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The effect of folic acid throughout pregnancy among pregnant women at high risk of pre-eclampsia: A randomized clinical trial

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ABSTRACT

Background: Pre-eclampsia is a serious hypertension disease that occurs during pregnancy. Folic acid (FA) supplementation has been reported to reduce pre-eclampsia risk in pregnant women. Here, we aimed to assess whether treatment of high doses of FA in pregnant women with high pre-eclampsia risk could prevent the onset of pre-eclampsia.

Methods: We conducted a randomized clinical trial in 1576 women who had pre-eclampsia or eclampsia in their last pregnancy and had a pregnancy plan. Subjects were randomized into two groups. The low dose (LD) group (n = 788) received 0.4 mg of FA daily from the first 3 months of pregnancy until the entire pregnancy, and the high dose (HD) group (n = 788) received 4 mg of FA per day. We followed up the subjects until production.

Results: The plasma homocysteine (homocysteine) and FA levels were significantly higher in the HD group that in the LD group. Severe gestational hypertension, early onset pre-eclampsia (< 32 weeks' gestation), severe pre-eclampsia, and newborns' Apgar score < 7 at 5 min were remarkably decreased in the HD group compared with the LD group. Further, the incidence of pre-eclampsia was reduced in the HD group with compliance > 50%.

Conclusion: This study has provided evidence that a high dosage of FA supplement from 3 months before pregnancy until the entire pregnancy reduces the recurrent pre-eclampsia.

1. Introduction

Pre-eclampsia is one of the most serious complications during pregnancy and increases the risk of premature death of the mother and the fetus [1]. The symptoms of pre-eclampsia are mainly hypertension during pregnancy and proteinuria [2]. To date, the etiology of pre-eclampsia is still unclear and needs to be further elucidated. The currently recognized pathogenesis of pre-eclampsia is mainly caused by multifactorial activation and damage of vascular endothelial cells [3]. Patients with pre-eclampsia history have an increased risk of cardiovascular disease in the future compared with normal pregnant women, suggesting that pre-eclampsia and cardiovascular disease may have a common pathogenesis [4].

Homocysteine is an independent risk factor for cardiovascular disease [5]. About 20–30% of patients with coronary artery and peripheral vascular disease develop hyperhomocysteinemia [6]. When the level of homocysteine is increased, it is easily oxidized to form homocysteine compounds, and at the same time, hydrogen peroxide and superoxide ion radicals are generated, which damages vascular endothelial cells and causes a series of vascular damage [7]. It has been reported that homocysteine is one of the causes of the onset of hypertensive disorders in pregnancy [8].

The main factors affecting homocysteine levels in the body include two aspects: genetic and food deficiencies [9]. Nutritional folic acid (FA) deficiency is the leading cause of acquired homocysteine. FA, also known as pteroyl glutamic acid, is a water-soluble B vitamin which exists in the form of FH4 in human body [10]. It is involved in protein and fat metabolism and fetal neural tube cell division and growth [11]. FA is an important coenzyme of cell DNA synthesis and plays an important role in placental production and fetal growth and development [12]. A sufficient amount of FA could reduce the incidence of megaloblastic anemia and abortion, promote fetal growth and development, expand placental blood vessels, and increase placental blood supply [13]. There is emerging evidence that supplementation with high doses of or long-term use of FA could effectively prevent pre-eclampsia [14]. However, further and more in-depth research is needed to provide more direct evidence for this theory. In this article, we report the results of clinical trials conducted in women who had pre-eclampsia or eclampsia during the last trimester to investigate whether supplementation with high doses of FA would reduce the subsequent development of pre-eclampsia and its adverse outcomes.

