Original Article

Comparison of high dose and low dose folic acid supplementation on prevalence, onset and severity of preeclampsia

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Abstract

Background: Folic acid supplementation had previously mentioned as a protective factor against the onset of preeclampsia (PE). In this study, we aimed to compare the effect of high dose (5 mg daily) and low dose (1 mg daily) of folic acid supplementation on prevalence, onset and severity of PE.

Materials and Methods: Pregnant women who were in the first trimester and referred to prenatal care university hospitals of Isfahan, Iran during October 2013–May 2015 were included in this study, then they were randomly divided into two groups of 5 mg and 1 mg (treated with daily 5 mg and 1 mg of folic acid, respectively), both groups received folic acid from the first trimester of pregnancy to 42 days after termination. Blood pressure, body mass index (BMI), and some urine and blood biochemistry parameters were measured. SPSS-22 used for statistical analysis.

Results: A total of 943 pregnant women participated in the study (450 women in 1 mg group and 450 women in 5 mg group). Incidence rate of PE was 3.8% in 1 mg group and 2.4% in 5 mg group. In a comparison of preeclamptic patients in 1 mg and 5 mg group, no significant differences were seen regarding age, BMI, laboratory data, the severity of the disease, and onset (early or late) ($P > 0.05$).

Conclusion: Although our findings support that administration of high dose folic acid may decrease the prevalence of PE, there is not enough data to support that higher amount of folic acid administration can reduce the severity of presentation's signs or ameliorate the laboratory data and the onset of PE.

Key Words: Folic acid, preeclampsia, pregnancy

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INTRODUCTION

Preeclampsia (PE) is characterized with hypertension and proteinuria after 20 weeks of gestational age, which can involve at least 5% of all pregnancies.[1] PE is considered to be an important cause of mortality and increased risk of future progression of cardiovascular disease, in both mother and fetus.[1-3] The main pathologic process of the disease remains unclear. However, inefficient placental perfusion was mentioned as a causational factor.[4] In nonsevere PE, hypertension defined as blood pressure of 140–159 mmHg systolic or 90–109 mmHg diastolic

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that occurs after 20 weeks of gestation in a woman with previously normal blood pressure, as well proteinuria, defined as urinary excretion of 0.3 g protein or more in a 24-h urine protein sampling.[5] Severe PE is defined when any of the following criteria are present: Systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on two occasions, at least 4 h apart while the patient is on bed rest (unless antihypertensive therapy is initiated before this time), progressive renal insufficiency (serum creatinine concentration >1.1 mg/dl or a doubling of the serum creatinine concentration in the absence of other renal disease), thrombocytopenia (platelet count <100,000/μl), evidence of hepatic dysfunction as indicated by abnormally elevated blood concentration of liver enzymes (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses (probably caused by subcapsular hepatic hemorrhage or stretching of Glisson capsule, or both), new-onset cerebral or visual disturbances such as headache and scotomata ("spots" before the eyes), pulmonary edema.[5,6]

Eclampsia as a probable outcome of PE may involve about 5–8% of those PE patients, and is characterized with generalized seizures.[7]

A large number of death related to pregnancy hypertensive disorder could be prevented.[8] There are some researches showed an association between multivitamin and mineral supplementation and risk of PE in pregnant women.[9-13] Previous studies also showed that there is a relationship between the elevated level of total homocysteine and PE, miscarriage, stillbirth and intrauterine growth restriction.[14-21] Serum levels of Vitamin B12 and folic acid are lowered in the preemlamptic patients.[22] Folic acid is generally accepted to have an effect on reduction of about 70% of neural tube defects cases,[23,24] and It is believed that it has also a role in the reduction of homocysteine level.[25-27] Thus, there could be a potential effect of folic acid supplementation on reduction of blood's circulating homocysteine and subsequent PE.

In this study, we aimed to evaluate the dose-dependent effect of folic acid on prevalence, onset and severity of PE.

