

Comparison study on the effect of prenatal administration of high dose and low dose folic acid

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ABSTRACT

الأهداف: تقييم الجرعة العالية والمنخفضة من حمض الفوليك على مستويات تركيز هيوموسيتسين Hcy خلال الثلاثة أشهر الأولى من الحمل والولادة، ومن أجل فحص اتحاد مستويات مصل Hcy ومقدمة الارتعاج.

الطريقة: أجريت دراسة سريرية عشوائية مجهولة الطرف، مدينة تابريرز - إيران، خلال الفترة ما بين عام 2005م وحتى 2008م، تلقت 246 امرأة حامل في مجموعتين متشابهتين حمض الفوليك يومياً منذ بداية الحمل وحتى الولادة (5mg) يومياً في المجموعة الأولى، و(0.5mg) يومياً في المجموعة الثانية. تمت مقارنة حدوث ارتفاع ضغط الدم، المتغيرات المخبرية في مستويات مصل Hcy واللاكتيت ديهيادرورجينيس وحمض اليوريك بالإضافة إلى مستويات اليوريا، الكرياتين، والبروتين بين المجموعتين.

النتائج: لم يكن هنالك أي نوع من ارتفاع ضغط الدم في كلتا المجموعتين. بلغ ضغط الدم الانقباضي (mm Hg) في الأشهر الثلاثة الأولى من الحمل وعند الولادة في المجموعة الأولى والثانية 114.01±8.78 - 114.16±9.05 و 117.24±6.91 - 117.23±11.48 على التوالي ($p=0.32$). بلغ الضغط الانبساطي 73.30±8.90 - 74.90±7.45 و 76.69±8.62، 76.46±5.58، على التوالي ($p=0.42$)، على الرغم من انخفاض مستوى (Hcy) ($\mu\text{mol/L}$) بشكل ملحوظ عند وقت الولادة في المجموعة الأولى (11.81±3.85) انخفض إلى (6.44±1.88) و المجموعة الثانية (2.99±7.44) انخفض إلى (9.08±3.24)، إلا أن هذا الهبوط كان ملحوظاً بشكل أكبر في المجموعة الأولى ($p<0.001$).

خاتمة: أظهرت النتائج أن التزويد بحمض الفوليك خلال الحمل بغض النظر عن مقدار الجرعة قد يحد من اضطرابات ارتفاع ضغط الدم وإنقاص مستوى مصل (Hcy)، على الرغم من انخفاضه بشكل أكثر وضوحاً في المشكل.

Objective: To evaluate the effect of high dose and low dose folic acid on the levels of homocysteine (Hcy) concentration during the first trimester of pregnancy

and at delivery, and to examine the association of Hcy serum levels and preeclampsia.

Methods: In a single blinded randomized clinical trial, which was conducted in Tabriz, Iran, from 2005-2008, 246 nulliparous pregnant women in 2 similar groups, received folic acid daily from early pregnancy until delivery (5 mg/day in group one and 0.5 mg/ day in group 2). The incidence of hypertension and laboratory changes in the levels of serum Hcy, lactate dehydrogenase, and uric acid in addition to the levels of urine creatinine and protein were compared between the groups.

Results: There was no presence of any type of hypertension in each group. The systolic blood pressures (BP) (mm Hg) at the first trimester were 114.01±8.78 for group one, 114.16±9.05 for group 2, and at delivery, 117.24±6.91 for group one, and 117.23±11.48 for group 2 ($p=0.32$). The diastolic BP at the first trimester were 74.90±7.45 for group one, 73.30±8.90 for group 2, and at delivery 76.46±5.58 for group one, and 76.69±8.62 for group 2 ($p=0.42$). Although the level of Hcy ($\mu\text{mol/L}$) decreased significantly at the delivery time in group one (11.81±3.85 decreased to 6.44±1.88), and 2 (9.08±3.24, decreased to 7.44±2.99), this decrement was more significant in the first group ($p<0.001$).

Conclusion: The results show that folic acid supplement throughout pregnancy, irrespective of the dosage, could eliminate hypertensive disorders, and decreases serum level of Hcy, although it is reduced more significant in the first group.

