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Original Article

Effects of targeted supplementation of folic acid during pregnancy on serum folic acid levels of women with different genetic characteristics

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Abstract: Objective: The aim of this study was to investigate the effects of various folic acid supplementation doses on serum folic acid levels in pregnant women with different genetic characteristics. Methods: Pregnant women (n=1371) receiving regular perinatal care, from July 2015 to August 2017, were divided into non-risk, low-risk, moderate-risk, and high-risk groups based on folic acid utilization ability. According to folic acid supplementation dosage, the non-risk group was subdivided into non-intervention and 0.4 mg groups, while low-risk, moderate-risk, and high-risk groups were subdivided into 0.4 mg and 0.8 mg groups. Folic acid levels in peripheral blood during early, middle, and late pregnancy were examined using direct chemiluminescence and enzymatic methods. Results: Among pregnant women with similar genetic characteristics receiving various folic acid doses, serum folic acid levels did not differ between 0.4 mg and 0.8 mg subgroups of low-risk and moderate-risk groups in early pregnancy but differed significantly between all other groups ($P<0.05$). In pregnant women with different genetic characteristics receiving the same dosage of folic acid, serum folic acid levels at various stages of pregnancy showed significant differences ($P<0.05$) between the non-risk, low-risk, moderate-risk, and high-risk 0.4 mg groups but did not differ significantly ($P>0.05$) between low-risk, moderate-risk and high-risk 0.8 mg groups. Conclusion: At various stages of pregnancy, individuals with distinct genetic characteristics have different folic acid demands. Therefore, timely, appropriate, and individualized folic acid supplementation throughout pregnancy is particularly important.

Keywords: Pregnant women, folic acid utilization ability, serum folic acid

Introduction

Folic acid, also known as pteroylglutamic acid, is a type of B vitamin. Folic acid can be converted into various active coenzyme forms. Folic acid participates in the transfer of one-carbon units, *in vivo,* and plays important roles in the biosynthesis of purines, pyrimidines, nucleic acids, and proteins as well as in cell division and growth. Folic acid is necessary to sustain normal life processes. The most common recommendation for supplemental dosage of folic acid is 0.4 mg per day. However, the optimal dose of folic acid remains unknown. In the present study, risk classification was performed based on allele frequencies of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and methionine synthase reductase (MTRR) gene (these two genes encode key enzymes in the folic acid metabolic pathway), allowing assessment of folic acid utilization rates [1]. The present study attempted to determine whether differential supplementation of folic acid is necessary based on risk levels. In addition, the present study investigated whether demand for folic acid remained the same at different stages of pregnancy and explored how to supplement folic acid in a more scientific and individualized manner.

Subjects and methods

Subjects

Study subjects were selected from 18 to 35-year-old Han women, with a naturally conceived

singleton pregnancy, receiving regular perinatal care between July 2015 and August 2017. After approval by the Ethics Committee, a total of 1,840 women meeting the inclusion criteria were enrolled in the present study. All subjects carefully reviewed and signed the informed consent document. In addition, subjects were given guidance regarding diet and nutrition during pregnancy. A questionnaire survey was adopted to collect general information of the study subjects, including age, height, and weight. There were no statistically significant differences in age, height, and weight between the groups. Among the 1,830 women, 229 were lost to follow up and 240 dropped out of the study during the follow up period. Finally, 1,371 women were included. Early pregnancy, mid-pregnancy, and late pregnancy were defined according to the eighth edition of "Obstetrics and Gynecology" (edited by Xie et al.) [2] as 0-13^{+6} weeks, 14-27^{+6} weeks, and beyond 28 weeks, respectively.

Inclusion criteria were as follows: ① Healthy without pregnancy complications at time of enrollment; ② Having no history of adverse pregnancy; and ③ Completing follow ups as required and receiving regular guidance on a healthy diet. Exclusion criteria were as follows: ① Patient had undergone gastrointestinal surgery or suffered from severe digestive system disorders or hepatic/renal dysfunction; ② Patient suffered from coronary heart disease, diabetes, thyroid diseases, malignant tumor, or diseases of the hematologic and immune system; ③ Patient had recently taken medications capable of affecting the absorption of folic acid and vitamin B_{12} (such as proton pump inhibitor-based and H_2 receptor blocker-based acid-suppressive drugs), contraceptives, or aspirin; and ④ Patient had a previous pregnancy affected by neural tube defects or first-degree relatives had a pregnancy affected by neural tube defects.