MATERIALS AND METHODS

This double-blinded randomized clinical trial study was done by the grant of Isfahan University of Medical Sciences (Research Number #393587 and it has been registered in www.irct.ir with the code of IRCT201507313944N4) on a group of pregnant women, went to obstetric clinics of Beheshti and Alzahra Hospitals (University Referral Medical Science Hospitals of Isfahan-Center of Iran) during October 2013–May 2015.

This study aimed at testing the effect of 1 mg versus 5 mg folic acid taken daily throughout the pregnancy. All of the pregnant women have a living fetus in the uterine cavity in the first trimester with willingness for participation in this study, were included. The study had a double-blinded randomized design, where both investigators and participants were blinded to the study group assignment. We informed all of the participants about the research protocol and process and then asked them to sign a participation written consent. After that, using computerized randomization system, we assigned them into two groups of taking 5 mg or 1 mg folic acid pills. Participant level randomization was stratified by the research site, to assure balance in representation [Figure 1].

For randomization, each participant has received an exclusive (random) number which was the same/different from the sequence numbers of pill boxes. Each of participants received a randomized sequential number (case number), too. We referred any of participants by her special case number to the Beheshti or Alzahra Hospital drug store, to receive the pillbox by the same number of her case number, regardless to the 5 mg or 1 mg dose of folic acid. Patients and drug store's stuff were both blind about a dose of the drug. The folic acid pills were manufactured by Jalinous Company Pharmaceutical Industries in Iran. Perioral the production the manufacturer was asked to develop quality control tests for each of pills, include the quantity of folic acid material and its dosage in the pills, and also the content uniformity and dissolution test to

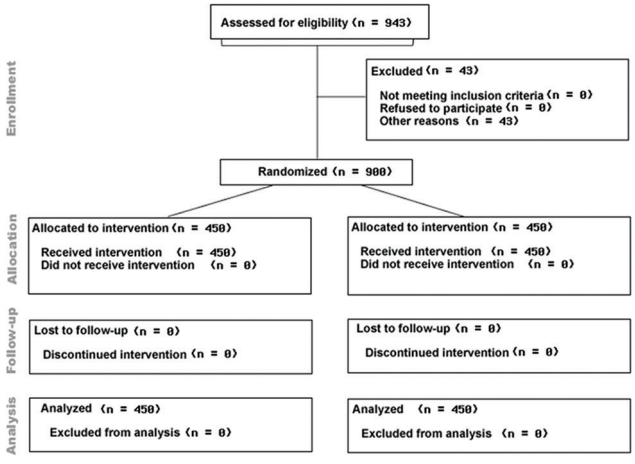

Figure 1: Study flowchart

ensure that the folic acid material is consistent across pills and it dissolve adequately in a standard time.

All participants were asked to take a single pill of 1 mg or 5 mg of folic acid daily throughout the first trimester of pregnancy since delivery. The first assessment of women was done about complete medical and obstetrical history, physical examination, demographic data (age, weigh, height, body mass index [BMI], and gravidity), calculating gestational age, vital sign (especially systolic and diastolic blood pressure) and laboratory data including urine analysis, platelet count, liver function tests (including aspartate transaminase and alanine transaminase) and serum's creatinine and making prenatal card.

Participants were followed up every month in first and second trimester to check on their health status, providing new pill boxes for the next 2 months, and collect old pillboxes. The follow-up surveys were continued during the years of the study (October 2013–May 2015), and every 2 weeks until 36 weeks and weekly in the last month of pregnancy up to delivery. Participants were also asked to no to use multivitamins during the study. The study protocol, informed consent, manual of operations, and data collection forms were approved by the Ethics Committees of the Isfahan University of Medical Sciences Ethic Committee. The study participants were pregnant women who have received prenatal cares at the study clinics since the first visit to delivery and followed up at least 42 days after childbirth in these clinics. If they have any of the following situations they do not include in our study: Presence or history of a disorder in which administration of folic acid is contraindicated, renal diseases, using anti-epileptic drugs (since the metabolism of anti-epileptic drugs requires a great deal of folic acid), malignancy, alcohol abuse, multiple birth (presence of three living fetus or more) and known hypersensitivity to folic acid (rashes, dermatologic, and gastroenterological discomforts). The followings considered as exclusion criteria: Fetal death during the study, major abnormality of the fetus, women who were planning to move outside of the catchment area of the study within the pregnancy duration.

