Folic acid intake during pregnancy reduced the incidence of pregnancy-induced hypertension in Tibetan Nationality of Tibet Plateau: a population based cohort study

Xiaohong Xu (✉ 1849649017@qq.com)
Beijing Obstetrics and Gynecology Hospital

Ming Wang
Beijing Obstetrics and Gynecology Hospital

Ziyi Zhao
Beijing Obstetrics and Gynecology Hospital

Zhuoma Pubu
Lasha People's Hospital

Yang Ge
Lhasa People's Hospital

Zhuoma Bianba
Lhasa People's Hospital

Research article

Keywords: Pregnancy Induced Hypertension, Folic Acid, Homocysteine, Anemia, Birth Defect.

DOI: https://doi.org/10.21203/rs.3.rs-40850/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Objective The objective of this study was to investigate the effect of maternal supplementation with folic acid on pregnancy induced hypertension (PIH) and fetal outcomes in Tibetan Nationality of Tibet Plateau.

Design A retrospective cohort study.

Setting Lasha People’s Hospital, China Participants Pregnant mothers of Tibetan Nationality on Tibet Plateau from Jan 2012 to Jan 2016.

Primary and secondary outcomes Negative maternal and fetal outcomes between mothers who received folic acid supplement and mothers did not. Frequency of pregnancy induced hypertension were main outcome.

Results 6700 pregnant women were included in the study, 18.37% (1231/6700) mothers (group A) received folic acid supplement during pregnancy and 24.37% (300/1231) of them (group A1) having supplement of folic acid longer than 3 months. 1230 mothers did not receive folic acid supplement were selected as control (group B) through propensity score matching. In comparison, mothers of group A have a lower frequency of PIH than group B (5.8 % vs. 19.3 %, P<0.001), including gestational hypertension (1.87 % vs. 4.47 %), mild Pre-eclampsia (2.11 % vs. 5.69 %), severe Pre-eclampsia (1.87 % vs. 8.54 %) and Eclampsia (0 % vs. 0.57 %). However, Longer duration of folic acid supplement did not reduced the incidence of PIH. Before delivery, mothers of group A had less anemia (31.7 % vs. 20.1 %, P <0.001) and much higher hemoglobin (122.3±16.02 g vs. 115.5±16.76 g, P <0.001) than group B. After delivery, more preterm infants, more neonatal asphyxia, lighter weight, shorter height, smaller head circumference and pediatric admission were observed in infants of group B than group A.

Conclusion Among pregnant women of Tibetan Nationality on Tibet Plateau, folic acid supplement could reduce the incidence of pregnancy induced hypertension, anemia and related negative outcomes of infants.

Background

Pregnancy induced Hypertension (PIH) is a common hypertensive disorders during pregnancy, which include gestational hypertension, preeclampsia and eclampsia. It is estimated that PIH complicates 6-10% of pregnancies and preeclampsia complicates 2-8% pregnancies globally. PIH are one of the leading causes of maternal and perinatal mortality worldwide, which are responsible for 9% of deaths hypertensive disorders in Africa and Asia, even almost 26% of maternal deaths in Latin America and the Caribbean. Also, pregnancy induced hypertension is a risk factor for diabetes and cardiovascular disease in later life. Preeclampsia is thought to be caused by superficial placenta invasion, however, other risk factors for preeclampsia remain unclear.

Folic acid (FA) are required for methionine-Hcy metabolism and intake deficiency can result in increased Homocysteine(Hcy) concentrations. Hyperhomocysteinemia affects the walls of blood vessels, causing endothelial changes and smooth muscle proliferation which further lead to cardiovascular disease and vasculopathy. It has been assumed that hyperhomocysteinemia may contribute to the development of placental microvascular diseases and affecting the endothelium adversely, then elevated levels of blood homocysteine is a risk of gestational hypertension and preeclampsia. A small but growing body of evidence has suggested that folic acid supplementation could decrease the incidence of preeclampsia and gestational hypertension. However, other studies thought that daily consumption of 400 μg folic acid during early pregnancy cannot prevent the occurrence of gestational hypertension and preeclampsia. Now, the 0.4mg folic acid were free distributed in many part of China. Whether folic acid supplementation alone in pregnancy can prevent the occurrence of gestational hypertension/ preeclampsia remains uncertain in several meta-analysis.

Tibetan people live at a high altitude of average 3,650 metres above the sea level, where people have to adapt to the unavoidable environmental stress of high altitude hypoxia for the reduced atmospheric pressure. People lived in high altitude
have large quantitative differences in numerous physiological and molecular traits involved in oxygen delivery. The genetic variance avail themselves of essentially the full range of oxygen-using metabolism as populations at sea level\textsuperscript{18}. An average increase of 1 lnHcy (log transformation of total Hcy level) was associated with an increase of 3.78mmHg of systolic blood pressure (BP) and 3.02mmHg of diastolic BP among Tibetans in China\textsuperscript{19}.