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Hypertensive disorders are a leading cause of maternal and neonatal morbidity and mortality, and the second leading cause of maternal deaths in developing countries.¹ Berg et al² reported 16% of deaths from pregnancy-induced hypertension (PIH) from 1991-1997 in the United States. Gestational hypertension occurs in approximately 3.7% of pregnancies.¹ Although preeclampsia represents one of the most important complications of pregnancy (5-7% of low-risk pregnancies), little is known of its etiology.³ Despite considerable research, how pregnancy incites, or aggravates hypertension remains unsolved. Indeed, hypertensive disorders remain among the most significant and intriguing unsolved problems in obstetrics. An association was found between the elevated plasma homocysteine (Hcy) level, and preeclampsia.⁴ Despite a large number of investigations conducted to understand the place of Hcy metabolism in the physiology of human pregnancy, and the role of hyperhomocysteinemia in the obstetric complications, such as pre-eclampsia, abortion, and recurrent pregnancy loss, a cause and effect relationship has not yet been proven. The current hypothesis for the pathogenesis of preeclampsia is that factors produced by the poorly perfused placenta enter the systemic circulation, and alter vascular sensitivity to the circulating pressors, activate coagulation, and reduce vascular integrity, resulting in the pathophysiologic changes of preeclampsia.⁵ However, which factors produced by the poorly perfused placenta are responsible for the development of preeclampsia, and how they interact with the maternal predisposing factors to induce the clinical syndrome of preeclampsia remain elusive.⁵ Although, Milman et al⁶ reported that plasma Hcy increased gradually during pregnancy and postpartum from 11-20⁶ $\mu\text{mol/L}$, however, Cikot et al⁷ reported a constant level. Other authors reported a decrease in the concentration of plasma Hcy during pregnancy.^{8,9} Hyperhomocysteinemia is an independent risk factor for atherosclerosis in women who are not pregnant. Cotter et al¹⁰ showed that elevation at the levels of serum Hcy in early pregnancy, and around midpregnancy,¹¹ will increase the risk of preeclampsia. Although clinical studies substantiate this association, and the majority of evidence suggests a positive association, some authors have not shown elevated serum Hcy levels to be a useful predictor.^{12,13} Although hyperhomocysteinemia is a known risk factor for venous and arterial thrombosis,^{14,15} whether or not the increased concentration of Hcy causes vascular disease,¹⁶ whether the current routine evaluation of hyperhomocysteinemia as a part of thrombophilia workup truly reflects the increased risk of thrombosis, or whether it is merely a secondary result of endothelial activation, remains the subject of intense debate. A good folate status may play a critical role in

lowering elevated homocysteine concentrations. It has been reliably shown that plasma Hcy concentrations can be reduced by folic acid supplementation. At the time of this study, a large number of clinical trials are examining the effect of such reduction on the clinical outcome.^{17,18} Folate is a water-soluble vitamin with a low potential toxicity, belongs to the vitamin B group, and involved in a large number of biochemical processes, particularly in the metabolism of Hcy. Dietary or genetically low levels of serum folate will result in mild hyperhomocysteinemia, which was associated with various pathologies. As shown in several researches, and with the recognition of frequent association with early onset pre-eclampsia and hyperhomocysteinemia, it is essential to establish the efficacy and safety of folic acid supplementation in the prophylaxis of hypertensive disorders during pregnancy, hence, reducing the frequency of pre-eclampsia/eclampsia in women with hyperhomocysteinemia. Recent studies have found that supplementation of multivitamins containing folic acid was associated with the reduced risk of preeclampsia.¹⁹ The potential benefit of folic acid supplementation is reduction of plasma total Hcy.²⁰ Taparia et al²¹ have shown a protective role of folic acid during the early embryonic development to prevent some congenital defects, but still the exact mechanism is not well known. It has also been proposed that the patients who receive folic acid have a reduced risk to develop preeclampsia. Placental and systemic endothelial functions may be improved directly or indirectly by lowering the blood Hcy levels.²²⁻²⁶ The role of increased plasma Hcy in pregnant women, who later develop toxemia of pregnancy, preeclampsia/eclampsia, is still controversial.²⁷ Further research is required to determine whether the disturbance of Hcy metabolism is the cause, or only is increased secondarily to such pathologies, and whether it is necessary to keep the levels of folate in the high range throughout pregnancy. These observations prompted us to speculate that administration of high dose folic acid throughout pregnancy in women could reduce Hcy levels, therefore, pregnancy induced hypertension. In order to test this hypothesis, a single-blind randomized clinical trial was conducted. The objectives of the present research were to determine the plasma Hcy concentrations during the first trimester of pregnancy and at delivery, and also to evaluate the efficacy of low doses (0.5 mg/day) and high doses folic acid (5 mg/day) in reducing the incidence of PIH, in addition, to evaluate the effect of high doses of folic acid administration on these values, and in the prevention of preeclampsia.

