Original Article

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Folic acid: The key to a healthy pregnancy – A prospective study on fetomaternal outcome

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Abstract

Objectives: The objective of the study is to study the fetomaternal outcome associated with folic acid deficiency in pregnancy. **Materials and Methods:** This hospital-based observational study was conducted in the Department of Obstetrics and Gynaecology at Base Hospital, Delhi Cantt, and a total of 351 participants were enrolled who were fulfilling the inclusion criteria. The plasma folic acid level of the selected patients was measured in the booking visit by automated chemiluminescence assay. The cutoff levels of folic acid were taken at 8.6 ng/mL. Based on these values, the study population was divided into two groups, one with folic acid values <8.6 ng/mL and the other with values ≥8.6 ng/mL. Plasma Vitamin B12 levels were measured to check for any concurrent deficiencies. Obstetric outcomes included first- and second-trimester miscarriages, development of anemia, gestational hypertension/preeclampsia, gestational diabetes mellitus, hypothyroidism, placental abruption, and intrauterine fetal growth restriction (FGR). Furthermore, the period of gestation at delivery, fetal weights, APGAR scores at 5 min were documented. The study also considered fetal neural tube defects, intrauterine fetal demise for data collection. Collected data were analyzed statistically to find the association of the above-mentioned outcomes with levels of folic acid. **Results:** The rate of preterm deliveries was significantly higher in the folic acid group with levels <8.6 ng/mL (16.94%). The incidence of small for gestational age/FGR was higher in the folic acid group with levels <8.6 ng/mL (27.11%) compared to the high folic acid group with levels ≥8.6 ng/mL (13.38%). The differences in the incidence of anemia, gestational hypertension, gestational diabetes, and preeclampsia between the two groups were not statistically significant and no cases of intrauterine fetal demise or placental abruption were observed in either group. Moreover, there was no significant difference in the relative risk of low Apgar scores at 5 min between the two groups. **Conclusion:** The present study suggests that low folic acid levels during pregnancy are associated with a higher risk of adverse pregnancy outcomes such as anemia, miscarriages, preterm delivery, and FGR. Therefore, adherence to nutritional recommendation of folic acid supplementation during pregnancy is essential to prevent these adverse outcomes.

Keywords: *Anemia, Fetal growth restriction, Folic acid, Preterm delivery*

Introduction

Folic acid, also known as folate or Vitamin B9, is a water-soluble vitamin essential for vital biochemical processes in the body. It exists in various interconvertible forms, including folate, dihydrofolate, and tetrahydrofolate. It acts as a coenzyme in one-carbon transfer reactions, involved in the synthesis of nucleotides, amino acids, and other essential biomolecules [1,2]. It also plays a crucial role in the formation of red blood cells and brain function and is especially important during pregnancy as it aids in embryonic formation and neural tube closure through epigenetic mechanisms [3-5].

Over the course of pregnancy, there is a steady decline in maternal plasma folate concentration to about 50% of

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non-pregnant levels in response to physiological hemodilution, alterations in renal function, and hormonal changes [6-8]. The study conducted by Toteja *et al*. [9] found that 62.8% of pregnant women in India had deficient serum folic acid levels below 3 ng/mL. The deficiency is more prevalent in vegetarian women due to high levels of phytates that bind to folic acid, inhibiting its absorption. A study conducted in southern India concluded that folic acid, Vitamin B12, and iron deficiency often coexist and were important public health problems in India affecting more than 50% of women of childbearing age, hence recommended that these should be supplemented together [10].

Several studies suggest a link between folate deficiency and increased risk of spontaneous abortion, recurrent pregnancy loss, and stillbirth. A Swedish study found low blood folate to be a significant independent risk factor for spontaneous abortion, while high folate status was associated with a nonsignificant decrease in risk [11,12]. However, small sample sizes, the presence of other confounding risk factors, and unrepresentative study samples have contributed to the lack of definitive conclusions. In summary, the evidence suggesting the role of folate in recurrent miscarriages is not robust.

Numerous epidemiological studies have examined the relationship between folic acid supplementation and the risk of developing preeclampsia, but the findings have been inconclusive. While some studies have found that folic acid supplementation can reduce the occurrence of preeclampsia and gestational hypertension [13,14], research by Li *et al*., found no such correlation [15].

