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Maternal anaemia and folate intake in early pregnancy

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ABSTRACT

Background The World Health Organization recommends that women take 400 µg of folate supplementation daily throughout pregnancy. We examined the relationship between total folate intake from the diet and supplements at the first prenatal visit and haematological indices at this visit and subsequently.

Methods Women were recruited at their convenience and in addition to clinical and sociodemographic details, detailed questionnaires on dietary intakes and supplementation consumption were completed under supervision. A full blood count and serum and red blood cell (RBC) folate levels were taken.

Results Of the 502 women studied, 97.5% had inadequate total dietary folate intake at the first visit, but, 98.2% were taking folic acid (FA) supplementation. Only 1.8% ($n = 9$) had anaemia at their first visit (with no case of macrocytosis). Subsequently, 212 women had a further Hb sample in the third trimester and 8.5% ($n = 18$) were anaemic and 43.4% (89/205) were anaemic postnatally. There was a relationship between the development of anaemia postnatally and lower RBC folate levels at the first visit ($P = 0.02$).

Conclusions In a country where FA food fortification remains voluntary, these findings support the recommendation that women should start FA supplementation before pregnancy and continue FA after the first trimester.

Keywords anaemia in pregnancy, folic acid supplementation

Introduction

The associations between maternal anaemia and an increase in maternal and foetal mortality and morbidity are well established, particularly in the developing world.[1,2] Anaemia is more likely to develop as pregnancy advances due to increasing feto-maternal cellular requirements and physiological changes in folate metabolism. To adjust for haemodilution during pregnancy, anaemia may be defined as a haemoglobin <11.0 g/dL in the first trimester, <10.5 g/dL in the second and third trimesters and <10.0 g/dL postnatally.[3,4]

The prevalence of anaemia in pregnancy varies widely due to the variation in maternal dietary intakes of micronutrients and in the consumption of supplementary vitamins. In pregnant women, the prevalence of anaemia was 6.1% in the USA, 18.7% in Europe and 55.8% in Africa with inadequate iron intake the commonest cause.[5] As iron deficiency may result in a microcytic anaemia, it is recommended by the World Health Organization (WHO) that in regions with a high prevalence of anaemia, all pregnant women should take 60 mg of iron during pregnancy to prevent iron deficiency anaemia.[6]

Inadequate folate intake may result in a macrocytic anaemia but the recommended nutrient intake values during pregnancy vary widely between countries.[7] In 1991–92, two landmark RCTs demonstrated that folic acid (FA) supplementation prevents neural tube defects (NTDs). Subsequently, national recommendations advised women to start FA before pregnancy and to continue for the first trimester.[8] Many countries also implemented either voluntary or mandatory FA food fortification policies because periconceptual FA supplementation

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rates were too low.[9] Fortification alone, however, is not deemed to be adequate to achieve the desired levels with the continuing intake of a supplement considered a more reliable method, especially in women following a low carbohydrate or gluten free diet.[10]

There is a dearth of recent information as to what impact the implementation of public health measures on supplementation and fortification with FA have had on development of a macrocytic anaemia during pregnancy particularly in well-resourced settings. It is notable that in a review of national guidelines from 20 European countries the focus was on FA supplementation in the first trimester to prevent NTDs but, no recommendations were made for the rest of pregnancy to prevent other potential adverse pregnancy outcomes, despite established dietary deficiencies of folate in adult women.[8] WHO advises 400 μg of FA supplementation to continue throughout pregnancy in response to fetomaternal cellular requirements and evidence of dietary deficiency.[6]

In a recent prospective observational study, we found that women needed to start FA supplementation before they became pregnant to achieve RBC folate measurements associated with a decreased risk of NTDs.[11] In this secondary analysis we examined the relationship between maternal intake of both dietary folate and FA supplementation in early pregnancy with haematological indices both at the first prenatal visit and as pregnancy advanced.