Methods. A single blind, randomized trial was carried out in a population of nulliparous women who attended the antenatal out-patient clinic of Alzahra

Hospital, a tertiary care teaching hospital in Tabriz, East Azarbayejan Province, North West of Iran, from April 2005 to February 2008, to compare the effect of 2 different doses of folic acid (5 mg/day for group one, and 0.5 mg/day for group 2), to prevent pregnancy induced hypertension. After giving their informed consent, only healthy mothers with a singleton pregnancy, aged between 20-30 years old, without any history of medical problems including heart disease, chronic hypertension, diabetes mellitus, chronic renal disease, and collagen vascular diseases were included in the study. Mothers who had preterm delivery for other reasons except PIH, who did not come for delivery to the assigned teaching hospital, who had abortion, or were taking medications other than calcium and ferrous sulfate, were excluded. The serum concentrations of Hcy and uric acid, lactic dehydrogenase, and blood nitrogen urea were measured enzymatically and by radioimmunoassay at the first visit, and again at delivery. The plasma level of creatinine, platelet count, urine creatinine, and urine protein were also measured. Severe preeclampsia was defined as blood pressure (BP) $\geq 160/110$ mm Hg, proteinuria ≥ 2.0 g/24 hours, or $\geq 2+$ dipstick, serum creatinine >1.2 mg/dl, unless known to be previously elevated, platelets <100.000 mm³, microangiopathic hemolysis (increased lactate dehydrogenase [LDH]), elevated alanine aminotransferase (ALT), or aspartate aminotransferase (AST), persistent headache or other cerebral, or visual disturbance and persistent epigastric pain. The total Hcy concentration in plasma was measured according to axis homocystein enzyme immunoassay technology (EIA)(homocysteine EIA; FHCY100, Axis-Shield, UK). Blood samples for Hcy was collected in tubes containing ethylenediaminetetra acetic acid (EDTA), and centrifuged within one hour. The plasma was stored at 2-8°C, until the assay was performed within 72 hours after collection. Demographic and perinatal information were abstracted from the medical records. Blood pressure, weight, fundal height, fetal growth, edema, fetal heart rate, and type of delivery were recorded for each mother. From 16 weeks' gestation, calcium (1g/d) plus ferrous sulfate (60 mg/day) were administered to the participants. The study was approved by the Drug Applied Research Center of Tabriz University of Medical Sciences. Permission was obtained from the university ethics committee and all participants were given adequate information and consent was obtained from each volunteer.

The sample size of the study was determined by Cochran formula, with a confidence of 95%, where t is the confidence level, p is the ratio of the number of subjects with preeclampsia ($p=0.05$), q is the ratio of the participants that did not show preeclampsia ($q=0.95$), and $d=0.03$ of the obtained value for p . Therefore, the

number of subjects in the present study was calculated as: $(1.96)^2 \times 0.05 \times 0.95 / (0.03)^2 = 203$. The measured values were given as mean \pm standard deviation. Intragroup comparison was carried out using the one-way ANOVA test, and independent t-test was used to compare means between the 2 groups. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated for the independent predictors. For all statistical analyses, the differences were considered statistically significant at $p < 0.05$. The statistical analysis was performed by Statistical Package for Social Sciences program (SPSS version 14.0 for Windows).