Research methods and grouping

Oral mucosal epithelial cells were collected from the study subjects. Fluorescence-based quantitative polymerase chain reaction (PCR) and DNA sequencing were performed to examine polymorphisms in the MTHFR gene (C677T and A1298C loci) and MTRR gene (A66G locus). Folic acid utilization ability was evaluated based on the function of each gene locus and frequencies of homozygous and heterozygous alleles at these loci. Risk was categorized into 7 levels. Specifically, level 0 (no-risk) indicated that folic acid utilization ability was normal. Levels 1 and 2 (low-risk) indicated slightly reduced ability to utilize folic acid. Levels 3, 4 and 5 (moderate-risk) indicated poor ability to utilize folic acid. Levels 6 and 7 (high degree of risk) indicated extremely poor ability to utilize folic acid.

Study subjects were divided into the following 4 groups, according to results of genetic polymorphism analysis: no-risk group (340 women), low-risk group (125 women), moderate-risk group (358 women), and high-risk group (548 women). Each group was further divided randomly into 2 subgroups. Specifically, the no-risk group was divided into 0.4 mg folic acid group (170 women) and non-intervention group (170 women). Based on the dosage of folic acid supplementation, low-risk, medium-risk, and high-risk groups were further divided into 0.4 mg group (60, 186 and 276 women, respectively) and 0.8 mg group (65, 172 and 272 women, respectively). Direct chemiluminescence and enzymatic methods were employed to examine serum levels of folic acid in the above groups in early, middle, and late pregnancy.

Statistical methods

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Measurement data that conformed to a normal distribution are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was employed to compare the means among groups. Non-normally distributed measurement data are represented using M (min-max). Differences between the groups of measurement data were examined using Kruskal-Wallis rank sum test, while count data were compared using X^2 test. *P* values of less than 0.05 indicated that differences were statistically significant.

Results

Changes in serum folic acid levels in pregnant women with distinct genetic characteristics after oral administration of various doses of folic acid

Serum folic acid levels were compared between no-risk women supplemented with 0.4 mg folic acid during pregnancy and no-risk women not

Table 1. Changes in serum folic acid levels in the no-risk group during early, middle, and late pregnancy

	Serum folic acid level (ng/ml)		
	0.4 mg group	Non-intervention group	P
Early pregnancy	20.10 (18.23-24)	17.45 (7.15-24.00)	<0.001
Mid-pregnancy	19.96 (13.42-24)	15.47 (4.93-24)*	<0.001
Late pregnancy	19.01 (10.58-24)	13.84 (3.21-24)*	<0.001
F/X^2	3.975	54.804	
	0.137	<0.001	

Note: *indicates a statistically significant difference compared to serum folic acid levels in early pregnancy (P<0.05).

Table 2. Changes in serum folic acid levels in the low-risk group during early, middle, and late pregnancy

Group	Serum folic acid level (ng/ml)		
	0.4 mg group	0.8 mg group	P
Early pregnancy	20.98 (6.95-24)	21.70 (10.95-24)	0.298
Mid-pregnancy	17.39 (3.76-24)*	21.01 (5.07-24)	0.010
Late pregnancy	16.30 (3.86-24)*	19.79 (4.02-24)	0.001
F/X^2	17.58	5.11	
	<0.001	0.078	

Note: *indicates a statistically significant difference compared to serum folic acid levels in early pregnancy (P<0.008).

Table 3. Changes in serum folic acid levels in the moderate-risk group during early, middle, and late pregnancy

	Serum folic acid level (ng/ml)		
	0.4 mg group	0.8 mg group	P
Early pregnancy	18.07 (13.33-24.00)	20.78 (7.80-24.00)*	<0.001
Mid-pregnancy	17.82 (12.07-24.00)	20.49 (13.12-24.00)*	<0.001
Late pregnancy	16.54 (10.50-24.00)	19.80 (11.90-24.00)*	<0.001
F/X^2	31.132	4.53	
	<0.001	0.104	

Note: *indicates a statistically significant difference compared to serum folic acid levels in early pregnancy (P<0.0167).

been subjected to folic acid intervention. As shown in **Table 1**, at all stages of pregnancy (early, middle, and late pregnancy), the 0.4 mg group had higher serum levels of folic acid than those of the non-intervention group. Differences were statistically significant (P<0.05). In the non-intervention group, serum folic acid levels exhibited a gradual downward trend during pregnancy. In contrast, serum folic acid levels showed no significant change in the 0.4 mg group during pregnancy (P<0.05).