Materials and methods

We studied women presenting for prenatal care after ultrasound confirmation of an ongoing pregnancy <18 weeks gestation. The exclusion criteria were women aged <18 years old and women who could not understand English. Women were recruited conveniently at their first prenatal visit by a single researcher (SC). Their height and weight were measured before calculating body mass index (BMI). Clinical and sociodemographic details were computerized as part of the standard medical records by a trained midwife using a barcode system.

The women also completed a detailed questionnaire under supervision which included questions on the duration of FA use, multivitamin use and the brand and dosage of supplements. Their total dietary intake was calculated using Nutritics Version 3.7 (University Edition). The dietary data were compiled based on an average daily intake for each food listed in a retrospective four day dietary history which was completed under supervision. Care was taken to specify particular food brands due to the varying content with respect to voluntary FA fortification.[12] Dietary folate equivalent was calculated for the dietary and supplemental intakes where 1 μg of folate from food is equal to 1 μg DFE. For FA in fortified food or supplements 1 μg of FA is equal to 1.7 μg DFE, as folate from food is 50% bioavailable compared to 85% bioavailability from supplementation (85/50 = 1.7).[13]

In addition, a routine full blood count was taken and blood for other biomarkers including plasma folate, whole blood folate and RBC folate levels. A microbiological assay was used to measure maternal folate.[14]

The hospital laboratory results system was searched for any further full blood count results taken over the course of the pregnancy and the gestation at phlebotomy recorded (if multiple samples were taken, the sample with the highest Hb value from each trimester was recorded). Delivery information was computerized under midwifery supervision immediately after birth before discharge from the delivery suite.

Data was anonymized and coded in SPSS (IBM SPSS Statistics version 20). Continuous data were assessed for normality by visual inspection of the histogram and box-plot and calculation of kurtosis and skewness. Descriptive statistics were used to describe the characteristics of the study population and where necessary, continuous variables were recoded into categorical variables for further analysis. Independent samples *t*-test was used to assess the difference between the mean Hb in the third trimester according to prepregnancy folic use. Linear regression was used to assess for relationships between prepregnancy folic acid use and duration of use and RBC folate levels at the first prenatal visit and Hb at term. Multiple regression was performed to control for potential confounding factors where necessary. Tests for statistical significance were performed with significance set at the 95% level.

Approval was granted by the Hospital's Research and Ethics Committee and written consent was obtained.

Results

The characteristics of the study population are shown in Table 1. The study population was similar to the overall hospital population as reported in the Annual Clinical Reports.[15] The population studied was predominantly of white European ethnicity (including Irish) (n = 446, 93.0%(415)) and 30 of the women reported living in Ireland ≤5 years (n = 388, 7.7%). One-third (n = 456, 30.5%) reported finishing in full time education at ≤18 years of age and of the 308 women providing the relevant information, the rates of relative income poverty and consistent poverty were 23.9% and 4.2%, respectively (Table 1).

Table 2 shows the dietary intakes and vitamin supplements consumption reported by women at their first antenatal visit. Despite voluntary food fortification, only 2.5% of

Table 1 Characteristics of the study population (n = 502)

Characteristic	
Age (years) (mean ± SD)	30.7 ± 5.5
BMI (kg/m^2) (mean ± SD)	26.0 ± 5.5
Nulliparous (%)	42.6% (n = 214)
Current smokers (%)	12.9% (n = 65)
Obese (%)	19.3% (n = 97)
Ethnicity (%) (n = 446)	
White European including Irish	93.0% (n = 415)
Afro Carribean	1.8% (n = 8)
Asian	1.4% (n = 6)
Other	3.8% (n = 17)
Finished full time education at ≤18 years (n = 456)	30.5% (n = 139)
Relative income poverty[a] (%) n = 308	23.9% (n = 74)
Consistent poverty[a] (%)	4.2% (n = 13)
Lived in Ireland ≤ 5 years (%) n = 388	7.7% (n = 30)
GA at first visit (weeks) (mean ± SD)	12.5 ± 2.4
Preterm delivery[b] (before 37.0 weeks) (%)	4.0% (19)
Birth weight[b] (g) (mean ± SD)	3422.0 ± 573.3
Birth weight[b] < 2.5 kg (%)	5.2% (26)
Birth weight[b] > 4.5 kg (%)	1.8% (9)
Caesarean delivery[a] (%)	28.4% (134.0)

