Serum Folate and Homocysteine Concentrations in Women with the First Early Spontaneous Pregnancy Loss

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The overall goal of the study was to evaluate serum folate and homocysteine concentrations for the pregnant women with the first early spontaneous pregnancy loss. To our best knowledge, this is the first published report on serum folate and homocysteine in pregnant women whose pregnancy was complicated with early spontaneous abortion. In Lithuania this is the first study conducted to investigate the folate and homocysteine status of pregnant women. **Subjects and methods.** In a case-control study, we measured fasting serum folate and homocysteine in 27 pregnant women who had first early spontaneous pregnancy loss and compared concentrations with those of 28 with elective abortion. **Results.** Serum folate was 8.55 ± 2.55 in women with spontaneous pregnancy loss and 8.83 ± 2.32 ng/ml in control group. Serum homocysteine was 7.32 ± 2.04 mmol/l and 7.27 ± 1.99 µmol/l, respectively. Serum folate and homocysteine values for both groups of women were within the population-based reference range. Four women with early pregnancy loss and three women in control group had homocysteine concentrations above 10 mmol/l. **Conclusions.** Women with the first early spontaneous pregnancy loss had lower serum folate levels and their homocysteine tended to be higher compared with the control group, but the differences did not reach statistical significance. Serum homocysteine inversely correlated with serum folate in both groups and this correlation was statistically significant. Serum folate and homocysteine concentrations for the women with spontaneous pregnancy loss and elective abortion were within the population-based reference range.

Key words: folate, homocysteine, hyperhomocysteinemia, metabolism, spontaneous abortion

INTRODUCTION

Early pregnancy loss is found in 15–20% of couples desiring pregnancy. Despite various efforts to find an etiologic factor for early pregnancy loss, more than half of these cases remain unexplained.

The first studies that investigated the relationship between folate deficiency and early pregnancy loss were published in the previous decades. The consistent conclusion was that low folate concentrations might predispose spontaneous abortion. More recent reports did not find lower folate concentrations, but they investigated folate concentrations in those women after but not during their pregnancies. Other sensitive markers of disturbed folate metabolism associated with early pregnancy loss were identified, one of which was elevated plasma homocysteine concentration. Subsequent studies have shown a relation between homocysteine and folate metabolism and the risk of pregnancy complications such as neural tube defects and other malformations, pregnancy loss, fetal death, intrauterine growth retardation or prematurity, placental abruption and infarction, and pre-eclampsia (1–7).

Although the majority of cases of hyperhomocysteinemia are thought to be caused by an interplay between the dietary and genetic factors, the genetic disorders are associated with the highest levels of homocysteine, with cystathionine β-syntha-

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se and 5, 10-methylenetetrahydrofolate reductase (MTHFR) deficiency being the most common (8). There is evidence that marginal folate status exacerbates the effects of an underlying genetic defect in the mother, the fetus, or both (9).

The proposed pathogenetic mechanism of folate deficiency and hyperhomocysteinemia causing pregnancy complications partly converge. The common mechanisms proposed are vascular effects, decreased synthesis and methylation of DNA and RNA, the excitotoxic effect of amino acids and impaired fatty acid synthesis (10).

Homocysteine may impair the vascularization of the placenta and thereby its function. Vascular damage associated with hyperhomocysteinemia reflects endothelial cell injury and/or dysfunction. In addition to causing injury, homocysteine has been shown to increase thromboxane-mediated platelet aggregation, reduce nitric oxide production, enhance low density lipoprotein oxidation, inhibit cell surface thrombomobulin expression and protein-C activation, enhance lipoprotein-fibrin binding, promote smooth muscle cell proliferation and collagen accumulation (8, 10, 11). In a study of women with recurrent early pregnancy loss, total plasma homocysteine was significantly and negatively correlated with the circumference and maximum diameter of chorionic villous blood vessels (12).

At this time it is not well established whether disturbed homocysteine metabolism and folate deficiency are associated with early spontaneous abortion. To our best knowledge, this is the first published report on serum folate and homocysteine in pregnant women whose pregnancy was complicated with early spontaneous abortion. In Lithuania this is the first study conducted to the investigate folate and homocysteine status of pregnant women.

OBJECTIVE

The overall goal of the study was to evaluate serum folate and homocysteine concentrations for the pregnant women with the first early spontaneous pregnancy loss.

SUBJECTS AND METHODS

Patients. Between November 1999 and December 2000 women who reported early spontaneous pregnancy loss within 12 weeks of menstrual age after conception (excluding ectopic and molar pregnancies) were assessed at the University Hospital of Vilnius. The study group was compared with the control group of women who had elective abortion within 12 weeks of menstrual age after conception.

