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Plenary Session VI – State of the art lecture

Chairpersons:
J. Tomkinson, London, United Kingdom
D.V.I. Fairweather, London, United Kingdom
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Life cycle of women

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The life cycle of women (conception, pregnancy, childhood, puberty, womanhood, menopause and death) was presented in a live performance using piano and video clip instrumentation. Made possible by technical assistance of Jan Acket, Van Amstel Video and Organon International, Oss, The Netherlands.

Elevated folate levels in amniotic fluid after oral supplementation

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Summary

During oral supplementation of 5 mg folic acid and 1 μg vitamin B12, the daily folate levels in plasma, red cells and midtrimester amniotic fluid were significantly higher in ten pregnant women during the 15th–18th week of menstrual age as compared to ten non-supplemented women serving as controls. In the control group as well as in the supplemented group, the folate concentrations in amniotic fluid

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fluid were found to be lower than in the corresponding maternal plasma and red cells. Of all women investigated there was a significant positive correlation between maternal plasma folate concentrations and amniotic fluid levels ($r = 0.72$, $p < 0.001$) and no correlation between red cell folate concentrations and amniotic fluid levels ($r = 0.30$, $p = 0.22$).

Oral supplementation of vitamin B12 did not elevate maternal blood concentrations and amniotic fluid levels. Vitamin B12 levels in amniotic fluid in this group and controls were always higher than in plasma.

These data suggest that the orally administrated dosages of folic acid unlike those of vitamin B12 augment both plasma, red cells and amniotic fluid levels.

Pregnancy; Supplementation; Folic acid; Vitamin B12; Amniotic fluid; Maternal blood

Introduction

Over the last decades much attention has been paid to the role of folate and vitamin B12 levels in early pregnancy, the period of organ development. Both vitamins are known to be involved in DNA synthesis. A deficiency of either of these vitamins could result in a defective cell proliferation, through which major malformations could develop. Various investigations suggest a correlation between low maternal plasma levels of folate and vitamin B12 during organogenesis and the development of congenital anomalies in the foetus, especially neural tube defects (NTD) [1,2]. Periconceptional supplementation of multivitamins, including folic acid and vitamin B12 in women who gave birth to a child with a neural tube defect could possibly lead to a reduction in the recurrence rate of NTD [3]. Also oral supplementation of only folic acid probably has a preventive effect [4]. Whether periconceptional supplementation of vitamins is always beneficial or can even have deleterious effects is a matter of controversy [5,6]. The way vitamin supplementation affects plasma concentrations is probably important in this regard, however, scarcely studied up till now [7]. Furthermore, the concentration of vitamins in the direct environment of the developing foetus, i.e., the amniotic fluid, could be of significance as well. The aim of the present investigation was to study folate and vitamin B12 concentrations in maternal blood and midtrimester amniotic fluid in orally supplemented and unsupplemented women. The study was performed during the 15th–18th week of menstrual age in uneventful pregnancies. Folate concentrations in maternal blood were measured in plasma as well as in red cells. Plasma concentration only represents the folate status at the moment of collection, whereas the folate concentrations in red cells represent the folate status of the foregoing 6 weeks [8].

Materials and methods

Study population

Two groups of Caucasian women participated in the study after given informed consent. No patient had any evidence of intestinal disease nor was she restricted in

TABLE I

Characteristics of study groups

	Group I (controls)	Group II (supplemented)
Number of patients (n)	10	10
Age (years) mean (SD)	29.9 (5.4)	28.8 (4.1)
Nulliparous (n)	8	6
Menstrual age at amnioncentesis (days) mean (SD)	113 (10.3)	112 (7.7)

her dietary intake. Amnioncentesis was indicated because of an increased risk of NTD. Folate and vitamin B12 levels in maternal plasma, red cells and amniotic fluid were studied in ten women without previous supplementation and were taken as control values (group I). At least 8 weeks before sampling, daily oral doses of 5 mg folic acid and 1 μg vitamin B12 were supplemented to a study group of ten pregnant women (group II) during the first half of their pregnancy. All women were delivered of a healthy infant. Population characteristics are given in Table I.