SD, standard deviation; BMI, body mass index; GA, gestational age.
[a]These measures were based upon questions from the European Union—Survey on Income and Living Conditions (EU-SILC) 2011.[16]
[b]Data on delivery outcomes were confined to 472 women as 30 women were lost to further follow up and did not deliver in the hospital.

Table 2 Dietary and supplement intakes and laboratory measurements of the study population

Dietary intakes[a]	
Median total dietary folate intake (μg) (IQ range)	235.2 μg (IQ range: 143.6)
Median dietary folate equivalent (μg DFE) (IQ range)	255.1 μg DFE (IQ range: 185.2)
WHO RNI dietary folate equivalent (μg DFE)	600 μg DFE
Meeting dietary folate recommendations (%)	2.5% (n = 10)
Meeting folate recommendations (based on dietary and supplemental intake) (%)	100.0%
Median dietary vitamin B12 intake	4.0 μg (IQ range: 2.7)
WHO RNI for dietary vitamin B12	2.6 μg
Meeting dietary vitamin B12 recommendations (%)	77.3% (n = 303)
Median dietary iron intake	9.3 mg (IQ range: 4.4)
EFSA PRI for dietary Iron	27–30 mg
Meeting dietary iron recommendations (%)	2.0% (n = 8)
Supplement intake	
Taking folic acid prepregnancy (%)	42.8% (n = 215)
Taking folic acid at the first antenatal visit (%)	98.2% (n = 493)
Laboratory measurement (n = 502)	
Serum folate (nmol/L) (median, IQ range)	34.6 (IQ range: 18.1)
Deficient based on serum folate level (%)[b]	1.0% (n = 5)
Red blood cell folate (nmol/L) (mean, SD)	1137.5 (±443.0)
Deficient based on red blood cell folate level[c]	0.0%
Red blood cell folate <906 nmol/L (%)[d]	34.3% (n = 172)
Serum vitamin B12 (pmol/L) (median, IQ range)	205.0 (IQ range: 110.5)

SD, standard deviation; IQ range, interquartile range; WHO, World Health Organization; RNI, recommended nutrient intake (per day); EFSA, European Food Safety Authority; PRI, population reference intake (per day).
[a]Dietary intakes reported are based on analysis of the food diaries which were complete for 398 women in the study cohort.
[b]World Health Organization cut off for folate deficiency based on serum folate <6.8 nmol/L.
[c]World Health Organization (WHO) cut off for folate deficiency based on red blood cell folate <226.5 nmol/L.
[d]The reported threshold concentration for red blood cell folate is reported as 906 nmol/L by Daly *et al.*[17] Above this concentration, the risk of neural tube defects (NTDs) was reduced to <8 NTDs/10 000 live births.

women had a total dietary folate considered adequate for pregnancy. The median total dietary folate intake reported at the first visit (n = 398) was 235.2 μg (interquartile range: 143.6 μg). At the time of the first prenatal visit, 98.2% of women were taking FA supplementation with only 1.0% deficient based on red blood cell (RBC) folate level and 0.0% deficient based on serum folate level. However, 34.3% of women had a RBC folate level below 906 nmol/L (Table 2).