After the purposes and procedures of the study were explained, women who agreed to participate were enrolled if they met the following eligibility criteria: 20 – 30 years of age, apparently healthy, no bad habits, no pregnancy complications, and no unusual dietary practices. Women were excluded if they had vitamin B supplementation within 3 months before conception or used oral contraceptives. All pregnancies had been confirmed histologically or by ultrasound imaging.

Twenty-seven women with the early pregnancy loss and twenty-eight with elective abortion were randomly selected and underwent a full-scale examination.

Laboratory evaluation. Blood samples were taken from subjects who had fasted overnight and immediately centrifuged for 7 min 2000 xg. Serum was frozen at – 20 °C until analysis. Serum folate was analyzed by enzyme immunoassay in the microtitre format by using the original IMMULITE method on the DBC Analyzer. Serum homocysteine was analyzed by enzyme immunoassay on the IMX (ABBOTT). The main point of this method is complete reduction of disulphide homocysteine, mixed disulphide (cysteine-homocysteine) and releasing the protein-bound homocysteine, followed by enzymatic conversion of homocysteine to S-adenosylhomocysteine and quantification of S-adenosylhomocysteine by an enzyme linked immunoassay in the microtitre format.

Laboratory evaluation was done at the Laboratory Diagnostic Centre of Vilnius University Hospital.

The study was approved by the Lithuanian Medical Ethic Committee and written informed consent was obtained from all subjects before participation.

Statistical analysis

The statistical analyses were performed using the statistical programme SPSS for Windows (version 10.0). Values of parameters with standard deviations and 95% confidence intervals were calculated. Values in text are means ± SD. The mean folate and homocysteine concentrations were compared by the use of Student t test for parametric variables. For all analyses, p values less than 0.05 were considered statistically significant. The Pearson correlations were used to describe the associations between homocysteine folate concentrations. The smoother uniform bivariate correlation was used to describe precise step-by-step correlation. Interdependence between folate and homocysteine concentration was calculated using linear regression and 95% confidence interval.

RESULTS AND DISCUSSION

The summary statistics concerning serum folate concentrations by groups are summarized in Table 1. The overall mean folate concentration of women with pregnancy loss (8.55 ± 2.55 ng/ml) was lower than that of women with elective abortion (8.83 ± ± 2.32 ng/ml), though the relation was not statistically significant (p = 0.62) (Fig.1).

elective abortion (7.27 ± 1.99 μmol/l), although the comparison was not significant (p = 0.92) (Fig. 2).

The summary statistics concerning association between serum folate and homocysteine concentrations by groups are shown in Table 3.

The values of serum folate are within the range of 3–17 ng/ml (13). Serum folate concentration is considered a sensitive index of the recent folate status, because it is highly influenced by current dieta-

Table 1. **Summary statistics concerning serum folate and homocysteine concentrations by groups**

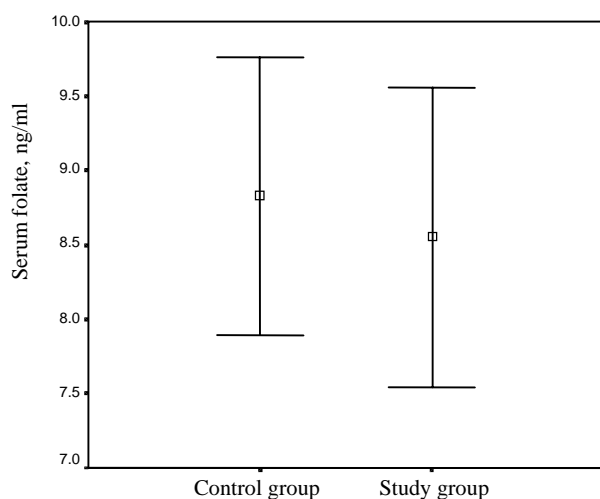| Serum folate, ng/ml	Mean	Standard deviation	Standard error	Range
Women with pregnancy loss (n = 27)	8.55	2.55	0.49	4.20–14.00
Women with elective abortion (n = 28)	8.83	2.32	0.45	4.30–14.00

