Folate and Homocysteine Levels in Pregnancy

M.A. Megahed and I.M. Taher

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 analyzed 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 unrecognized 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 metabolized via two main pathways: remethylation to methionine or transulfuration 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 methylenetetrahydrofolate 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 transulfuration to cystathionine requires vitamin B_{6}.6

Plasma homocysteine is normally lower during pregnancy7 and Vollset et al.8 reported that hyperhomocysteinaemia may be an important marker for, and
Materials and methods

Both verbal and written informed consents were 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 and homocysteine determination were collected in potassium EDTA and plasma was stored at -20°C until analysis. Blood samples for vitamin B12, serum folate and albumin were collected in plain tubes. After coagulation, samples were centrifuged for 10 min at 3000 g to separate the serum, and this was stored at -20°C until analysis.

Tests for hemoglobin and hematocrit were performed to exclude anemia. Liver function tests included serum aminotransferases (ALT and AST).

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 hemoglobin concentration, hematocrit 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 B12 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. 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 hyperhomocysteinemia; however, increased homocysteine concentration can be corrected easily by low-dose folate supplementation. It is recognized that hyperhomocysteinemia 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.

Khong and Hague demonstrated deficient trophoblastinduced physiological vascular changes, acute atherosis, intrauterine endovascular trophoblasts in the third trimester, infarction, retroplacental hematoma 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 hyperhomocysteinemia.

Higgins et al. 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 etiology 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. 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-adenosylhomocysteine.\(^{23}\) 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 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 utilization 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 atherosclerosis 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 postpartum 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, 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.\(^{33,34,35}\) 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.\(^{36}\) 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 inter-

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

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

* Significantly different from the control group (P < 0.001).
** Significantly different from the control group (P < 0.05).
pregnancy 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 folate acid supplementation. Mandatory folate fortification of foods needs to be defined and monitored.

References

Questions for STEP Participants

Answer questions only on the official STEP answer sheet. If you do not have the official STEP answer sheet, a year’s supply can be obtained (at no cost), simply by writing to: STEP Program Answer Sheets, American Medical Technologists, 710 Higgins Road, Park Ridge, IL 60068-5765, or by fax: 847/823-0458, or by e-mail: paula.simoncini@amt1.com.

In addition to marking your answers, be sure to include all the required information on the answer sheet and a processing fee of $3.00 per article.

In the following, choose the one best answer for each question.

1. Homocysteine is a demethylated derivative of methionine.
   A. True
   B. False

2. Folate is a member of the B-vitamin family.
   A. True
   B. False

3. An accumulation of circulating homocysteine can be caused by a breakdown in the metabolic pathway of remethylation to methionine.
   A. True
   B. False

4. Plasma homocysteine is generally lower during pregnancy.
   A. True
   B. False

5. The results of the study explained in this article suggest that pregnant females with short interpregnancy intervals are less likely to develop folate deficiency.
   A. True
   B. False

6. This study indicates that from the 5th month of pregnancy onwards, concentrations of folate in the body decrease.
   A. True
   B. False

7. The study speculates that one possible mechanism for the reduction in homocysteine levels during pregnancy is utilization by the fetus.
   A. True
   B. False

8. Results indicated that pregnancy following a short interpregnancy interval is more likely to cause an increase in folate levels.
   A. True
   B. False

9. Hyperhomocysteinaemia is associated with several obstetric complications.
   A. True
   B. False

10. Since women are so well educated on this subject, it is not necessary to develop an awareness education program.
    A. True
    B. False