THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE

http://informahealthcare.com/jmf
ISSN: 1476-7058 (print), 1476-4954 (electronic)

J Matern Fetal Neonatal Med, Early Online: 1–5
 DOI: 10.3109/14767058.2015.1023189

informa healthcare

ORIGINAL ARTICLE

5-Methyl-tetrahydrofolate in prevention of recurrent preeclampsia

Gabriele Saccone[1], Laura Sarno[1], Amanda Roman[2], Vera Donadono[1], Giuseppe Maria Maruotti[1], and Pasquale Martinelli[1]

[1]*Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy and* [2]*Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, PA, USA*

Abstract

Objective: To evaluate the efficacy of 5-methyl-tetrahydrofolate (5-MTHF) supplementation in prevention of recurrent preeclampsia.

Methods: Retrospective cohort of women who received daily oral 5-MTHF 15 mg supplementation as prophylactic treatment since first trimester for recurrent preeclampsia were compared with women who did not. All asymptomatic singleton gestations with prior preeclampsia (in the previous pregnancy) were included. Women with chronic hypertension were excluded. The primary outcome was the incidence of preeclampsia.

Results: Three hundred and three singleton gestation met the inclusion criteria: 157 received 5-MTHF, while 146 did not (control group). Women who received 5-MTHF had a significantly lower incidence of recurrent overall preeclampsia (21.7% versus 39.7%; odds ratio (OR) 0.57, 95% confidence interval (CI) 0.25, 0.69), severe preeclampsia (3.2% versus 8.9%; OR 0.44, 95% CI 0.12–0.97) and early-onset preeclampsia (1.9% versus 7.5%; OR 0.34, 95% CI 0.07–0.87) compared to control. The intervention group delivered about 10 d after the control and had higher birth weight.

Conclusion: This retrospective study showed that women with prior preeclampsia who received daily oral 5-MTHF 15 mg supplementation had a significantly lower incidence of overall preeclampsia, severe preeclampsia and early-onset preeclampsia. Randomized controlled trials are needed to confirm our findings.

Keywords

Folic acid, hypertension, prevention, recurrent, supplementation

History

Received 9 February 2015
Revised 19 February 2015
Accepted 23 February 2015
Published online 17 March 2015

Introduction

Preeclampsia is a leading cause of maternal and neonatal mortality and morbidity, complicating up to 5% of all pregnancies [1]. Earlier preeclampsia is one of the most important risk factors for preeclampsia [2]. The reported rate of recurrent preeclampsia ranges from 11.5% to 65% [2]. Many research has been published about this topic and it is still a challenge for obstetricians [3,4].

Data from observational studies showed that folic acid (FA), which is normally used to prevent neural tube defects [5], may have a role in the prevention of pregnancy complications such as preterm birth and small for gestational age [6,7]. In 2014, Kim et al. showed that the concentration of folate in maternal blood was inversely associated with the risk of developing preeclampsia [8]. However, so far the effect of FA on preeclampsia is still controversial [9–12].

5-Methyl-tetrahydrofolate (5-MTHF) is the predominant form of dietary folate and the only species normally found in the circulation [13]. 5-MTHF is also available commercially as a crystalline form of the calcium salt, which has the stability required for use as supplement [14]. A recent study found that 5-MTHF is more effective than FA in improving folate status and that it has a better bioavailability [14]. For this reason, 5-MTHF could be a good alternative to FA [15,16].

The aim of this study is to evaluate the efficacy of daily oral 5-MTHF 15 mg supplementation in prevention of recurrent preeclampsia in asymptomatic singleton gestations with prior preeclampsia.

