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Cobalamin Deficiency During Pregnancy Expressed as Elevated Urine Methylmalonic Acid Levels Determined by a Photometric Assay

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Aim: Deficiency of cobalamin and folate during pregnancy is associated with megaloblastic anemia. Lower levels of folate and vitamin B12 were reported in mothers whose offspring had neural tube defects compared to unaffected controls. Increased methylmalonic acid (MMA) levels are a sensitive indicator of mild vitamin B12 deficiency and elevated homocysteine levels denote vitamin B12 or folate deficiency. We aimed to evaluate cobalamin state in pregnancy by using urine MMA levels determined by an economic spectrophotometric method.

Materials and Methods: For this cross-sectional study, plasma total homocysteine (tHcy), serum folate and vitamin B12, and urine MMA levels were measured in 186 uncomplicated pregnant women (30 in first, 58 in second and 98 in third trimester). MMA measurements were made in first morning urine samples with normalizing by creatinine concentrations.

Results: The tHcy concentrations were not elevated (<11.0 mmol/L) and folate levels were not found reduced (<5.0 ng/ml) in almost all subjects, whereas 36% of the pregnant women had elevated urine MMA levels (>6.0 mmol/mol creatinine). Nearly half of the group (99 women) had low vitamin B12 concentrations (180 pg/ml). We found higher folate levels in second and third trimesters than in first trimester. No other parameter was found different according to trimester.

Conclusions: The photometric urine MMA determination method showed an increased functional cobalamin deficiency that could not be indicated by tHcy levels during pregnancy. By using this economic spectrophotometric method, urine MMA should be measured in every pregnant patient with or without low serum vitamin B12 before treatment with vitamin B12 injections is instituted.

Key Words: Cobalamin deficiency, pregnancy, urine MMA

Gebelik Sırasında Oluşan Kobalamin Eksikliğinin, Fotometrik Olarak Ölçülen Yüksek İdrar Metilmalonik Asit Seviyeleri ile Ortaya Konması

Amaç: Gebelik sırasındaki kobalamin ve folat eksikliği megaloblastik anemi ile ilişkilidir. Gebelerdeki vitamin B12 ve folat düşüklüğü, bu annelerden doğan çocuklarda nöral tüp defektlerine neden olduğu bildirilmiştir. Yüksek metilmalonik asit seviyeleri orta dereceli bir vitamin B12 eksikliğinin hassas bir göstergesidir ve yüksek homosistein seviyeleri vitamin B12 veya folat eksikliğini gösterir. Bu çalışmada amacımız; gebelerdeki kobalamin durumunu, ekonomik bir spektrofotometrik metotla metilmalonik asit (MMA) seviyelerini ölçerek değerlendirmektir.

Yöntem ve Gereç: Kesitsel çalışmamıza alınan 186 komplikasyonsuz gebenin (30'u ilk trimestr, 58'i ikinci trimestr ve 98'i üçüncü trimestrda idi) plazma total homosistein seviyeleri (tHcy), serum folat ve vitamin B12 seviyeleri ve idrar metilmalonik asit seviyeleri ölçüldü. Sabah ilk idrar numunelerinin MMA seviyeleri idrar kreatin seviyeleri ile normalize edildi.

Bulgular: Hemen hemen tüm gebelerdeki tHcy seviyeleri normal sınırlarda idi(<11.0 mmol/L) ve folat konsantrasyonları düşük değildi (<5.0 ng/mL) . Fakat tüm gebelerin %36'sında idrar MMA seviyeleri yüksek olarak tespit edildi(> 6.0 mmol/mol creatinine). Neredeyse tüm gebelerin yarısının (99 gebe) vitamin B12 seviyeleri düşüktü (180 pg/mL). Ayrıca ikinci ve üçüncü trimestrdaki folat seviyeleri birinci trimestrdan daha yüksekti. Ölçülen parametrelerin hiçbirisi arasında bir farklılık saptanmadı.

Sonuç: Fotometrik idrar MMA ölçümü, gebelik sırasında gelişebilecek olan fonksiyonel kobalamin eksikliğini tHcy'nin tespit edemediğini göstermiştir. Vitamin B12 seviyeleri düşük olsun yada olmasın vitamin B12 tedavisi öncesinde tüm gebelerden bu ekonomik fotometrik metot kullanılarak idrar MMA seviyeleri ölçülmelidir.

