Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2024**; 51(9): 202
https://doi.org/10.31083/j.ceog5109202

Original Research

The Effect of Folic Acid Supplementation in the First Trimester of Pregnancy on Perinatal Outcomes: A Retrospective Cohort Study

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Academic Editor: Michael H. Dahan
Submitted: 31 May 2024 Revised: 15 July 2024 Accepted: 25 July 2024 Published: 10 September 2024

Abstract

Background: The purpose of this study is to compare the perinatal outcomes of pregnant women who received folic acid (FA) supplements to those who did not, using a large sample size. **Methods**: This study was conducted retrospectively at Konya Training and Research Hospital between 2016 and 2020. The study included pregnant women who took FA and those who did not. We obtained sociodemographic data and perinatal outcomes of all pregnant women from the electronic record system. We learned about folic acid usage from the e-prescription tracking system. **Results**: Out of the total sample size of 2393 patients, n = 861 (35.97%) received FA supplements during pregnancy, while n = 1532 (64.02%) did not. The gestational age at birth in the group receiving FA was higher than in the group not receiving FA (271.59 $\pm$ 13.83 days *vs.* 270.09 $\pm$ 15.27 days, $p = 0.005$). The group that did not take FA had more neonatal intensive care unit (NICU) admissions than the group that did (5.09% *vs.* 2.90%, $p = 0.011$). In the group that took FA, there were n = 17 (1.97%) patients with gestational diabetes (GDM), whereas in the group that did not use FA, there were n = 15 (0.98%) patients with GDM ($p < 0.001$). **Conclusions**: The group taking FA supplements had a higher gestational age at birth and GDM, while admission to the NICU were lower compared to the group not taking FA supplements.

Keywords: folic acid; first trimester; gestational diabetes mellitus; perinatal outcome

1. Introduction

Folic acid (FA) is essential for DNA methylation and the synthesis of nucleic acids and proteins in the body. Consequently, its demand increases during pregnancy, a period characterized by heightened cellular proliferation and fetal growth. FA, the synthetic form of folate, a B vitamin derivative, is obtained from dietary supplements and FA-fortified foods. It has a higher bioavailability compared to folate. Folate cannot be synthesized in the body and is naturally found in vegetables and fruits, but most of it is destroyed during cooking [1]. FA supplementation of 400 µg per day is recommended by the World Health Organization (WHO) during the preconception period to prevent neural tube defects (NTD) [2]. It is recommended at least 1 month before conception, especially in the low-risk group for NTD [3]. At least 3 months before planned pregnancy, individuals in the high-risk group with a history of NTD in the mother, father, or newborn child are recommended to take 5 mg/day of medications containing active folate and B12 during the first trimester. It is also recommended to take 800 µg/day during the ongoing 2nd and 3rd trimesters and lactation [4,5].

Many studies have found that folate deficiency is associated with heart defects, urinary tract issues, limb abnormalities, cleft palate, low birth weight, and preeclampsia [6,7]. High concentrations of homocysteine in the blood are neurotoxic, teratogenic, and vasculotoxic. FA deficiency leads to increased homocysteine levels, which are believed to contribute to NTDs, abortions associated with placental vasculopathy, placental abruption, and preeclampsia [8,9].

A recent study revealed that folic acid supplementation during pregnancy can reduce the risk of childhood acute lymphoblastic leukemia by 60% [10]. Some studies have revealed that elevated maternal folate levels and FA supplementation are associated with gestational diabetes (GDM). It has been proven in a study conducted with 1058 patients in Shanghai that high levels of folate measured in maternal erythrocytes in the early period of pregnancy increase the risk of GDM [11]. A report summarizing evidence from studies conducted between July 2015 and July 2021, with ongoing surveillance until February 2023, indicates that folic acid supplementation before and during pregnancy significantly reduces the risk of neural tube defects. Findings from twelve observational studies, involving over 1.2 million participants, showed no significant harms, such as multiple gestation, autism, or maternal cancer, thus supporting previous recommendations for folic acid supplementation to prevent birth defects [12]. As shown above, a deficiency or excess of FA is linked to various health problems or abnormalities. The purpose of this study is to compare the perinatal outcomes of pregnant women who received FA supplements to those who did not in the first trimester of pregnancy, using a large sample size.