Materials and methods

This is a retrospective study using data collected prospectively from clinical records of women with prior preeclampsia who were referred to the Division of High Risk Pregnancies (University of Naples Federico II) from January 2009 to August 2013. Started from January 2009, we offered daily oral 5-MTHF 15 mg supplementation and low dose aspirin starting on the 1st trimester assessment prior to 14 weeks of gestation to all women with singleton pregnancy and diagnosis of preeclampsia in the immediately previous pregnancy. All women were tested for inherited thrombophilia. Data of

Address for correspondence: Pasquale Martinelli, MD, Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy. Tel: 0039/0817462966. E-mail: martinel@unina.it

the pregnancy affected with preeclampsia and the subsequent pregnancy were collected and retrospectively analyzed.

Women were encouraged to breastfeed their infants. All data were registered in a dedicate database. Data were collected with the patient consent and were anonymized before analysis.

In this retrospective study, women who received 5-MTHF 15 mg daily as prophylactic treatment for recurrent preeclampsia were compared with women who declined this prophylactic treatment. All women included in the study received low dose of aspirin [17,18].

Exclusion criteria included women with prior gestational hypertension but not preeclampsia, more than one prior pregnancy, one prior preeclampsia but not in the immediately previous pregnancy, chronic hypertension, multiple gestations, MTHFR mutations (due to the related alteration of homocysteine level) and inherited or acquired thrombophilia, lack of 1st trimester assessment in our hospital, women who delivered at a different hospital and those who received assisted reproductive technology. Anomalous and aneuploidy fetuses were also excluded.

The primary outcome was designed *a priori* (i.e. the incidence of overall preeclampsia). Secondary outcomes included incidence of severe preeclampsia, early-onset (i.e. preeclampsia requiring delivery before 34 weeks) and late-onset preeclampsia (i.e. preeclampsia requiring delivery at or after 34 weeks), and neonatal outcomes including birth weight, admission to neonatal intensive care unit, respiratory distress syndrome (RDS) (grade 1–4), intra-ventricular hemorrhage (grade 1–4), culture-proven sepsis and necrotizing enterocolitis (grade 1–4). Diagnosis and management of preeclampsia and severe preeclampsia were based on ACOG and SMFM guidelines [19,20]. Preeclampsia (mild preeclampsia) is defined as a blood pressure elevation ($\geq$140/90 on two occasions 4 h apart or $\geq$160/110 once), after 20 weeks of gestation, with proteinuria ($\geq$300 mg on 24-h protein or >0.3 protein/creatinine ratio) or any of the following if proteinuria not present: platelets <100 000; creatinine >1.1 (or doubling of creatinine in absence of other renal disease); doubling of AST or ALT [20]. Preeclampsia with severe features (severe preeclampsia) is defined as preeclampsia with any of the following: blood pressure $\geq$160/110, 4 h apart on bed rest (unless on anti-hypertensive); platelets <100 000; doubling of AST or ALT; creatinine >1.1 (or doubling of creatinine in absence of other renal disease); pulmonary edema; new cerebral or visual disturbances [20].

Our data were collected prospectively from 2009, but we retrospectively classified preeclampsia based on the new guidelines [20]. Data on neonatal outcomes were obtained by neonatal folders. We planned sub-group analysis in women without chronic medical conditions.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) v. 19.0 (IBM Inc., Armonk, NY). Data were shown as means ± standard deviation or as number (percentage). Categorical variables were compared using the chi-square or Fischer exact test. Within-group comparison was undertaken using Wilcoxon and Mann–Whitney tests. p value < 0.05 was considered statistically significant. Results of the variables statistically significant were presented as odds ratio (OR) with 95% of confidence interval (CI). The study was performed following the STROBE guidelines [21].

Results

Three hundred and three singleton gestations with a prior preeclampsia in the immediately previous pregnancy met the inclusion criteria and were included in this study.

The two groups were similar in terms of maternal demographics: both of them had preeclampsia at the similar gestational age in the past and with similar phenotype (Table 1). Of the 303 women included, 157 (51.8%) received daily oral 5-MTHF 15 mg, while 146 (48.2%) did not (control group). The control group declined 5-MTHF mostly for economic reasons. All women received low dose of aspirin (100 mg daily) starting <14 weeks of gestation. No women received low molecular weight heparin during pregnancy. About one-third of women in each group had other maternal medical condition (e.g. diabetes mellitus, vasculitides, lupus), but no differences were found between the two groups.