Anahtar Sözcükler: kobalamin eksikliği, gebelik, idrar MMA

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Introduction

Deficiency of vitamin B12 is frequently accompanied by megaloblastic anemia and occasionally neurological or psychiatric disorders (1). Although approximately 20% of women show a physiologic drop in serum vitamin B12 level during pregnancy, the decreased vitamin B12 in serum may not indicate true megaloblastic anemia (2). The steady fall in vitamin B12 throughout gestation is thought to be caused by active transport across the placenta and, to a lesser degree, by hemodilution. Some of these variations may be caused by hormonally induced changes in transcobalamin binder production, because vitamin B12 levels return to normal within a few weeks of delivery without the need for supplementation (3); however, this phenomenon of low vitamin B12 is commonly seen in obstetric practice.

Evaluation of cobalamin deficiency requires direct measurement of metabolites related to the vitamin-B12-dependent pathways. Vitamin B12 and folic acid catalyze the remethylation of homocysteine (Hcy) to methionine. Hyperhomocysteinemia may be caused by a deficiency in cobalamin or folate metabolites. Furthermore, studies have shown that methylmalonyl-CoA, a metabolite of various amino acids, accumulates when its mutase is blocked by deficiencies in its vitamin B12 co-factors or in the presence of congenital abnormalities of the mutase (4).

The measurement of serum or urine concentrations of methylmalonic acid (MMA) and plasma Hcy metabolites related to vitamin B12 deficiency has been used to establish vitamin B12 deficiency at the biochemical level (5-8). Using a combination of metabolite concentrations, serum vitamin concentrations, clinical characteristics, and the response to therapy, it has been demonstrated that every vitamin B12-deficient patient who will respond to therapy has an elevation of MMA and/or Hcy, whereas findings of normal concentrations of both metabolites rule out clinical vitamin B12 deficiency (9). In line with these findings, the drop in vitamin B12 serum concentration in pregnancy may not reflect B12 deficiency; thus, to establish true B12 deficiency in pregnancy, the concentrations of serum Hcy and serum or urine MMA should be used.

Since it has been reported that many factors affect serum MMA concentrations, urine MMA concentrations have been recommended for diagnosing tissue or functional cobalamin deficiency (10). However, most of the urine MMA determination methods are expensive and require a gas chromatography mass spectroscopy (GC/MS). Recently, an economic and reliable spectrophotometric method for urine MMA was reported (6).

The aim of the present study was to assess vitamin B12 concentrations in normal pregnant women and to examine whether low vitamin B12 concentrations are associated with changes in the plasma total Hcy (tHcy) and urine MMA concentrations (using photometric method) as biochemical indices of vitamin B12 deficiency

Materials and Methods

Patients

One hundred and eighty-six uncomplicated pregnant women (30 in first, 58 in second and 98 in third trimester) were selected for this cross-sectional study. The participants' ages were between 19-35 years. The pregnant women met the following criteria: (i) normal diet; (ii) singleton pregnancy; (iii) no history of anemia before the present pregnancy; and (iv) no evidence of malabsorption. Patients with diseases known to cause anemia (renal disease, thalassemia, etc.) or those maintaining a vegetarian diet were excluded. As was normal obstetric practice in the institution, all women had begun to take iron and folic acid supplements since their initial booking visit. Daily folic acid supplementation was given to all pregnant women until the third trimester.

Analytical Methods

Venous blood was drawn after an overnight fast and immediately stored at 4–7°C and all analyses were performed on the same day. The first urine in the morning was used for MMA measurements. The urinary MMA measurements were based on the reaction between MMA and diazotized *p*-nitroaniline and utilized a scanning spectrophotometer (Philips UV/VIS scanning spectrophotometer, PU 8700 series, UK) (6). Urine MMA concentrations were normalized with urine creatinine levels and expressed as mmol/mol creatinine. The folate and vitamin B12 measurements were obtained by commercial kits using a microparticle enzyme immunoassay method via the Abbott AxSYM System

Automated Immunoassay Analyzer (Abbott Laboratories, Abbott Park, Illinois 60064 USA). The tHcy concentrations were measured by a commercial kit (Chromsystems) in HP Agilent 1100 HPLC system.

