Folate and homocysteine levels in pregnancy

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ABSTRACT

This study aims to determine serum folate and plasma homocysteine levels in healthy pregnant women following a live birth and compare them with healthy non-pregnant women. Fifty healthy gravid multiparous women are included in the study and 25 normal non-pregnant female subjects act as controls (group I). The pregnant women are divided into two groups according to interpregnancy interval: group II (six months or less); group III (18–24 months). Venous blood samples are analysed for red blood cell folate and homocysteine, vitamin B_{12}, serum folate and albumin, and serum aminotransferases (ALT and AST). There was a significant decrease in red cell folate and serum folate in group II compared to the control group ($P<0.001$). Serum vitamin B_{12} showed no significant difference. Plasma homocysteine and serum albumin showed significant decreases in both groups II and III compared to the control group. ($P<0.001$) There was significant positive correlation between homocysteine and serum albumin in the three studied groups. ($r=0.42$, $P<0.001$; $r=0.45$, $P<0.001$; $r=0.51$, $P<0.001$, respectively). There was significant negative correlation between red cell folate and homocysteine in the three studied groups. ($r=-0.48$, $P<0.001$; $r=-0.53$, $P<0.001$; $r=-0.49$, $P<0.001$, respectively). Two cases in group II showed signs of intrauterine growth retardation. The results suggest that pregnant females with short interpregnancy intervals are more likely to develop folate deficiency. Educational strategies are required to increase folate awareness among women to promote the benefits of folic acid supplementation. Mandatory folate fortification of foods should be defined and monitored.

KEY WORDS: Folic acid. Homocysteine. Pregnancy.

Introduction

Folate, a member of the B-vitamin family, is a polyglutamate compound. Folate plays an important role in two biochemical cycles: one involving DNA biosynthesis and the other involving one-carbon metabolism (DNA, lipid and protein methylation).[1] In addition, folate is a substrate for the enzymatic conversion of many amino acids and in vitamin metabolism. Folate is needed for normal embryonic development and growth, and deficiency has been associated with the development of neural tube defects and low birthweight. Maternal folate deficiency remains a frequent and mostly unrecognised disorder, and is associated with recurrent miscarriage, placental abruption and intrauterine growth restriction.[2]

Recent reports indicate that concentrations of folate in maternal serum, plasma and red blood cells decrease from the fifth month of pregnancy onwards, and continue to decrease during the weeks after pregnancy such that by the second to third post-partum month a third of all mothers can have subnormal concentrations of folate in serum and red blood cells.[3] By the sixth post-partum month, 20 % of mothers remain deficient of folate.[4]

Homocysteine is a sulphur-containing amino acid that is a demethylated derivative of methionine. Homocysteine is metabolised via two main pathways: remethylation to methionine or transulphuration to cystathionine and then to cysteine. A defect in either leads to an accumulation of circulating homocysteine.

The defect may be congenital, due to an inborn error of cystathionine-B- synthetase, or to homozygosity for a C→T mutation of nucleotide 677 in the methylene-tetrahydrofolate reductase (MTHFR) gene.[5] Other reasons for mild hyperhomocysteinaemia are nutrient-related: deficiencies of folate, vitamin B_{12} or vitamin B_6 cause homocysteine to accumulate because remethylation to methionine requires folate and vitamin B_{12}, and transulphuration to cystathionine requires vitamin B_6.[6]

Plasma homocysteine is normally lower during pregnancy,[7] and Vollset *et al*.[8] reported that hyperhomocysteinemia may be an important marker for, and possibly a cause of or contributor to, complications and an adverse outcome of pregnancy. Thus, the purpose of this study is to determine serum folate and plasma homocysteine levels in normal pregnant women following short (six months or less) or long (18–24 months) interpregnancy intervals.

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Materials and methods

Both verbal and written informed consents was received from all participants in the study.

Fifty healthy gravid multiparous women were included who were non-smokers, had no history of hypertension, no personal or family history of deep venous thrombosis, no prior significant illnesses, no vitamin deficiency, and were not receiving any medication. None of subjects studied had a history of liver disease, or signs and symptoms of nutritional deficiency. Women with a history of neural tube defects were excluded.

Twenty-five normal non-pregnant control female subjects of comparable age and socioeconomic state, and not receiving hormonal contraception, were included as a control group (group I). The pregnant women were divided according to the length of interpregnancy interval into group II (six months or less) and group III (18–24 months).

A detailed history was taken and thorough clinical examination and ultrasonography were performed. The women were asked not to take routine daily multivitamin supplementation two days before the date of sampling. Venous blood samples were taken only once, at 36 weeks' gestation in groups II and III, and from those in the control group.