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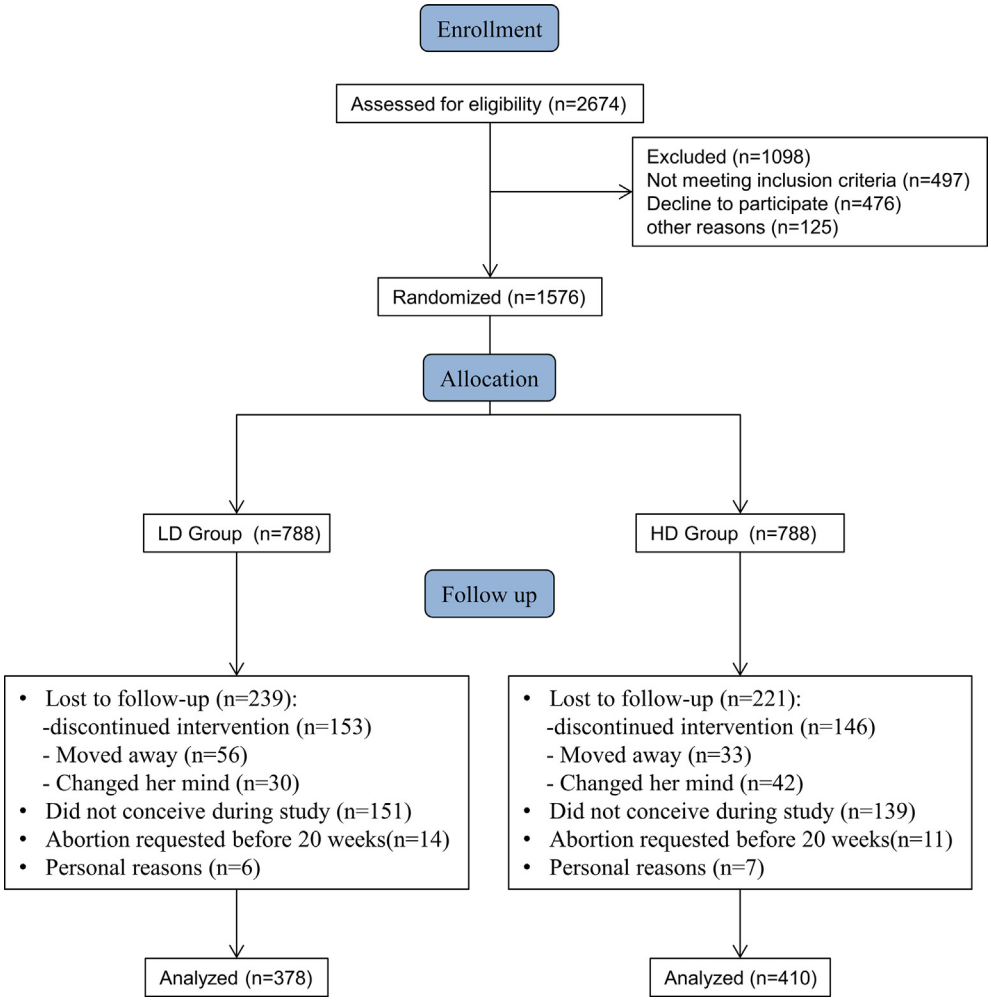

Fig. 1. Schematic procedures of this study.

2. Methods

2.1. Study design and participants

The Second Hospital of Hebei Medical University ethical committee approved the use of the regimens. All participants signed an informed consent form. A randomized clinical trial of women who had pre-eclampsia or eclampsia in their last pregnancy and had a pregnancy plan were conducted. We mainly seek qualified female subjects through community health service staff through interviews, telephone consultations or posting advertisements. We initially recruited 2764 participants in our clinical trial. Except for 1098 patients who were excluded at the beginning of the study because of unable to meet the inclusion criteria (n = 497), decline to participate (n = 476), and some other reasons (n = 125), the rest 1576 eligible patients joined in the next stage of our trial and were divided randomly and equally into 2 groups including the low dose (LD) group (n = 788) and the high dose (HD) group (n = 788). Some lost to the follow-up or drooped out for some reasons shown in Fig. 1, leaving a final sample of n = 378 in the LD group and n = 410 in the HD group. Inclusion and exclusion criteria were determined during the interview by participating obstetricians.

Inclusion criteria:

1. A woman who has had a pre-eclampsia or eclampsia or HELLP syndrome during her last pregnancy
2. There are plans for re-pregnancy in the near future;
3. Women of childbearing age over 18 years of age;
4. The daily FA intake before randomization is less than 1.1 mg;
5. Willing to undergo pregnancy test and production at the designated hospital.

Exclusion criteria:

1. Below 18 years old;
2. Unmarried;
3. Smoking and drinking before pregnancy;
4. Being pregnant;
5. Have previously or currently demonstrated intolerance to high dose (4 mg) FA disease or symptoms;
6. Have other kinds of diseases, such as urolithiasis, kidney disease, and thyroid-related diseases or related gastrointestinal diseases;
7. HIV, Hep-B or Hep-C positive patients;
8. Those who have taken illegal drugs within 6 months before pregnancy;
9. Those who are allergic to FA;
10. Disagree to sign a written consent form, such as an unwillingness to produce in a designated hospital.