Women who received 5-MTHF had a significantly lower overall incidence of recurrent preeclampsia (21.7% versus 39.7%; OR 0.57, 95% CI 0.25–0.69), mild preeclampsia (18.5% versus 30.8%; OR 0.37, 95% CI 0.18–0.74), severe preeclampsia (3.2% versus 8.9%; OR 0.44, 95% CI 0.12–0.97), early-onset preeclampsia (1.9% versus 7.5%; OR 0.34, 95% CI 0.07–0.87), RDS (6.4% versus 15.7%; OR 0.38, 95% CI 0.14–0.57) and intubation (1.8% versus 6.8%; OR 0.25, 95% CI 0.07–0.89) compared to controls. The intervention group delivered about 10 d after control (259 versus 249 d; mean difference 10.00 d, 95% CI 3.80–16.20) and had higher birth weight (2983 versus 2518 g; mean difference 465.00 g, 95% CI 246.29–683.71) (Table 2). Given the possibility of dissimilar baseline risks between the two comparison groups, a comparability score is advised in order to adjust the analysis for differences in the confounding interventions impact. The analysis of primary outcome (i.e. incidence of recurrent preeclampsia) based on comparability score had no effect on the overall OR estimate but affected the 95% CI (reweighted OR 0.57, 95% CI 0.27–0.88). However, the results were still statistically significant. No women reported adverse drug reactions and none of them required admission to intensive care unit. We reported two stillbirths in the control group in women who developed HELLP syndrome.

Among women without medical conditions (188/303, 62.1%), we found that women who received 5-MTHF had a significantly lower incidence of overall preeclampsia (20.2% versus 39.3%; OR 0.51, 95% CI 0.32–0.82), severe preeclampsia (3.0% versus 11.2%; OR 0.10, 95% CI 0.03–0.33) and early-onset preeclampsia (2.0% versus 10.1%; OR 0.20, 95% CI 0.04–0.90) compared to controls. No differences were found in terms of maternal demographics between the two groups (Table 3).

Discussion

Our retrospective cohort of singleton gestations with prior preeclampsia showed that women who received daily oral 5-MTHF 15 mg supplementation had a significantly lower incidence of overall recurrent preeclampsia, severe preeclampsia, mild preeclampsia, early-onset preeclampsia,

Table 1. Maternal demographic characteristics.

	5-MTHF 157 (51.8%)	Control 146 (48.2%)	*p* value
Age			
Mean ± SD	31.5 ± 5.8	30.9 ± 6.0	0.590
BMI			
Mean ± SD	28.6 ± 7.7	26.1 ± 4.7	0.510
>30	34 (21.7%)	31 (21.1%)	0.975
Smoking	25 (15.9%)	24 (16.4%)	0.954
Race			
Caucasian	157 (100%)	146 (100%)	1.00
Gravidity			
Median (range)	2.3 (1–4)	2.1 (1–4)	0.745
Family history of hypertension	57 (36.3%)	55 (37.7%)	0.688
Chronic disease*	58 (36.8%)	57 (39.1%)	0.840
Diabetes mellitus	15 (9.6%)	12 (8.2%)	0.621
Celiac disease	20 (12.7%)	25 (17.1%)	0.098
Vasculitis	1 (0.6%)	0	0.845
LES	3 (1.9%)	2 (1.3%)	0.882
Addison	1 (0.6%)	0	0.845
CREST syndrome	1 (0.6%)	2 (1.3%)	0.329
Crohn's disease	5 (3.2%)	2 (1.3%)	0.129
Graves' disease	4 (2.5%)	3 (2.1%)	0.519
Hashimoto's thyroiditis	8 (5.1%)	11 (7.5%)	0.144
GA at the prior delivery			
Mean ± SD (days)	243.7 ± 23.8	248.1 ± 27.6	0.712
Mean ± SD (weeks)	34.8 ± 3.4	35.4 ± 3.9	0.654
Prior severe PE	26 (16.6%)	27 (18.4%)	0.423
Prior mild PE	131 (83.4%)	119 (81.6%)	0.745

5-MTHF, 5-methyl-tetrahydrofolate; SD, standard deviation; GA, gestational age; PE, preeclampsia.
*No women had more than one diagnosis of chronic disease.