Venous blood was drawn from the antecubital vein. Samples for red blood cell folate[9] and homocysteine[10] determination were collected in potassium EDTA and plasma was stored at –20°C until analysis. Blood samples for vitamin B_{12},[9] serum folate[9] and albumin[11] were collected in plain tubes. After coagulation, samples were centrifuged for 10 min at 3000 x*g* to separate the serum, and this was stored at –20°C until analysis.

Tests for haemoglobin[12] and haematocrit[13] were performed to exclude anaemia. Liver function tests included serum aminotransferases (ALT and AST).[14]

Student's t-test was used for comparisons between the groups. Correlations between homocysteine and both serum albumin and red cell folate were also examined.

Results

Table 1 illustrates the haemoglobin concentration, haematocrit and ALT and AST activities. No significant change in these parameters was seen in groups II and III compared to the control group. Table 2 demonstrates that there was a significant decrease in red cell folate and serum folate in group II compared to the control group ($P<0.001$). Serum vitamin B_{12} exhibited no significant difference. Plasma homocysteine ($P<0.001$) and serum albumin ($P<0.05$ and $P<0.001$, respectively) showed significant decreases in both pregnant groups compared with the control group.

There was a significant positive correlation between homocysteine and serum albumin in the three groups studied (r=0.42, $P<0.001$; r=0.45, $P<0.001$; r=0.51, $P<0.001$, respectively), and a significant negative correlation between red cell folate and homocysteine in the three groups studied (r=–0.48, $P<0.001$; r=–0.53, $P<0.001$; r=–0.49, $P<0.001$, respectively). Two cases in group II showed signs of intrauterine growth retardation.

Discussion

It has been reported that a relative folate shortage may be equally as damaging as a deficiency.[15] Such an observation could explain the presence of cases of intrauterine growth retardation in group II in the present study, as it is documented that folate deficiency results in impairment of cell proliferation and folate-dependent vitamin and amino acid metabolism.

The remethylation of homocysteine into the amino acid methionine is blocked by a lack of folate, which results in hyperhomocysteinaemia;[16] however, increased homocysteine concentration can be corrected easily by low-dose folate supplementation. It is recognised that hyperhomocysteinaemia produces thrombogenesis, vasodilation and endothelial damage, and is associated with cardiovascular and cerebrovascular disease, as well as recurrent miscarriage, placental abruption, pre-eclampsia, intrauterine growth restriction and perinatal death.

Table 1. Haemoglobin, haematocrit and serum aminotransferase activities in the studied groups (means ± SE).

		Pregnant women	
	Group I	Group II	Group III
	(*n*=25)	(*n*=25)	(*n*=25)
Haemoglobin (g/dL)	12.9 ± 0.4	11.8 ± 0.4	11.9 ± 0.35
Haematocrit (%)	43.2 ± 1.5	42.1 ± 1.6	42.5 ± 1.5
ALT (U/L)	20.6 ± 0.78	21.2 ± 0.8	19.1 ± 0.68
AST (U/L)	22.1 ± 0.8	21.9 ± 0.7	20.8 ± 0.6

Khong and Hague[17] demonstrated deficient trophoblast-induced physiological vascular changes, acute atherosis, intrauterine endovascular trophoblasts in the third trimester, infarction, retroplacental haematoma formation, and accelerated villous maturity. Uteroplacental vascular thrombosis was also seen. They attributed these features to a combination of increased apoptosis, endothelial damage and thrombosis secondary to folate deficiency and hyperhomocysteinaemia.

Higgins *et al.*[18] demonstrated that folate catabolism is increased significantly during normal pregnancy. The rate of folate catabolism peaks in the third trimester and falls sharply in the days following delivery. This corresponds with the period of maximal increase in fetal mass. This peak rate is more than twice the rate found in the non-pregnant group.

Alteration in methionine metabolism in humans due to folate or vitamin B_{12} shortage may play a role in the aetiology of neural tube defect, recurrent miscarriage, placental infarct and placental abruption.[19] The causes of these complications of pregnancy may be traced to the first gestational weeks.[20]

Methionine is essential for cell proliferation and DNA and transfer RNA (tRNA) methylation. It is converted to S-adenosylmethionine and, following decarboxylation, this methyl donor is the source of the 3-carbon moieties of the polyamines spermidine and spermine. In addition, S-adenosylmethionine is involved in the methylation of DNA.[21] The homocysteine derived from methionine is normally present in blood in low concentration, and elevated intracellular and extracellular levels may be cytotoxic; however, whether or not elevated circulating levels of homocysteine are embryotoxic remains unknown.[22]

The results obtained in the present study are consistent with previous reports.[7] The decrease in homocysteine in the pregnant groups could be attributed to the significant decrease in albumin and the significant positive correlation between albumin and homocysteine (Table 2).