The incidence of anaemia was 1.7% based on a cut-off of <11.0 g/dL in the 420 women who presented in the first trimester. The incidence of anaemia in the women who attended for their first visit in the second trimester (n = 82) was 2.4% based on a cut-off of <10.5 g/dL. There were 18 cases of anaemia (8.5%) in the 212 women who had a full blood count taken in the third trimester. Postnatally there were 89 cases of anaemia based on a cut-off of 10.0 g/dL (representing 43.4% of the women who had a full blood count postnatally). There were no cases of macrocytic anaemia identified in any trimester or postnatally (Table 3).

Women who were anaemic in the third trimester and postnatally had lower rates of preconceptual folic acid use compared to those who did not have anaemia (third trimester; FA use in those with anaemia—27.8% versus FA use in those who were not anaemic—45.6% (not significant) and postnatally; FA use in those with anaemia—43.8% versus FA use in those who were not anaemic—50.9% (not significant)) (Supplementary Table S1).

Table 3 Full blood count parameters measured in each trimester and postnatally

	First trimester[a] *(n = 420)*	*Second trimester*[a] *(n = 82)*	*Third trimester*[a] *(n = 212)*	*Postnatally (n = 205)*
Mean gestation at phlebotomy (antenatal—weeks, postnatal—days) (±SD)	11.7 (±1.4)	16.6 (±2.4)	39.2 (±1.2)	1.9 (±1.2)
Mean Haemoglobin (g/dL) (±SD)	12.9 (±0.9)	13.0 (±0.8)	12.1 (±1.2)	10.2 (±1.5)
Mean MCV (fl) (±SD)	88.7 (±4.1)	88.7 (±4.2)	88.7 (±5.8)	87.4 (±5.7)
Anaemic[b] (%)	1.7% (*n* = 7)	2.4% (*n* = 2)	8.5% (*n* = 18)	43.4% (*n* = 89)
Microcytic anaemia[c] (%)	0.2% (*n* = 1)	0.0%	3.8% (*n* = 8)	8.8% (*n* = 18)
Macrocytic anaemia[c] (%)	0.0%	0.0%	0.0%	0.0%

SD, standard deviation; MCV, mean corpuscular volume.

[a]Cut-offs for gestation; first trimester <14 weeks, second trimester ≥14 weeks, <28 weeks, third trimester ≥28 weeks. Of the 502 patients, 420 were in their first trimester at the time of phlebotomy and 82 were in their second trimester. 212 of the 502 patients had a sample taken in the third trimester between 37 and 42 weeks.

[b]Cut off for anaemia in the first trimester; haemoglobin (Hb) <11 g/dL, second and third trimester Hb <10.5 g/dL and postnatal Hb <10.0 g/dL.

[c]Microcytic, MCV < 80 fl; macrocytic, MCV >100 fl.

There was a positive relationship identified between a higher haemoglobin postnatally and a higher RBC folate level at the first prenatal visit. For women with a Hb > 10.0 g/dL (n = 116) postnatally, the mean RBC folate at the first prenatal visit was 1202.3 (± 444.4) nmol/L compared to a mean RBC folate of 1060.1 (± 429.1) nmol/L in those with a Hb < 10.0 g/dL postnatally ($P = 0.02$) (Supplementary Table S1).

The overall percentage taking FA prepregnancy was 42.8% (Table 2). The use of FA prepregnancy was lower in those women reporting that they completed full time education at ≤18 years of age ($P < 0.001$) and amongst those reporting relative income poverty ($P < 0.01$). There was no relationship between prepregnancy FA use and ethnicity or years lived in Ireland. RBC folate at the first prenatal visit and Hb in the third trimester had a positive relationship with the prepregnancy use of FA ($P < 0.001$ and $P < 0.005$, respectively). In particular, women who started FA before pregnancy were more likely to have a higher RBC folate at the first prenatal visit (B: 390.1, 95% CI: 316.9–463.2, $P < 0.001$), and a higher Hb in the third trimester (B: 0.487, 95% CI: 0.171–0.804, $P = 0.003$).