Serum folic acid levels were compared throughout the pregnancy between low-risk women supplemented with 0.4 mg folic acid and low-risk women supplemented with 0.8 mg folic acid. As shown in **Table 2**, there were no significant differences in serum folic acid levels between the 0.4 mg group and 0.8 mg group during early pregnancy (P>0.05). During mid-pregnancy and late pregnancy, serum folic acid levels were statistically significantly lower in the 0.4 mg group than the 0.8 mg group (P<0.05). In the 0.4 mg group, serum folic acid levels exhibited a downward trend during pregnancy (P<0.05). In contrast, the 0.8 mg group showed no significant changes in serum folic acid levels during pregnancy (P>0.05).

Serum folic acid levels were compared throughout the pregnancy between moderate-risk women supplemented with 0.4 mg folic acid and moderate-risk women supplemented with 0.8 mg folic acid. As shown in **Table 3**, there were no significant differences in serum folic acid levels between the 0.4 mg group and 0.8 mg group in early pregnancy (P> 0.05). During mid-pregnancy and late pregnancy, serum folic acid levels were statistically significantly lower in the 0.4 mg group than the 0.8 mg group (P< 0.05). In the 0.4 mg group, serum folic acid levels exhibited a downward trend during pregnancy (P<0.05). In contrast, the 0.8 mg group showed no significant changes in serum folic acid levels during pregnancy (P>0.05).

Serum folic acid levels were compared between high-risk women supplemented with 0.4 mg folic acid and high-risk women supplemented with 0.8 mg folic acid throughout the pregnancy. As shown in **Table 4**, serum folic acid levels were statistically significantly lower in the 0.4 mg group than the 0.8 mg group during early, middle, and late pregnancy (P<0.05). In the 0.4 mg group, serum folic acid levels exhibited a downward trend during pregnancy. In contrast, the 0.8 mg group showed no signifi-

Table 4. Changes in serum folic acid levels in the high-risk group during early, middle, and late pregnancy

	Serum folic acid level (ng/ml)		
	0.4 mg group	0.8 mg group	P
Early pregnancy	18.98 (13.33-24.00)	20.40 (8.90-24)	<0.001
Mid-pregnancy	17.31 (9.8-24.00)*	20.25 (7.54-24.00)	<0.001
Late pregnancy	14.85 (2.86-24.00)*,#	18.81 (10.1-24.00)*,#	<0.001
F/X^2	131.94	72.41	
	<0.001	<0.001	

Note: #, indicates a statistically significant difference compared to serum folic acid levels in mid-pregnancy (P<0.0167). *, indicates a statistically significant difference compared to serum folic acid levels in early pregnancy (P<0.0167).

cant changes in serum folic acid levels throughout pregnancy.

Examination of differences in serum folic acid levels in pregnant women with distinct genetic characteristics receiving the same dose of folic acid supplementation

The no-risk group, low-risk group, moderate-risk group, and high-risk group all received 0.4 mg folic acid supplementation during pregnancy. Serum folic acid levels were then compared between the groups at various stages of pregnancy. As shown in **Table 5**, significant differences were observed between the groups during early pregnancy (P<0.001). Comparison between the groups showed that serum folic acid levels were higher in the no-risk group than moderate-risk and high-risk groups. Compared to the high-risk group, the low-risk group also exhibited increased serum levels of folic acid. All of the differences described above were statistically significant (P<0.008). In contrast, no statistically significant differences existed among the other groups. Differences were also found between the groups during mid-pregnancy (P<0.001). During mid-pregnancy, the non-intervention group exhibited higher serum folic acid levels than the other three groups, while the moderate-risk group had higher serum folic acid levels than the high-risk group (P<0.008). In contrast, there were no statistically significant differences among the other groups. In addition, differences existed between the groups in late pregnancy (P< 0.001). During late pregnancy, serum folic acid levels were statistically significantly higher in the no-risk group and moderate-risk group than in the high-risk group (P<0.008).

The low-risk group, moderate-risk group, and high-risk group also received 0.8 mg folic acid supplementation during pregnancy (**Table 6**). There were no statistically significant differences in serum folic acid levels among the groups during early, middle, and late pregnancy (P>0.05).