In vitro studies in the rat suggest that the embryotoxic effect of L-homocysteine is due to inhibition of methyl donation by S-adenosylmethionine.[23] Also, the effect of homocysteine toxicity on vascular endothelium of the spiral or yolk sac arteries cannot be excluded. The development of neural tube defect might be explained partly by the decreased availability

Table 2. Red cell folate, serum folate, vitamin B_{12}, homocysteine and serum albumin in the studied groups (means ± SE).

		Pregnant women	
	Group I	Group II	Group III
	(*n*=25)	(*n*=25)	(*n*=25)
Red cell folate (ng/mL)	396.6 ± 14.4	325.1 ± 11.8*	379.4 ± 13.1
Serum folate (ng/mL)	16.5 ± 0.6	11.2 ± 0.4*	15.0 ± 0.5
Vitamin B_{12} (pmol/L)	195.1 ± 6.8	187.3 ± 6.4	189.1 ± 6.7
Homocysteine (μmol/L)	8.2 ± 0.3	5.2 ± 0.2*	5.4 ± 0.2*
Serum albumin (g/L)	42.1 ± 1.5	35.2 ± 1.3**	34.9 ± 1.1*

* Significantly different from the control group ($P < 0.001$).
** Significantly different from the control group ($P < 0.05$).

of methionine, folate and cobalamin, and the subsequent derangement of methionine metabolism during early human pregnancy, resulting in decreased DNA synthesis and distorted cell proliferation. The prevention of neural tube defect by periconceptional folate supplementation might be explained, in part, by the correction of disturbed methionine metabolism.[24]

A further possible mechanism for the reduction in homocysteine level during pregnancy is utilisation by the fetus. A decreasing plasma homocysteine concentration gradient exists from the maternal vein to the umbilical artery, suggesting incorporation of homocysteine into the fetal metabolic cycle.[25]

Hyperhomocysteinaemia is associated with obstetric complications such as placental abruption, pre-eclampsia, neural tube defect, stillbirth and recurrent miscarriage.[26] The mechanisms involved remain unknown but there is experimental evidence to indicate that hyperhomocysteinaemia causes endothelial dysfunction.[27]

In vitro studies suggest that this dysfunction is mediated through the generation of potent reactive oxygen species, in particular hydrogen peroxide.[28] *In vivo*, homocysteinaemia alters the effect of many clotting proteins on the endothelial cell surface, leading to a prothrombotic environment.[29] Thus, it is conceivable that hyperhomocysteinaemia could affect placental function or maternal uteroplacental perfusion via any of these mechanisms.[30]

Studies of athersclerosis have shown that a graded risk of vascular disease is associated with increasing homocysteine level;[31] thus, reduction of high-normal levels of homocysteine may provide benefit. Potentially, folic acid supplementation in pregnancy could reduce the risk of obstetric complications related to high levels of homocysteine.[(30)]

Folate deficiency can be explained by the hypothesis proposed by Smits and Essed.[32] They suggest that maternal folate concentration decreases from the fifth month of pregnancy, and continues to do so during the first post-partum months, irrespective of lactation. If a subsequent pregnancy commences after a sufficiently long restorative period, the probability of maternal folate deficiency is equivalent to that of a first pregnancy. However, they concluded that commencement of a further pregnancy before complete folate restoration has taken place will result in a higher risk of maternal folate deficiency.

Concentrations of other micronutrients such as zinc and vitamins A, B_6 and B_{12} also fall during pregnancy but they return to normal within a few weeks following delivery and/or do not affect the outcome of pregnancy.[3,4,33,34] Thus, folate deficiency appears to be the most important nutritional factor associated with the higher risk of poor pregnancy outcome after a short interpregnancy interval.[32]

Furthermore, a two-fold increase in the risk of neural tube defect was observed for pregnancies conceived within six months of a previous live birth.[35] Also, the effect of short interpregnancy interval on the risk of smallness for gestational age also has been documented.[36]

In conclusion, pregnancy following a short interpregnancy interval is more likely to involve folate deficiency, and thus supplementation is highly recommended. Also, the value of health education about the prevention of folate deficiency, which is associated with the occurrence of neural tube defect, should be stressed.

Clearly, educational strategies are required to increase folate awareness among women and to promote the benefits of folic acid supplementation. Mandatory folate fortification of foods needs to be defined and monitored. □

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