Observational studies have provided indirect evidence suggesting the role of folate in the timing of labor, with low folate levels being associated with spontaneous preterm delivery, as a result of abnormal inflammatory response triggered by infections or hemorrhages [16-18]. Folic acid plays a crucial role in normal immune function and its deficiency often leads to an impaired cell-mediated and humoral immunity [19]. In addition, polymorphonuclear leukocytes in folate-deficient individuals exhibit decreased bactericidal and phagocytic capabilities increasing susceptibility to the infections [20]. Studies have shown that the dietary supplementation with folic acid can improve immune function and decrease circulating inflammatory biomarkers such as α1-acid glycoprotein and C-reactive protein in such individuals.

These inconsistent observations prompted a review of the role of folate in pregnancy, in relation to the prevention fetal growth restriction (FGR), miscarriages, pre-eclampsia, placental abruption, and preterm labor. Our research aimed at enhancing our comprehension of the correlation between folic acid levels with various obstetric outcomes.

Materials and methods

This hospital-based observational study was conducted in the Department of Obstetrics and Gynaecology at Base Hospital, Delhi Cantt. Institutional ethical committee Base Hospital and Army College of medical Sciences had approved the study (IEC/08/2020/03) and informed consent of the study participants was taken. The study was conducted in accordance with the Declaration of Helsinki.

The sample size of 300 for the study size was calculated according to the formula:

$$N = (z1-\alpha/2)^2 \times p \times (1-p)\ \delta 2$$

Where, p (prevalence of Target Condition) = 0.1, δ (Precision) = 0.025 (2.5%), α (Type I error) = 5%, $Z1\text{-}\alpha/2 = 1.96$, and Confidence interval (CI) - 95%.

Inclusion criteria

All pregnant women in their first or early second trimester with spontaneous conceptions and were willing to participate in the study.

Exclusion criteria

Multifetal pregnancy

Patients conceived after with assisted reproductive technique, i.e., *in vitro* fertilization.

Patients with chronic hypertension, pregestational diabetes, or thrombophilias.

A total of 351 participants fulfilling the inclusion criteria were enrolled for the study. Nature and purpose of the study were explained to the enrolled women in detail and informed consent was obtained. Baseline demographic characteristics of the study population were recorded which included maternal age and gravidity. A history was taken in detail which included dietary habits and clinical evaluation was done.

The plasma folic acid level of the selected patients was measured in the booking visit by automated chemiluminescence assay. The cutoff levels of folic acid according to the pathological definition was taken at 8.6 ng/mL. Based on these values, the study population was divided into two groups, one with folic acid values <8.6 ng/mL and the other with values >8.6 ng/mL. Forty-three study participants were lost to follow-up midway due to various reasons and 308 were then followed up until the end of pregnancy, and various fetomaternal outcomes were recorded. Routine folic acid supplements were given to the study group and no intervention was done. Plasma Vitamin B12 levels were measured by automated chemiluminescence assay to check for any concurrent deficiencies. Obstetric outcomes included first- and second-trimester miscarriages, development of anemia, gestational hypertension/preeclampsia, gestational diabetes mellitus (GDM), hypothyroidism, placental abruption, and intrauterine FGR. Furthermore, the period of gestation at delivery, fetal weights, APGAR scores at 5 min were documented. The study also considered fetal neural tube defects, intrauterine fetal demise for data collection. Collected data were analyzed statistically to find association of the above-mentioned outcomes with levels of folic acid.

Statistical analysis

Data were coded and recorded in MS Excel Software. The Chi-square test was used for group comparisons and SPSS v 23 (SPSS downloaded, IBM, Delhi, India) was used for analysis. $P < 0.05$ was considered statistically significant.

Results

In this study, 308 pregnant women were included to investigate the association between folic acid levels with demographic variables including food habits, co-existing Vitamin B12 deficiency, and miscarriages.