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2. Materials and Methods

This study was conducted retrospectively at Konya Training and Research Hospital between 2016 and 2020. All participants provided written informed consent with guarantees of confidentiality. A total of 2393 birth records of pregnant women were reviewed retrospectively. The study was approved by the Karatay University Clinical Research Ethics Committee (2024/009) and investigated pregnant patients who delivered between 2016 and 2020. For each woman, the number of visits, details of examinations, laboratory results from each visit, as well as birth and surgical reports, newborn assessments, and prescription records were reviewed and documented from the hospital computer system. Patients with systemic diseases who gave birth before the 24th week of pregnancy and for whom data were unavailable were not included in the study.

Factors such as maternal age, number of pregnancies, live births, miscarriages, and the number of antenatal follow-ups were assessed. The use of FA supplements prescribed through the e-prescription system during pregnancy was documented. Gestational age at birth, delivery method, Apgar scores at 1 and 5 minutes, birth weight, and, if necessary, neonatal intensive care unit (NICU) admission were recorded. In addition, the results of the oral glucose tolerance test (OGTT), hemoglobin (Hb), hematocrit (HCT), and platelet levels were recorded for the mother. The need for blood transfusion and length of hospitalization were also documented. Gestational age was determined using the last menstrual period (LMP). When the LMP was unknown, it was calculated using the first-trimester ultrasonography head-rump length (CRL) measurement. In cases with no follow-up, the gestational age was estimated based on the last ultrasonography measurement. Births before the 37th week, as determined by the last menstrual period, were classified as premature births according to the International Classification of Diseases system [13]. A 50-gram OGTT was administered to the patients. Those with glucose levels exceeding 145 mg/dL were considered at risk and referred for a two-step 75-gram OGTT. Participants with glucose levels over 200 mg/dL in the 75-gram OGTT were diagnosed with GDM. Individuals with one elevated glucose value in the 75-gram OGTT were also classified as having GDM.

Statistical Analysis

Descriptive statistics display numerical variables, such as the mean and standard deviation, as well as categorical variables, such as frequency and percentage. Chi-square and Fisher's exact tests were used to analyze categorical variables. For numerical variables, the *t*-test (a type of analysis of variance (ANOVA) test) was used, with pairwise comparisons conducted using the Tukey test. Statistical analysis was performed using the R 4.2.2 software (R Core Team, 2023, Vienna, Austria). A *p*-value less than 0.05 was considered statistically significant.

Table 1. Average data of all patients.

Variable	N = 2393[1]
Age (year)	27.73 ± 5.26
Gravida (n)	4.17 ± 1.93
Parity (n)	2.79 ± 1.62
Length of hospitalization (day)	1.50 ± 1.11
Birthweight (grams)	3151.87 ± 536.60
Apgar score 1. minute	7.09 ± 0.69
Apgar score 5. minute	8.93 ± 0.59
NICU admission (n/%)	
Yes	103.00 (4.30%)
No	2290.00 (95.70%)
Fetal anomaly (n/%)	
Yes	387.00 (16.17%)
No	2006.00 (83.83%)
Obstetric complication (n/%)	
Yes	333.00 (13.92%)
No	2060.00 (86.08%)
Hb (g/dL)	11.58 ± 1.53
HCT (%)	35.38 ± 4.09
Folic acid intake (n/%)	
Yes	861.00 (35.97%)
No	1532.00 (64.02%)
Antenatal follow-up (n)	3.48 ± 1.63
Preterm labor (n/%)	
Yes	100.00 (4.18%)
No	2293.00 (95.82%)
Blood transfusion (n/%)	
Yes	154.00 (6.44%)
No	2238.00 (93.56%)
Delivery type (n/%)	
Cesarean	1011.00 (42.25%)
Vaginal delivery	1382.00 (57.75%)
OGTT (n/%)	
GDM	32.00 (1.34%)
Normal	195.00 (8.15%)
No OGTT	2166.00 (90.51%)

[1]Mean ± SD; n (%); NICU, neonatal intensive care unit; Hb, hemoglobin; HCT, hematocrit; OGTT, oral glucose tolerance test; GDM, gestational diabetes; SD, standard deviation.