Fig. 1. Mean concentrations of serum folate in groups (95% CI)
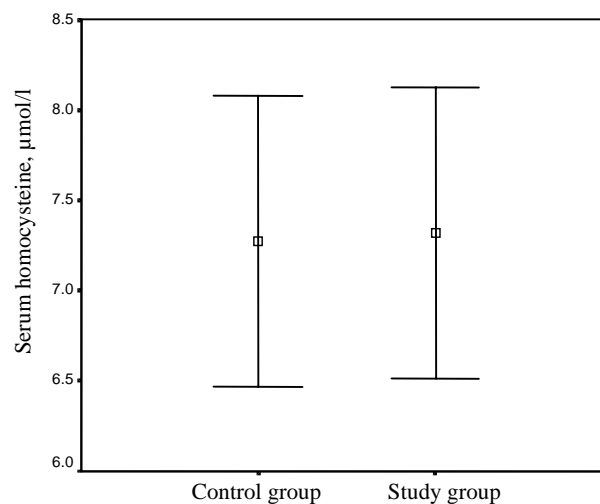

Fig. 2. Mean serum homocysteine concentrations in groups (95% CI)

The summary statistics concerning serum homocysteine concentrations by groups are summarized in Table 2. The overall mean homocysteine concentration of women with pregnancy loss (7.32 ± ± 2.04 μmol/l) was higher than that of women with

ry intake. However, under metabolic conditions in which dietary intake is consistent, serum folate concentration should reflect the overall folate status of the individual (9).

Table 2. **Summary statistics concerning serum homocysteine concentrations by groups**

Serum homocysteine, μmol/l	Mean	Standard deviation	Standard error	Range
Women with pregnancy loss (n = 27)	7.32	2.04	0.39	4.25-11.30
Women with elective abortion (n = 28)	7.27	1.99	0.39	4.90-2.68

Table 3. **Summary statistics concerning association between serum folate and homocysteine concentrations by groups**

	Cases (n = 27)	Controls (n = 28)	Mean difference	Standard deviation	Standard error	p	95% CI of difference
Folate, ng/ml	8.55 ± 2.5	8.83 ± 2.32	0.28	2.88	0.55	0.62	–0.86–1.42
Homocysteine, μmol/l	7.32 ± 2.04	7.27 ± 1.99	0.05	2.55	0.49	0.92	–1.06–0.96

The reference limits based upon results from a general population of serum homocysteine are within the range of 5–15 μmol/l (8, 10). Hyperhomocysteinemia is generally defined as fasting homocysteine levels above 15 μmol/l (8).

In our study, serum folate and homocysteine values for both groups of women were within the population-based reference range. Four women with early pregnancy loss and three women in the control group had homocysteine concentrations higher than 10 μmol/l.

Pregnant women are a special group whose homocysteine levels are considerably lower. In a recent study, the mean total homocysteine level was 5.6 μmol/l during the first, 4.3 μmol/l during the second, and 5.5 μmol/l during the third trimester (14, 15). An ideal or desirable level of homocysteine remains the main target of metabolic studies, because current epidemiological studies have demonstrated that the risk for vascular disease starts to increase with homocysteine values of 10.5–11.7 μmol/l (16).

An important aspect is the involvement of several B-vitamins, in particular folate and cobalamin, in homocysteine metabolism. Pregnancy is associated with progressively reduced serum and erythrocyte folate levels and increased urinary folate excretion.

An inverse, statistically significant, association was observed in our study between serum homocysteine and folate in both groups (the Person correlation coefficient in the study group was 0.63 and in the control group 0.71; p < 0.01) (Figs. 3, 4). The mean concentration of homocysteine (CH) (μmol/l) can be expressed by the formula CH = 11.64–0.51 concentration of folate (CF) (ng/ml) in the study group and by the formula CH = 12.67–0.61 CF in the control group, applying linear regression and 95% confidential interval. Precise step-by-step correlation shows a nonlinear correlation in both groups (Figs. 5, 6).
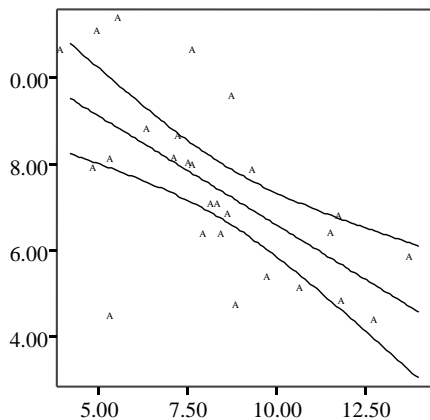

