal physiological conditions. Di-methyl-tetrahydrogen folic acid reductase (MTHFR), cystathionine beta-synthase (CBS) and methionine synthetase (MS) are important metabolic enzymes during transformation of methionine. FA and VB_{12} are necessary co-enzymes for metabolism of those key enzymes. Hcy is one intermediate product for methionine and cysteine but does not participate in protein syntehssi. Hcy can be methylated to generate methionine via methylation under assistance by FA and VB_{12} [7]. Deficiency of FA and VB_{12} frequently impedes the metabolism of Hcy toward methionine, leading to high blood levels of Hcy [14]. The sulfide group within Hcy can undergo autooxidation, producing peroxides and oxygen free radicals, thus damaging vascular cell structure and functions. As one important anti-oxidants, glutathione effectively prevents the oxidative damage of reactive oxygen on vascular endothelium, and can exert protection on vessels via interaction with nitric oxide (NO) [15]. High blood level of Hcy can interfere with synthesis of glutathione, antagonize anti-oxidative and protective effects of glutathione. High level of Hcy can damage vascular endothelial cells, disrupting expression or function of vascular constriction regulatory factor in endothelium, leading to a series of pathological changes in vascular structure, eventually causing unfavorable endpoints of pregnancy such as hypertension or other adverse effects [16]. Hcy can change function of blood clotting factors and enhance formation of thrombosis via producing peroxides and superoxide to damage vascular endothelium, and accelerating platelet adhesion and aggregation [17]. High serum Hcy can also inhibit the formation of chorion, decrease chorionic villi number, and affect fetal perfusion, thus causing fetal implantation and frequently causing unfavorable endpoints of pregnancy [6].

This study showed lower serum FA and VB_{12} levels in early pregnant women compared to non-pregnant ones, whilst Hcy content was elevated but without statistical significance in both scenarios. Compared to non-pregnant group, middle to late stage pregnant women had significantly lower serum FA and VB_{12} levels, whilst Hcy content was remarkably enhanced. These results showed that insufficient serum levels of FA and VB_{12} in late pregnant women caused elevated Hcy level, probably due to higher estrogen level in late pregnant women, with faster purine metabolism and division of fetal cells, all of which enhanced requirement of FA and VB_{12}, whilst weakened gastrointestinal functions impair absorption. In those pregnant women with threatening miscarriage, anemia, myocardial injury and complication of pregnant hypertension, serum FA and VB_{12} levels were significantly lower than normal pregnant women, whilst Hcy content was elevated. This study also observed abnormal serum FA, VB_{12}, and Hcy levels in threatening miscarriage women, probably due to the formation of thrombosis for inducing miscariage via producing peroxides and superoxide to damage vascular endothelium, and changing blood clotting functions. This study also observed abnormal levels of FA, VB_{12}, and Hcy in patients with myocardial injury and hypertension, probably due to the independent risk factor of cardiovascular disease by high serum Hcy level, sharing common mechanism Eskes et al [18]. In neonatal anoxic ischemic encephalopathy, pregnant women had significantly decreased serum levels of FA, VB_{12}, whilst Hcy content was significantly elevated. χ^2 analysis showed that deficiency of FA or VB_{12}, or abnormally elevated Hcy level elevated the risk of neonatal anoxic ischemic encephalopathy or FGR. Moreover, FA, VB_{12}, and Hcy levels are closely correlated with birth weights probably due to the high blood-clotting status caused by high blood Hcy level, which further affects blood/oxygen supply of placental tissues and causes oxidative injury of vascular endothelium, causing lower chorionic villi number [19] and compromising on fetal perfusion [20], eventually causing intra-uterus hypoxia/anoxic, leading to neonatal anoxic ischemic encephalopathy or FGR. Scholl et al [21] and Gomes et al [22] showed that FA deficiency could significantly increase the risk of FGR and lower birth body weight, as consistent with our results. This study focused on the correlation between serum FA, VB_{12}, whilst Hcy content and pregnant complication and neonatal

healthy status, which has not been widely reported before.

Conclusion

Deficiency of FA, VB_{12}, and elevated Hcy level can cause pregnant complications including hypertension, threatening miscarriage, myocardial injury and anemia, and enhances the risk of FGR and neonatal anoxic ischemic encephalopathy.

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Disclosure of conflict of interest

None.

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