3. Results

A total of 2393 live births patients were included in the study. The mean age of the participants was 27.73 ± 5.26 years. The average gestational age at delivery was 270.63 ± 14.79 days, and the average weight of the patients at birth was 3151.87 ± 536.60 grams. Table 1 includes all the demographic and clinical parameters of the patients.

Out of the patients who gave birth, n = 861 (35.97%) took folic acid supplements in the first trimester of pregnancy, while n = 1532 (64.02%) did not. Table 2 presents the comparison of variables based on the intake of FA supplements. Yes, means the group of patients taking folic acid supplements, no means the group of patients not taking folic acid supplements.

Table 2. Comparison of groups that took and did not take folic acid supplements.

Variable	Yes, N = 861[1]	No, N = 1532[1]	p[2]
Age (year)	27.50 ± 5.13	27.87 ± 5.33	0.10
Gravida (n)	4.14 ± 1.87	4.19 ± 1.97	0.58
Parity (n)	2.67 ± 1.53	2.86 ± 1.66	0.005
Gestational age at birth (day)	271.59 ± 13.83	270.09 ± 15.27	0.005
Birthweight (grams)	3173.16 ± 518.58	3139.91 ± 546.27	0.14
Apgar 1. minute	7.11 ± 0.61	7.07 ± 0.74	0.94
Apgar 5. minute	8.96 ± 0.48	8.92 ± 0.65	0.45
Length of hospitalization (day)	1.42 ± 0.83	1.54 ± 1.23	0.021
NICU admission (n/%)			0.011
Yes	25.00 (2.90%)	78.00 (5.09%)	
No	836.00 (97.10%)	1454.00 (94.91%)	
Fetal anomaly (n/%)			0.34
Yes	131.00 (15.21%)	256.00 (16.71%)	
No	730.00 (84.79%)	1276.00 (83.29%)	
Obstetric complication (n/%)			0.64
Yes	116.00 (13.47%)	217.00 (14.16%)	
No	745.00 (86.53%)	1315.00 (85.84%)	
Hb (g/dL)	12.37 ± 1.26	11.14 ± 1.49	<0.001
HCT (%)	37.10 ± 4.18	34.41 ± 3.71	<0.001
Antenatal follow-up (n)	4.69 ± 1.20	2.80 ± 1.42	<0.001
Preterm labor (n/%)			0.20
Yes	30.00 (3.48%)	70.00 (4.57%)	
No	831.00 (96.52%)	1462.00 (95.43%)	
Blood transfusion (n/%)			<0.001
Yes	19.00 (2.21%)	135.00 (8.82%)	
No	842.00 (97.79%)	1396.00 (91.18%)	
Delivery type (n/%)			0.88
Cesarean	362.00 (42.04%)	649.00 (42.36%)	
Vaginal delivery	499.00 (57.96%)	883.00 (57.64%)	
OGTT (n/%)			<0.001
GDM	17.00 (1.97%)	15.00 (0.98%)	
Normal	123.00 (14.29%)	72.00 (4.70%)	
No OGTT	721.00 (83.74%)	1445.00 (94.32%)	

[1]Mean ± SD; n (%).

[2]Welch Two Sample *t*-test; Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test.

There were significant differences in gestational age at birth between the groups that took FA supplements and those that did not. The gestational age at birth for patients who took FA was 271.59 ± 13.83 days, whereas the gestational age at birth for patients who did not take FA was 270.09 ± 15.27 days (p = 0.005). No significant difference was found in birth weight between patients who took FA supplements and those who did not (p = 0.14). In the NICU, the admission rate of patients who did not take FA supplements was significantly higher than that of patients who took FA supplements (n = 25.00 *vs.* n = 78.00 patients, p = 0.011). There were no significant differences between the two groups in terms of fetal anomalies and obstetric complications (p = 0.34, p = 0.64).