Because the aforementioned studies showed different results between the occurrence of preeclampsia or gestational hypertension and the consumption of folic acid alone during early pregnancy. Besides, little is known about the role of folic acid in Tibetan women, then more comparative study is needed to enhance the body of knowledge and determine whether there is the need to explore folic acid supplement on pregnancy induced hypertensions in Tibetan Nationality of Tibet Plateau.

**Methods**

**Study Design :**

This was a retrospective cohort study performed from January 2012 to January 2016 at the Lasha People's Hospital, which provided health care service for 50 percent pregnant women of Tibet Plateau. The trial was approved by the ethics review committee of Lasha People's Hospital (approval number: SYLL2217001). Subjects were screened for the following eligibility criteria: age between 20 and 45 years; all participants had singleton pregnancies and progressed into the third trimester. Patients who had a history of chronic diseases before pregnancy, such as primary hypertension, diabetes mellitus, kidney and liver diseases were excluded.

Mothers received folic acid supplement during early pregnancy were assigned into group A and did not received folic acid supplement were assigned into group B, respectively.

**Data Collections:**

Using an electronic medical record system and paper charts, the following data from the clinic and inpatient services at Lasha People's Hospital were collected for analysis: baseline information before delivery including age, ways of conception, history of pregnancy or delivery; pregnancy and obstetric complications; pertinent physical findings; and laboratory information including sonography results. The study only reported on maternal and fetus and infant complications without details on patient symptoms and laboratory abnormalities. Data were assessed at the following time points: 4-week intervals from baseline information to delivery; postpartum weeks 6-8. Data were allocated to the more recent time point if the tests were performed between two time points.

Maternal habitation at recruitment was categorized into two groups: countryside and town/city. Educational level was dichotomized into illiteracy, elementary education (grade school/junior high school), Secondary education (high school/technical secondary school), Higher education (college/university). Habitation altitude was grouped into low altitude (<1500), middle altitude (1500:3500), and high altitude (≥3500). Occupation was rough classified into light physical labor (sitting work and no need overtime), moderate physical labor (continuous work of arms and/or legs) and Heavy physical labor (long time work with load). 5 min Apgar score was dichotomized using the cut-off value 7.

Propensity score matching for each group was computed for each case determined by multivariable logistic regression analysis. Patient demographics, tumor characteristics, and treatment patterns were entered in the propensity score model (PSM). One-to-one propensity score matching between the postoperative radiation group and the ovarian conservation group was performed through an automated algorithm with the propensity score difference cutoff being 1%.

**Outcome Measurements**

The primary outcomes were the frequency of pregnancy induced hypertension (including gestational hypertension, pre-eclampsia, eclampsia, HELLP syndrome) between two groups. The secondary assessment was other negative maternal and
fetal outcomes between two groups, including birth defects, fetal growth retardation, and intrapartum complications (e.g., fetal distress, neonatal death, premature delivery and postpartum hemorrhage). Safety reports in newborns or infants were tabulated using data acquired from infants during the prenatal period or the postnatal period up to 72 hours (e.g., ultrasonography examination, reports of birth defects and APGAR scores, measurements from growth charts, and development milestones).

The incidence rates of GH and PE were described according to the characteristics of participants, including maternal age, gravid, parity, education, habitation altitude and folic acid supplementation.

**Statistical Analysis**

Based on the studies, we estimated 5.9% of patients with GH or PE in Tibetan women\textsuperscript{20} and OR of preeclampsia with folic acid supplementation in comparison to no folic acid supplementation were 0.78 (95% CI 0.63, 0.98)\textsuperscript{21}, the number of patients needed was calculated to be 309, with a significance level of 0.05 (one-tailed) and detection power of 0.90. Considering a 10% drop-outs rate, a sample size of patient enrollment more than 340 was a reasonable estimation for each group.

The intention to treatment analysis was defined as analysis included all enrolled patients, including those with protocol deviations. We included all enrolled infants to perform ITT analysis of the PIH rates. Data of mothers who were lost to follow-up were still included in the analysis and counted as having no PIH. Baseline characteristics and laboratory results were summarized for two groups by means of descriptive statistics, including percentage, means±standard deviation (SD), and 95% CI. For the quantitative variable, the t-test was used to compare group differences. For categorical variables, the chi-square test was used for group comparisons. Significance level was set at P < 0.05; all data were analyzed by SPSS 23.0 (SPSS, IBM., NewYork).

