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IS LOW IRON STATUS A RISK FACTOR FOR NEURAL TUBE DEFECTS?

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Abstract

Background—Folic acid supplements can protect against neural tube defects (NTDs). Low folate and low vitamin B_{12} status may be maternal risk factors for having an NTD affected pregnancy. However, not all NTDs are preventable by having an adequate folate/ B_{12} status and other potentially modifiable factors may be involved. Folate and vitamin B_{12} status have important links to iron metabolism. Animal studies support an association between poor iron status and NTDs but human data are scarce. We examined the relevance of low iron status in a nested NTD case-control study of women within a pregnant population-based cohort.

Methods—Pregnant women were recruited between 1986 and 1990, when vitamin or iron supplementation in early pregnancy was rare. Blood samples, taken at an average of 14 weeks gestation, were used to measure ferritin and hemoglobin in 64 women during an NTD affected pregnancy and 207 women with unaffected pregnancies.

Results—No significant differences in maternal ferritin or hemoglobin concentrations were observed between NTD affected and non-affected pregnancies (case median ferritin 16.8μg/L and hemoglobin 12.4g/dL versus 15.4μg/L and 12.3g/dL in controls). As reported previously, red cell folate and vitamin B_{12} concentrations were significantly lower in cases. Furthermore, there was no significant association of iron status with type of NTD lesion (anencephaly or spina bifida)

Conclusions—We conclude that low maternal iron status during early pregnancy is not an independent risk factor for NTDs. Adding iron to folic acid for periconceptional use may improve iron status but is not likely to prevent NTDs.

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Keywords

ferritin; iron; hemoglobin; neural tube defects

INTRODUCTION

Iron deficiency is the most common nutrient deficiency in pregnancy and the most common cause of anemia (Haider and others, 2013; Iannotti and others, 2005; Pathak and others, 2007). Fetal iron requirements place heavy demands on maternal stores (King, 2003) and numerous studies have linked low maternal iron status to low birth-weight and pre-term birth (Haider and others, 2013). In the fetal compartment, iron has extensive involvement in growth and development as a cofactor in hemoproteins and iron cluster enzymes involved in fetal energy metabolism, cell proliferation and organogenesis (Fretham and others, 2013; Zohn and Sarkar, 2010). Iron is required by the growing embryo from the earliest stages in pregnancy, as demonstrated by the existence of specific receptor complexes for transfer of iron across the visceral yolk sac prior to the development of the fetal blood system (Donovan and others, 2005; Young and others, 1997; Zohn and Sarkar, 2010).

Neural tube defects (NTDs) are the most common anomalies of the central nervous system with over 250,000 affected pregnancies each year and a birth prevalence from approximately 6 to 60 cases per 10,000 births world-wide (Botto and others, 2006; Dolk and others, 2010; Heseker and others, 2009; Moore and others, 1997; Rosenthal and others, 2013). The etiology of NTDs is complex, with both genetic and environmental factors implicated (Molloy and others, 2009a) and the cause of many NTDs remains to be determined. Providing folic acid to women in the periconceptional period has resulted in a large decrease in NTD rates; however, between 30% of 50% NTDs cannot be prevented by folic acid (Botto and others, 1999; De Wals and others, 2007; Heseker and others, 2009; MRC, 1991; Williams and others, 2005). Thus there is a need to continue research to identify additional risk factors and alternative interventions for preventing NTDs. It is possible that a number of other nutritional factors may contribute to NTD etiology (Mills and Carter, 2009; Mosley and others, 2009). For example, there is now reasonably strong evidence that low vitamin B_{12} status is involved, and in particular, may exacerbate risk in conjunction with low folate status (Molloy and others, 2009b; Ray and others, 2007; Zhang and others, 2009). Other micronutrients that have been implicated include zinc, vitamin C and choline but the evidence for an association is conflicting and weak (Carmichael and others, 2010; Cengiz and others, 2004; Hinks and others, 1989; Schorah and others, 1983; Shaw and others, 2004; Shaw and others, 2009)

Studies using genetically mutated animal models confirm that iron status is important in embryonic development (Donovan and others, 2005; Hoyle and others, 1996) and especially in the development of the neural tube (Mao and others, 2010). Several case controls studies have proposed that low iron status may contribute to risk of having an NTD affected pregnancy (Felkner and others, 2005; Groenen and others, 2004) although other studies have not found an association (Weekes and others, 1992). Therefore the aim of this case-control

study was to investigate the relationship between iron status and NTDs during early pregnancy in women carrying an NTD affected fetus and in non-affected pregnant controls.