2.2. Intervention

After the subjects were recruited and randomized, the LD group was supplemented with 0.4 mg of FA per day, and for the HD group, 4 mg of FA was added daily until production. To improve compliance, FA was provided free of charge by the study group and was 0.4 mg per dose for

Table 1
Baseline characteristics of participants at trial entry (pre pregnancy sample) and of the final sample (analyzed sample) in the study group.

	All participants at trial entry		Participants analyzed	
	LD group (n = 788)	HD group (n = 788)	LD group (n = 378)	HD group (n = 410)
Maternal age, years	30.7[4.9]	30.4[5.1]	29.4[5.2]	30.5[5.3]
Prepregnancy BMI, kg/m^2				
< 18.5 kg/m2	35(4.4)	34(4.3)	23(6.1)	29(7.1)
18.5–24.9 kg/m2	507(64.3)	494(2.7)	329(87.0)	356(86.8)
24.9–29.9 kg/m2	139(17.6)	143(18.1)	19(5.0)	18(4.4)
≥30 kg/m2	107(13.6)	117(14.8)	7(1.9)	7(1.7)
Blood pressure, mm Hg				
Systolic	114.3[12.4]	113.6[13.2]	114.3[12.4]	113.6[13.2]
Diastolic	68.5[10.7]	68.9[9.6]	68.5[10.7]	68.9[9.6]
Previous severe pre-eclampsia	497(63.1)	506(64.2)	249(65.9)	261(63.7)
Previous eclampsia	291(36.9)	282(35.8)	129(34.1)	149(36.3)
Previous livebirth	509(64.6)	517(65.6)	201(53.2)	228(55.6)

Data were expressed as n (%) or mean [SD]. LD, low dose. HD, high dose.

the LD group and 1 mg per dose for the HD group. The investigators guided the subjects to use the drug rationally, and each subject issued a form to record the daily FA intake for later calculation of compliance. Compliance is defined as the total dose of actual FA intake divided by the total dose of FA that should theoretically be taken, which is ultimately converted to a percentage.

2.3. Follow-ups

All subjects received the appropriate dose of FA daily from the time they were assigned to the group until production. All subjects, once assigned to the group, were given a comprehensive examination and were asked to go to the clinical trial center (specifically, a hospital) every 4 weeks for later examination. After confirming the pregnancy, the subjects were asked to check at least once in the first trimester of pregnancy, once every month during the middle trimester of pregnancy, once every two weeks from 28 weeks to 36 weeks, and once a week after 36 weeks until smooth production. Demographic data and other relevant information were obtained during the first visit to the prenatal period or by reviewing relevant medical data. As for the final data analysis work, the analyst was unaware of the entire group work and the source of the final test sample from the beginning.

2.4. Primary and secondary outcome measurements

Pre-eclampsia is the primary outcome measure. The definition and measurement of pre-eclampsia were based on previous research [15]. Plasma homocysteine levels and plasma FA levels were measured by Human homocysteine ELISA Kit and Human Folic acid ELISA Kit (Biorbyt, San Francisco, CA, USA) according to the manufacturers' instructions. The definition and measurement of secondary outcomes were based on previous research [16] and the items for secondary outcomes were listed in.

2.5. Data analyses

According to the literature [15], for women who have had previous pre-eclampsia or eclampsia, the probability of recurrence is about 25%. Assuming that the probability of recurrence could be reduced from 25% to 15% after taking high doses of FA, according to the significance level $\alpha = 0.05$ and the statistical power is set to $\beta = 0.2$, we need about 540 subjects. Noncompliance, withdrawn, loss to follow up, and other unanticipated events were not considered in the condition. If we consider that only about 50% of women will eventually conceive and 15% of loss to follow-up, we will need about 1270 participants. Recruitment is stopped when it is expected to reach the primary sample size.

Categorical variables were described as n (%). Quantitative variables were described by mean ± standard deviation. In the analysis of the demographic characteristics of the subjects, we separately counted the subjects who initially entered the clinical trial and the subjects who completed the clinical trial to ensure that the subjects who were not normally pregnant or lost to follow-up were removed and did not affect the randomness of the group and the balance between groups. For baseline demographic characteristics, the categorical variables were analyzed by Chi-square tests, and the quantitative variables between the two groups were compared by independent samples *t*-test. For the plasma homocysteine levels and FA levels in the primary outcome, a comparative analysis was performed by *t*-test. Other categorical parameters were compared as risk ratios (RR) with 95% CIs. The analysis was done with SPSS 17.0 software.

