Serum Homocysteine, Folate, and Vitamin B12 Levels in Pregnant and Non-Pregnant Women

GEBELERDE VE GEBE OLMAYAN KADINLARDA SERUM HOMOSİSTEİN, FOLAT VE VİTAMİN B12 DÜZEYLERİ

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Abstract

Objective: To assess the serum homocysteine (tHcy) status of the second trimester pregnant women, aged 18-42 years, and to compare the results with the age-matched non-pregnant women.

Material and Methods: Forty-one pregnant women (24-28 weeks of gestation) aged 18-42 years and 44 healthy non-pregnant women aged 19-35 years were included in the study. tHcy, folate, vitamin B_{12} and albumin levels were measured and correlated to homocysteine values.

Results: The second trimester pregnant women's homocysteine levels were significantly lower while folate and vitamin B_{12} levels were significantly higher than non-pregnant women ($p< 0.001$). The overall mean homocysteine concentration of the pregnant subjects (3.6 ± 1.59 µmol/L) was significantly lower than those observed in the non-pregnant control group (9.64 ± 1.87 µmol/L) ($p< 0.001$). Homocysteine levels were positively correlated with albumin levels and negatively correlated with folate and vitamin B_{12} levels in both pregnant and non-pregnant groups.

Conclusion: The decrease in homocysteine levels during pregnancy seems to be associated with physiologic low levels of albumin and with using prenatal multivitamin supplementation.

Key Words: Homocysteine, pregnancy, folic acid, vitamin B_{12}, albumin

Özet

Amaç: 18-42 yaş arası 2. trimester gebelerdeki serum homosistein, (tHcy) folat ve vitamin B_{12} düzeylerinin aynı yaş grubundaki gebe olmayan kadınlarla karşılaştırılması.

Gereç ve Yöntemler: Çalışmaya 18-42 yaş arası 41 gebe (24-28 haftalık) ve 19-35 yaş arası 44 sağlıklı gebe olmayan kadın dahil edildi. tHcy, folat, vitamin B_{12} ve albumin düzeyleri çalışılarak homosistein düzeyleriyle karşılaştırıldı.

Bulgular: İkinci trimester gebelerdeki tHcy düzeyleri gebe olmayan kontrol grubuna göre anlamlı olarak düşük iken folat ve vitamin B_{12} düzeyleri anlamlı olarak yüksekti ($p< 0.001$). Gebelerin ortalama homosistein konsantrasyonu (3.6 ± 1.59 µmol/L), gebe olmayan kontrol grubuna göre (9.64 ± 1.87 µmol/L) anlamlı düzeyde düşük olarak gözlendi ($p< 0.001$). Hem gebe hem de gebe olmayan grupta homosistein düzeyi albumin ile pozitif korelasyon gösterirken folat ve vitamin B_{12} ile negatif korele idi.

Sonuç: Gebelikte homosistein düzeyleri düşük olarak gözlendi. Bunun sebebi albuminin fizyolojik düşüklüğü ve prenatal multivitamin desteğiyle ilişkili olabilir.

Anahtar Kelimeler: Homosistein, gebelik, folat, vitamin B_{12}, albumin

Turkiye Klinikleri J Med Sci 2006, 26:121-125

Homocysteine is an intermediary metabolite of the essential amino-acid methionine.[1] It is metabolized by one of two pathways. The majority of homocysteine is catabolized into cystathionine by the vitamin B_6-dependent enzyme cystathione-β-syntase (CBS). A significant proportion of homocysteine is regenerated into methionine by methionine synthase. This enzymatic reaction involves methylenetetrahydrofolate reductase (MTHFR) and the cofactor vitamin B_{12}.[2] Folate provides the methyl group for methionine synthase and when folate intake is inadequate, plasma homocysteine concentrations increase. This fact can be used to assess folate status.[3] Elevated fasting homocysteine levels are associated with lower circulating concentrations or lower intake of folate and vitamin B_{12} and are amenable to treatment with these vitamins.[4-7]

Geliş Tarihi/*Received:* 02.06.2005 **Kabul Tarihi/*Accepted:*** 08.11.2005

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An elevated level of fasting tHcy is a risk factor for many diseases ranging from cardiovascular to pregnancy-related conditions.[8] Plasma homocysteine level is considered a functional indicator of folate status and may have important implications in individuals at risk for developing folate deficiency, such as pregnant women.[3]

Hyperhomocysteinemia has also been associated with complications in pregnancy, such as neural tube defects, repeated miscarriages, abruptio placentae, fetal death, preeclampsia and intrauterine growth retardation.[9,10] Pregnant women have high folate requirements because of increased folate utilization and catabolism.[11] Several studies in fact reported that tHcy concentrations were lower during pregnancy compared with the non-pregnant state.[12,8] According to Andersson et al, tHcy concentrations were 29-60% lower in pregnant than in non-pregnant women and reached their lowest values during the second trimester of pregnancy.[12,2]

In the current study, we assessed the serum homocysteine status of second trimester pregnant women, aged 18-42, and compared the data with those obtained in age matched non-pregnant women.