Both groups were on a routine perinatal clinical follow-up for management of any discomfort (rapid weight gain, headache, epigastric pain, and visual impairment), assessment of vital signs and evaluation of laboratory data (as mentioned above). There were no reported side-effects related to administration of folic acid in this study.

943 participants were included in the study, of which, 43 people were finally excluded due to incomplete information and irregular visiting period, migration to other cities, no recursion.

Finally, all collected data were analyzed using IBM SPSS Statistics for Macintosh, Version 22.0, Chicago, USA software. Paired *t*-test, Fisher's test and Chi-square tests were used for statistical analysis of the data; $P < 0.05$ was considered as statistically significant difference.

RESULTS

Nine-hundred participants were included in the study. The mean of body height and weight were 161.7 ± 5.86 and 65.33 ± 10.24, respectively. Of all patients, 883 (98.1%) were singleton pregnancy and 17 (1.9%) were twin pregnancy. As shown in Table 1, there were no significant differences between 1 mg and 5 mg groups regarding BMI, age, gravidity ($P > 0.05$). There were 55 (6.1%) patients who had a positive history of PE, two of them had the same situation again during our study, and both of them were in 1 mg group. Chi-square test did not reveal statistical difference about the history of PE ($P > 0.05$).

The prevalence of preeclampsia was 3.1% (28 out of 900) in all participants, of which, it was 3.8% (17 out of 450) in 1 mg group and 2.4% (11 out of 450) in 5 mg group. The independent *t*-test showed no significant statistical difference about the prevalence of PE ($P > 0.05$).

Overall there were 28 individuals who fulfilled the diagnostic criteria of PE during the study, among them, 17 out of 28 (60.7%) were in 1 mg group, and 11 out of 28 (39.3%) were in 5 mg group. The independent *t*-test showed no significant statistical difference about the prevalence of PE ($P > 0.05$). Chi-square test showed no difference between groups regarding platelet count ($P > 0.05$). 96.8% of participants (872 out of 900) had normal blood pressure in the whole study duration, which includes the 97.6% (439 out of 450) of 5 mg group and 96.3% (433 out of 450) of 1 mg group. In 5 mg group, 2.4% (11 out of 450) had nonsever PE (a systolic blood pressure more than 140 mmHg and <160 mmHg or diastolic one more than 90 mmHg and <110 mmHg), whereas, 3.7% (17 out of 450) in 1 mg group had the same amount of diastolic blood pressure.

In other words, 3.5% (16 out of 450) of 1 mg group had nonsever PE and only one individual 0.2% had systolic blood pressure higher than 160 mmHg that mentioned as sever PE. The sever preeclamptic participant in 1 mg group 5.9% (1 out of 28) had a headache, visual impairment, epigastric pain. The Fisher's test showed

no significant statistical difference about measured blood pressure and presentation of sever sign between groups ($P > 0.05$).

Nonsevere PE in 1 mg group was 94.1% (16 out of 17) and in 5 mg group 100% (11 out of 11) of patients with PE criteria have nonsevere PE.

We used the Fisher's test to compare the severity of PE between two groups, according to this analysis there were no statistical difference between 57.1% (16 out of 28) in 1 mg group and 39.2% (11 out of 28) in 5 mg group, nonsevere preeclamptic women ($P > 0.05$).

In 1 mg group, 2% (9 out of 450) suffered PE before 34 weeks, as well 1.8% (8 out of 450) experience it after 34 weeks, likewise in 5 mg group, 0.4% (2 out of 450) have it before 34 weeks, 2% (9 out of 450) after 34 weeks. Before 34 weeks, 52.9% (9 out of 17) in 1 mg group as well, 18.2% (2 out of 11) in 5 mg group developed the PE presentations and after 34 weeks 81.8% (8 out of 11) in 1 mg group also, 32.1% (9 out of 28) in 5 mg group experienced the PE. Fisher's test showed no statistically significant differences regarding the onset of PE among two groups.