Study design

The experimental protocol was approved by the ethics committee of the university hospital. All pregnancies were dated by the last menstrual period and ultrasound investigation. Amniotic fluid samples were obtained by routine transabdominal amnioncentesis during the 15th–18th week of menstrual age and collected in dry tubes of 4 ml. Five minutes after amnioncentesis venous maternal blood samples for haematocrit were collected in EDTA vacutainer tubes 4 ml and for folate or vitamin B12 measurements in heparinized vacutainer tubes 10 ml (Monoject, Sherwood Medical, St Louis, MO, U.S.A.). All amniotic fluid samples were free from blood contamination. Blood and amniotic fluid were immediately stored at $-20\,^{\circ}$C. All samples were assayed within 2 years after collection.

Laboratory determinations

Concentrations of folate and vitamin B12 in plasma and amniotic fluid were determined by using the Dualcount SPB (Solid Phase Boil) radioassay (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.).

The method included a heat denaturation step of endogenous protein (15 min at $100\,^{\circ}$C, pH 9.3) of the unknown samples and the calibrators (zero dose standard and six calibrators containing between 40–1800 pmol vitamin B12 and 1–55 nmol folate per liter of a protein matrix). At this stage the duallabel tracer solution (cobalt-57 vitamin B12 and iodine-125 folate) was added. It contained dithiothreitol and potassium cyanide (pH 9.3) to release the vitamin B12 and folate in the sample from carrier proteins. Next, a suspension of the solid phase binders for vitamin B12 and folate (purified hog intrinsic factor, and β-lactoglobulin, respectively, coated onto microcrystalline cellulose particles) was added and incubated for 60 min at

room temperature and pH 9.3. At this pH, intrinsic factor is fully active and the folic acid binder has equal affinity for 5-methyltetrahydrofolic acid (MTHF) and this stable form, pteroylglutamic acid (PGA, also used as the calibrator preparation). Following bound-free separation by centrifuging (15 min at $3000 \times g$) and decanting the supernatant, the radioactivity of the bound (precipitate) fraction was counted and the quantitation of the analytes was performed using the logit-log calculation facilities present in this laboratory (Ria Calc, Wallac Oy, Turkur, Finland).

Red blood cell folate (RBF) levels were calculated from folate measurements in heparinized blood hemolysate and plasma. Hemolysates were made in three dilutions in assay buffer ($21\times$, $10\times$ and $5\times$) to which 1% ascorbic acid was added. The calculation was done according to the equation:

$$RBF = DF \times R/H - S(1 - H)/H$$

where RBF is the red blood cell folic acid level (nmol/l); R the hemolysate folic acid level (nmol/l); DF the dilution factor of hemolysate (i.e., 21); S the plasma folic acid level (nmol/l); and H the hematocrit (l/l). The minimum detectable concentration of RBF thus calculated was 48 nmol/l, since the lowest detectable plasma level is 1.1 nmol folate per litre and the highest hematocrit found was 0.48. The minimum detectable plasma concentration for vitamin B12 was 40 pmol/l as calculated at the B/B_0 response level of 0.90. Assay precision in terms of intra- and inter-assay variabilities for means of duplicate determinations was calculated from 24 consecutive assay runs with two different plasma pools. At mean plasma pool levels of 20 and 40 nmol of folate per litre and of 540 and 800 pmol of vitamin B12 the coefficients of variation (CV) for intra- and inter-assay precision were always $< 5\%$, and $< 10\%$, respectively, with each of the two vitamins.

Statistical analysis

The results of both groups were statistically analysed using Wilcoxon's two sample rank test for unpaired samples. In order to test the correlations between folate and vitamin B12 in maternal blood and amniotic fluid Spearmans coefficient of correlation (r) was computed. Unless indicated otherwise, results are expressed as median and range.

Results

The levels of folate in maternal plasma and midtrimester amniotic fluid as found in the non-supplemented control group (group I) and in women who received folic acid and vitamin B12 supplements (group II) are given in Table II.

In group I, red cell folate concentrations were higher as compared to plasma levels. Detectable folate levels were found in amniotic fluid as well, which appeared to be slightly lower than in corresponding plasma samples. In the supplemented group folate concentrations in plasma, red cells and amniotic fluid were significantly elevated in comparison to the control group. In all women a significant positive correlation was found between plasma and amniotic fluid folate levels ($n = 18$, $r = 0.72$, $p < 0.001$, Fig. 1). Red cell folate was not correlated with amniotic

TABLE II

Folate levels in maternal plasma, red cells and amniotic fluid (nmol/l)

	Group I (controls)	Group II (supplemented)	P-value
Plasma folate	n = 9	n = 9	
median	17	60	< 0.001
range	8– 38	34– 290	
Red cell folate	n = 9	n = 9	
median	470	1400	0.03
range	120–600	140–1800	
Amniotic fluid			
folate	n = 10	n = 10	
median	13.5	33	0.001
range	4– 23	17– 48	

P values indicate the significance of differences between group I and group II.

fluid folate (n = 18, r = 0.30, p = 0.22) and plasma folate and red cell folate concentrations tended to be positively correlated (n = 18, r = 0.42, p = 0.09, Fig. 1).