Material and Methods

Study Group and Design

This cross-sectional study was performed at Ankara Numune Hospital, from October 2004 to February 2005. It included 41 pregnant women (24-28 weeks) aged 18-42 (mean 27.41 ± 2.62) years and 44 healthy non-pregnant women aged 19-35 (mean 27.7 ± 2.11) years. The study and control groups comprised subjects who were non-smokers, had no history of hypertension, diabetes, renal-cardiac diseases and coagulation defects. Informed consent was obtained from all participants. All subjects were questioned on the use of folic acid or prenatal vitamins and the brand of vitamin and duration of use were recorded.

Laboratory Analysis

Venous blood samples were obtained after fasting overnight for 8-10 hours and a resting period of 20 min. Blood samples for vitamin B_{12}, serum folate and albumin were collected in serum separator tubes. After 30 minutes they were centrifuged for 10 minutes at 3000 g and were assayed after being separated. Homocysteine specimens were centrifuged at 4°C for 10 minutes at 3000 g and sera were stored at -20°C until assayed.

Serum vitamin B_{12} and serum folate were analyzed on the Abbott Axsym System immunoassay analyzer by using the manufacturer's reagents. (Abbott Laboratories Diagnostics Division, Abbott Park, IL) Serum albumin was analyzed on the Abbott Aeroset System autoanalyzer (Abbott Laboratories Diagnostics Division, Abbott Park, IL). Homocysteine assays were carried out by HPLC methods (Hewlett-Packard Agilent 1100 Series, Waldbronn, Germany). HPLC system consists of an isocratic pump, autosampler, thermostated column compartment, vacuum degasser, and fluorescence detector. The autosampler injected 20 µL aliquots of final analytical solutions. The backpressure of column is about 100 bars at a flow rate of 1.7 ml/min and the temperature of column is 20-25ºC. The absorbance was measured between 385-515 nm. Identification of peaks was based on comparison of retention times. The intra- and inter-assay coefficients of variation for homocystein were 2% and 4.2%, respectively. The limit of detection was 4 µmol/L; reference ranges for homocysteine: 5.5-17 µmol/L.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS version 10 for Windows) was used for statistical analyses. The normality of the distribution of all variables was assessed by the Kolmogorov-Smirnov test. Differences between 2 groups in continuous variables that had a Gaussian distribution (homocysteine, serum folate, and albumin) were evaluated by independent-t test; for continuous variables that had no Gaussian distribution (B_{12}) were evaluated by Mann-Whitney U test. Pearson's correlation analyses were used to calculate correlations between parameters. Statistical significance was set at $p < 0.01$. Descriptive statistics are expressed as mean +/- standart deviation.

Pregnant women had taken a prenatal vitamin supplement that contained 800 µg/d folate, 4 µg/d vitamin B_{12} and 2.6 mg/d Vitamin B_6.

Results

Ages of the subjects and serum homocysteine, folate, vitamin B_{12} and albumin levels are shown on Table 1.

There were no significant differences between the 2 groups with regard to age. In the control group, homocysteine levels were significantly higher, while folate and vitamin B_{12} levels were significantly lower than in pregnant women ($p < 0.01$) (Figure 1). Compared with non-pregnant controls, albumin levels decreased ($p < 0.001$) when pregnancy progressed. There was a positive correlation between albumin and homocysteine concentrations in both groups ($r = 0.846$, $p < 0.001$) (Figure 2).

Table 1. The homocysteine, vitamin B_{12}, folate, and albumin levels and ages of the study and control groups (mean ± SD) and the significance of differences between them.

	Control (non-pregnant women) n: 44	Pregnant women (24-28 weeks) n: 41	p-value*
Age (years)	27.7 ± 2.11	27.41 ± 2.62	NS (0.815)
Homocysteine (µmol/L)	9.64 ± 1.87	3.6 ± 1.59	< 0.001
Albumin (g/L)	44.07 ± 4.36	35.05 ± 5.79	< 0.001
Vitamin B12 (pmol/L)	220 ± 116	285 ± 80	< 0.001
Serum folate (nmol/L)	14.96 ± 5.49	31.83 ± 6.69	< 0.001

*: Statistical significance α= 0.01 NS: Non-significant.