3. Results

3.1. Study design

The flow diagram of the study and the reasons for dropping out were shown in Fig. 1 and the detailed description were presented in the methods section. Participants were divided randomly and equally into 2 groups including the low dose (LD) group (n = 788) and the high dose (HD) group (n = 788). Some lost to the follow-up or drooped out for some reasons shown in Fig. 1, leaving a final sample of n = 378 in the LD group and n = 410 in the HD group. The LD group was supplemented with 0.4 mg of FA per day, and for the high dose group, 4 mg of FA was added daily until production.

3.2. Baseline characteristics of participants

The baseline characteristics including the maternal age, prepregnancy BMI, blood pressure, previous severe pre-eclampsia, previous eclampsia, and previous livebirth were initially carried out by these participants and were shown in Table 1. At the time of initial grouping, there was no significant difference between LD and HD group in baseline characteristics. The withdrawal of some people or the loss to follow-up did not break the balance between the two groups, and there was no significant difference in baseline characteristics between the subjects who performed the final analysis.

3.3. Compliance data

We evaluated the compliance of the participants (Table 2). Our calculation method is to divide the total actual FA consumption by the total theoretical dose. We found that there were 276 participants (73.0%) in the LD group and 304 participants (74.1%) in the HD group with compliance > 80%. Further, 325 participants (86.0%) in the LD

Table 2
Compliance of participants analyzed.

	LD group (n = 378)	HD group (n = 410)	Risk ratio (95% CI)	p value
Compliance > 80%	276(73.0)	304(74.1)	0.98(0.80–1.19)	0.827
Compliance > 50%	325(86.0)	347(84.6)	1.02(0.88–1.25)	0.799

Data were expressed as n (%). LD, low dose. HD, high dose.

Table 3
Plasma levels of homocysteine and Folic acid of participants with compliance over 50%.

	LD group (n = 378)	HD group (n = 410)	p value
Plasma homocysteine level[a,] μmol/L	11.72[2.10]	8.69[1.51]	< 0.001
Plasma Folic acid level[a,] nmol/L	8.17[1.62]	10.74[1.94]	< 0.001
Plasma homocysteine level[b,] μmol/L	12.23[3.08]	9.21[2.31]	< 0.001
Plasma Folic acid level[b,] nmol/L	7.26[1.52]	9.12[1.87]	< 0.001

Data were expressed as mean [SD]. LD, low dose. HD, high dose. homocysteine, homocysteine.
[a] Participants with Compliance > 80%.
[b] Participants with Compliance > 50%.

group and 347 participants (84.6%) in the HD group showed the compliance > 50%. Next, we measured the plasma homocysteine and FA levels of participants with compliance over 80% or 50% (Table 3). We found that the plasma homocysteine levels were significantly lower in the HD group than in the LD group, demonstrating that high dosage of FA supplement further decreased the plasma homocysteine levels.

3.4. Primary and secondary outcomes

The primary and secondary outcomes according to study group were shown in Table 4. For the primary outcome, the prevalence of pre-eclampsia showed no significant difference between the LD group and the HD group (37 [9.8%] vs 42 [10.2%]). The measured items of secondary outcomes are described in the methods section. Among the secondary outcomes, severe gestational hypertension, early onset pre-eclampsia Table 4 (< 32 weeks' gestation), severe pre-eclampsia, and newborns' Apgar score < 7 at 5 min were remarkably decreased in the HD group. Next, we examined the incidence of pre-eclampsia in participants with compliance over 50% or 80% (Table 5). Our data showed that the incidence of pre-eclampsia was significantly decreased in the HD group. Moreover, the incidence of pre-eclampsia was further reduced in the HD group with compliance > 80% compared with that compliance > 50%.

Table 5
The incidence of pre-eclampsia in participants with compliance over 50%.

	LD group [n/N (%)]	HD group [n/N (%)]	Risk ratio (95% CI)	p value
Participants with compliance > 50%				
Pre-eclampsia†	63/325(19.4)	44/347(12.7)	1.53(0.44–3.51)	0.016*
Participants with compliance > 80%				
Pre-eclampsia†	49/276(17.8)	31/304(10.2)	1.75(0.45–4.35)	0.009**

Data were expressed as n/N (%). LD, low dose. HD, high dose. *p < 0.05. **p < 0.01. †Pre-eclampsia here includes the ordinary pre-eclampsia and severe pre-eclampsia.