Discussion

The United States was the first to recommend that women in the periconceptional period should take a daily supplement containing 0.4 mg folic acid to prevent occurrence of neural tube defects. This recommended dose was soon adopted by many countries. Daily et al. found that risk of neural tube defects was reduced to a minimum when the erythrocyte folate concentration in pregnant women reached or exceeded 906 mol/L [3]. However, 8-12 weeks was generally required to reach an erythrocyte folate level of 906 mol/L if the women consumed 0.4 mg folic acid per day. In contrast, this folate level (906 mol/L) could be achieved within 4.2 weeks when women were supplemented with 0.8 mg folic acid daily [4]. There remains controversy surrounding the dosage of periconceptional folic acid supplementation. At present, the American Academy of Family Physicians (AAFP) and U.S. Preventive Services Task Force (USPSTF) recommend that women planning a pregnancy consume 0.4-0.8 mg folic acid each day or take a daily multivitamin supplement containing 0.4-0.8 mg of folic acid to prevent occurrence of neural tube defects [5-6]. The Society of Obstetricians and Gynecologists of Canada (SOGC) recommends that pre-pregnant, pregnant, and lactating women take multivitamin supplements containing 0.4-1.0 mg of folic acid daily. The Obstetrics Group of Obstetrics and Gynecology Branch of Chinese Medical Association has released guidelines for pre-pregnancy and prenatal care. These guidelines recommend that pregnant women take a daily supplement of 0.4-0.8 mg folic acid to prevent occurrence of neural tube defects [7].

Frequencies of MTHFR C677T and A1298C mutations and MTRR A66G mutations vary depending regional and racial differences. MTHFR is a key enzyme in the metabolism of folic acid-homocysteine (Hcy). MTHFR determines whether folic acid derivatives are used in DNA and

Table 5. Differences in serum folic acid levels between no-risk, low-risk, moderate-risk, and high-risk groups supplemented with 0.4 mg folic acid at various stages of pregnancy

Group	Serum folic acid level		
	Early pregnancy	Mid-pregnancy	Late pregnancy
No-risk group	20.10 (18.23-24)	19.96 (13.42-24)	19.01 (10.58-24)
Low-risk group	20.98 (6.95-24)	17.39 (3.76-24)[a]	16.30 (3.86-24)
Moderate-risk group	18.07 (13.33-24.00)[a]	17.82 (12.07-24.00)[a]	16.54 (10.50-24.00)
High-risk group	18.98 (13.33-24.00)[a,b]	17.31 (9.8-24.00)[a]	14.85 (2.86-24.00)[a,c]
F/X^2	5.326	78.80	173.13
	<0.001	<0.001	<0.001

Note: [a]indicates a statistically significant difference compared to the no-risk group (P<0.008); [b]indicates a statistically significant difference compared to the low-risk group (P<0.008); [c]indicates a statistically significant difference compared to the moderate-risk group (P<0.008).

Table 6. Differences in serum folic acid levels between low-risk, moderate-risk, and high-risk groups supplemented with 0.8 mg folic acid at various stages of pregnancy

Group	Serum folic acid level		
	Early pregnancy	Mid-pregnancy	Late pregnancy
Low-risk group	21.70 (10.95-24)	21.01 (5.07-24)	19.79 (4.02-24)
Moderate-risk group	20.78 (7.80-24.00)	20.49 (13.12-24.00)	19.80 (11.90-24.00)
High-risk group	20.40 (8.90-24.00)	20.25 (7.54-24.00)	18.81 (10.1-24.00)
F/X^2	2.38	0.85	3.76
P	0.305	0.653	0.149

RNA synthesis or in Hcy remethylation and DNA methylation [8]. MTRR is another key enzyme in folic acid-Hcy metabolism. Using vitamin B12 as a coenzyme, MTRR participates in the remethylation of Hcy to methionine (Met) [9]. A variety of mutations occur in the gene encoding MTHFR. Among mutations, the two major ones are C677T and A1298C polymorphisms. The C677T polymorphism of the MTHFR gene refers to the C to T transition at nucleotide 677. This mutation reduces the activity of MTHFR, resulting in increased serum Hcy levels and/or DNA hypomethylation. A1298C polymorphism of the MTHFR gene consists of an A to C transversion at nucleotide 1298. This mutation also reduces MTHFR activity. However, decline in MTHFR activity caused by A1298C mutation is less dramatic than that caused by C677T mutation. Simultaneous heterozygous mutations at the two loci significantly reduce MTHFR activity, resulting in decreased serum concentrations of folic acid and an increased serum Hcy levels [10]. C677T and A1298C polymorphisms of the MTHFR gene and A66G polymorphism of the MTRR gene cause variations in folic acid utilization ability. Therefore, the principle of individualization should be applied when determining dosages for folic acid supplementation.