Fig. 3. Correlation between serum folate and homocysteine concentrations in women with the first early spontaneous pregnancy loss
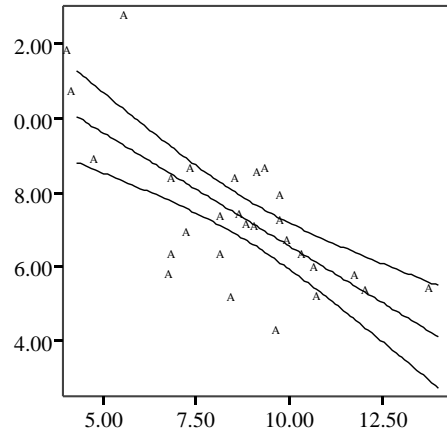

Fig. 4. Correlation between serum folate and homocysteine concentrations in women with elective abortion
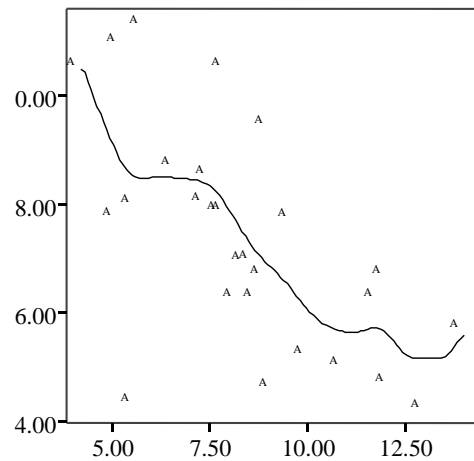

Fig. 5. Precise step-by-step correlation between serum folate and homocysteine concentrations in women with the first early spontaneous pregnancy loss
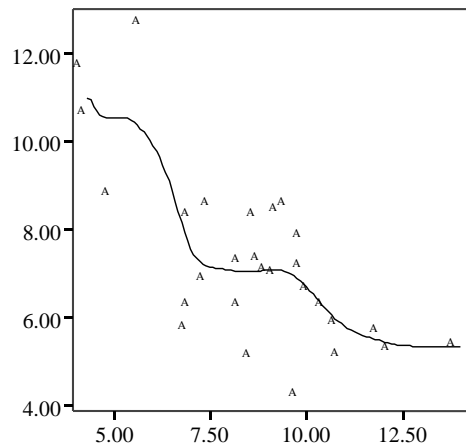

Fig. 6. Precise step-by-step correlation between serum folate and homocysteine concentrations in women with elective abortion

An inverse correlation between plasma homocysteine and folate established in our study is in excellent agreement with previous studies (11, 17, 18).

Serum homocysteine is considered a functional indicator of intra-individual folate and cobalamin status. The observed inverse associations between folate intake and serum homocysteine concentrations and the possible associations with adverse health effects from elevated maternal homocysteine levels provide a rationale for assessing the adequacy of folate intake to maintain normal homocysteine concentrations during pregnancy. Supplementation with folate, alone or in combination with cobalamin and vitamin B6 is an efficient means to reduce serum homocysteine, even in subjects without overt vitamin deficiencies (17).

It is not well established whether disturbed homocysteine metabolism and folate deficiency are associated with early spontaneous abortion. But there is growing evidence that elevated homocysteine concentrations and folate deficiency is a risk factor for recurrent early pregnancy loss. Raziel et al. (6) found hyperhomocysteinemia in 31% of the patients. Authors concluded that combinations of gene mutations and hyperhomocysteinemia, which are associated with an increased thrombotic risk, are more common in recurrent pregnancy loss patients compared with controls. Quere et al. (19) in a retrospective study identified 12 of 100 hyperhomocysteinemic women with recurrent miscarriages. Low plasma folate concentrations were found in 15%.

Nelen et al. (7) identified with MEDLINE – search case – control studies published between January 1992 and November 1999 in order to quantify the risk of recurrent early pregnancy loss in the presence of elevated fasting or after load homocysteine concentrations. Studies published in the English language concerning two or more pregnancy losses before 16 weeks' menstrual age were included. Pooled risk estimates of 2.7 (1.4 to 5.2) and 4.2 (2.0 to 8.8) were calculated for fasting and after load plasma homocysteine concentrations, respectively. These data support hyperhomocysteinemia as a risk factor for recurrent early pregnancy loss.

Quere et al. (19) described a case report concerning a woman with five consecutive fetal losses. The patient was hyperhomocysteinemic, homozygous for the C677T mutation in the methylene tetrahydrofolate reductase gene, and had plasma folate deficiency. Folic acid and pyridoxine administration normalized the homocysteine concentration and favored a successful pregnancy.