4. Discussion

An important theoretical basis for the occurrence of pre-eclampsia is the placental insufficiency and its vascular discrepancy caused by an increase in the content of homocysteine [17]. Most scholars believe that

Table 4
Primary and secondary outcomes according to study group.

	LD group (n = 378)	HD group (n = 410)	Risk ratio (95% CI)	p value
Pre-eclampsia	37 (9.8)	42 (10.2)	0.96 (0.76–1.19)	0.684
Gestational hypertension	243 (64.3)	259 (63.2)	1.01 (0.85–1.18)	0.516
Severe gestational hypertension	126 (33.3)	89 (21.7)	1.54 (0.67–3.70)	0.021*
Gestational proteinuria	90 (23.8)	102 (24.9)	0.95 (0.80–1.15)	0.544
Early onset pre-eclampsia (< 32 weeks' gestation)	73 (19.3)	52 (12.7)	1.52 (0.53–4.20)	0.039*
Severe pre-eclampsia	64 (16.9)	40 (9.8)	1.69 (0.55–4.80)	0.011*
Renal failure (creatinine > 120 mmol/L)	11 (2.9)	9 (2.2)	1.35 (0.60–3.09)	0.324
Liver failure	9 (2.4)	9 (2.2)	1.05 (0.78–1.35)	0.473
Placental abruption	8 (2.1)	10 (2.4)	0.88 (0.60–1.24)	0.457
Pulmonary oedema	1 (0.3)	0 (0)	–	–
Pregnancy loss at any gestation	67 (17.7)	60 (14.6)	1.20 (0.72–2.14)	0.156
ICU admission > 24 h	4 (1.1)	5 (1.2)	0.91 (0.73–1.11)	0.524
Caesarean section	211 (55.8)	232 (56.6)	0.96 (0.84–1.22)	0.447
Hospital stay 7d or more after birth	27 (7.1)	25 (6.1)	1.15 (0.82–1.60)	0.258
HELLP syndrome	13 (3.4)	15 (3.7)	0.92 (0.68–1.32)	0.249
Maternal death	1 (0.3)	0 (0)	–	–
Preterm birth (< 37 weeks' gestation)	57 (15.1)	53 (12.9)	1.15 (0.73–1.60)	0.223
Early preterm birth (< 32 weeks' gestation)	15 (4.0)	14 (3.4)	1.17 (0.79–1.22)	0.314
Birthweight < 2500 g	43(11.4)	36 (8.8)	1.29 (0.56–2.98)	0.105
Apgar score < 7 at 5 min	5 (1.3)	3 (0.7)	1.85 (0.44–4.32)	0.013*
Perinatal death or admission to neonatal ICU for 24 h or more	13 (3.4)	11 (2.7)	1.25 (0.64–1.68)	0.472
Stillbirth	5 (1.3)	6 (1.5)	0.88 (0.67–1.66)	0.287

Data were expressed as n (%). LD, low dose. HD, high dose. *p < 0.05. HELLP syndrome = haemolysis, elevated liver enzymes, and low platelet count.

the increase in homocysteine levels leading to pre-eclampsia is associated with peroxidative damage of vascular endothelial cells, which ultimately leads to a variety of adverse pregnancy outcomes [18]. Studies have shown that homocysteine levels are reduced during normal pregnancy, and could be reduced to 50%–60% of pre-pregnancy levels in 20–32 weeks of pregnancy, whereas increased slightly in the third trimester [19]. Before the occurrence of pre-eclampsia, the patient's plasma homocysteine concentration was slightly higher than that of normal pregnant women [19]. In the second trimester, the risk of pre-eclampsia increased by 3.2 times in patients with elevated plasma homocysteine levels [19]. Hyperhomocysteinemia has been shown to be associated with female spontaneous abortion, placental thrombosis and placental abruption, and obesity and type 2 diabetes in the mothers and the offspring [6]. Long-term exposure of endothelial cells to higher levels of homocysteine results in a decrease in nitric oxide production in the body [20]. This leads to a decrease in platelet inhibition and platelet adhesion and aggregation, which ultimately leads to vascular disease affecting the blood flow to the uterus and eventually leading to pre-eclampsia [20].