METHODS

Between March 1986 and March 1990, blood samples were collected from 56 049 women attending their first antenatal clinic in the 3 major Dublin maternity hospitals. This corresponds to approximately 70% of births in these hospitals during the study period. Blood was collected into K_2 EDTA, plasma was separated and samples were stored below −20°C. Mothers who delivered an NTD affected infant within this period were identified and the bio-bank was searched for plasma samples collected during the affected pregnancy. A total of 81 samples with sufficient plasma for analysis were retrieved. Using a nested case-control design, 247 plasma samples were retrieved relating to women who delivered infants without major malformations. Details on maternal pregnancy history, including maternal age, weight, gestation at first antenatal clinic, gestation at delivery, supplement use, hemoglobin at first antenatal clinic, previous pregnancy outcomes and delivery details of the study pregnancy were obtained from hospital records. Ethical approval was obtained for the study and participants consented to giving a research blood sample. Human subject approval was also obtained from the office of the US National Institutes of Health. These study participants have been the subject of several studies since the collection was completed, diminishing the available plasma (Daly and others, 1995; Kirke and others, 1993; Mills and others, 1995; Molloy and others, 2009b). For the current study, plasma from 64 case mothers and 207 control mothers was available for ferritin assay.

Ferritin levels in the samples were measured using a Microparticle Enzyme Immunoassay (MEIA) (Abbott AxSYM Plus platform). The samples were analysed as four batches in a continuous run of assays. Cases and controls were randomly distributed across assay batches and the individuals performing the assay were blinded as to the status (case or control) of the samples. Six ferritin controls were included in each run. Folate and vitamin B12 were measured by microbiological assays, using a chloramphenicol resistant strain of Lactobacillus Casei for folate and a colistin resistant strain of Lactobacillus Leichmannii for vitamin B12 as described previously for this nested case-control cohort (Kirke and others, 1993; Molloy and others, 2009b). Homocysteine was measured by HPLC linked to fluorescence detection as described in a previous publication on these samples (Mills and others, 1995).

Statistical methods

The statistical analysis was performed using the SAS (Version 9.3) and any P values <0.05 were considered significant. Data were not normally distributed and are presented as medians and interquartile (25th and 75th) ranges. Case control comparisons were carried out on log transformed data. Associations between variables were assessed on log transformed data using a Pearson correlation coefficient. Differences in proportions were assessed using a Fisher's Exact test. Ferritin concentrations less than 12 μg/L were considered to indicate iron deficiency (Felkner and others, 2005; Shields and others, 2011). Logistic regression analysis was carried out to assess significant predictors of NTDs.

RESULTS

Among the NTD cases, 25 (39%) had anencephaly, 34 (53%) had spina bifida and 5 (8%) had other NTD defects (encephalocele, iniencephaly or combined anomalies). Demographic details of cases and controls are shown on Table 1. There were no folic acid supplement users but 7.8% of cases and 6.3% controls (5 cases and 13 controls) had reported some iron use prior to the first antenatal clinic and blood sample, with no significant difference between the proportion of users within cases and controls (p=0.87). Details of blood ferritin, hemoglobin and B vitamin status are given in Table 2. There was a weak positive correlation between ferritin and hemoglobin among all 271 subjects (r=0.22; p=<0.001) and between vitamin B12 and both iron status markers (r=0.15; p=0.015 for ferritin and r=0.23; p<0.001 for hemoglobin). This remained significant in controls when the data were sub-divided by case-control status but not in cases. There were no associations of iron status markers with serum or red cell folate.