As shown in Table 1 and Figure 1, of them, 242 (78.57%) had folic acid levels >8.6 ng/mL and 66 (21.43%) had levels ≤8.6 ng/mL. The results showed no significant difference in folic acid levels across different age groups (P = 0.52) and gravidity categories (P = 0.06). It was found that women who had folic acid <8.6 ng/mL also had a concurrent deficiency of Vitamin B12, and this association was deemed statistically significant (44.62% vs. 59.09%, P = 0.03). The relative risk (RR) for co-existing Vitamin B12 deficiency was 1.33, connoting that women with folic acid levels of <8.6 ng/ml have 1.33 times higher risk of having B12 deficiency compared to those with folic acid levels of ≥8.6 ng/ml. The proportion of vegetarian women with folic acid levels >8.6 ng/ml was higher compared to nonvegetarian women, although the difference was not statistically significant (P = 0.46).

Women with folic acid levels <8.6 ng/mL had a higher proportion of first/second-trimester miscarriages (7.57% vs. 2.06%) and the difference was statistically significant (P = 0.02). The RR for first/second-trimester miscarriages in this dataset is 2.48, suggesting that women with folic acid levels of <8.6 ng/mL have 2.48 times higher risk of miscarriage compared to those with folic acid levels of ≥8.6 ng/mL.

Table 2 and Figure 2 exhibits the subgroup analysis after deducting first- and second-trimester miscarriages and suggests that 18.8% of women with folic acid levels ≥8.6 ng/mL developed anemia (Hb <10 gm %) in the second trimester compared to 25.4% in the folic acid group <8.6 ng/mL. However, the RR of anemia was not statistically significant (RR = 0.74, 95% CI = 0.43–1.27, P = 0.25).

The differences in the incidence of anemia, gestational hypertension, gestational diabetes, and preeclampsia between the two groups were not statistically significant. No cases of intrauterine fetal demise or placental abruption were observed in either group. The number of cases of neural tube defect was too low to calculate statistical significance. There were no differences in the incidence of large for gestational age and Apgar score at 5 min <5 between the two groups.

The rate of preterm deliveries was significantly higher in the folic acid group (<8.6 ng/mL) (16.94%) compared to the normal folic acid group (7.53%) (RR = 0.45, 95% CI = 0.22–0.93, P = 0.03). The incidence of small for gestational age (SGA)/FGR was higher in the folic acid group (<8.6 ng/mL) (27.11%) compared to the high folic acid group (≥8.6 ng/mL) (13.38%) with a significant RR (RR = 0.49, 95% CI = 0.26–0.93, P = 0.03). Finally, there was no significant difference in the RR of low Apgar scores at 5 min (RR = 0.16, 95% CI = 0.02–1.43, P = 0.55) between the two groups.

Discussion

Among dietary factors, folate and Vitamin B12 are essential nutrients required in early pregnancy, which are metabolically interlinked in one-carbon metabolism. The two are necessary for DNA methylation and production of nucleotides which in turn are needed for increased cellular replication and fetal growth [1]. Specifically, folate is the donor of one-carbon units for the re-methylation of homocysteine to methionine and then to S-adenosylmethionine [21]. Folate along with Vitamin B12 as a cofactor is necessary to maintain normal levels of homocysteine, as high levels of homocysteine are known to cause several pregnancy complications owing to its pro-inflammatory effect. Thus, both these nutrients (folate and Vitamin B12) are closely intertwined in this important metabolic function and deficiency of any of the two can potentially lead to pregnancy-related complications [22].

Furthermore, folate is an extremely important micronutrient for pregnant women due to its role in preventing birth defects [23]. Given the evidence, the USA Public Health Service recommends daily supplementation of 0.4–0.8 mg of folic acid for all pregnant females [24]. The primary aim of our study was enhancing our comprehension of the association between folic acid levels and maternal and fetal health outcomes.

Table 1: Folic acid level analysis by demographic characters, food habits, B12 deficiency, and first/second-trimester abortions

	Folic acid levels		Total	P
	≥8.6 ng/mL (n=242)	<8.6 ng/mL (n=66)	(n=308)	
Age				
18–24	68 (28.09)	16 (24.24)	84 (27.2)	0.52
25–29	108 (44.62)	34 (51.51)	142 (46.10)	
30–35	54 (22.31)	11 (16.66)	65 (21.10)	
>35	12 (4.95)	5 (7.57)	17 (5.51)	
Gravidity				
Primigravida	115 (47.52)	23 (34.84)	138 (44.8)	0.06
Multigravida	127 (52.47)	43 (65.15)	170 (55.19)	
Co-existing Vitamin B12 deficiency				
B12 ≤193 pg/mL	108 (44.62)	39 (59.09)	143 (46.42)	0.03
Food habits				
Vegetarian	98 (40.49)	30 (45.45)	128 (41.5)	0.46
Nonvegetarian	144 (59.50)	36 (57.57)	180 (58.44)	
First/second-trimester miscarriages	5 (2.06)	5 (7.57)	10 (3.24)	0.02