Results. A total of 246 nulliparous pregnant women were included in the study and 200 participants met the inclusion criteria. The consort of the participants is shown in Figure 1. The demographic characteristics of the study population are summarized in Table 1. The results of independent t-test shows no significant differences between groups in the mean age of patients. In the group who received 5 mg/day folic acid, serum levels of Hcy at the first trimester was 11.8 ± 3.85 $\mu\text{mol/l}$, and at delivery was 6.44 ± 1.88 $\mu\text{mol/l}$. The mean serum levels of Hcy in group one was significantly lower at term in comparison to group 2 ($t=0.86$, $df=189.7$, $p < 0.001$). The result of laboratory parameters are shown in Table 2. The results of independent t-test to compare means between the 2 groups are also shown in Table 2. In group 2, at the time of delivery, serum concentrations of uric acid, and lactic dehydrogenase, were significantly higher than in group one. There was also no significant difference between groups in the mean arterial pressure (Table 3). In addition, there was no significant difference between groups in the weight at birth, and neonate hospitalization (Table 4). However, in the 2 stages of pregnancy, serum creatinine, urea levels, and platelet counts did not exhibit a significant change between the 2 groups. There was no clinically apparent case of any type of hypertension in each group. In addition, the results show that there was no significant difference between groups in the one-minute Apgar score of neonates (Table 5).

Discussion. The prevention of PIH and preeclampsia/eclampsia is a challenging goal. By promoting and improving maternal health and well being prior to the pregnancy, many risk factors can be mitigated with a good preconception and prenatal care. The role of folic acid and Hcy in the pathogenesis of PIH has been recently described.¹⁸ However, their relationship with preeclampsia/eclampsia has not well established.²⁸ Several studies were conducted to show an association between the level of Hcy and PIH disorders,^{8-10,12,13,28} Preeclampsia in nulliparous women