Table 2. Primary and secondary outcomes.

	5-MTHF 157 (51.8%)	Control 146 (48.2%)	*p* value
Incidence of overall PE	34 (21.7 %)	58 (39.7%)	**0.019**
Mild PE	29 (18.5%)	45 (30.8%)	**0.022**
Severe PE	5 (3.2%)	13 (8.9%)	**0.041**
Early-onset PE	3 (1.9%)	11 (7.5%)	**0.033**
Late-onset PE	31 (19.7%)	47 (32.2%)	**0.023**
GA at delivery			
mean ± SD (d)	259.1 ± 23.6	249.7 ± 30.7	**0.047**
HELLP	1 (0.6%)	3 (2.1%)	0.179
Cesarean delivery	90 (57.3%)	82 (56.2%)	0.769
Birth weight			
mean ± SD (g)	2983 ± 987	2518 ± 955	**0.001**
NICU admission	15 (9.5%)	23 (15.7%)	0.094
RDS	10 (6.4%)	23 (15.7%)	**0.032**
Intubation	3 (1.8%)	10 (6.8%)	**0.038**
IVH	3 (1.8%)	4 (2.7%)	0.443
Sepsis	3 (1.8%)	2 (1.1%)	0.634
NEC	2 (1.3%)	3 (2.3%)	0.584

5-MTHF, 5-methyl-tetrahydrofolate; SD, standard deviation; PE, preeclampsia; GA, gestational age; NICU, admission to neonatal intensive care unit; RDS, respiratory distress syndrome; IVH, intra-ventricular hemorrhage; Sepsis, culture-proven sepsis; NEC, necrotizing enterocolitis.
Boldface data: statistically significant.

late-onset preeclampsia, RDS and neonatal intubation compared to women who did not. The 5-MTHF group delivered about 10 d later than control group and had higher birth weight.

One of the strengths of our study is the inclusion of a specific population, i.e. singleton gestations with prior preeclampsia. This is the sub-group of women at greatest risk for recurrence preeclampsia [2,3]. Primary and secondary outcomes were established *a priori*. We planned sub-group analysis in women without medical chronic conditions. This may be the first study in the literature evaluating the efficacy of oral 5-MTHF supplementation (initiated in the first trimester) for the prevention of preeclampsia. No similar publication were found by a systematic review: searches were performed in MEDLINE, OVID, Scopus, Sciencedirect.com, ClinicalTrials.gov and EMBASE with the use of a

Table 3. Maternal demographic characteristics among women without chronic medical conditions.

	5-MTHF 99 (52.7%)	Control 89 (47.3%)	*p* value
Age			
Mean ± SD	29.7 ± 6.6	28.9 ± 5.4	0.788
BMI			
Mean ± SD	28.8 ± 6.7	27.3 ± 5.1	0.714
>30	25 (25.3%)	31 (21.1%)	0.975
Smoking	25 (15.9%)	17 (19.1%)	0.343
Race			
Caucasian	99 (100%)	89 (100%)	1.00
Gravidity			
Median (range)	1.7 (1–3)	2.3 (1–4)	0.245
Family history of hypertension	15 (15.2%)	9 (10.1%)	0.273
GA at the prior delivery			
Mean ± SD (d)	239.5 ± 21.8	244.3 ± 25.6	0.533
Mean ± SD (weeks)	34.2 ± 3.1	34.9 ± 3.7	0.497
Prior severe PE	14 (14.2%)	15 (16.9%)	0.714
Prior mild PE	85 (85.8%)	74 (83.1%)	0.736

5-MTHF, 5-methyl-tetrahydrofolate; SD, standard deviation; GA, gestational age; PE, preeclampsia.

combination of keywords related to ''folic acid'', ''pre-eclampsia'', ''hypertension'' and ''5-methyl-tetrahydrofolate'' from inception of each database to December 2014.