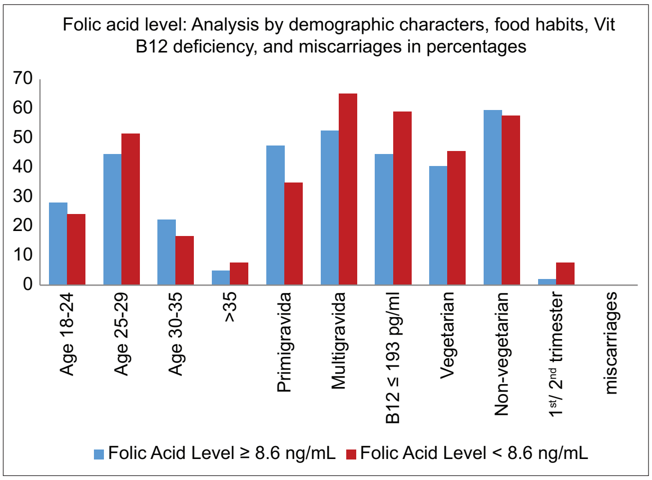

Figure 1: Folic acid level: analysis by demographic characters, food habits, Vitamin B12 deficiency, and miscarriages in percentages

Table 2: Subgroup analysis after excluding abortions

Variables	Folic acid levels ≥8.6 ng/mL (*n*=239)	Folic acid levels <8.6 ng/mL (*n*=59)	*P*
Anaemia (Hb <10 g %)	45 (18.8)	15 (25.4)	0.25
Gestational hypertension	20 (8.36)	6 (10.16)	0.66
Gestational diabetes	61 (25.52)	11 (18.64)	0.26
Preeclampsia	25 (10.46)	8 (13.5)	0.49
Term deliveries	221 (92.46)	49 (83.05)	0.02
Preterm deliveries	18 (7.53)	10 (16.94)	
Neural tube defect	0	1 (1.69)	-
SGA/FGR (weight <10th centile)	32 (13.38)	16 (27.11)	0.03
AGA (between 10th and 90th centile)	196 (82.0)	40 (67.79)	
LGA (weight >90th centile)	11 (4.60)	3 (5.08)	
APGAR at 5 min <5	2 (0.83)	3 (5.08)	0.55

SGA: Small for gestational age, FGR: Fetal growth restriction, LGA: Large for gestational age, AGA: Appropriate for gestational age, Hb: Hemoglobin

In prospective cohort study by Gaskins *et al*. [25], conducted on 15,950 pregnant women, concluded that the risk of spontaneous abortion was 20% lower among women in the highest category of supplemental folate intake (>730 µg/day) than in the lowest (0 µg/d) category suggesting that folic acid levels <8.6 ng/mL are associated with higher risk of miscarriages. Our study substantiates the fact wherein women with folic acid levels <8.6 ng/mL had a higher proportion of first/second-trimester miscarriages (7.57% vs. 2.06%) and the difference was statistically significant (*P* = 0.02). Our dataset suggests the potential connection between folic acid levels and the likelihood of experiencing a first- or second-trimester miscarriage. The RR value of 2.48 indicates that women with folic acid levels lower than 8.6 ng/mL have a 2.48 times greater risk of miscarriage than those with levels of 8.6 ng/ml or higher denoting a significant correlation between folic acid levels and the risk of miscarriage.