While women who started FA prepregnancy had the same mean haemoglobin at the first prenatal visit compared to those who did not take prepregnancy FA (12.9 ± 0.8 versus 12.9 ± 0.9 g/dL), they had an increase in their mean haemoglobin in the third trimester ($n = 212$, 12.3 ± 1.1 versus 11.9 ± 1.2 g/dL, $P = 0.003$). Multiple regression was performed to demonstrate the relationship between taking folic acid prepregnancy and a higher haemoglobin in the third trimester. The relationship between taking FA prepregnancy and Hb persisted in the third trimester in the first model after controlling for age, BMI, current smoking and parity (B: 0.348, 95% CI: 0.010–0.686, $P = 0.04$). As BMI and smoking status did not show a significant relationship with Hb in the third trimester, they were omitted in the second model (B: 0.361, 95% CI: 0.028–0.694, $P = 0.03$) (Table 4).

Discussion

Main findings of this study

Even with voluntary folate food fortification, almost all women studied (97.5%) had a total dietary folate intake below that recommended by WHO for pregnancy and breastfeeding,[18,19] yet, no woman presenting for prenatal care had a macrocytic anaemia. The absence of macrocytic anaemia may reflect good compliance with FA supplementation in early pregnancy.

In total, 98.2% were taking FA supplementation at the time of phlebotomy. However, women who started FA after they became pregnant had a lower RBC folate at presentation and were more likely to have a lower Hb in the third trimester. Of the women who had a FBC postpartum nearly half were anaemic. This study highlights the potential benefits of women starting FA before pregnancy and continuing supplementation throughout pregnancy, even in a developed country, to prevent maternal anaemia and possibly blood transfusion.

The primary analysis related to this work demonstrated that the median time of commencement of FA in the group that took prepregnancy FA (42.8%) was 12 weeks before pregnancy and in the remaining women, the median duration of commencement was 5 weeks post the last menstrual period (coinciding with the time point of a positive pregnancy test).[11] It was possible in this analysis to demonstrate that those who commenced FA ≥ 4 weeks prepregnancy were

Table 4 Multiple regression analysis showing the relationship between taking folic acid prepregnancy and higher haemoglobin in the third trimester controlled for maternal age, BMI, smoking status and parity

Variable	*Coefficient (B)*	*SE*	*95% CI*	P[a]
Model 1				
Folic acid prepregnancy	0.348	0.171	0.010–0.686	0.04
Maternal age	0.034	0.017	0.000–0.068	0.05
Maternal BMI	0.005	0.014	–0.022–0.032	0.71
Smoking status	–0.117	0.265	–0.640–0.407	0.66
Nulliparas	0.412	0.176	0.075–0.768	0.02
Model 2				
Folic acid prepregnancy	0.361	0.169	0.028–0.694	0.03
Maternal age	0.036	0.017	0.002–0.069	0.04
Nulliparas	0.407	0.172	0.067–0.747	0.02

SE, standard error; CI, confidence interval; BMI, body mass index.
[a]Significance value set at 95% level.

more likely to achieve the optimal RBC folate level of >906 nmol/L (86.4 versus 53.3%, $P < 0.001$).

This study has demonstrated a relationship between use of FA prepregnancy and a higher haemoglobin in the third trimester and the relationship persisted upon controlling for maternal age, BMI, smoking status and parity. Theoretically, a higher haemoglobin in the third trimester should reduce the need for blood transfusion due to haemorrhage and should improve maternal well-being postpartum.

In view of the dietary inadequacies of folate, despite voluntary food fortification, our findings strengthen the case for women continuing FA supplementation throughout pregnancy, as recommended by WHO, especially if they only started during pregnancy.[6]

Considering the strengths of the study, the hospital provides care for women from all socioeconomic groups across the urban-rural divide. All women had sonographic confirmation of gestational age at the first prenatal visit. Clinical and sociodemographic data were computerized at the first visit, thus minimizing recall bias. Maternal weight and height were measured before calculating BMI, and not self-reported at the first visit. Information on dietary intake and supplementation use (including the dose and brand of each supplement) were collected at the same visit by a SC. Biomarkers were also measured at the same visit. The laboratory is validated for the analysis of plasma folate, whole blood folate and RBC folate. Pregnancy outcomes were computerized immediately after delivery.