For further evaluation of disease, we categorized all PE presentations (28 cases) into two groups of 5 mg (11 individuals) and 1 mg (17 individuals). As shown in Table 2, independent *t*-test revealed no significant differences between groups regarding age, BMI, gravidity, twin pregnancy, the onset of the disease that described as early (before 34 weeks of gestation) and late (after 34 weeks of gestation) and severity of the disease ($P > 0.05$).

As shown in Table 3, more than 90% of patients in both preeclamptic groups had proteinuria of 1+ or more, Fisher's test did not reveal statistical significant difference between groups regarding degree of proteinuria, platelet count, blood pressure, severity of disease, impaired liver function tests, creatinine and 24 h urine protein ($P > 0.05$). Just 11.8% of all patients had episodes of severe signs of PE (including a headache, visual impairment, and epigastric pain) and it's beneficial to say that, however, all of mentioned patients were in the 1 mg group, but there was no difference between groups regarding presentations of severe signs using Fisher's test ($P > 0.05$).

There was just one person in 1 mg group who had rapid weight gain (more than 4 kg in a month), and there was no difference between groups in the field of rapid weight gain ($P > 0.05$). There was no one with previous drug history or concurrent disease in patients with PE.

DISCUSSION

The main goal of this study was to evaluate whether there is a difference between the administration of high dose (5 mg/daily) and low dose (1 mg/daily) of folic acid during pregnancy, on prevalence, onset, and severity of PE in a population of 900 pregnant Iranian women.

The prevalence of PE in our research was 3.1%. In 2014, the Bilano *et al.* performed a secondary analysis of the WHO Global Survey on Maternal and Perinatal Health. The survey was a multicountry, facility-based cross-sectional study. A global sample consisting of 24 countries from three regions and 373 health facilities was obtained via a stratified multi-stage cluster sampling design. Maternal and offspring data were extracted from records using standardized questionnaires. Multi-level logistic regression modeling was conducted with random effects at the individual, facility, and country levels. The prevalence of PE/eclampsia in the Mori João *et al.*

Table 1: BMI, age, gravidity, history of PE and twin or single pregnancy in 1 mg and 5 mg groups

Dose of folic acid	Number of patients	BMI	Age (year)	Gravidity (mean)	History of PE (number)	Twin/single pregnancy
5 mg	450	25±3.51	28.2±5.73	1.94±1	23	1±0.17
1 mg	450	25±3.53	28±4.81	1.97±1	32	1±0.1
Total	900	25±3.52	28.1±5.3	1.96±1	55	1±0.1
P		0.6	0.5	0.7		0.2

BMI: Body mass index, PE: Preeclampsia

Table 2: Age, BMI, gravidity, twin pregnancy, timing of the disease onset and severity of the disease in preeclamptic patients divided in 1 mg and 5 mg groups

Dose of folic acid	Age (year)	BMI	Gravidity (mean)	Twin pregnancy	Onset of the PE (%)		Severity of PE (%)	
					Early	Late	Non severe	Severe
5 mg	26.45±5.1	24±4	1.36±0.7	1.1±0.3	2 (18.2)	9 (81.8)	11 (100)	0 (0)
1 mg	27.47±6.1	26.5±5.5	1.8±0.9	1	9 (52.9)	8 (47.1)	15 (88.2)	2 (11.8)
P	0.6	0.1	0.1	0.2	0.06		0.2	

BMI: Body mass index, PE: Preeclampsia

Table 3: Urine analysis, platelet count, blood pressure, 24 h urine protein, LFT and serum creatinine of patients presented with PE in 1 mg and 5 mg groups