**Results**

**Study Population and Baseline values**

A total of 6902 pregnant patients were retrospectively screened and 202 patients were excluded due to other reasons of multiple gestation and other ethnicity. Among 6700 patients were included in the final study, 18.37% (1231/6700) patients received folic acid supplement during pregnancy were assigned into group A and 81.63% (5469/6700) patients did not receive folic acid supplement. 24.37% (300/1231) patients having supplement of folic acid longer than 3 months were further assigned into group A1 and 75.63% (931/1231) having supplement of folic acid shorter than 3 months were assigned into group A2. Compared to the mothers without folic acid supplement, the mothers with folic acid supplement were older (28.59±3.83 vs. 28.23±4.90, P=0.015), having fewer multipara (49.6% vs. 70.2%, p=2.53*10\textsuperscript{-43}) and living in lower altitude (3706.95±449.42 vs. 3800.39±475.14, P=3.26*10\textsuperscript{-10}). More mothers in folic acid supplement group had higher level of Education, engaged in jobs with less physical labor and lived in town or city (see table 1). 1230 mothers did not received folic acid supplement were selected through PSM (based on age, educational levels, living altitude, multipara or not, occupation and residence) and enrolled group B. The patients who were screened and enrolled into the different study groups are shown in Fig. 1. After propensity score matching, there was no statistical significance of the baseline between group A and group B. The baseline characteristics of maternal showed in the table 1.

The mean (±SD) duration of folic acid supplement was 2.07±1.41 months in group A, 3.87±1.74 months in group A1 and 1.48±0.51 months in group A2. From baseline visit to the last visit at postpartum week 6, there was no patients drop-out from our study. Thus, the on-protocol analyses also represented the outcomes of the intention-to-treat analyses in our study.

**The incidence of maternal and infant complications**

As show in table 2, a significantly higher frequency of pregnancy induced hypertension (19.3% vs. 5.8%, p=7.33*10\textsuperscript{-19}, RR=3.294) were observed in group B than in group A, including Gestational hypertension (4.47% vs. 1.87%), Mild Pre-
eclampsia (5.69% vs. 2.11%), Severe Pre-eclampsia (8.54% vs. 1.87%) and Eclampsia (0.57% vs. 0%). There were other 13 itemized complications of mothers, including maternal symptoms and laboratory abnormalities, reported in this study, and they are shown in Table 3. More anemia (31.7% vs. 20.1%, \(P=4.5 \times 10^{-11}\)) and much lower hemoglobin (122.3±16.02 vs. 115.5±16.76, \(P=4.99 \times 10^{-24}\)) were found in group B than in group A before delivery. Besides, the incidence of placental abruption (0.4% vs. 1.0%) and oligohydramnios (2.8% vs. 4.1%) seems less in group A than in group B, but there was no significant difference between two group. There were no differences between the incidence rates of other maternal complications including postpartum hemorrhage and pathological pregnancy (Table 3).

In the term of infants complications, more preterm delivery were observed in the infants of group B than group A (8.0% vs. 1.0%, \(P=3.85 \times 10^{-12}\)) and resulted in a shorter gestational ages (38.74±1.93 vs. 39.10±1.12, \(P=1.61 \times 10^{-8}\)). Therefore, the infants of group B had a lighter weight (3001.33±558.92 vs. 3186.04±400.43, \(P=1.06 \times 10^{-20}\)), shorter height (49.44±2.75 vs. 50.14±1.83, \(P=1.62 \times 10^{-15}\)), smaller head circumference (33.97±1.36 vs. 33.55±1.84, \(P=4.75 \times 10^{-9}\)) and lighter placenta weight (612.91±107.17 vs. 594.31±118.07, \(P=4.4 \times 10^{-5}\)) than group A at birth. For the above reason, more full-term low birth weight infants (8.5% vs. 3.0%, \(P=6.02 \times 10^{-9}\)) were found in group B than in group A. Besides, a significantly higher frequency of asphyxia of newborn (9.0% vs. 2.1%, \(P=7.55 \times 10^{-14}\)) and Pediatric admission (17.1% vs. 4.1%, \(P=8.29 \times 10^{-26}\)) in group B than in group A. There were no differences in the incidence of newborn pathological jaundice (0.8% vs. 0.6%, \(P=0.466\)) and pneumonia (0.4% vs. 0.2%, \(P=0.478\)) between two group.

The incidence of fetal/infant congenital deformities (1.1% vs. 0.7%, \(P=0.391\)) and newborn death (0.4% vs. 0.1%, \(P=0.102\)) seems more in group B than in group A, but there were no significant differences between two group. 9 infants were reported congenital deformities in the newborns of group A: 1 infant had hydrocephalus, 6 infants had polydactyly, 1 had unilateral external acoustic meatus atresia and 1 had unilateral cleft palate. 13 congenital deformities were found in the newborns of group B: 4 infants had polydactyly, 4 cleft lip and palate, 1 external acoustic meatus atresia, 1 anencephalus, 1 strephyropodia, 1 anal atresia and 1 unknown subcutaneous edema. 1 infants of group A were died of hydrocephalus and 5 infants of group B were died of the following reasons: 1 severe preeclampsia, 1 placenta abruption caused by severe preeclampsia, 1 persistent occipitotransverse position, 1 unknown newborn asphyxia and 1 anencephalus.