What is already known on this topic

In a study in the USA, for the first time, data on the reported dietary supplement use and folate status among pregnant women sampled in the National Health and Nutrition Examination Survey (NHANES) was analysed in 1296 women from 1999 to 2006 after FA food fortification became mandatory.[20] When surveyed about taking FA either as a single or multivitamin in the previous 30 days, only 55–60% reported they consumed FA in the first trimester, 76–78% in the second and 88% in the third. The majority were taking 800 μg as a single supplement. The median RBC folate was 1255 nmol/L in the first trimester and increased as pregnancy advanced. No information, however, was presented on maternal haematological indices.

A mandatory food fortification programme in the USA has been shown to increase serum folate with a corresponding 19% decline in the incidence of NTDs reported consequently.[21,22] A Canadian study based on 95 women of childbearing age demonstrated that mandatory food fortification with FA increased the mean folate consumption by 96 μg/day (within the range predicted by the food fortification programme; 70–130 μg/day for adults aged ≥ 19 years). No one within this cohort met the dietary folate equivalent for FA without supplementation. Only 14% of women exceeded the optimal RBC folate of 906 nmol/L associated with a reduced risk of NTDs.[17,23]

A systematic review of 31 studies involving 17 771 women examined FA supplementation during pregnancy and pregnancy outcomes.[24] The studies were undertaken 30–45 years previously in both developed and developing countries using differing dosages of supplementation and differing definitions of anaemia. The review found no impact on the mother of FA before delivery on anaemia (eight studies), haemoglobin levels (12 studies), serum folate levels (eight studies) or RBC folate levels (four studies). FA

supplementation during pregnancy did, however, reduce the incidence of megaloblastic anaemia by 79% (four studies; RR = 0.21 95% CI: 0.11–0.038). As many of the studies were 30–45 years old, they were conducted before widespread supplementation and fortification practices were introduced.

The authors had intended to carry out a subgroup analysis based on different durations of FA supplementation but were unable to do so because not all studies included baseline haemoglobin levels and furthermore, the duration and timing of FA supplementation during pregnancy varied. Such information was available for our study as well as information on FA dosage and other supplements consumed.

What this study adds

Detailed dietary analysis in this study has shown that dietary folate intakes are inadequate for almost all women. Owing to supplementation, we have shown that serum and RBC folate are adequate by the first prenatal visit with a positive correlation between duration of use and these levels. Beyond the role of folate in preventing NTDs, folate is an integral vitamin to fetomaternal cellular development throughout pregnancy. We believe this study provides evidence that women should continue supplementation after the first trimester and postpartum to avoid anaemia and blood transfusion.

Limitations of this study

Limitations of the study include the fact that this study was undertaken in a country where fortification of food with folate is voluntary and not mandatory. Also, we do not have information as to how many women continued either single supplementation of FA or iron or multivitamins after the first trimester.

Supplementary data

Supplementary data are available at the *Journal of Public Health* online.

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Conflicts of interest

The authors have no conflict of interest to declare.

Authors' contributions

EOM contributed to the conception and design of the study, collected data, performed data analysis and wrote and edited the article. SC conducted the primary study involving patient recruitment, issuing questionnaires, analysis of food diaries and gathering data and contributed to the conception and design of this study and drafting of the article. RK was involved in the conception and design of this study, data analysis and contributed to writing and editing. CR and AM were involved in conception and design of the study, drafting sections of the article and approving the final submitted version. MT contributed to the conception and design of the study, analysis of data and writing and editing of the article.

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