Statistical Analysis

The distributions of all variables were tested using normality plots and the Kolmogorov-Smirnov test, which quantifies the discrepancy between data distribution and ideal Gaussian distribution. The data passed the normality tests and comparison of each trimester's laboratory parameters was performed by using Student's t test. The relationships between laboratory parameters were evaluated by Pearson correlation coefficient. All statistical calculations were performed using SPSS for Windows (ver. 11.0) software.

Results

The mean blood vitamin B12, folate, tHcy and urine MMA concentrations of each group are listed in Table 1. Second and third trimester folate concentrations were found statistically higher than first trimester values ($P < 0.01$). No other parameter was found significantly different from the other. There was no correlation between laboratory parameters.

When the threshold for functional cobalamin deficiency is selected >6 mmol/mol creatinine for urine MMA levels, 20.0% of the first, 34.5% of the second and 41.8% of the third trimester group were defined as cobalamin deficient, whereas none of the pregnant women had tHcy levels higher than 12 mmol/L, as the threshold for cobalamin deficiency (Figure 1).

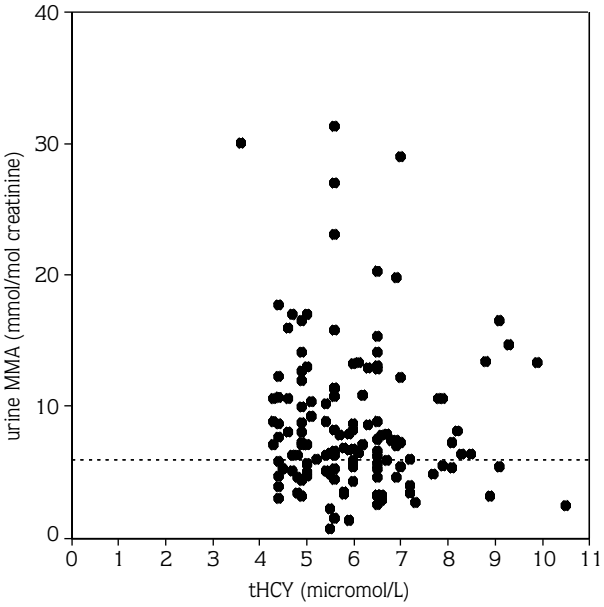

Figure 1. The distribution of urine MMA values versus tHcy concentrations in all pregnant women. The dotted line shows the cut-off point (6 mmol/mol creatinine) for elevated urine MMA concentration.

According to urine MMA levels, 67 of 186 (36%) pregnant women had cobalamin deficiency. When vitamin B12 and folate levels were compared with urine MMA levels, it was clearly seen that there were 36 false-positive cases according to vitamin B12 measurements and 63 and 40 false negative cases with folate and vitamin B12 determinations, respectively (Figure 2).

Table 1. Mean ± SD values of biochemical parameters related to cobalamin metabolism in each trimester and comparisons of those values between groups.

	First trimester (n =30) Mean ± SD	Second trimester (n=58) Mean ± SD	Third trimester (n=98) Mean ± SD
Vitamin B12 (pg/ml)	172 ± 53	200 ± 71	173 ± 92
Folate (ng/ml)	10.6 ± 2.9	13.6 ± 2.8[a]	13.9 ± 3.2a
tHcy (µmol/L)	7.47 ± 2.03	6.18 ± 1.25	6.03 ± 1.41
Urine MMA (mmol/mol creatinine)	6.59 ± 4.07	8.39 ± 5.55	8.40 ± 5.37

[a] $P < 0.01$, versus first trimester.