Subgroup analysis stratified by the time of folic acid supplement

To further evaluate the role of folic acid supplement on reducing the incidence of PIH, subset analysis was performed based on the dosing course of folic acid. Patients with dosing course longer than 3 months were assigned into group A1, and those without into group A2. Compared to patients in group A2, longer dosing course of folic acid seems to further reducing the incidence of PIH but without significant difference (6.0% vs. 5.4%, \(P=0.662\))(see suppl table 1).

Discussion

In this study, we report on retrospective data on folic acid supplement for reducing pregnancy induced hypertension in selected Tibetan Nationality of Tibet Plateau. To our knowledge, this is the first and largest study involving 2461 subjects in this special population. Our results indicate that folic acid supplement during early stage of pregnancy could reduce the incidence of pregnancy induced hypertension and antepartum anemia. In combination with regularly antepartum examination, a significant reduction of preterm delivery and asphyxia of newborn was observed in mothers received folic acid supplement, compared to those did not receive.

Whether folic acid supplementation in pregnancy can reduce the incidence PIH remains uncertain for the inconsistency of previous studies\textsuperscript{16-17}. The prevalence of gestational hypertension/preeclampsia are associated with maternal age, parity, ethics, education, socioeconomic status, history of preeclampsia and chronic hypertension, antenatal care among other factors\textsuperscript{22-23}. The inconsistency of findings may be attributable to different heterogeneity of population, sample size, education, socioeconomic status, and different antenatal care. Among 6700 Tibetan mothers screened in our study, only 18.37% patients received folic acid supplement during pregnancy. The mothers received folic acid supplement were older.
(28.59±3.83 vs. 28.23±4.90) and lived in lower altitude (3706.95±449.42m vs. 3800.39±475.14m). Less of them were illiteracy (3.6% vs. 29.1%), multipara (49.6% vs. 70.2%), heavy physical labor (29.1% vs. 61.5%) and lived in countryside (34.4% vs. 63.4%). All the above features might reflect a more easily acquiring of prenatal education or sanitary condition. After adjusted the age, parity, living altitude, education, occupation and residence through propensity score matching, our results indicated that folic acid supplement from 3 months before conception to first trimester could reduce the incidence of PIH from 19.3% to 5.8% in this special population. The folic acid supplement could reduce PIH from Gestational hypertension (4.47% vs. 1.87%), mild Pre-eclampsia (5.69% vs. 2.11%), severe Pre-eclampsia (8.54% vs. 1.87%) to Eclampsia (0.57% vs. 0%) comprehensively. However, longer duration of folic acid supplement did not reduced the incidence of PIH.

Previous studies only reported on the incidence of PIH without details on maternal and fetus and infant complications. Our study also showed that less anemia (20.1% vs. 31.7%) and much lower hemoglobin (122.3±16.02 vs. 115.5±16.76) were found in group B than in group A before delivery. There is evidence that natural selection is ongoing in the Tibetan population, where women estimated to have genotypes for high oxygen saturation of hemoglobin (and less physiological stress) have higher offspring survival. Due to the higher incidence of PIH, more preterm infants were observed in mothers did not receive folic acid supplement than mothers received (8.0% vs. 1.0%) and resulted in a shorter gestational ages (38.74±1.93 vs. 39.10±1.12). More preterm and mother complications further led to more full-term low birth weight infants (8.5% vs. 3.0%), neonatal asphyxia (9.0% vs. 2.1%) and Pediatric admission (17.1% vs. 4.1%). In antepartum examinations, folic acid was mainly used to prevent the Neural tube defects. The incidence of fetal/infant congenital deformities (1.1% vs. 0.7%) and newborn death (0.4% vs. 0.1%) seems more in mothers did not receive folic acid supplement than mothers received, but no significant differences were found.

Inflammation and oxidative stress is considered to play a role in the pathophysiology of preeclampsia. Hyperhomocysteinemia might damage the vascular endothelium of the developing placenta by promoting oxidative stress, thereby increasing contractile response and the production of procoagulants and vasoconstrictors. Folic acid supplementation might reduce the risk of gestational hypertension or preeclampsia through reduce elevated homocysteine. However, other studies thought hyperhomocysteinemia may be a consequence rather than a cause of hypertensive disorders of pregnancy. The concentration of blood folic acid level were affected by many factors except folic acid supplement, like dietary habit. In their pastoral pattern, 85% did not consume packaged vegetables or consumed this food group less than once per month; whereas onions and garlic were consumed as condiments in minimal amounts as parts of culinary dishes. Besides, maternal and fetal MTHFR C677T polymorphism may be associated with a moderately increased risk of gestational hypertension, and there is a suggestion that this association may be diminished among women receiving folate supplementation during Pregnancy.