The most important limitation of our study is that this is a retrospective, non-randomized comparison. *A priori* power analysis could not be assessed due to its retrospective nature [22]. The number of women included were small due to the restrictive inclusion criteria; however, the confidence intervals of odds ratio are quite narrow [23]. The confidence intervals are more statistically useful than *post-hoc* power calculations [23]. The level of dietary intake of folate could not be assessed by blood analysis. Compliance with therapy was not measured. No cost-effectiveness analysis was assessed. No data regarding weight gain during pregnancy were available. The control group refused 5-MTHF because of financial reasons; this raises the strong possibility that the women in this group were most likely of lower socio-economical class and maybe of higher baseline risk for developing preeclampsia. This could also explain the unusually high recurrence rate of preeclampsia in the control group. All women received low-dose aspirin as well, it was difficult to evaluate if the outcome is due to an additive effect or due only to 5-MTHF.

The biological plausibility to explain our results is not completely clear. 5-MTHF is the most active form of reduced FA circulating plasma, which enters directly into the metabolic process of folate and so far no potential adverse and toxic effects of oral 5-MTHFR supplementation have been reported in the literature [14–16,24]. Folate is involved in the metabolism of homocysteine and methionine. Homocysteine, a metabolite of the methionine, can be re-methylated into methionine, and the required methyl group is obtained from 5-methyltetrahydrofolate. Folate deficiency can interfere with homocysteine re-methylation, leading to high concentration of homocysteine [25]. Hyperhomocysteinemia seems to be involved in oxidative stress and endothelial cell dysfunction that are both associated with preeclampsia [26]. Moreover, it is known that folate plays a role in placental development and it has been reported to be positively associated with intrauterine growth restriction [27–29]. In contrast, hyperhomocysteinemia has been related to be inversely associated with placental and fetal growth [30].

Large well-designed placebo-controlled randomized trials, including evaluation of maternal serum and umbilical cord folate levels and calculating cost and cost-effectiveness of 5-MTHF, are needed to confirm our findings. We observed that with an α of 0.05 and 80% power, a sample size of 200 women in each group is required to detect a reduction in the incidence of recurrent preeclampsia from 39.7% to 21.7%.

Acknowledgements

We would like to acknowledge Biagio Simonetti, PhD, for his assistance with the statistical analysis.

Declaration of interest

The authors report no conflicts of interest. No financial support was received for this study.

References

1. World Health Organization (WHO). Make every mother and child count. Geneva: World Health Report; 2005.
2. Barton JR, Sibai BM. Prediction and prevention of recurrent preeclampsia. Obstet Gynecol 2008;112:359–72.
3. Sibai BM. Prevention of preeclampsia: a big disappointment. Am J Obstet Gynecol 1998;179:1275–8.
4. Caritis S, Sibai B, Haulth J, et al. Low dose aspirin to prevent preeclampsia in women at high risk. National Institute of Child Health and Human Development Network of Maternal Fetal Medicine Units. N Eng J Med 1998;338:701–5.
5. Cheschier N, ACOG Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin. Neural tube defects. Number 44, July 2003 (Replaces committee opinion number 252, March 2001). Int J Gynaecol Obstet 2003;83:123–33.
6. Hodgetts V, Morris R, Francis A, et al. Effectiveness of folic acid supplementation in pregnancy on reducing the risk of small-for-gestational age neonates: a population study, systematic review and meta-analysis. BJOG 2015;122:478–90.
7. Mantovani E, Filippini F, Bortolus R, Franchi M. Folic acid supplementation and preterm birth: results from observational studies. Biomed Res Int 2014;2014:481914.
8. Kim MW, Ahn KH, Ryu KJ, et al. Preventive effects of folic acid supplementation on adverse maternal and fetal outcomes. PLoS One 2014;9:e97273.