Neither the maternal ferritin nor maternal hemoglobin concentrations were significantly different by case/control groups (Table 2). As previously reported, vitamin B12, plasma folate and red cell folate concentrations were lower in case mothers compared with controls (Kirke and others, 1993; Molloy and others, 2009b). Plasma homocysteine was marginally higher in cases. Reanalysis after removal of iron supplement users had no effect on the data. We carried out a logistic regression analysis to determine the effect of ferritin and other vitamins on risk of being a case mother (Table 3). There was no effect of ferritin or hemoglobin, whereas red cell folate and vitamin B12 remained significant factors in this reduced sample size analysis. Ninety eight women in total (36%) had ferritin concentrations less than 12 μg/L, which is a recognized cut-off for deficiency. Using a dichotomous logistic model and taking ferritin levels less than 12 μg/L as an indication of iron deficiency, there was also no significant effect of iron deficient status on risk of having an NTD affected pregnancy (Table 3). Finally, we looked at the relationship between ferritin status and NTDs in those with low and high folate status separately by sub-dividing the cohort into two groups, based on those with red cell folate above or below the median concentration found in controls (734 nmol/L). As expected, there were more NTD case mothers in the lower status (N=44) versus higher status group (N=20), but we found no significant case effects for ferritin in the lower (p=0.15) or higher (p=0.62) folate status groups.

DISCUSSION

We found no association between ferritin or hemoglobin concentrations measured during pregnancy and NTD risk. In fact ferritin showed a non-significant direct correlation with NTD risk (Table 3), making a protective effect unlikely. Although we cannot rule out an effect of low iron stores on development of defects during neural tube closure, the relevance of this to prevalence of NTDs is likely to be much lower than that for low folate or vitamin B12 status, which remain significant factors in this truncated data set from our previous studies (Kirke and others, 1993; Molloy and others, 2009b).

Iron is an important nutrient in pregnancy, and pregnant women are vulnerable to insufficient iron status. Serum ferritin determination is thought to be the most sensitive

indicator of iron deficiency with concentrations lower than 12 μg/L indicating depletion of body stores (Felkner and others, 2005). In a recent survey of iron status during healthy pregnancies, ferritin levels below 12 μg/L were found in 30% of pregnant women (Shields and others, 2011). It is unsurprising therefore that 36% of all women in this study fell below the threshold for depletion and 47% were lower than 15 μg/L, which is the World Health Organization cut-off for diagnosis of deficiency in adults (Pasricha and others, 2013).

Inconsistent findings in the literature make it uncertain whether low iron status is a risk factor for NTDs. Much of the experimental evidence supporting a role of iron in the prevention of NTDs is derived from studies of mice with hypomorphic mutations in the gene encoding the iron transporter ferroportin gene. These animals present with NTD-like congenital defects (Mao and others, 2010). Additional animal studies have shown that having a set of intact iron transport proteins is essential for early developmental processes (De Domenico and others, 2008; Donovan and others, 2005; Fraenkel and others, 2005; Zohn and others, 2007; Zohn and Sarkar, 2010). It is therefore clear that iron plays an important role in development of the fetus (King, 2003). However, this study failed to find a clear association between ferritin levels, an indicator of iron status, and human NTDs.

There are very few human studies that examined NTDs in relation to iron status. Felkner et al (Felkner and others, 2005) conducted an extensive study, drawing blood five to six weeks post-partum and collecting food frequency questionnaire data on cases and controls. They found a non-significant increase in risk associated with low iron intake and odds ratios of 1.8 for both low ferritin (<12 μg/L) and medium ferritin (12–29 μg/L) concentration compared with high ferritin concentration (≥30 μg/L). The odds ratio for medium, but not low, concentration was statistically significant. They noted that they could not “definitively demonstrate a causal effect for low serum ferritin”. Carmichael and co-workers (Carmichael and others, 2010) reported an increased risk for NTDs when pregnant women consumed a lower quality diet, i.e. lower iron, vitamin B6, calcium, vitamin A and folate. It is not clear, however, whether inadequate iron rather than inadequate folate was the critical factor. Chandler et al (Chandler and others, 2012) conducted an extensive analysis of dietary factors as risks for NTDs. They found that lower iron intake as reported by interview was associated with a significantly increased risk for anencephaly but not spina bifida in women who took folic acid supplements. They found no significant effect in non-users, or when the data were stratified by racial/ethnic groups or body mass index groups. The authors note that 816 comparisons were made, thus raising the possibility that the positive findings might have occurred by chance, particularly given the inconsistent pattern. In a Netherlands study, case mothers reported significantly lower intakes than control mothers of iron and five other nutrients on food frequency questionnaires (Groenen and others, 2004). Limitations of this study include an interval of several years between the event and completion of the questionnaire and uncertainty regarding which of the six nutrients might have caused the increased risk for NTDs.