At sea level, maternal uterine artery blood at a pO2 of 100 mmHg enters the intervillous space where it is stirred by oscillations of fetal villi and equilibrates with uterine vein blood at a pO2 of 45 mmHg. While in High altitude at 3600 m, maternal uterine artery pO2 is only 55 mmHg, the same gradient, need a lower uterine vein blood or an adaptive uterine artery pressure. Then lead to an altitude-related increase in the incidence of PE and GH. However, different to previous study in european, Tibetan women had higher rates of pre-eclampsia/gestational hypertension than han chinese women (5.9% vs 10.3%, P = 0.04). Also, altitude was found to be negatively associated with the risk of DM in tibetan people. Compared to individuals living at < 3500 meters, the risk of DM decreased by 65% for those living at 3500-3999 meters (P = 0.034) and by 89% for those living at ≥ 4000 meters (P = 0.015). The screening of Gestational Diabetes Mellitus were not commonly performed among Tibetans without symptoms or abnormal laboratory results. Our study did not include the patients of Diabetes Mellitus and other chronic diseases which is also the risk factor of PIH. Further prospective studies are need to include the data of blood Hcy levels, hcy polymorphism and chronic diseases in this population.

In conclusion, Folic acid supplement in the first trimester could reduce the incidence of pregnancy induced hypertension in Tibetan Nationality of Tibet Plateau. Continuing free distribution of 0.4mg folic acid and closely follow-up were highly recommended in this population.
Abbreviations

PIH, Pregnancy induced Hypertension; Hcy, Homocysteine; FA, Folic Acid; BP, Blood Pressure; PSM, propensity score model; GH, gestational hypertension; PE, preeclampsia.

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments. The study design was reviewed and approved by the Ethics Committee of Beijing YouAn Hospital, Capital Medical University (approval number: Jing-you-ke-lun-zi [2020]088-hao). Permission to access and to use these data was approved by the institution. The need for informed consent was waived.

Patient consent for publication: Not required.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: None declared.

Funding: This study is supported by a research grant from Science and Technology project of Tibet Autonomous Region (grant number: ZX2017ZY03) and Beijing Hospitals Authority Talent Training Plan (grant number: QMS20191706).

Author Contributions: Drs. Xu and Wang proposed the concept and designed the study. Drs. Xu, Bianba, and Wang obtained the research funding. Drs. Xu, Pubu, Yang Ge and Bianba contributed to the acquisition of data. Dr. Xu supervised the data collection. Dr. Wang performed the statistics, interpreted the data and wrote the manuscript with assistance from Dr. Xu and Zhao. All authors provided inputs for the manuscript. Dr. Xu performed critical revision of the manuscript and addressed the comments from the journal.

Acknowledgements: Not applicable.

Ethics approval This study was approved by the ethics review committee of Lasha People's Hospital (approval number: SYLL2217001).

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

References

1. Al-Jameil N, Aziz Khan F, Fareed Khan M, et al. A brief overview of preeclampsia. J Clin Med Res 2014;6:1-7.

2. Kintiraki E, Papakatsika S, Kotronis G, et al. Pregnancy-Induced hypertension. Hormones (Athens) 2015;14:211-23.

3. Perry H, Khalil A, Thilaganathan B. Preeclampsia and the cardiovascular system: An update. Trends Cardiovasc Med 2018;28:505-513.

4. Steegers EA, von Ddenszen P, Duvekot JJ, et al. Pre-eclampsia. Lancet 2010;376:631–44. (Level III)

5. ACOG Practice Bulletin No. 202: Gestational Hypertension and Preeclampsia. Obstet Gynecol 2019;133:e1-e25.
6. Garovic VD, Bailey KR, Boerwinkle E, et al. Hypertension in pregnancy as a risk factor for cardiovascular disease later in life. J Hypertens 2010; 28:826.

7. Magnussen EB, Vatten LJ, Smith GD, et al. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. Obstet Gynecol 2009; 114:961–970.

8. Roberts JM, Pearson G, Cutler J, et al. Summary of the NHLBI working group on research on hypertension during pregnancy. Hypertension 2003;41:437-445.

9. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338: 1042-50.

10. Antoniades C, Antonopoulos AS, Tousoulis D, et al. Homocysteine and coronary atherosclerosis: from folic acid fortification to the recent clinical trials. Eur Heart J 2009;30: 6-15.