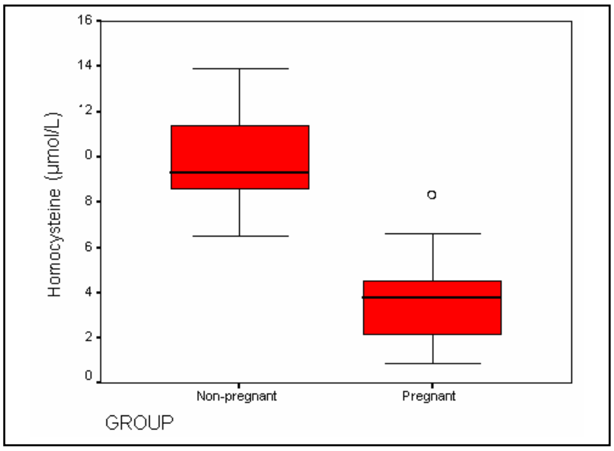

Figure 1. Homocysteine levels in pregnant and non-pregnant women. Data points represent mean values with 95% confidence intervals (CI).

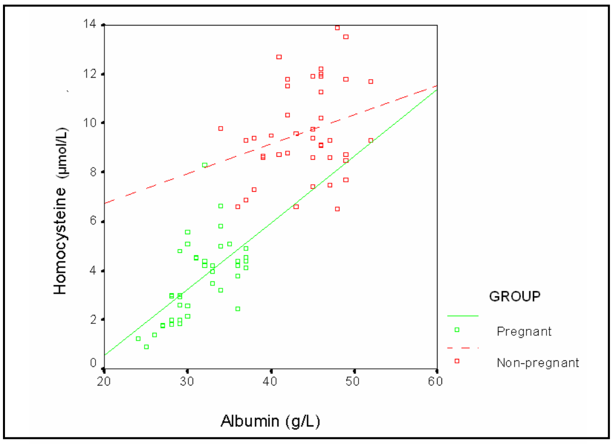

Figure 2. Correlation between homocysteine and albumin in pregnant and non-pregnant groups.

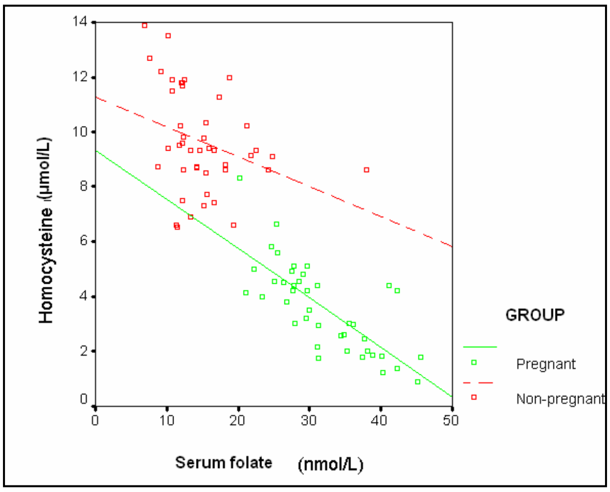

Figure 3. Correlation between homocysteine and serum folate in pregnant and non-pregnant groups.

A significantly negative correlation was obtained between homocysteine-folate and homocysteine-vitamin B_{12} levels in both groups ($r = -0.857$; $p < 0.01$, $r = -0.494$; $p < 0.01$ respectively) (Figures 3, 4).

Comment

In this study, we observed that serum homocysteine levels decreased during pregnancy. Plasma homocysteine concentrations in women in the second and third trimesters of pregnancies without complications are lower than values detected in non-pregnant women.[9,13] The mechanism behind the lowering of homocysteine levels in pregnancy is currently unknown.

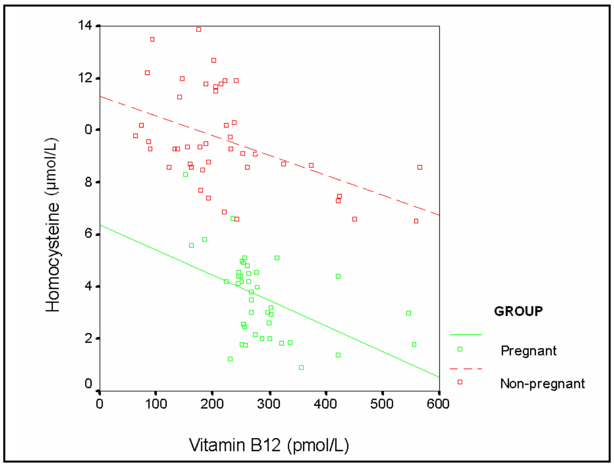

Figure 4. Correlation between homocysteine and vitamin B_{12} in pregnant and non-pregnant groups.