Other studies have not found an association between iron status and NTDs. In a medical record investigation, Banhidy et al. found that women who had anemia, (almost all of whom had iron deficiency), in the second and third months of pregnancy had no increased risk for having an affected child (odds ratio 1.0; 95%CI: 0.7–1.4) (Banhidy and others, 2010). The

authors had information on when the anemia was diagnosed, but not when it began. Thus, they could not be certain whether women who were first evaluated later in pregnancy were anemic during neural tube closure or not. Placental iron has also been measured in NTD cases and controls. In a study of 80 cases and 50 normal controls, (Liu and others, 2013), cases had a somewhat higher placental iron concentration (86 μg/g) than controls (82 μg/g). The case placentas were, however, collected at an earlier gestational age because of pregnancy terminations; this might have affected iron levels. Finally, Weekes and coworkers (Weekes and others, 1992) measured iron in amniotic fluid in cases and controls; they found no significant difference. They noted that amniotic fluid levels are approximately 10 to 30 percent the levels found in maternal serum. It is not clear how amniotic fluid levels at 14 to 22 weeks gestation would reflect the milieu in the first 28 days after conception, the critical period for neural tube closure.

There is some evidence that iron may affect other nutrient levels. Studies have indicated a relationship between folate and ferritin, in that ferritin can modulate folate availability via cellular one-carbon pathways (Oppenheim and others, 2001; Suh and others, 2000). It has been suggested, for example, that low iron status could cause altered utilization of folate, despite adequate folate intake and extracellular folate concentrations (O'Connor, 1991). While the results from our study failed to find a relationship between folate and ferritin, there was, however, a positive correlation between vitamin B_{12} and both markers of iron status. Whether or not ferritin and vitamin B_{12} have interacting roles is a question that remains to be answered.

Our study has several strengths. Notably, it is the first to investigate maternal ferritin levels during an NTD affected pregnancy. Moreover, as a prospective nested case-control study, it is likely to have had less bias than a standard case-control design. While previous case-control studies suggested lower iron status in women with a history of NTD affected pregnancy, ferritin levels were taken from blood collected postpartum (Felkner and others, 2005). However, as pregnancy has both acute and chronic influence on iron levels, it is impossible to definitively identify a link between low ferritin and the development of NTDs in this way (Suarez and others, 2012). The samples in the current study were taken at an average of 15 weeks gestation, and are thus closer the time of neural tube closure. Furthermore, neither the study subjects nor the investigators knew which women carried an NTD affected pregnancy at the time the sample was obtained. The study samples also have the advantage of being taken from a population of high NTD risk, at a time when prenatal supplementation was rare. Such samples would be difficult to obtain today and are more likely to indicate ferritin levels at neural tube closure, because they have not been altered by the intake of prenatal supplements.

Potential limitations to the study include the long storage period prior to analysis and the use of plasma EDTA samples. Only human serum or plasma collected in sodium heparin has been validated by the manufacturers for the AxSYM Ferritin assay, although it has been reported that identical ferritin values have been obtained from serum and plasma from blood treated with EDTA or heparin (Valberg, 1980). There is no reason to believe that the relationship between case and control ferritin values would change if the assay was conducted on serum samples.

In conclusion, our study provides important new information by measuring serum ferritin from samples collected during affected pregnancies. The data show no evidence to suggest a role for iron in the development of NTDs. As we reported previously, low red cell folate and vitamin B_{12} status consistently emerged as maternal risk factors for having an NTD affected pregnancy, and this reflects the current focus in prevention programs. However, more research is needed to elucidate the risks and benefits of increased maternal iron status. Our data indicate that adding iron to folic acid supplements for use by women at risk for becoming pregnant is unlike to increase the number of NTDs being prevented.