A systematic review and meta-analysis published in 2020 found that low levels of folic acid (<8.6 ng/mL) during pregnancy were associated with an increased risk of preterm birth (RR = 1.29, 95% CI = 1.05–1.60), low birth weight (RR = 1.24, 95% CI = 1.08–1.43), and small-for-gestational-age infants (RR = 1.14, 95% CI = 1.02–1.27) [26]. Our study's findings aligned with the aforementioned research. The rate of preterm deliveries was significantly higher in the low folic acid group (<8,6 ng/mL) compared to the normal folic acid group (RR = 0.45, 95% CI = 0.22–0.93, *P* = 0.03). The percentage of SGA/FGR was higher in the low folic acid group (27.11%) compared to the high folic acid group (>8.6 ng/mL) (13.38%) with a significant relative risk (RR = 0.49, 95% CI = 0.26–0.93, *P* = 0.03). Another study published in 2015, found that pregnant women who used folic acid supplements had a lower risk of developing preeclampsia compared to nonusers (odds ratio [OR] = 0.61, 95% CI: 0.43–0.87). Furthermore, the study also showed a significant dose–response relationship between the duration of folic acid supplementation during pregnancy and the reduction in the risk of preeclampsia among the women who used it [14].

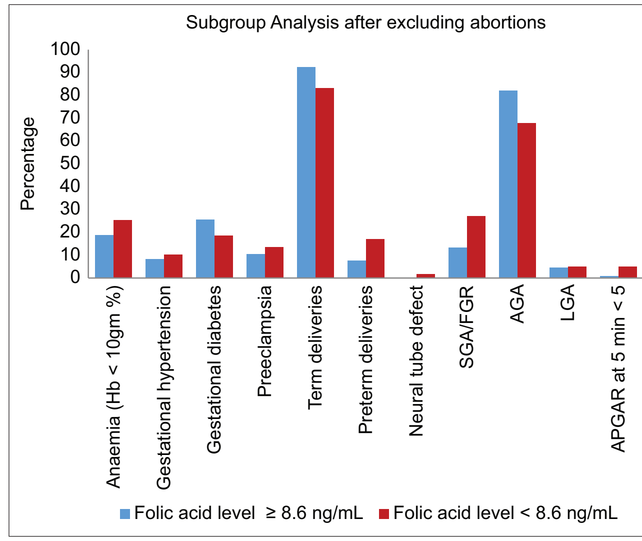

Figure 2: Subgroup analysis after excluding abortions

In our study, the differences in the incidence of anemia, gestational hypertension, preeclampsia, and gestational diabetes in low or normal folic acid level group were not statistically significant, whereas in study conducted by Xiaotian Chen *et al*. [27], higher maternal RBC folate and Vitamin B12 levels in early pregnancy are significantly associated with GDM risk, while the balance of folate/Vitamin B12 is not significantly associated with GDM.

Limitations

The limitations of the study are acknowledged. First, the study was conducted at a single center, which may limit the generalizability of the findings to other populations. Second, the sample size was relatively small, which may have affected the statistical power of the study and ability to detect significant associations. Furthermore, although the study controlled for several potential confounding factors, there may be other unmeasured confounders that could affect the observed associations.

Conclusion

The present study suggests that low folic acid levels

(<8.6 ng/mL) during pregnancy are associated with a higher risk of adverse pregnancy outcomes such as miscarriages, preterm delivery, and FGR. The differences in the incidence of anemia (P = 0.25), gestational hypertension (P = 0.66), gestational diabetes (P = 0.26), and preeclampsia (P = 0.49) between the two groups were not statistically significant. This single study has described the significant association between concurrent Vitamin B12 deficiency, First- and second-trimester abortions and preterm deliveries which makes this study unique and useful. Therefore, adherence to nutritional recommendations of folic acid supplementation during pregnancy is essential to prevent these adverse outcomes. Further studies are required to confirm these findings and to explore the optimal dose and duration of folic acid supplementation during pregnancy.