Result of analysis	Groups of study			
	1 mg	5 mg	Total	*P*
Protein urine dipstick (+)				
≤1+	1 (5.9)	1 (9.1)	2 (7.1)	0.7
≥2+	16 (94.1)	10 (90.9)	26 (92.9)	
Platelet count (/ml^3)				
≥100,000	17 (100)	10 (90.9)	27 (96.4)	0.2
<100,000	0 (0)	1 (9.1)	1 (3.6)	
Blood pressure (mmHg)				
≥140/90	16 (94.1)	11 (100)	27 (96.4)	0.6
≥160/110	1 (5.9)	0 (0)	1 (3.6)	
24-h urine protein (/mg)				
≥300	16 (94.1)	11 (100)	27 (96.4)	0.4
≥500	1 (5.9)	0 (0)	1 (3.6)	
LFT (mg/dl)				
Normal	14 (82.4)	11 (100)	25 (83.3)	0.1
Abnormal	3 (17.6)	0 (0)	3 (10.7)	
Serum creatinine (mg/dl)				
Normal	14 (82.4)	11 (100)	25 (89.3)	0.1
Abnormal	3 (17.6)	0 (0)	3 (10.7)	

LFT: AST and ALT, LFT: Liver function tests, AST: Aspartate transaminase, ALT: Alanine transaminase, PE: Preeclampsia

study population was 10,754 (4%), but they found much variation between and within countries. PE/eclampsia prevalence ranged from <1% in Angola to 8% in Brazil.[28]

Near to our findings, there is a local investigation in our local area (Iran) which showed that the prevalence of hypertensive disorder between a population of 2300 pregnant women was 3.3% of which 96% fulfilled PE criteria.[29]

According to the WHO Global Survey on Maternal and Perinatal Health, and in attention to this fact that the prevalence of PE is higher in low and middle-income countries than Western, we suggest that this study results support the hypothesis that folic acid supplementation associated with reduced risk of PE.

Our findings do not support that administration of high dose (5 mg) folic acid can decrease severity rate of PE. Patients in 1 mg and 5 mg groups' evaluations did not reveal any difference in blood pressure and regarding laboratory data, all of the following parameters were similar in both groups: Platelet count, serum creatinine, liver function tests, 24-h urine analysis; although high doses of folic acid can reduce the risk of PE, there is no proved finding on efficacy of supplementation on reducing signs and laboratory data of the disease. In any case, we did not find a significant relationship between severity of PE between those supplemented with high dose and those treated with a low dose. We have not significant differences which support that administration of folic acid can retard the onset of PE. Patients in 1 mg and 5 mg groups' evaluations did not reveal any difference in onset of PE.

There are cohort studies that support that use of multivitamin containing folic acid has a protective role on PE,[9,30,31] by contrast some previous studies failed to find a protective role for folic acid in relation to PE.[32-34]

A study by Wen *et al*. on a population on 2951 pregnant women showed that supplementation of multivitamin containing folic acid in the second trimester is associated with increased serum folate, decrease homocysteine level and reduced risk of PE.[35] A recent chines study revealed that folic acid supplementation can significantly decrease risk of PE, but in contrast to our data they found an association between intake of folic acid supplementation and mild late-onset presentation of the disorder.[36] A study designed by Bodnar *et al*. showed a 45% decreased risk of PE during pregnant women who intake multivitamin compared with those nonusers. Interestingly, the reduction rate were 71% among those lean multivitamin users compared with lean nonusers, thus they concluded that a regular administration of multivitamin during the preconceptional period would lead to decreased presentation of the disease.[9] Statistical data analysis of another study by Catov *et al*. revealed that a regular 12-weeks periconceptional use of a multivitamin was associated with reduced risk of PE between normal weight women, although no association was found between use of folate-only supplementation and reduced risk of PE.[34] The main difference of previous studies which led to various results may come from the different type of administration of folic acid that needs a better attention of researchers to find a unique protocol of folic acid supplementation.

A low number of participants, noncontrolled dietary program, unmatched participants about socioeconomic aspects and selection of cases from only two medical centers can be determined as the limitations of this study.

By conclusion, it could be said that although our findings support that administration of high dose folic acid may decrease the prevalence of PE but there is not enough data to support that higher amounts of folic acid administration can reduce the severity of presentation's signs or ameliorate the laboratory data and the onset of PE.

The clinical importance of these statistically significant changes is necessary to be validated in well-controlled

prospective studies, distributed in different area or province in our country.

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Conflicts of interest

There are no conflicts of interest.

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