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References

Banhidy F, Acs N, Puho EH, Czeizel AE. Iron deficiency anemia: Pregnancy outcomes with or without iron supplementation. Nutrition. 2011 Jan; 27(1):65–72. [PubMed: 20381313]

Botto LD, Lisi A, Bower C, Canfield MA, Dattani N, De Vigan C, De Walle H, Erickson DJ, Halliday J, Irgens LM, Lowry RB, McDonnell R, Metneki J, Poetzsch S, Ritvanen A, Robert-Gnansia E, Siffel C, Stoll C, Mastroiacovo P. Trends of selected malformations in relation to folic acid recommendations and fortification: an international assessment. Birth defects research Part A, Clinical and molecular teratology. 2006; 76(10):693–705.

Botto LD, Moore CA, Khoury MJ, Erickson JD. Neural-tube defects. N Engl J Med. 1999; 341(20): 1509–1519. [PubMed: 10559453]

Carmichael SL, Yang W, Shaw GM. Periconceptional nutrient intakes and risks of neural tube defects in California. Birth defects research Part A, Clinical and molecular teratology. 2010; 88(8):670–678.

Cengiz B, Soylemez F, Ozturk E, Cavdar AO. Serum zinc, selenium, copper, and lead levels in women with second-trimester induced abortion resulting from neural tube defects: a preliminary study. Biological trace element research. 2004; 97(3):225–235. [PubMed: 14997023]

Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. JAMA. 1995; 274(21):1698–1702. [PubMed: 7474275]

De Domenico I, McVey Ward D, Kaplan J. Regulation of iron acquisition and storage: consequences for iron-linked disorders. Nature reviews Molecular cell biology. 2008; 9(1):72–81.

De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, Evans JA, Van den Hof MC, Zimmer P, Crowley M, Fernandez B, Lee NS, Niyonsenga T. Reduction in neural-tube defects after folic acid fortification in Canada. N Engl J Med. 2007; 357(2):135–142. [PubMed: 17625125]

Dolk H, Loane M, Garne E. The prevalence of congenital anomalies in Europe. Adv Exp Med Biol. 2010; 686:349–364. [PubMed: 20824455]

Donovan A, Lima CA, Pinkus JL, Pinkus GS, Zon LI, Robine S, Andrews NC. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell metabolism. 2005; 1(3):191–200. [PubMed: 16054062]

Felkner MM, Suarez L, Brender J, Scaife B, Hendricks K. Iron status indicators in women with prior neural tube defect-affected pregnancies. Maternal and child health journal. 2005; 9(4):421–428. [PubMed: 16315101]

Fraenkel PG, Traver D, Donovan A, Zahrieh D, Zon LI. Ferroportin1 is required for normal iron cycling in zebrafish. The Journal of clinical investigation. 2005; 115(6):1532–1541. [PubMed: 15902304]

Fretham SJ, Carlson ES, Georgieff MK. Neuronal-specific iron deficiency dysregulates mammalian target of rapamycin signaling during hippocampal development in nonanemic genetic mouse models. The Journal of nutrition. 2013; 143(3):260–266. [PubMed: 23303869]

Groenen PM, van Rooij IA, Peer PG, Ocke MC, Zielhuis GA, Steegers-Theunissen RP. Low maternal dietary intakes of iron, magnesium, and niacin are associated with spina bifida in the offspring. The Journal of nutrition. 2004; 134(6):1516–1522. [PubMed: 15173422]

Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. BMJ (Clinical research ed). 2013; 346:f3443.

Heseker HB, Mason JB, Selhub J, Rosenberg IH, Jacques PF. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. Br J Nutr. 2009; 102(2):173–180. [PubMed: 19079944]

Hinks LJ, Ogilvy-Stuart A, Hambidge KM, Walker V. Maternal zinc and selenium status in pregnancies with a neural tube defect or elevated plasma alpha-fetoprotein. British journal of obstetrics and gynaecology. 1989; 96(1):61–66. [PubMed: 2466480]

Hoyle C, Henderson DJ, Matthews DJ, Copp AJ. Transferrin and its receptor in the development of genetically determined neural tube defects in the mouse embryo. Developmental dynamics: an official publication of the American Association of Anatomists. 1996; 207(1):35–46. [PubMed: 8875074]