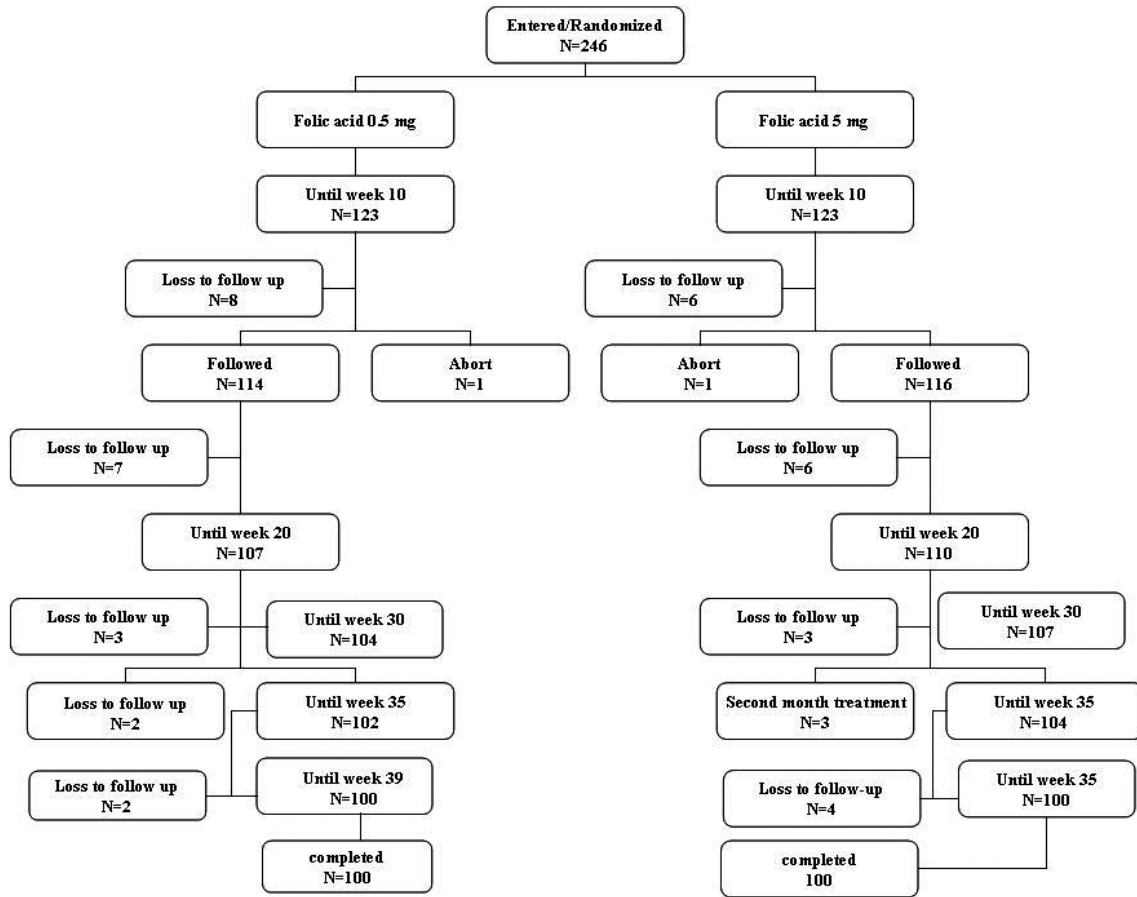


Figure 1 - The consort of participants.

Table 1 - Demographics and clinical variables.

Variables	Group 1 (mean±SD)	Group 2 (mean±SD)	t	df	P-value
Age (years)	24.28 ± 2.86	25.07 ± 3.52	1.73	194	0.08
Weight (kg)*	65.40 ± 8.92	64.43 ± 7.14	0.83	185.13	0.40
Weight (kg)†	77.05 ± 7.16	77.05 ± 7.16	1.29	194	0.19
BMI	24.94 ± 3.10	24.78 ± 2.68	0.37	193	0.70

Differences were considered statistically significant at $p < 0.05$, *at first prenatal visit, †at delivery, BMI - body mass index

Table 2 - The results of laboratory parameters in participants who took high dose and low dose folic acid.

Parameters	First trimester		Delivery		t	df	P-value
	High dose	Low dose	High dose	Low dose			
Homocysteine (µmol/l)	11.81 ± 3.85	9.08 ± 3.24	6.44 ± 1.88	7.44 ± 2.99	0.86	189.07	<0.001
Serum creatinine (mg/dl)	0.66 ± 0.063	0.70 ± 0.078	0.64 ± 0.077	0.68 ± 0.084	0.36	188.42	0.718
Urine creatinine (mg/dl)	71.99 ± 68.20	111.50 ± 56.7	23.51 ± 34.10	79.57 ± 68.64	1.93	197	0.045
LDH (U/L)	257.49 ± 66.2	255.83 ± 61.6	284.67 ± 74.81	262.75 ± 81.43	1.94	197	0.043
Urea (mg/dl)	15.09 ± 5.97	19.53 ± 5.64	15.27 ± 3.76	18.29 ± 5.88	1.59	197	0.112
Uric acid	3.58 ± 0.88	3.86 ± 0.83	4.24 ± 0.61	4.83 ± 1.24	1.90	182.03	0.049
Platelet count/µl	251.60 ± 6.43	25.25 ± 20.454	246.70 ± 1.406	223371 ± 4384	0.21	197	0.83
Urine protein (mg/24 hour)	25.9 ± 33.10	33.96 ± 36.55	44.29 ± 66.14	105.85 ± 109.1	5.22	175.10	<0.001

Differences were considered statistically significant at $p < 0.05$, LDH - lactate dehydrogenase

Table 3 - The results of participant's blood pressure at the first trimester and at delivery.

Blood pressure	First trimester		Delivery		t	df	P-value
	High dose	Low dose	High dose	Low dose			
Systolic BP	114.01 ± 8.78	117.24 ± 6.91	114.16 ± 9.05	117.23 ± 11.48	0.99	195	0.32
Diastolic BP	74.90 ± 7.45	76.46 ± 5.58	73.30 ± 8.90	76.69 ± 8.62	0.80	182.08	0.42

Differences were considered statistically significant at $p < 0.05$, BP - blood pressure (mm Hg)

Table 4 - The results of neonatal demographic parameters and hospital stay.

Parameters	Low dose	High dose	t	df	P-value
Neonatal height (cm)	50.76 ± 2.13	50.90 ± 5.30	0.277	196	0.78
Neonatal weight (g)	3236.01 ± 337.19	3331 ± 367.74	1.91	197	0.057
Head circumference (cm)	34.95 ± 1.33	34.81 ± 1.20	0.78	196	0.43
Hospitalization (day)	1.85 ± 1.91	1.71 ± 0.84	0.63	193	0.52

Differences were considered statistically significant at $p < 0.05$

Table 5 - The results of neonatal Apgar Score and types of delivery.