An important metabolic pathway of homocysteine in vivo is the re-synthesis of methionine via the methylation pathway [21]. This reaction requires N5,10-methylenetetrahydrofolate reductase (MTHFR) to catalyze the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [22]. FA is a cofactor for this enzyme, and a methyl donor is provided by FA to regenerate methionine. Therefore, FA might be crucial in the onset and the progression of pre-eclampsia. Studies have shown that pregnant women with hyperhomocysteinemia and low folate status have a multiple risk of developing pre-eclampsia relative to the control group [15]. A large amount of data indicates that oral FA intake could reduce the incidence of pre-eclampsia. In one study, subjects taking high doses of FA daily (3–9 mg daily) were able to significantly reduce the rate of preterm labor and early onset of pre-eclampsia [23]. In another study, continued usage of FA in the early stages of second trimester significantly reduced the risk of pre-eclampsia. Recently, studies have reported that when pregnant women are given low-dose or high-dose FA throughout pregnancy, although the expression of homocysteine is different between the two groups, there is no pre-eclampsia occurs in both groups [24]. Therefore, it is believed that the administration of FA during pregnancy could reduce the level of homocysteine and prevent the occurrence of pre-eclampsia. Studies have also suggested that FA administration during the second trimester could reduce the risk of pre-eclampsia [25]. However, Fernández et al believe that the increase of homocysteine in early pregnancy is not correlated with the occurrence of pre-eclampsia, and the early administration of pre-eclampsia could not reduce the incidence of pre-eclampsia [26]. There are controversial reports on whether FA could reduce the incidence of pre-eclampsia, therefore, a randomized clinical trial of high dose or low dose FA administration in pregnant women with high pre-eclampsia risk was conducted in our research. For the dosage selection of FA, the low dose (0.4 mg/day) and high dose (4 mg/day) of FA was adopted from protocols published before [27,15,23]. Although the most common recommendation in Chinese is 0.4 mg/day, the question of the optimal dose of FA remains. Thus, the aim of this project is to assess the effects of a higher dose of peri-conceptional folic acid supplementation on reducing the occurrence of pre-eclampsia. Our trial exhibited a significant reduction in recurrent pre-eclampsia with high dose FA supplementation throughout pregnancy. This trial is fundamentally different from previous trials of FA to prevent pre-eclampsia. First, we recruited participants who had pre-eclampsia or eclampsia during the last pregnancy to study the recurrence rate of the participants. Actually, the incidence of pre-eclampsia is approximately 5% of pregnant women. Therefore, if pregnant women are randomly recruited, when the sample size is not large enough, there may be no pregnant women showing pre-eclampsia.

Among the secondary outcomes, we found that severe gestational hypertension, early onset pre-eclampsia (< 32 weeks' gestation), severe pre-eclampsia, and newborns' Apgar score < 7 at 5 min were remarkably decreased in the HD group compared with the LD group. Studies have shown that the serum concentration of homocysteine in patients with severe pre-eclampsia before delivery is higher than 3 days after delivery, 42 days after delivery and normal late pregnancy women. Serum homocysteine levels were significantly lower in patients with severe pre-eclampsia 42 days postpartum than at 3 days postpartum [28]. It can be seen that the serum homocysteine concentration in patients with severe pre-eclampsia decreased after delivery, and the decrease of serum homocysteine concentration increased with the prolongation of postpartum period and the relief of the disease. It is indicated that the occurrence of severe pre-eclampsia is related to the increase of serum homocysteine level in pregnant women, and the serum homocysteine concentration is related to its severity. In the present study, we found that high dose FA administration further decreased the plasma homocysteine levels in participants.

For the limitation of the study, a relatively small number of disabled patients were recruited for better compliance to treatment and imaging follow-up, which may limit the generalization of the findings. In addition, throughout the pregnancy, this study did not limit pregnant women's use of other drugs that could treat and alleviate pre-eclampsia. Because the participants we recruited were all pregnant women with a history of pre-eclampsia, the participants showed a high incidence of morbidity, including severe pre-eclampsia. It is not known whether FA is related to other therapeutic drugs, and whether FA could play a role in treating pre-eclampsia, which warrants further investigation.

5. Conclusion

In conclusion, we showed that high dose FA supplementation that commenced from 3 months before pregnancy until the entire pregnancy showed a significant reduction in recurrent pre-eclampsia compared with low dose FA.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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