11. Cheng RJ, Huang SY, Su SY, et al. Prognostic value of cardiovascular disease risk factors measured in the first-trimester on the severity of preeclampsia. Medicine (Baltimore) 2016; 95: e2653.

12. Hernandez-Diaz S, Werler MM, Louik C, et al. Risk of gestational hypertension in relation to folic acid supplementation during pregnancy. Am J Epidemiol 2002;156:806–812.

13. Bodnar LM, Tang G, Ness RB, et al. Periconceptional multivitamin use reduces the risk of preeclampsia. Am J Epidemiol 2006;164:470–477.

14. Wen SW, Chen XK, Rodger M, et al. Folic acid supplementation in early second trimester and the risk of preeclampsia. Am J Obstet Gynecol 2008;198:45.e1–45.e7.

15. Li Z, Ye R, Zhang L, et al. Folic Acid Supplementation During Early Pregnancy and the Risk of Gestational Hypertension and Preeclampsia. Hypertension 2013;61:873-879.

16. Hua X, Zhang J, Guo Y, et al. Effect of folic acid supplementation during pregnancy on gestational hypertension/preeclampsia: A systematic review and meta-analysis. Hypertension in Pregnancy 2016;35: 447-460.

17. Yang X, Chen H, Du Y, et al. Periconceptional folic acid fortification for the risk of gestational hypertension and preeclampsia: a meta-analysis of prospective studies. Maternal and Child Nutrition 2016;12:669–679.

18. Beall CM. Two routes to functional adaptation: Tibetan and Andean high-altitude natives. PNAS 2007;104(Suppl 1):8655-60.

19. Sun P, Wang Q, Zhang Y, et al. Association between homocysteine level and blood pressure traits among Tibetans: A cross-sectional study in China. Medicine (Baltimore)2019;98:e16085.

20. Suellen Miller, Carrie Tudor, Vanessa Thorsten, et al. Comparison of maternal and newborn outcomes of Tibetan and Han Chinese delivering in Lhasa, Tibet. J Obstet Gynaecol Res 2008;34: 986–993.

21. Bulloch RE, Lovell AL, Jordan VMB, et al. Maternal folic acid supplementation for the prevention of preeclampsia: A systematic review and meta-analysis. Paediatr Perinat Epidemiol 2018;32:346–357.

22. Miller EC, Cao H, Wen SW, et al. The risk of adverse pregnancy outcomes is increased in preeclamptic women who smoke compared with nonpreeclamptic women who do not smoke. Am J Obstet Gynecol 2010;203:334 e331–e338.

23. Tinker SC, Cogswell ME, Devine O, et al. Folic acid intake among U.S. women aged 15–44 years, National Health and Nutrition Examination Survey, 2003–2006. Am J Prev Med 2010;38:534–542.
24. US Preventive Services Task Force. Folic Acid Supplementation for the Prevention of Neural Tube Defects: US Preventive Services Task Force Recommendation Statement. JAMA 2017;317:183-189.

25. Roberts JM, Escudero C. The placenta in preeclampsia. Pregnancy Hypertens 2012;2: 72–83.

26. Roberts JM, Cooper DW. Pathogenesis and genetics of preeclampsia. Lancet 2001;357:53–6.

27. Kemse NG, Kale AA, Joshi SR. A Combined Supplementation of Omega-3 Fatty Acids and Micronutrients (Folic Acid, Vitamin B12) Reduces Oxidative Stress Markers in a Rat Model of Pregnancy Induced Hypertension. PLoS ONE 2014; 9: e111902.

28. Scorsatto M, Uehara SK, Luiz RR, et al. Fortification of flours with folic acid reduces homocysteine levels in Brazilian women. Nutr Res 2011;31:889–895.

29. Chuang CZ, Boyles A, Legardeur B, et al. Effects of riboflavin and folic acid supplementation on plasma homocysteine levels in healthy subjects. Am J Med Sci 2006;331:65–71.

30. Steegers-Theunissen RP, Van Iersel CA, Peer PG, et al. Hyperhomocysteinemia, pregnancy complications, and the timing of investigation. Obstet Gynecol 2004;104:336–343.

31. Peng W, Liu Y, Liu Y, et al. Major dietary patterns and their relationship to obesity among urbanized adult Tibetan pastoralists. Asia Pac J Clin Nutr 2019;28:507-519.

32. Hernández-Díaz S, Wu XF, Hayes C, et al. Methylene tetrahydrofolate reductase polymorphisms and the risk of gestational hypertension. Epidemiology 2005;16:628-34.

33. Battaglia FC, Meschia G. Review of studies in human pregnancy of uterine and umbilical blood flows. Dev. Period Med 2013;17: 287–292.