Variable	Group 1 n (%)	Group 2 n (%)	t	df	P-value
<i>Apgar</i>			1.41	1	0.37
Apgar minute 1 (9/10)	96 (96)	92 (92)			
Apgar minute 1 (8/10)	4 (4)	8 (8)			
<i>Type of deliver</i>			2.57	1	0.11
Vaginal delivery	45 (45)	51 (56.7)			
Cesarean	55 (55)	39 (43.3)			

Differences were considered statistically significant at $p < 0.05$

with elevated Hcy is 7.7-12.90 times more common than in normal controls.^{23,29,30} The association of hyperhomocysteinemia and preeclampsia has been suggested initially by Dekker et al.⁴ These authors demonstrated that hyperhomocysteinemia was 7 times more common in women with a history of severe preeclampsia. According to these authors, women with the early-onset of preeclampsia showed an 18% incidence of hyperhomocysteinemia compared with a 2.5% incidence in the normal population.⁴ Cotter et al^{10,11} and Noris et al³¹ reported 3-4 fold risk of preeclampsia in women with elevated serum Hcy levels. In another study on patients with severe early onset preeclampsia, 19% of preeclamptic patients had hyperhomocysteinemia compared with 4.5% of normal controls.³² Although most clinical studies substantiate this association, they have not shown elevated serum

Hcy levels to be a successful predictor.^{12,13} Homocysteine is a thiol (SH)-containing amino acid, which is involved in several key metabolic processes, including methylation and sulphuration pathways.^{33,34} Elevated plasma Hcy has been implicated in vascular changes through direct toxic and oxidative stress mechanisms, and can damage the decidual blood vessels, and result in faulty placentation resulting in blunted vasorelaxation mechanisms.^{35,36} Several studies carried out scientific work to show the role of folic acid and Hcy in PIH. In spite of the advantages of folate supplementation in age-related diseases, and several developmental abnormalities, data around the possible preventive effects on preeclampsia is less.³⁷ Whether it is a cause or effect, is still a matter of debate.³⁸ Evidence shows that plasma total Hcy depends on many physiological, pathological, and genetic determinants, which are closely

interrelated to each other.²⁷ In addition, reproductive failure from repeated miscarriages, neural tube defects, and placental abruption have been associated with hyperhomocysteinemia.³⁹ A genetic defect in Hcy synthesis and metabolism may be responsible.^{21,40-46}

The role of folic acid and Hcy in pregnancy is becoming clearer. Järvenpää et al⁴⁷ suggested the fortification of mineral water, and showed that improving folate status decreases plasma homocysteine concentration in pregnant women. There appears to be no evidence from the available data to clarify the possible consequences of long-term high-dose folic acid supplementation, especially those related to the methionine cycle, where folate participates as a substrate.^{21,47} Fernandez et al⁴⁸ in a study evaluated the plasma Hcy concentration and its relationship with the development of preeclampsia in women who had been administered folic acid prenatally. These authors did not find significant differences in Hcy concentrations, or the frequency of hyperhomocysteinemia in the different stages of pregnancy, between the women with normal gestation and those who developed preeclampsia. The sample size in this study was small, for that reason, the validity of such information remains unclear.⁴⁸ In our study, despite the levels of Hcy are decreased at delivery in the pregnant women who had been receiving folic acid 5 mg/d throughout the pregnancy in comparison to those who received 0.5 mg/d, blood pressure had not been increased in any group, and neonatal outcome was good in each group ($p>0.5$). Randomized trials of folic acid supplementation in the prevention of recurrent abruption,⁴⁹ and preeclampsia,¹⁹ have been conducted. Bodnar et al¹⁹ in a study, reported a 45% reduction in the preeclampsia risk in patients who had used multivitamins containing folic acid at less than 16 weeks' gestation (OR=0.55; 95% CI, 0.32-0.95).