Iannotti LL, O'Brien KO, Chang SC, Mancini J, Schulman-Nathanson M, Liu S, Harris ZL, Witter FR. Iron deficiency anemia and depleted body iron reserves are prevalent among pregnant African-American adolescents. The Journal of nutrition. 2005; 135(11):2572–2577. [PubMed: 16251613]

King JC. The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. The Journal of nutrition. 2003; 133(5 Suppl 2):1732S–1736S. [PubMed: 12730491]

Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. Q J Med. 1993; 86(11):703–708. [PubMed: 8265769]

Liu J, Jin L, Zhang L, Li Z, Wang L, Ye R, Zhang Y, Ren A. Placental concentrations of manganese and the risk of fetal neural tube defects. Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS). 2013; 27(4):322–325.

Mao J, McKean DM, Warrier S, Corbin JG, Niswander L, Zohn IE. The iron exporter ferroportin 1 is essential for development of the mouse embryo, forebrain patterning and neural tube closure. Development (Cambridge, England). 2010; 137(18):3079–3088.

Mills JL, Carter TC. Invited commentary: Preventing neural tube defects and more via food fortification? American journal of epidemiology. 2009; 169(1):18–21. discussion 22–13. [PubMed: 18953060]

Mills JL, McPartlin JM, Kirke PN, Lee YJ, Conley MR, Weir DG, Scott JM. Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet. 1995; 345(8943):149–151. [PubMed: 7741859]

Molloy AM, Brody LC, Mills JL, Scott JM, Kirke PN. The search for genetic polymorphisms in the homocysteine/folate pathway that contribute to the etiology of human neural tube defects. Birth defects research Part A, Clinical and molecular teratology. 2009a; 85(4):285–294.

Molloy AM, Kirke PN, Troendle JF, Burke H, Sutton M, Brody LC, Scott JM, Mills JL. Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. Pediatrics. 2009b; 123(3):917–923. [PubMed: 19255021]

Moore CA, Li S, Li Z, Hong SX, Gu HQ, Berry RJ, Mulinare J, Erickson JD. Elevated rates of severe neural tube defects in a high-prevalence area in northern China. Am J Med Genet. 1997; 73(2): 113–118. [PubMed: 9409858]

Mosley BS, Cleves MA, Siega-Riz AM, Shaw GM, Canfield MA, Waller DK, Werler MM, Hobbs CA. Neural tube defects and maternal folate intake among pregnancies conceived after folic acid fortification in the United States. American journal of epidemiology. 2009; 169(1):9–17. [PubMed: 18953063]

MRC; MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet. 1991; 338(8760):131–137. [PubMed: 1677062]

O'Connor DL. Interaction of iron and folate during reproduction. Progress in food & nutrition science. 1991; 15(4):231–254. [PubMed: 1784737]

Oppenheim EW, Adelman C, Liu X, Stover PJ. Heavy chain ferritin enhances serine hydroxymethyltransferase expression and de novo thymidine biosynthesis. J Biol Chem. 2001; 276(23):19855–19861. [PubMed: 11278996]

Pasricha SR, Drakesmith H, Black J, Hipgrave D, Biggs BA. Control of iron deficiency anemia in low- and middle-income countries. Blood. 2013; 121(14):2607–2617. [PubMed: 23355536]

Pathak P, Kapil U, Yajnik CS, Kapoor SK, Dwivedi SN, Singh R. Iron, folate, and vitamin B12 stores among pregnant women in a rural area of Haryana State, India. Food and nutrition bulletin. 2007; 28(4):435–438. [PubMed: 18274171]

Ray JG, Wyatt PR, Thompson MD, Vermeulen MJ, Meier C, Wong PY, Farrell SA, Cole DE. Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. Epidemiology. 2007; 18(3):362–366. [PubMed: 17474166]

Rosenthal J, Casas J, Taren D, Alverson CJ, Flores A, Frias J. Neural tube defects in Latin America and the impact of fortification: a literature review. Public health nutrition. 2013:1–14.