34. Palmer SK, Moore LG, Young D, et al. Altered blood pressure course during normal pregnancy and increased preeclampsia at high altitude (3100 meters) in Colorado. Am. J. Obstet. Gynecol 1999;180: 1161–1168.

35. Shaopeng Xu, Qing Wang, Jie Liu, et al. The prevalence of and risk factors for diabetes mellitus and impaired glucose tolerance among Tibetans in China: a cross-sectional study Oncotarget 2017; 68: 112467–112476.

Tables

Table 1. The Baseline Values of study mothers (n=6700)
| Variables                        | Before Matching                  | After Matching                  |
|---------------------------------|----------------------------------|---------------------------------|
|                                 | Group A (n=1231)                | Group B (n=5469)                |
|                                 | Group A (n=1231)                | Group B (n=1230)                |
| P-value                         | t=2.42, P=0.015                 | t=0.67, P=0.505                 |
| Age (years)                     | 28.59±3.83                      | 28.71±4.58                      |
|                                 | t=0.67, P=0.505                 |                                 |
| multipara                       | 611(49.6)                       | 611                             |
|                                 | 3839(70.2)                      | 597                             |
|                                 | X²=190.45, p=2.53*10^-43        |                                 |
| History of abortion             | 0.33±0.73                       | 0.36±0.75                       |
|                                 | t=0.812, P=0.417                | t=1.15, P=0.249                 |
| Altitude (m)                    | 3706.95±449.42                  | 3706.95±449.42                  |
|                                 | t=6.30, P=3.26*10^-10          | t=1.39, P=0.163                 |
|                                 |                                 |                                 |
| Educational level               |                                 |                                 |
| illiteracy                      | 44 (3.6)                        | 44 (3.6)                        |
|                                 | 1590(29.1)                      | 82(6.7)                         |
|                                 | Z=29.69, P=9.06*10^-194         | Z=1.79, P=0.074                 |
| elementary education            | 162(13.2)                       | 162(13.2)                       |
|                                 | 1743 (31.9)                     | 143(11.6)                       |
| Secondary education             | 56(4.5)                         | 56(4.5)                         |
|                                 | 400 (7.3)                       | 70 (5.7)                        |
| Higher education                | 969(78.7)                       | 969(78.7)                       |
|                                 | 1736(31.7)                      | 935 (76)                        |
| Occupation                      |                                 |                                 |
| none                            | 90 (7.3)                        | 90 (7.3)                        |
|                                 | 539(9.9)                        | 76(6.2)                         |
|                                 | Z=19.22, P=2.66*10^-82         | Z=1.64, P=0.102                 |
| Light physical labor            | 508 (41.3)                      | 508 (41.3)                      |
|                                 | 723 (13.2)                      | 479(38.9)                       |
| Moderate physical labor         | 275(22.3)                       | 275(22.3)                       |
|                                 | 845 (15.5)                      | 304(24.7)                       |
| Heavy physical labor            | 358 (29.1)                      | 358 (29.1)                      |
|                                 | 3362(61.5)                      | 371(30.2)                       |
| Residence                       |                                 |                                 |
| countryside                    | 424(34.4)                       | 424(34.4)                       |
|                                 | 3466(63.4)                      | 456 (37.1)                      |
|                                 | X²=345.41, p=4.23*10^-77        | X²=1.85, p=0.174                |
| Town/city                       | 807(65.6)                       | 807(65.6)                       |
|                                 | 2003(36.6)                      | 774(62.9)                       |

Data are mean±standard deviation or n (%) unless otherwise specified.

Bold indicates significant P value.

Table 2. Incidence of Pregnancy-induced Hypertension in Pregnant Mothers of Tibetan Nationality Received Folic Acid Supplement or Not (n=2461)
| Condition                  | Group A (n=1231) | Group B (n=1230) | RR(95%CI)       | P-value                  |
|---------------------------|------------------|------------------|------------------|--------------------------|
| PIH                       | 72(5.8)          | 237(19.3)        | 3.294(2.051-4.339) | $x^2=78.67, P=7.33*10^{-19}$ |
| Gestational hypertension  | 23(31.9)         | 55(24.2)         | 2.393(1.462-3.919) |                          |
| Mild pre-eclampsia        | 26(36.1)         | 70(26.4)         | 2.694(1.706-4.256) |                          |
| Severe pre-eclampsia      | 23(31.9)         | 105(46.3)        | 4.569(2.890-7.224) |                          |
| HELLP*                    | 0(0)             | 5(0.41)          | -                | $Z=2.46, P=0.014$        |
| Eclampsia                 | 0(0)             | 7(3.1)           | -                |                          |

*PIH: pregnancy induced hypertension; HELLP: hemolysis, elevated liver enzymes and low platelets.

Bold indicates significant P value.