These results may be related to the low levels of folate in the rest of the pregnancy. Therefore, considering a protective role for folic acid, in our study in the group who continued folic acid in a dose of 5 mg/day throughout pregnancy, the levels of Hcy were lower than the group who received 0.5 mg/d ($p<0.001$) (Table 2), and BP was not increased to the levels eclampsia/preeclampsia (Table 3) ($p>0.5$). In addition, according to our data, the results of taking 5 mg folic acid/day throughout the pregnancy were successful (Tables 2 and 3), and high dose folic acid consumption had good maternal and neonatal outcome (Tables 4 and 5). This was comparable with other studies, which only used folic acid in a dose of 5 mg at the first trimester, or early second trimester, and for the remainder of pregnancy, folic acid has only been continued in a dose of 0.5 mg/day.^{17,19,48,50-52} Charles et al⁵³ in the re-analysis of a large, well-conducted randomized placebo controlled

trial assessed the effects of a larger dose of folic acid (200 mg/day, and 5 mg/day) on the birth weight, gestational age at delivery, or stillbirth/neonatal death, but not preeclampsia, and showed no significant effect. All of these studies used high dose folic acid in the first trimester and were not examined thereafter.⁵³ In our study, there was no major congenital abnormality, and the rate of abortion and preterm delivery was not different in each group (Figure 1). Achon et al⁵⁰ evaluated the possible effects of high dose folic acid (folic acid-supplemented, 40 mg/kg diet versus control, 2 mg folic acid/kg diet), in virgin and pregnant Wistar rats, and found that gestation outcome was adequate in both groups regardless of the dietary supplementation. Similar to our study, serum homocysteine levels were reduced in supplemented rats.⁵⁰ Recently, Wen et al⁵⁴ prescribed multivitamins containing folic acid successfully in the second trimester, and their results showed a reduction in the rate of preeclampsia.

The value of treating elevated homocysteine concentrations with folic acid and vitamin B6 and B12 supplements, to reduce cardiovascular morbidity is under active research. Hyperhomocysteinemia caused by the mutation 677 CT of methylenetetrahydrofolate reductase (MTHFR), can be corrected with folic acid administration.⁵⁵ In another study, there was no difference in folic acid and vitamin B12 levels between pooled normal and preeclamptic groups, but these levels were significantly lower in patients with the 677 CT mutation of MTHFR.⁵⁶ Similarly in a systematic review by Mignini et al,⁵⁷ folate and B12 concentrations were lower in the preeclamptic women compared with the normotensive women, but the differences did not also reach significance. Powers et al⁵⁸ did not find any differences in the folic acid concentrations between preeclamptic and normal pregnant women, too.⁵⁸ Both folate and betaine are important predictors of total Hcy during pregnancy. Wallace et al⁵⁹ showed that betaine is a significant predictor of total Hcy in pregnant women with low serum folate, and low serum methionine concentrations.

In addition to preeclampsia, hyperhomocysteinemia was also associated with complications in pregnancy such as neural tube defects, repeated miscarriages, abruptio placentae, fetal death, intrauterine growth retardation,^{17,24,27,60} and thereafter, and may predispose the child to congenital heart disease (CHD).⁶¹ Also, an association has been reported between the elevated level of prenatal homocystein, Down's syndrome,⁴¹ adult cardiovascular disease,⁶² and schizophrenia.⁶³ Elevated third-trimester homocystein levels may elevate schizophrenia risk through developmental effects on brain structure, and function and/or through subtle damage to the placental vasculature that compromises

oxygen delivery to the fetus.⁶³ These data may imply that the hyperhomocysteinemic mothers and their children should be targeted for nutritional interventions. According to Wald et al,⁶⁴ the minimal effective dose of folic acid that can lower the serum homocysteine level by approximately 3 mmol/L is 0.4 mg per day, an effect that is associated with a decrease of approximately 15% in the risk of ischemic cardiac events, and a decrease of approximately 25% in the risk of stroke, deep venous thrombosis, and pulmonary embolism. There is no evidence that folic acid fortification at these levels poses a risk to health. According to our finding in this study, consumption of high dose folic acid was more effective than low dose folic acid in lowering the levels of Hcy, although the levels of BP were not different significantly in each group (Table 3). Czeizel et al,⁶⁵ showed a role for a high dose (for example 6 mg) of folic acid, in the prevention of cleft lip and palate, and recommended it for the reduction of recurrent orofacial clefts during early pregnancy under medical control. These authors showed that preconceptional daily supplementation with multivitamins including physiologic doses (<1 mg) of folic acid can not reduce the birth prevalence of isolated cleft lip±cleft palate and cleft palate. Only the high pharmacological doses (for example, 6 mg/day) of folic acid alone, in the critical period of the primary and the secondary palate development seem to be effective for the reduction of orofacial clefts. This strategy has been used to prevent congenital abnormalities and the underlying pathology around the effect on Hcy metabolism still remain unsolved. Hence, continuing to consume folic acid in a dose of 5 mg throughout the pregnancy is not only logical, but is also preventative. The possible concern associated with folic acid supplementation is on the possible rare side effects, for example, in women with pernicious anemia arise because of a misplaced worry that it might mask vitamin B12 deficiency, by partially correcting the associated anemia while allowing associated neurologic dysfunction to progress. According to Mills et al,⁶⁶ all of the patients with a vitamin B12 deficiency have no anemia and folic acid fortification does not cause a masking effect. Recently, Smith et al⁶⁷ showed that in pregnant women, high folate levels and low vitamin B-12 status may be associated with an increased risk of insulin resistance, and obesity in their children.