Preterm labor rates did not show a significant difference between the groups (p = 0.20). Patients receiving FA supplementation showed significantly higher Hb (12.37 ± 1.26 g/dL) and HCT values (37.10 ± 4.18) compared to those who did not (Hb 11.14 ± 1.49 g/dL, HCT 34.41 ± 3.71; $p < 0.001$). The blood transfusion requirement of patients who did not receive FA supplements was found to be higher than that of those who received FA supplements. It was determined that out of 154 patients who received blood transfusions, n = 135.00 (8.82%) did not receive FA support, and this difference was found to be statistically significant ($p < 0.001$). Patients taking FA had a higher incidence of GDM (17.00, 1.97%) compared to those not using FA (15.00, 0.98%) ($p < 0.001$).

4. Discussion

This study was conducted using the birth records of 2393 pregnant women. The study concluded that GDM was more prevalent in the group that took FA supplements. Additionally, gestational age at birth, Hb, and HCT levels were higher, and consequently, the need for blood transfusion was reduced in the group that received FA. This article is one of the rare studies in the literature that has comprehensively investigated the pregnancy and perinatal outcomes of FA supplementation with a large patient population.

At present, numerous medical societies and the WHO do not recommend the routine use of dietary supplements. It is believed that maintaining a balanced diet before and during pregnancy can ensure adequate mineral and vitamin intake [14].

FA plays a crucial role in cell division and fetal development, but it cannot be synthesized in the body. In addition, since it has been proven to prevent neural tube defects, the WHO recommends 400 µg of folic acid supplementation starting 4–12 months before pregnancy and continuing until the 12th week of pregnancy [15]. The WHO recommends folic acid supplementation to prevent both anemia and NTD. During pregnancy, the increased demand for folic acid means deficiency can lead to megaloblastic anemia [16]. Daily oral iron and folic acid supplementation with 30–60 mg of elemental iron and 400 µg (0.4 mg) FA is recommended for pregnant women to prevent maternal anemia, puerperal sepsis, low birth weight, and preterm birth [2]. In our study group, the Hb value of patients who took FA supplements was 12.37 $\pm$ 1.26 g/dL, while the average Hb value of those who did not take FA supplements was 11.14 $\pm$ 1.49 g/dL. This difference was statistically significant ($p < 0.001$). At the same time, supporting this situation, the need for blood transfusion was evident in n = 19.00 (2.21%) patients in the group taking FA supplements, while it was observed in n = 135.00 (8.82%) patients in the group not taking FA supplements ($p < 0.001$). Research shows that iron and FA supplementation during pregnancy can reduce the risk of anemia [17,18].

The average birth weight of the patients who took FA was 3173.16 $\pm$ 518.58 grams, while the mean birth weight of those who did not take it was 3139.91 $\pm$ 546.27 grams in our study. This difference was not statistically significant ($p = 0.14$). Petry *et al.* [19], in their study with 974 patients (776 with mothers who supplemented with FA and 198 who did not), found that the baby's birth weight and subcutaneous fat tissue did not differ between patients who used FA and those who did not. In a study involving 466 women taking 200 µg/day of FA and 485 women taking 5 mg/day of FA, researchers found that the dosage of FA supplementation did not affect the mean birth weight, placental weight, or gestational age [20]. In their article on FA supplementation and newborn weights, Lin *et al.* [21], compared patients who used only FA during the preconception period with those who used multiple micronutrients in addition to FA. They found a reduced risk of small for gestational age (SGA) in patients who used both of these drugs compared to those who did not take any supplements. However, they found no effect on large for gestational age (LGA) with these drugs [21]. In our study, we found that the need for NICU admission was significantly lower in patients who consumed FA compared to those who did not ($p = 0.011$). The reason for this can be explained by the fact that the gestational age at birth of the group taking FA supplements was higher than that of those who did not.

Our study found no statistically significant difference in the incidence of fetal anomalies between the FA consumption group and the non-consumption group ($p = 0.34$). Similarly, the article on congenital hydrocephalus found no significant association between FA supplementation and the risk of developing this specific condition. This indicates that FA, despite its known benefits in preventing certain conditions like neural tube defects, might not influence the occurrence of congenital hydrocephalus [22]. A systematic review and meta-analysis conducted for this study conclude that there is no strong evidence of an association between FA intake alone and a reduced risk of oral clefts. While multivitamin use shows some protective effects, especially against cleft lip with or without cleft palate, this might be confounded by other lifestyle factors associated with multivitamin use [23].