Many reasons have been proposed for the decreased levels of homocysteine in pregnancy, including hemodilution, physiological decreased albumin levels during pregnancy, or a relationship with maternal folic acid supplementation during pregnancy.[13] According to Walker et al decreasing homocysteine levels in pregnancy occurs in association with the physiologic fall in albumin during pregnancy, as well as with folic acid supplementation. We evaluated the albumin levels of 2 groups and observed a significant positive correlation between homocysteine and albumin levels (Table 2). This finding is not unexpected, because 70% to 80% of homocysteine is albumin bound.[2] However, Glorimar et al reported that the changes in tHcy concentrations were due to the period of pregnancy and were not related to a reduction in serum albumin concentration.[14]

Plasma free amino acid concentrations express the balance between uptake and utilization; they are influenced by hormonal factors and the availability of vitamins and cofactors involved in intermediary metabolism.[15] To take prenatal multivitamins containing 800 µg/d folic acid might be the reason of decreasing levels of homocysteine in pregnant women. Brouwer et al showed that doses of folic acid as low as 250 µg/d, on average, in addition to usual dietary folate intake significantly decreased plasma tHcy concentrations in healthy and young women.[16] In another study, daily consumption of folic acid-fortified breakfast cereals and the use of folic acid supplements appear to be the most effective factor in reducing homocysteine concentrations.[17] Our results indicated that there was a significant negative correlation between serum folate and homocysteine levels in both groups (Table 2, Figure 3). In the pregnant group, while the mean homocysteine level was low, an increased mean folate concentration was detected. On the other hand, several studies suggest that taking folic acid supplementation during pregnancy is not the main cause of decreasing homocysteine levels.[3,8,14]

Vitamin B_{12} is a cofactor of methionine synthase, which catabolizes homocysteine to methionine. Elevated vitamin B_{12} levels may reduce homocysteine concentrations. Our pregnant women had higher vitamin B_{12} levels than those in the non-pregnant group and we observed a negative correlation between vitamin B_{12} and homocysteine (Table 2, Figure 4). In accordance with our study, Bondevik et al found that the majority of elevated homocysteine values were most likely caused by an impaired cobalamin status.[18] However Walker et al and Glorimar et al could not show any correlation between vitamin B_{12} and homocysteine levels in second trimester pregnant women and non-pregnant control group.[2,14]

Table 2. Pearson's correlation coefficients (r) among the variables.

	Homocysteine (µmol/L)	Serum folate (nmol/L)	Vitamin B_{12} (pmol/L)	Albumin (g/L)
Homocysteine (µmol/L)	1.000			
Serum folate (nmol/L)	-0.857**	1.000		
Vitamin B_{12} (pmol/L)	-0.494**	0.319**	1.000	
Albumin (g/L)	0.846**	-0.535**	-0.137	1.000

**: Correlation is significant at the 0.01 level.

We could not evaluate the vitamin B_6 levels in our groups. Glorimar et al found no correlation between vitamin B_6 and homocysteine concentrations.[14]

Homocysteine levels might be expected to decrease during pregnancy due to hemodilution. Gestational blood volume starts to increase at 6-10 weeks, proceeds rapidly during the second trimester, and peaks at 30-34 weeks, the average total increase is 1.2-1.5 L. Hematocrite during the second trimester of pregnancy is expected to be as 33-39%, in agreement with the observations in pregnant women.[19] However Murphy et al's study showed that the pregnancy-induced reduction in homocysteine concentration was significantly greater in terms of percentage than the reduction in the hematocrite value.[8]

Many complex hormonal changes occur in pregnancy and there is increasing evidence that female hormones seem to decrease homocysteine levels. According to Dierkes et al, from the gender-related variables, homocysteine correlated significantly with fat-free mass and testosterone and inversely with estradiol.[20] Kim et al showed a significant decrease in plasma homocysteine concentrations in male rats that were administered estradiol, and Giri et al showed that oral 17β-estradiol reduced homocysteine concentrations by 11% in healthy elderly men.[21,22] Homocysteine concentrations reduced in postmenopausal women with elevated values after they received hormone replacement therapy.[23]

In conclusion, we observed lower homocysteine concentrations in pregnant women than those in age-matched non-pregnant control group in this study. The reason of decreasing homocysteine levels in pregnancy seems to be multifactorial due to taking prenatal multivitamin, physiologic fall in albumin, hemodilution and hormonal changes.

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