Serum folate has been considered an indicator of recent folic acid intake. Lao et al. showed that serum folic acid levels were the highest in early pregnancy. However, maternal serum folic acid levels decrease gradually as the gestational age increases and reaches its minimum in the puerperium [11]. A study conducted by an international scholar showed that fetal growth-induced increase in the number of rapidly dividing cells and growth of placenta enhances the maternal demand for folic acid. In addition, blood volume increases in pregnant women, which dilutes folic acid levels in the blood. As a result, blood folic acid concentration is reduced during pregnancy [12]. Mcnulty et al. found that supplementation of folic acid during pregnancy would maintain the serum folate concentration [13]. This present study revealed that serum folic acid levels declined with increased gestational age in the non-intervention no-risk group and at-risk groups (low-/moderate-/high-risk groups) who took a daily supplement of 0.4 mg folic acid. In contrast, serum folic acid levels showed no significant changes throughout the

pregnancy in the no-risk group supplemented with 0.4 mg folic acid and in at-risk groups (low-/moderate-/high-risk groups) supplemented with 0.8 mg folic acid. The above results indicate that continuous supplementation of an appropriate dose of folic acid could prevent decrease in serum folic acid levels.

Folic acid cannot be synthesized by the body and needs to be obtained from external sources. Naturally occurring folate is very unstable, susceptible to sunlight and heat, and is readily oxidized. Therefore, humans cannot obtain a large amount of folate from diet alone. The present study found that the serum folic acid concentration was higher in the no-risk group supplemented with 0.4 mg folic acid than in those not given folic acid supplements. This finding suggests that preventive supplementation of folic acid should be applied to pregnant women who are not at risk. The present study did not detect significant differences in serum folic acid levels between low-risk women supplemented with 0.4 mg folic acid and low-risk women supplemented with 0.8 mg folic acid during early pregnancy. In contrast, during middle and late pregnancy, low-risk women supplemented with 0.8 mg folic acid showed higher serum levels of folic acid than low-risk women supplemented with 0.4 mg folic acid. The above findings indicate that supplementation with 0.4 mg of folic acid was sufficient for the low-risk group at early pregnancy. Women in early pregnancy may need relatively low amounts of folic acid. As gestational age increases, growth and development of the fetus lead to an increased demand for folic acid. Therefore, the dose of folic acid supplements should be increased in middle and late pregnancy. The present study also showed that the serum folic acid concentration was higher in moderate-risk and high-risk women supplemented with 0.8 mg folic acid during pregnancy than in moderate-risk and high-risk women supplemented with 0.4 mg folic acid. Moderate-risk and high-risk women have a reduced ability to utilize folic acid. Therefore, a folic acid dose of 0.8 mg per day was required to meet the needs of these women during pregnancy. Insufficient intake of folic acid during pregnancy may cause certain birth defects. One study revealed that folic acid can prevent neural tube defects that occur during early and mid-pregnancy [14]. At present, there is not sufficient evidence to confirm whether folic acid also prevents other congenital defects [15]. However, folic acid supplementation is still recommended for preventing occurrence of congenital heart disease and similar diseases.

Folic acid is an essential substance for nucleic acid and amino acid synthesis, cell division, tissue growth, and DNA methylation. Folic acid provides methyl groups for Hcy metabolism. MTHFR is a key enzyme in folic acid-Hcy metabolism. One study showed that serum folic acid levels were negatively correlated with Hcy levels [16]. Supplementation of folic acid reduces the serum Hcy concentration [17] and prevents occurrence of hyperhomocysteinemia (HHcy). The absolute or relative deficiency of folic acid during pregnancy is a direct cause of HHcy. MTHFR C677T and A1298C mutations and the MTRR A66G mutation reduce the activity of key enzymes, leading to inhibition of conversion of Hcy to methionine and DNA hypomethylation. Hcy accumulates in the body, resulting in the development of HHcy [18]. *In vivo*, Hcy accumulation leads to the production of a large amount of reactive oxygen species (ROS). ROS may seriously damage vascular endothelial cells, causing placental vascular disease and coagulation system disorders [19]. In early pregnancy, Hcy accumulation may result in miscarriage [20] and arrested embryonic development [21]. In middle and late pregnancy, Hcy accumulation may cause concurrent gestational hypertension, gestational diabetes, preterm birth, and fetal growth restriction [22]. Therefore, appropriate supplementation of folic acid during pregnancy and regular monitoring of serum folic acid levels can prevent occurrence of HHCY and reduce pregnancy complications.

Conclusions

In summary, individual differences exist in folic acid utilization ability due to MTHFR C677T and A1298C polymorphisms and MTRR A66G polymorphism. In addition, the demand for folic acid varies at different stages of pregnancy. Appropriate supplementation of folic acid will prevent decreases in the serum folic acid concentration, reduce occurrence of HHcy, and improve outcomes of pregnancy.

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Disclosure of conflict of interest

None.

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