There are studies in the literature that show conflicting results regarding the relationship between excessive FA intake and GDM. Some studies suggest that an excess of FA increases the risk of GDM. Two studies have shown that supplementing FA reduces the risk of GDM. Chen *et al.* [24], conducted a study in which they assessed the dietary intake of 1464 patients with GDM and 8092 non-GDM controls. They found that a diet rich in vitamins, especially high in folate, was associated with a reduced risk of GDM [24]. Li *et al.* [25], in their prospective cohort study, found that pre-pregnancy supplemental folate intake was significantly associated with a reduced risk of GDM.

Our study revealed that 1.97% of patients with GDM used FA, compared to 0.98% who did not. This difference was statistically significant ($p < 0.001$). There are studies in the literature that support our findings. A 2016 prospective cohort study involving 1938 participants identified a higher risk of gestational diabetes mellitus linked to daily folic acid supplementation during the first trimester. (adjusted odds ratio [aOR]: 2.25, 95% confidence interval (CI): 1.35–3.76) [26]. Chen *et al.* [11], studied a total of 1058 pregnant women to investigate the relationship between the levels of vitamin B12 and FA in red blood cells and GDM. They observed that the levels of FA and B12 in the red blood cells of patients with GDM were found to be high (with *p*-values of 0.045 and 0.002, respectively). They revealed that higher maternal red blood cell (RBC) folate and vitamin B12 levels in early pregnancy are significantly associated with the risk of GDM [11]. This finding should not

mislead the literature into thinking that FA supplementation increases the risk of GDM. Additional studies are needed. Excessive intake of FA has been linked to potential risks such as GDM. Studies indicate that high FA levels can affect cell functions via the folate receptor signaling pathway. For instance, optimal FA doses (0.1 M) have been found to enhance pancreatic stem cell transformation into insulin-secreting cells, promoting cell survival and proliferation. In contrast, high doses (1 M) have shown adverse effects, reducing cell survival and growth compared to ideal levels [27].

Limitations of the study: While we are aware that these patients were given FA supplements, there is a lack of clear information regarding whether they discontinued taking them in the 12th week of pregnancy. FA supplements are sometimes mistakenly used by pregnant women until the later stages of pregnancy. This may lead to an excess of FA. Stopping FA at 12 weeks is important as this could be the confounder re GDM. On the other hand, the rate of fetal anomalies was high, with 15% in the group taking FA and 16% in the group not taking FA; but we do not know if they were major or minor fetal anomalies, because in our hospital system, anomalies are recorded as present or absent, so it is not possible to distinguish between major or minor fetal anomalies. Obstetric complications are the same way. At the same time, we did not have information about the dietary intake status of FA for these patients. Gestational diabetes, being a metabolic issue, is influenced by many personal factors. The most significant limitation in our study was the lack of information on the body mass index of the patients involved. Finally, this study is not a large cohort study; it has a small patient population.

5. Conclusions

In summary, our study found higher gestational age at birth, higher Hb and HCT levels, and a higher incidence of GDM in the group that took FA supplements compared to those who did not. Furthermore, the FA supplementation group showed lower NICU admissions and blood transfusion requirements. Maternal FA supplementation is crucial during pregnancy. It reduces NTD and improves perinatal outcomes by preventing anemia. However, the process affecting newborn outcomes is multifactorial, influenced by many factors, not just folic acid supplementation. A single parameter cannot definitively establish a cause. From this study, we cannot conclude that FA increases the risk of GDM, further studies are needed to clarify this. However, future adjustments in FA supplementation for pregnant women at high risk for GDM could be important for maternal and neonatal health. In this regard, this study may serve as a crucial step for enabling more specific future research.

Availability of Data and Materials

Data is available from the corresponding author upon request.

Author Contributions

AOD designed the research study. DGK performed the research. AOD analyzed the data. DGK wrote the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The approval for the study was obtained from the Karatay University Clinical Research Ethics Committee (2024/009). All human subjects provided written informed consents.

Acknowledgment

We would like to thank the management and secretaries of Konya City Hospital for their help in data entry.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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