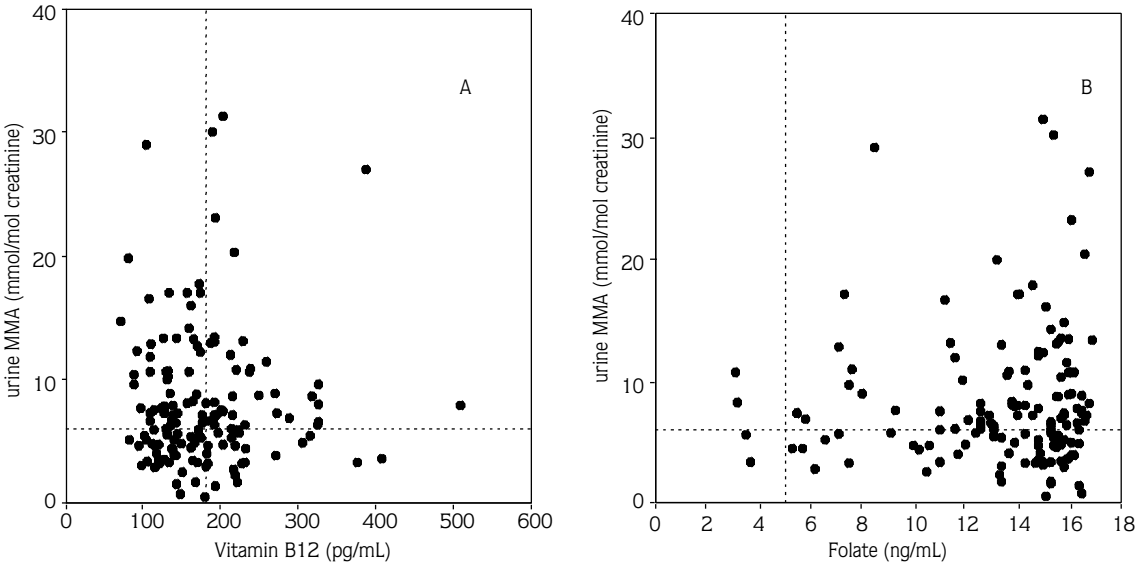

Figure 2. The distributions of urine MMA vs. vitamin B12 (A) and folate (B) for all pregnant women. Ninety-nine pregnant women have vitamin B12 levels lower than 180 pg/ml but only 4 women have folate levels lower than 5 ng/ml (dotted lines show cut-off values for vitamin B12, folate and urine MMA concentrations).

Discussion

In this study, we found a functional cobalamin deficient state expressed as elevated urine MMA levels during pregnancy. The tHcy and folate levels of pregnant individuals did not change pathologically, whereas 36% of urine MMA levels were found to be higher than the deficiency threshold. This implies that there is a functional (tissue level) cobalamin deficiency and this condition manifests itself by elevated urine MMA in pregnant women. Thus, neither vitamin B12 and folate nor tHcy as a metabolite seems useful for detecting cobalamin deficiency in pregnancy. The decrease in serum B12 concentrations noted by several studies during pregnancy does not reflect a true tissue depletion of B12 (megaloblastic anemia) (11). In a recent comparison of 50 pregnant patients with low serum B12 concentrations and 25 pregnant patients with normal concentrations (12), no significant difference in either serum MMA or total Hcy was reported between the groups. However, we evaluated urine instead of serum MMA, which was not found suitable for MMA measurements.

In the cell, the coenzyme vitamin B12 should be converted to methylcobalamin and deoxyadenosylcobalamin in order to be active; however, these conversion mechanisms are not yet well understood. The latter compound is used in the conversion of methylmalonyl CoA to succinyl CoA, and lack of deoxyadenosylcobalamin causes elevated MMA in serum and urine. The increased urine MMA levels in our pregnant group may reflect an intracellular change causing diminished conversion of cobalamin to deoxyadenosylcobalamin.

Low tHcy levels in normal pregnancy were reported (13-17). The decreased tHcy levels might be related to increased folate supplementation during pregnancy (15). There is an increased requirement for folic acid in pregnancy and this has been shown to be associated with accelerated catabolism of folic acid (18). In mothers who have a neural tube defect (NTD) pregnancy, metabolic pathways of folic acid may be abnormal (19,20) and increased folic acid has the potential to overcome this. Hence, determination of folate status in pregnancy and the detection of mild deficiency are imperative. In this study, there was no elevation in tHcy levels and we also found no relationship between Hcy levels and folate. This may be the consequence of folate supplementation during pregnancy.

The usual means for delineating vitamin B12 malabsorption secondary to intrinsic factor deficiency versus other malabsorption factors (Schilling test) is

contraindicated in pregnancy because of the risk of radiation exposure. Therefore, therapy is usually instituted empirically in pregnant patients with low serum vitamin B12, and diagnostic testing is delayed until the postpartum period (21). This practice, however, may lead to over-treatment of pregnant patients without true vitamin B12 deficiency. Our results suggest that by using this economic spectrophotometric method, urine MMA should be measured in every pregnant patient with or without low serum vitamin B12 before treatment with vitamin B12 injections is instituted.

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