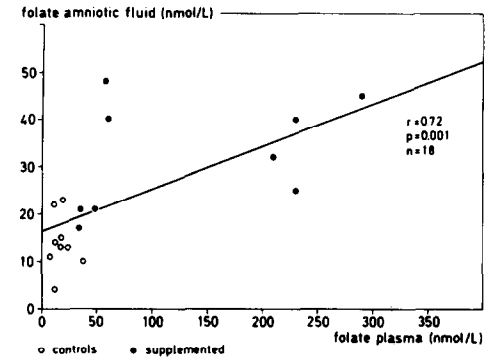
The vitamin B12 concentrations in plasma and amniotic fluid of groups I and II are shown in Table III. In both groups detectable vitamin B12 concentrations were found in amniotic fluid, which were higher to those of plasma. The median concentrations of vitamin B12 in plasma and amniotic fluid were equal in the supplemented group as compared to the control group, respectively (p = 0.96, and 0.47). In contrast to the folate levels, there was no significant correlation between vitamin B12 concentrations in plasma and amniotic fluid (n = 18, r = 0.32, p = 0.18, Fig. 1). The concentrations of folate in plasma, red cells and amniotic fluid were not correlated with vitamin B12 concentrations in plasma and amniotic fluid.

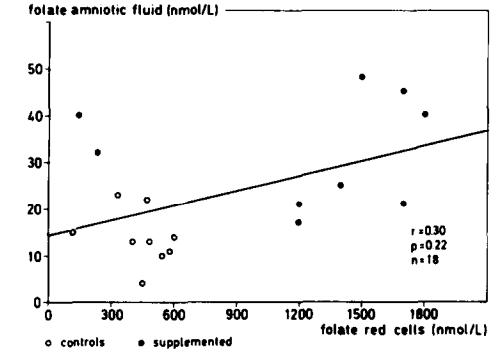
TABLE III

Vitamin B12 levels in maternal plasma and amniotic fluid (pmol/l)

	Group I (controls)	Group II (supplemented)	P-value
Plasma:			
Vitamin B12	n = 9	n = 9	
median	270	240	0.96
range	110–420	110– 590	
Amniotic fluid:			
Vitamin B12	n = 10	n = 10	
median	540	495	0.47
range	130–910	170–2400	

P values indicate the significance of differences between group I and group II.





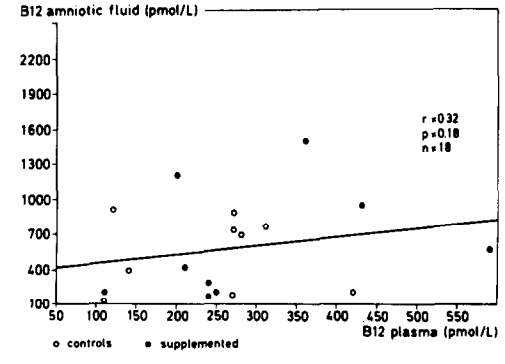


Fig. 1. The supplemented women (●) and controls (○) are taken together. The correlation between the concentration of folate in amniotic fluid and plasma (top), amniotic fluid and red cells (middle) and the vitamin B12 concentration in amniotic fluid and plasma (bottom) are given.

Discussion

Folate levels in maternal plasma and red cells significantly increased after a daily oral supplementation of 5 mg for more than 8 weeks. This indicates that women adequately absorb, metabolize and accumulate the monoglutamate form of folate during the first trimester of pregnancy. This confirms the results of Heseker and Schmidt [7], who studied the plasma and red cell levels in men and nonpregnant women after long-term supplementation with 1 mg of folate. They showed that after 4 weeks of supplementation, the mean folate concentration in plasma reached a maximum level and remained constant thereafter, and that the folate concentrations in red cells increased linearly in all subjects during supplementation. In contrast to these results, we showed that folate levels in plasma and red cells tended to be positively correlated. This confirms the results of Ek and Magnus [9], who studied plasma and red cell folate concentrations during normal pregnancy with iron, but without folate supplementation.