Data availability statement

The data supporting this study are available through the corresponding author on reasonable request. The dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Stover PJ. Physiology of folate and Vitamin B12 in health and disease. Nutr Rev 2004;62:S3-12.
2. Lucock M. Folic acid: Nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab 2000;71:121-38.
3. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet 1991;338:131-7.
4. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327:1832-5.
5. Liu X, Lv L, Zhang H, Zhao N. The epigenetic role of folate and DNA methylation in colorectal cancer development and progression. J Cancer Res Clin Oncol 2017;143:595-603.
6. Bruinse HW, van den Berg H. Changes of some vitamin levels during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1995;61:31-7.
7. Cikot RJ, Steegers-Theunissen RP, Thomas CM, de Boo TM, Merkus HM, Steegers EA. Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr 2001;85:49-58.
8. Bruinse HW, van der Berg H, Haspels AA. Maternal serum folacin levels during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1985;20:153-8.
9. Toteja GS, Singh P, Dhillon BS, Saxena BN, Ahmed FU, Singh RP, et al. Prevalence of anemia among pregnant women and adolescent girls in 16 districts of India. Food Nutr Bull 2006;27:311-5.
10. Singh S, Geddam JJ, Reddy GB, Pallepogula DR, Pant HB, Neogi SB, et al. Folate, Vitamin B12, ferritin and haemoglobin levels among women of childbearing age from a rural district in South India. BMC Nutr 2017;3:50.
11. Martin RH, Harper TA, Kelso W. Serum-folic-acid in recurrent abortions. Lancet 1965;1:670-2.
12. George L, Mills JL, Johansson AL, Nordmark A, Olander B, Granath F, et al. Plasma folate levels and risk of spontaneous abortion. JAMA 2002;288:1867-73.
13. Vanderlelie J, Scott R, Shibl R, Lewkowicz J, Perkins A, Scuffham PA. First trimester multivitamin/mineral use is associated with reduced risk of pre-eclampsia among overweight and obese women. Matern Child Nutr 2016;12:339-48.
14. Wang Y, Zhao N, Qiu J, He X, Zhou M, Cui H, et al. Folic acid supplementation and dietary folate intake, and risk of preeclampsia. Eur J Clin Nutr 2015;69:1145-50.
15. Li Z, Ye R, Zhang L, Li H, Liu J, Ren A. Folic acid supplementation during early pregnancy and the risk of gestational hypertension and preeclampsia. Hypertension 2013;61:873-9.
16. Bodnar LM, Himes KP, Venkataramanan R, Chen JY, Evans RW, Meyer JL, et al. Maternal serum folate species in early pregnancy and risk of preterm birth. Am J Clin Nutr 2010;92:864-71.
17. Malinow MR, Rajkovic A, Duell PB, Hess DL, Upson BM. The relationship between maternal and neonatal umbilical cord plasma homocyst(e)ine suggests a potential role for maternal homocyst(e)ine in fetal metabolism. Am J Obstet Gynecol 1998;178:228-33.
18. Cunningham F, Leveno K, Bloom S. Hematological disorders. In: Williams obstetrics: 23rd edition. New York: McGraw- Hill; 2009.
19. Courtemanche C, Elson-Schwab I, Mashiyama ST, Kerry N, Ames BN. Folate deficiency inhibits the proliferation of primary human CD8+ T lymphocytes *in vitro*. J Immunol 2004;173:3186-92.
20. Christian P, Jiang T, Khatry SK, LeClerq SC, Shrestha SR, West KP Jr. Antenatal supplementation with micronutrients and biochemical indicators of status and subclinical infection in rural Nepal. Am J Clin Nutr 2006;83:788-94.
21. Mandaviya PR, Joehanes R, Brody J, Castillo-Fernandez JE, Dekkers KF, Do AN, et al. Association of dietary folate and Vitamin B-12 intake with genome-wide DNA methylation in blood: A large-scale epigenome-wide association analysis in 5841 individuals. Am J Clin Nutr 2019;110:437-50.
22. Singh MD, Thomas P, Owens J, Hague W, Fenech M. Potential role of folate in pre-eclampsia. Nutr Rev 2015;73:694-722.
23. Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. Int J Epidemiol 2010;39(Suppl 1): i110-21.
24. U.S. Preventive Services Task Force. Folic acid for the prevention of neural tube defects: U.S. Preventive services task force recommendation statement. Ann Intern Med 2009;150:626-31.
25. Gaskins AJ, Rich-Edwards JW, Hauser R, Williams PL, Gillman MW, Ginsburg ES, et al. Maternal prepregnancy folate intake and risk of spontaneous abortion and stillbirth. Obstet Gynecol 2014;124:23-31.
26. Kc K, Shakya S, Zhang H. Gestational low folic acid has a greater impact on birth weight in nulliparous than multiparous women: A systematic review and meta-analysis. Public Health Nutr 2020;23:680-91.
27. Chen X, Zhang Y, Chen H, Jiang Y, Wang Y, Wang D, et al. Association of maternal folate and Vitamin B(12) in early pregnancy with gestational diabetes mellitus: A prospective cohort study. Diabetes Care 2021;44:217-23.