Charles et al⁵³ studied the baseline serum folate in women who booked for antenatal care prior to, and after 17 weeks gestation. They suggested further large trials assessing the role of folate supplementation, to clarify the role of folate in antepartum hemorrhage, pre-eclampsia, and low birthweight.⁵³ According to the results of this trial, the effect of folic acid taken throughout the pregnancy is unclear, and folic acid supplementation commenced after the first trimester of pregnancy,

confers any benefit, and supports the recommendation that periconceptual folate supplements should not be continued throughout the pregnancy. In this study, folic acid was only given at 400 µg per day. Leeda et al⁶⁸ showed that women with a history of pre-eclampsia may benefit from folic acid supplementation in subsequent pregnancies. Barton and Sibai,⁶⁹ in women with a history of previous preeclampsia, recommend more frequent monitoring for signs and symptoms of severe hypertension or pre-eclampsia. They also suggested a strategy to detect obstetric complications as early as possible, to reduce risk factors by optimizing maternal health before conception, and to detect obstetric complications as early as possible.⁶⁹

Stegers-Theunissen et al⁷⁰ suggested to perform a preconceptional cohort study before, during, and after pregnancy to answer the question of whether hyperhomocysteinemia is a risk factor for PIH or not, and how long after pregnancy one could perform the methionine loading test to identify hyperhomocysteinemia as a preconceptional risk factor for vascular-related pregnancy complications.⁷⁰

Our study is the first study to assess the prevalence of PIH disorders in pregnant women who took folic acid in a dose of 5 mg/day throughout pregnancy. Until now, most of investigations were on the effect of periconceptual use of folic acid on congenital abnormalities and its effect on the level of Hcy, hence atherosclerosis and endothelial dysfunction. Keeping in mind that pathophysiological changes gain momentum across gestation will not relieve until delivery and thereafter. So, if there is an effect, its use until delivery is justified. We did not use any additional vitamins in the studied groups, except for patients who had nausea or vomiting, we used vitamin B6 early in the pregnancy. The results of our study also did not show any adverse outcome in pregnant women who consumed 5 mg/day folic acid throughout the pregnancy, we measured the Hcy levels at the first prenatal visit. However, our study on this issue had several weaknesses. Our sample size was small. There was no other study to compare the effect of high dose folic supplementation throughout the pregnancy to compare the results. Future research is recommended with larger sample sizes, to increase the validity of the study findings with additional studies that examine the association between high dose maternal folate serum levels and Hcy levels, and cord blood concentrations of Hcy and folate and subsequent outcomes.

The results show that there is no difference between folic acid administration in a dose of 5 or 0.5 mg throughout the pregnancy with respect to hypertensive disorders, but is accompanied by improvement in clinical and laboratory outcomes with respect to Hcy levels. If future studies both replicate this association and support

a causal link, then the use of folic acid supplementation would merit evaluation as a strategy for the prevention of preeclampsia/eclampsia. Measuring Hcy level in early pregnancy routinely, may recognize the high risk patients for pre-eclampsia. According to the results of this study, high dose folic acid could be administered safely during pregnancy, and could be recommended for routine clinical practice. We therefore, suggest that it will be prudent to prescribe folic acid at 5 mg/day for pregnant women, for prevention of PIH in their second and third trimesters, in addition to the first trimester.

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