Comparison of the results of the plasma and the red cell concentrations of folate is hampered by differences in methodology. In most studies the results are based upon determinations of the folate content by a microbiological assay (with *Lactobacillus casei* as test organiser), while this study used a radioimmunochemical assay. The plasma folate concentrations in controls (group I), however, were mostly comparable with the levels determined in the second trimester by radioimmunochemical assay in the study of Bruinse et al. [10] (13.3 nmol/l) and lower than the concentrations in the study of Rolschau et al. [11] (37 nmol/l). These differences occur, because the radioimmunochemical assay depends on various factors such as the specificity and other assay reagents and characteristics.

Detectable folate levels were found in amniotic fluid, which increased after oral supplementation. This could suggest a placental transfer of folate from plasma to amniotic fluid. However, it cannot be ruled out that the placenta itself is capable of synthesizing folate. Amniotic fluid folate concentrations can also originate from the foetus, for example by excreting a surplus of folate into the amnion.

The supplemented patients received 5 mg folic acid which is usually prescribed in the Netherlands. This dose is higher than that applied in the surrounding countries where the recommended daily folate intake varies between 2 and 4 mg. The recommended daily intake of folate during pregnancy by the World Health Organization (1974) is 400 μg free, and 800 μg total folate [12]. Since a daily oral supplementation of 5 mg folic acid causes a 3-fold increase in folate levels in plasma and red cells, one should also be cautious of probable toxic maternal and foetal effects. As known, the folate level in foetal plasma and red cells are almost invariably higher than in maternal blood [11] and depend partly on specific folate-binding proteins (FBP). The concentrations of FBP seem to be hormone-regulated [13] and to stimulate folate transfer across the placenta. Simmer et al. [14] reported that folate supplementation in pharmacologic doses may interfere with the absorption of organic zinc, an essential compound for DNA synthesis. Other complications have been reported, such as an increased susceptibility to seizures in women supplemented with folic acid and receiving antiepileptic drugs because of epilepsy, and an interaction between folate and vitamin B12, resulting in the

aggravation of irreversible neurologic disorders. Therefore it appears necessary to consider the preventive as well as the possible deleterious effects of oral folate supplements before these preparations will be routinely prescribed.

Plasma vitamin B12 levels decrease progressively during pregnancy [10]. However, pregnancy is not a predisposing factor for a vitamin B12 deficiency. Factors thought to be involved are haemodilution, foetal demand, and most likely a change in vitamin B12 metabolism. Any vitamin B12 deficiency during pregnancy is due to an underlying condition: dietary deficiency, vegetarian, tropical spruw of pernicious anaemia. The increased vitamin B12 absorption in pregnancy compensates for the B12 supply to the foetus [15]. Our results suggest that vitamin B12 levels in maternal blood are equal in supplemented as well as in unsupplemented women. This is in agreement with the results of Metz et al. [16] and Edelstein et al. [17] who reported that inadequate dietary intake does not seem to play a role in the development of a vitamin B12 deficiency. The daily allowance during pregnancy is 3–4 μg vitamin B12. Therefore it is possible that the supplemented dose is too low to determine a difference in the vitamin B12 concentrations in maternal plasma and amniotic fluid of supplemented women and controls. The most likely explanation, however, is an inadequate absorption of the orally administrated dosage of vitamin B12 [18]. A vitamin B12 deficiency could be due to a change in the concentration of vitamin B12 binding proteins, transcobalamin I and II and it is possible, that hormonal influences may interfere [19]. It is well-known that vitamin B12 is transported across the placenta and cord serum vitamin B12 levels appeared to be generally twice as high as in corresponding maternal serum [20]. The placenta is able to accumulate high contents of vitamin B12 in the intervillous spaces [21]. Therefore it is likely that the higher levels of vitamin B12 in amniotic fluid as compared to maternal plasma could be due to synthesis of vitamin B12 by the placenta or points at fetal renal excretion of this vitamin in the amniotic fluid compartment. This could possibly explain our finding of relatively high amounts of vitamin B12 in amniotic fluid in both the supplemented and control group.

So far, no deleterious effects have been reported with regard to periconceptional vitamin B12 supplementation during pregnancy.

There were no correlations between the folate concentrations in plasma, red cells, amniotic fluid and vitamin B12 concentrations in plasma and amniotic fluid. However, further research is necessary to determine the effects of folic acid and vitamin B12 supplementation in the embryo and foetus during pregnancy.

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