Schorah CJ, Wild J, Hartley R, Sheppard S, Smithells RW. The effect of periconceptional supplementation on blood vitamin concentrations in women at recurrence risk for neural tube defect. Br J Nutr. 1983; 49(2):203–211. [PubMed: 6830748]

Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. American journal of epidemiology. 2004; 160(2): 102–109. [PubMed: 15234930]

Shaw GM, Finnell RH, Blom HJ, Carmichael SL, Vollset SE, Yang W, Ueland PM. Choline and risk of neural tube defects in a folate-fortified population. Epidemiology. 2009; 20(5):714–719. [PubMed: 19593156]

Shields RC, Caric V, Hair M, Jones O, Wark L, McColl MD, Ramsay JE. Pregnancy-specific reference ranges for haematological variables in a Scottish population. Journal of obstetrics and gynaecology: the journal of the Institute of Obstetrics and Gynaecology. 2011; 31(4):286–289. [PubMed: 21534746]

Suarez L, Felkner M, Brender JD, Canfield M, Zhu H, Hendricks KA. Neural tube defects on the Texas-Mexico border: what we've learned in the 20 years since the Brownsville cluster. Birth defects research Part A, Clinical and molecular teratology. 2012; 94(11):882–892.

Suh JR, Oppenheim EW, Girgis S, Stover PJ. Purification and properties of a folate-catabolizing enzyme. J Biol Chem. 2000; 275(45):35646–35655. [PubMed: 10978335]

Valberg LS. Plasma ferritin concentrations: their clinical significance and relevance to patient care. Canadian Medical Association Journal. 1980; 122(11):1240–1248. [PubMed: 6992966]

Weekes EW, Tamura T, Davis RO, Birch R, Vaughn WH, Franklin JC, Barganier C, Cosper P, Finley SC, Finley WH. Nutrient levels in amniotic fluid from women with normal and neural tube defect pregnancies. Biol Neonate. 1992; 61(4):226–231. [PubMed: 1610951]

Williams LJ, Rasmussen SA, Flores A, Kirby RS, Edmonds LD. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995–2002. Pediatrics. 2005; 116(3):580–586. [PubMed: 16140696]

Young D, Klemm AR, Beckman DA, Brent RL, Lloyd JB. Uptake and processing of 59Fe-labelled and 125I-labelled rat transferrin by early organogenesis rat conceptuses in vitro. Placenta. 1997; 18(7):553–562. [PubMed: 9290151]

Zhang T, Xin R, Gu X, Wang F, Pei L, Lin L, Chen G, Wu J, Zheng X. Maternal serum vitamin B12, folate and homocysteine and the risk of neural tube defects in the offspring in a high-risk area of China. Public health nutrition. 2009; 12(5):680–686. [PubMed: 18547453]

Zohn IE, De Domenico I, Pollock A, Ward DM, Goodman JF, Liang X, Sanchez AJ, Niswander L, Kaplan J. The flatiron mutation in mouse ferroportin acts as a dominant negative to cause ferroportin disease. Blood. 2007; 109(10):4174–4180. [PubMed: 17289807]

Zohn IE, Sarkar AA. The visceral yolk sac endoderm provides for absorption of nutrients to the embryo during neurulation. Birth defects research Part A, Clinical and molecular teratology. 2010; 88(8):593–600.

Table 1

Characteristics of pregnant case and control participants[1]

Characteristic	Anencephaly (*n* 25)[2]	Spina Bifida (*n* 34)	Other NTDs (*n* 5)[3]	Total Cases (*n* 64)	Control (*n* 207)
Maternal age (yrs)	26.2 (24.0,31.4)	27.7 (24.0,33.0)	28.5 (20.0,33.5)	26.8 (24.0, 32.6)	28.1 (24.4,33.0)
Median weeks gestation at sampling	14.3 (12.4,17.9)	14.4 (11.7,19.5)	12.7 (10.0,18.6)	14.3 (12.3, 19.1)	14.9 (11.9, 20.4)
Iron supplements at blood test *n* (%)[4]					
Yes	2 (8.0)	3 (8.8)	0 (0)	5 (7.8)	13 (6.3)
No	22 (88.0)	28 (82.4)	5 (100)	55 (85.9)	156 (75.4)
Possible	0 (0)	0 (0)	0 (0)	0 (0)	16 (7.7)
No information	1 (4.0)	3 (8.8)	0 (0)	4 (6.3)	22 (10.6)
Folate or other vitamin supplements at blood test *n* (%)[4]					
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	24 (96.0)	31 (91.2)	5 (100)	60 (93.7)	185 (89.4)
No information	1 (4.0)	3 (8.8)	0 (0)	4 (6.3)	22 (10.6)
Any medication at blood test *n* (%)[4]	0	3 (9.7)	0	3 (4.7)	8 (4.3)