The cases of HELLP were included in severe Pre-eclampsia

Table 3. Maternal and Infant adverse Events Reported in the Study (n=2461)
| Mothers                                      | Group A (n=1231) | Group B (n=1230) | P-value         |
|---------------------------------------------|------------------|------------------|----------------|
| Hemoglobin (g/L) at delivery                | 122.3 ± 16.02    | 115.5 ± 16.76    | t=10.22, P=4.99*10^-24 |
| Anemia at delivery                          | 248 (20.1)       | 391 (31.8)       | X^2=43.38, P=4.5*10^-11 |
| mild                                        | 153 (12.4)       | 222 (18)         |                |
| moderate                                    | 91 (7.4)         | 160 (13)         |                |
| severe                                      | 4 (0.3)          | 9 (0.7)          |                |
| Thrombocytopenia                            | 9 (0.7)          | 6 (0.5)          | X^2=0.601, P=0.438 |
| Intrahepatic cholestasis of pregnancy       | 33 (2.7)         | 30 (2.4)         | X^2=0.144, P=0.704 |
| Premature rupture of membranes              | 99 (8.0)         | 127 (10.3)       | X^2=3.845, P=0.050 |
| Placenta praevia                            | 9 (0.7)          | 10 (0.8)         | X^2=0.054, P=0.816 |
| Placental abruption                         | 5 (0.4)          | 12 (1.0)         | X^2=2.908, P=0.088 |
| Oligohydramnios                             | 35 (2.8)         | 50 (4.1)         | X^2=2.754, P=0.097 |
| Prolapse of umbilical cord                  | 0 (0)            | 1 (0.1)          | Fishers'test, P=0.5 |
| Postpartum haemorrhage                      | 211.91±123.33    | 219.78±105.81    | t=1.694, P=0.090 |
| Shock                                       | 1 (0.1)          | 2 (0.2)          | X^2=2.754, P=0.097 |
| Heart failure                               | 0 (0)            | 2 (0)            | X^2=0.501, P=0.479 |
| Upper respiratory infection                 | 6 (0.5)          | 5 (0.4)          | X^2=0.091, P=0.764 |
| Vaginitis (Bacterial vaginitis or Vulvovaginal candidiasis albicans) | 133 (10.8)       | 118 (9.6)        | X^2=0.985, P=0.321 |
| Infants                  | Group A (n=1231) | Group B (n=1230) | P-value  |
|-------------------------|------------------|------------------|----------|
| Gender (male)           | 638 (51.8)       | 609 (49.5)       | $X^2=1.320, P=0.251$ |
| Gestational age (weeks) | 39.10±1.12       | 38.74±1.93       | $t=5.674, P=1.61\times10^{-8}$ |
| Placenta weight         | 612.91±107.17    | 594.31±118.07    | $t=4.091, P=4.4\times10^{-5}$ |
| Cesarean Section (%)    | 328 (26.6)       | 374 (30.4)       | $X^2=4.27, P=0.039$ |
| Weight (kg)             | 3186.04±400.43   | 3001.33±558.92   | $t=9.423, P=1.06\times10^{-20}$ |
| Height (cm)             | 50.14±1.83       | 49.44±2.75       | $t=7.425, P=1.62\times10^{-13}$ |
| Head circumference (cm) | 33.97±1.36       | 33.55±1.84       | $t=5.863, P=4.75\times10^{-9}$ |
| Deformity               | 9 (0.7)          | 13 (1.1)         | $X^2=0.737, P=0.391$ |
| Preterm delivery        | 24 (1.9)         | 99 (8.0)         | $X^2=48.202, P=3.85\times10^{-12}$ |
| Full-term low birth weight | 37 (3.0)       | 104 (8.5)        | $X^2=33.83, P=6.02\times10^{-9}$ |
| Fetal macrosomia        | 34 (2.8)         | 21 (1.7)         | $X^2=3.132, P=0.077$ |
| Neonatal asphyxia       | 26 (2.1)         | 111 (9.0)        | $X^2=55.91, P=7.55\times10^{-14}$ |
| Neonatal pneumonia      | 3 (0.2)          | 5 (0.4)          | $X^2=0.503, P=0.478$ |
| Neonatal jaundice       | 10 (0.8)         | 7 (0.6)          | $X^2=0.531, P=0.466$ |
| Pediatric admission     | 50 (4.1)         | 210 (17.1)       | $X^2=110.238, P=8.29\times10^{-26}$ |
| Fetal death             | 1 (0.1)          | 5 (0.4)          | $X^2=2.676, P=0.102$ |

Data are mean±standard deviation or n (%) unless otherwise specified.

Bold indicates significant P value.

**Figures**
Figure 1
Disposition of Mothers and Infants

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.Subgroupanalysis.docx