Increased levels of homocysteine may lead to premature vascular disease, *i.e.* early damage to decidual or chorionic vessels that may cause disturbed implantation of the concepts. The damage caused by hyperhomocysteinemia results from a defective remethylation of homocysteine to methionine and not from the blockage of the homocysteine transsulfuration pathway. Malnutrition and malabsorbtion of folate and vitamin B12 or an inherited enzymatic defect, such as 5, 10-methylenetetrahydrofolate reductase deficiency, may also result in hyperhomocysteinemia (10).

On the other hand, folate deficiency may cause decreased synthesis and methylation of DNA and RNA and gene expression. Therefore folate deficiency could be related to early pregnancy loss independently of the disturbed homocysteine metabolism.

We observed an increase in serum homocysteine in our study group, and their folate levels tended to be lower compared with the control group, but this difference did not reach statistical significance, probably because of small samples or selection of controls. A single measurement of homocysteine does not exclude the possibility that these women could have had a genetic or other tendency to elevated levels of homocysteine – a fact that can be determined only with the aid of a methionine-loading test. Our findings therefore do not rule out the possibility of a transient fetal folate deficiency at an earlier stage of development. It is possible that a low concentration of folate is more significant before the pregnancy and during conception than during abortion.

Although a relationship between disturbed homocysteine and folate methabolism and early pregnancy loss is not well established, hyperhomocysteinemia in women with pregnancy complications should be identified because therapeutic normalization might permit a normal birth. In animal studies, folic acid supplementation seemed to improve survival of fetuses during early gestation and increase the number of living fetuses. However, we cannot conclude that folic acid supplementation prevents early pregnancy loss, and so far no data are available from randomized controlled trials on folic acid in women with a first spontaneous abortion.

Large-scale prevalence studies are needed to draw conclusions as to the causative relation between hyperhomocysteinemia and folate deficiency and spontaneous abortion. Further research should be focused on the pathophysiology of this relationship and on the clinical efficacy of B vitamin supplementation.

CONCLUSIONS

The results obtained in this laboratory evaluation show that serum homocysteine and folate concentrations for women with the first spontaneous early abortion and elective abortion were within the population-based reference range. Women with spontaneous abortion had higher homocysteine and their folate levels tended to be lower compared with the control group, but the difference did not reach sta-

tistical significance. Serum homocysteine inversely correlated with serum folate in both groups, and this correlation was statistically significant.

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NĖŠČIŲ MOTERŲ, KURIOMS ĮVYKO PIRMAS ANKSTYVAS SAVAIMINIS PERSILEIDIMAS, HOMOCISTEINO IR FOLATŲ KONCENTRACIJA KRAUJO SERUME

S a n t r a u k a

Tyrimo tikslas buvo nustatyti nėščių moterų, kurioms įvyko pirmas ankstyvas savaiminis persileidimas, homocisteino ir folatų koncentraciją kraujo serume, kadangi mokslinėje literatūroje tokių duomenų nėra. Lietuvoje tokie tyrimai buvo atlikti pirmą kartą. **Objektas ir metodai.** Tyrimo metu nustatėme 27 nėščių moterų, kurioms įvyko pirmas ankstyvas savaiminis persileidimas, ir 28 nėščiųjų, kurios nėštumą nutraukė savo noru, kraujo serumo folatų ir homocisteino kiekį. **Rezultatai.** Moterų, kurioms įvyko ankstyvas savaiminis persileidimas, folatų kiekis kraujo serume buvo 8,55 ± 2,55 ng/ml, nutraukusių nėštumą savo noru, – 8,83 ± 2,32 ng/ml. Kraujo serumo homocisteino kiekis moterų, kurioms įvyko savaiminis persileidimas buvo 7,32 ± 2,04 µmol/l, moterų, kurios nėštumą nutraukė savo noru, – 7,27 ± 1,99 µmol/l. Keturių moterų, kurioms įvyko savaiminis persileidimas, ir trijų, nutraukusių nėštumą savo noru, homocisteino kiekis kraujo serume buvo didesnis negu 10 µmol/l. **Išvados.** Nėščiųjų, kurioms įvyko ankstyvas savaiminis persileidimas, kraujo serumo homocisteino kiekis buvo didesnis, o folatų – mažesnis lyginant su kontroline grupe, tačiau šis skirtumas statistiškai nereikšmingas. Abiejų grupių moterų kraujo serumo homocisteino kiekis buvo atvirkščiai proporcingas folatų kiekiui ir ši koreliacija buvo statistiškai patikima. Tirtų nėščių moterų kraujo serumo homocisteino ir folatų kiekis buvo fiziologinės normos ribose.