[1] Values are medians (inter-quartile range);

[2] Anencephaly only or anencephaly + spina bifida;

[3] Encephalocele only or iniencephaly only or iniencephaly + spina bifida or iniencephaly + spina bifida + anencephaly;

[4] Information taken from hospital charts

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Table 2

Blood metabolite data at sampling[1]

Characteristic	Anencephaly (*n* 25)[2]	Spina Bifida only (*n* 34)	Others (*n* 5)[3]	Total Cases (*n* 64)	Control (*n* 207)	P[4]
Hemogobin (g/dL)	12.6 (11.9,13.2)	12.2 (11.5,13.1)	13.0 (11.8,13.5)	12.4 (11.5,13.2)	12.3 (11.7,13.1)	0.74
Plasma Ferritin (μg/L)	10.4 (6.7,32.5)	17.5 (9.7,33.1)	30.7 (16.3,36.3)	16.9 (8.8,32.6)	15.4 (9.1,25.7)	0.84
Plasma Ferritin<12 μg/L	13 (52%)[5]	9 (26%)[5]	0 (0%)[5]	22 (34%)	76 (37%)	0.77
Red cell folate (nmol/l)	624 (454,786)	540 (460,817)	354 (310,497)	566 (428,778)	734 (531,895)	**<0.001**
Plasma folate (nmol/l)	8.1 (4.6,12.6)	7.9 (5.5,12.1)	4.1 (2.8,11.1)	7.9 (5.0,12.2)	10.2 (5.9,15.6)	**0.016**
Vitamin B12 (pmol/L)	211 (164,250)	185 (161,247)	152 (145,168)	191 (158,243)	222 (182,273)	**0.023**
Homocysteine (μmol/l)	9.4 (7.8,11.2)	9.5 (8.3,11.7)	10.1 (9.5,16.0)	9.5 (8.1,11.4)	8.6 (7.3,10.7)	0.063
Creatinine (μmol/l)	40.5 (38.6,44.2)	40.1 (36.3,45.8)	40.3 (38.2,43.2)	40.3 (37.6,44.2)	43.4 (38.3,47.7)	0.10

[1] Values are medians (inter-quartile range);

[2] Anencephaly only or anencephaly + spina bifida;

[3] Encephalocele only or iniencephaly only or iniencephaly + spina bifida or iniencephaly + spina bifida + anencephaly;

[4] Differences between total cases and controls were assessed using Student's t-test on log transformed data for continuous variables and Fisher's Exact test for ferritin categories;

[5] Fisher's Exact p values for individual NTD types against controls are p=0.12 for anencephaly, p=0.33 for spina bifida and p=0.16 for others.

Table 3

Odds Ratios for ferritin concentration and other variables as independent risk factors for being a mother of an NTD-affected child using a logistic regression model

Variables	Maximum Likelihood Estimates	Standard Error	Wald Chi-Square	Odds Ratio Estimates	95% Confidence Limits		*P*
					Lower	Upper	
Hemoglobin	−0.00195	0.1461	0.0002	0.998	0.750	1.329	0.9894
Ferritin	0.00246	0.00697	0.1243	1.002	0.989	1.016	0.7244
Ferritin Deficient (<12 μg/L) vs Sufficient (≥12 μg/L)	0.1022	0.3001	0.1159	1.108	0.615	1.994	0.7335
Red Cell Folate	−0.00150	0.000523	8.2476	0.998	0.997	1.000	0.0041
Plasma Folate	−0.0354	0.0185	3.6683	0.965	0.931	1.001	0.0555
Vitamin B12	−0.00478	0.00213	5.0344	0.995	0.991	0.999	0.0248
Homocysteine	0.0380	0.0359	1.1248	1.039	0.968	1.114	0.2889
Creatinine	−0.0321	0.0198	2.6229	0.968	0.932	1.007	0.1053