9. Wen SW, Champagne J, Rennicks WR, et al. Effect of folic acid supplementation in pregnancy on preeclampsia: the folic acid clinical trial study. J Pregn 2013;2013:294312.
10. Theriaults S, Giguere Y, Masse J, et al. Absence of association between serum folate and preeclampsia in women exposed to food fortification. Obstet Gynecol 2013;122:345–51.
11. Salehi-Pourmehr H, Mohamad-Alizadeh S, Malakouti J, Farshbaf-Khalili A. Association of the folic acid consumption and its serum levels with preeclampsia in pregnant women. Iran J Nurs Midwifery Res 2012;17:461–6.
12. Li Z, Ye R, Zhang L, et al. Folic acid supplementation during early pregnancy and the risk of gestational hypertension and preeclampsia. Hypertension 2013;61:873–9.
13. Zhao R, Matherly LH, Goldman ID. Membrane transporters and folate homeostasis: intestinal absorpition and transport into systemic compartments and tissues. Expert Rev Mol Med 2009;11:e4.
14. Pietrizk K, Bailey L, Shane B. Folic acid and L-5-methyltetrahydrofolate: comparison of clinical pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2010;49:535–48.
15. Obeid R, Holzgreve W, Pietrzik K. Is 5-methyltetrhahydrofolate an alternative to folic acid for the prevention of neural tube defects? J Perinatal Med 2013;41:469–83.
16. Seremak-Mrozikiewicz A. Metafolin – alternative for folate deficiency supplementation in pregnant women. Ginekol Pol 2013;84:614–16.
17. Henderson JT, Whitlock EP, O Connor E, et al. Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: a systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med 2014;160:695–703.
18. Bujold E, Roberge S, Lacasse Y, et al. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. Obstet Gynecol 2010;116:402–14.
19. Publications Committee, Society for Maternal-Fetal Medicine, Sibai BM. Evaluation and management of severe preeclampsia before 34 weeks' gestation. Am J Obstet Gynecol 2011;205:191–8.
20. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 2013;122:1122–31.
21. Von Elm E, Altman DG, Egger M, et al., for the STROBE Initiative. The strengthening the reporting of the observational studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 2007;370:1453–7.
22. Peterson AM, Nau DP, Cramer JA, et al. A checklist for medication compliance and persistence studies using retrospective database. Value Health 2007;10:3–12.
23. Smith AH, Bates MN. Confidence limit analyses should replace power calculation in the interpretation of epidemiologic studies. Epidemiology 1992;3:449–52.
24. Henderson GI, Perez T, Schenker S, et al. Maternal-to-fetal transfer of 5-methyltethrahydrofolate by the perfused human placental cotyledon: evidence for a concentrative role by placental folate receptors in fetal folate delivery. J Lab Clin Med 1995;126:184–203.
25. Yang XL, Tian J, Liang Y, et al. Homocysteine induces blood vessel global hypomethylation mediated by LOX-1. Genet Mol Res 2014;13:3787–99.
26. Powers RW, Evans RW, Majors AK, et al. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. Am J Obstet Gynecol 1998;179:1605–11.
27. Steegers-Theunissen RP, Obermann-Borst SA, kremer D, et al. Periconceptional Maternal Folic acid use of 400 mg per Day is related to increased methylation of the IGF2 gene in the very young child. PLoS One 2009;4:e7845.
28. Scholl TO, Hediger ML, Schall JI, et al. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr 1996;63:520–5.
29. Goldenberg RL, Tamura T, Cliver SP, et al. Serum folate and fetal growth retardation: a matter of compliance? Obstet Gynecol 1992;102:777–85.
30. Van der Molen EF, Verbruggen B, Novakova I, et al. Hyperhomocysteinemia and other thrombotic risk factor in women with placental vasculopathy. BJOG 2000;107:785–91.