Plasma Homocysteine, Folate and Vitamin B_{12} in Different Trimester of Pregnancy

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Abstract:
The present study has been undertaken to investigate plasma creatinine, folate and vitamin B_{12} as underlying factors in the reduction of plasma homocysteine concentration in different trimesters of pregnancy. Under a cross-sectional design, 30 healthy nonpregnant as well as 130 pregnant women, at 3 different trimesters of pregnancy (43 in 1st trimester, 44 in 2nd trimester and 43 in 3rd trimester) were sampled for plasma levels of folate, vitamin B_{12}, homocysteine and creatinine. Plasma homocysteine and creatinine level were significantly ($p<0.014$) lower in pregnant women at different trimester (1st, 2nd and 3rd trimester) compared to nonpregnant women. Plasma folate was significantly higher in different groups of pregnant women compared to nonpregnant women. Significant negative correlation was found between plasma homocysteine and folate in nonpregnant ($r=-0.453$, $p<0.012$) and pregnant women at 2nd trimester ($r=-0.681$, $p<0.001$). There was found significant negative correlation between plasma homocysteine and vitamin B_{12} at 1st trimester group ($r=-0.322$, $p<0.035$) and 2nd trimester group ($r=-0.394$, $p<0.008$) but not in 3rd trimester. In present study, reduction of plasma total homocysteine appears to be a physiological response in pregnancy, and the level of plasma folate and vitamin B_{12}, but can not be explained by renal homodynamic changes.

Key words: Folate, vitamin B_{12}, homocysteine and pregnancy

Introduction: An elevated plasma total homocysteine (tHcy) concentration is regarded as a risk factor for atherosclerosis [1,2] and venous thrombosis [3]. Serious pregnancy complications, including pregnancy-induced hypertension, preeclampsia[4], placental abruption [5] and spontaneous abortion [5,6] and adverse pregnancy outcomes such as neural tube defects [7] and low birth weight[8] are associated with elevated homocysteine concentration. In normal pregnancy homocysteine concentration in plasma will decrease. THcy concentrations were 29–60% lower in pregnant women than in non pregnant women and reached their lowest values during the second trimester of pregnancy [9,10]. Murphy *et al.* 2002 [11] has shown that tHcy concentrations decreased gradually during early and mid pregnancy and reached a trough in later pregnancy, with no further decreases,

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which is consistent with other previous findings [9,10]. Various hypotheses explaining the decrease in tHcy concentration during pregnancy have been proposed, among these are hormonal influences on homocysteine metabolism [12,13], maternal dietary protein intake during pregnancy [12], pregnancy-associated hemodilution [9,10,13], or fetal utilization [9,13,14]. Murphy *et al.*2002 [11] demonstrated that the reduction of tHcy can not be accounted for by folate supplementations, plasma volume expansion, or a decrease in serum albumin. They suggested that low tHcy represents a physiologic adaptation to pregnancy, mediated by endocrine changes. An alternative mechanism for the pregnancy-related decrease in total plasma homocysteine concentration is the change in renal hemodynamics during pregnancy, glomerular filtration rate (GFR) and renal plasma flow increase markedly in pregnancy [15,16]. Homocysteine derived from the demethylation of the amino acid methonine is either further catabolished by trans-sulfuration to cysteine or remethylation to methonine. Remethylation of methonine requires the cofactors, folate and vitamin B_{12}. The concentration of homocysteine is regulated by many factors such as age, genetically determined metabolic enzyme alteration, nutritional factors like Vitamin B_6, B_{12} and folate. Folate is essential to maintain the normal levels of serum homocysteine. Folate plays an important role in two biochemical cycles; one involving DNA biosynthesis and in one carbon metabolism such as DNA, lipid and protein methylation[17], so the requirement of folate during pregnancy is increased. Folate in pregnancy, protects against a number of congenital malformations including neural tube defects[18], low birth weight, recurrent miscarriage, placental abruption and intrauterine growth restriction [19].
Vitamin B_{12} is an essential nutrient in the diet of humans due to its important function as a co-enzyme in the remethylation of homocysteine to methionine and in the conversion of methyl malonyl CoA to succynyl CoA.

Objective: This study was conducted to compare the folate, vitamin B_{12} and homocysteine status in pregnant and nonpregnant women and to investigate the dynamics of plasma homcysteine changes in pregnancy and its underlying causes.

Subjects and Methods:

Subjects: The study was approved by the Biomedical Research Group, and consent was obtained from each participant on recruitment. Healthy pregnant women (n=43 in 1st trimester, n=44 in 2nd trimester and n=43 in 3rd trimester, 18 to 35 years old) and nonpregnant women (n=30, 18 to 35 years old) were sampled. Pregnant women attending antenatal clinics at the BSMMU and Shurwardy hospital were recruited in the study during antenatal visit at 6-12 week, 13-24 week and 25-36 week of gestation, and the study was conducted as cross-sectional design. Age matched nonpregnant women were recruited as control from the staff of Biomedical Research Group, BIRDEM and the graduate students of Dhaka University. Trained interviewer collected information on socioeconomic data, previous obstetric history, vitamin supplementation (either folate or multivitamin), and height and weight.

Sample Collection and Analytical Methods:

Venous blood sample were drawn from antecubital vein. Fasting blood sample were collected in a haperiniazed test tube. Blood was allowed to stand for 10 minutes and then centrifuged for 10 minutes at a rate of 3000 rpm at 4^0C. to separate plasma, which was then stored at – 70^0C until analysis. Plasma homocysteine, folate, vitamin B_{12} and creatinine were measured by Fluorescence Polarization Immunoassay (FPIA) method in AxSym system[20], chemiluminesent ELISA[21], Microparticle Enzyme Immunoassay (MEIA) method[22] and alkaline picrate method[23] respectively.

STATISTICAL ANALYSIS

Data are expressed as median (range) for non-parametric values. Comparison between groups were done using Mann-Whitney U test for skewed data. The relationship between homocysteine and other variables were examined using Spearmen's nonparametric coefficient correlation analysis. Plasma vitamin concentrations were compared with published reference values to determine the proportions of nonpregnant and pregnant groups with biochemical evidence of vitamin deficiencies. Vitamin deficiency was defined as plasma concentrations < 6.8 nmol/l (3 ng/ml) for folate[24] and < 258 pmol/l (350 pg/ml) for vitamin B_{12} [25,26]. There is no standard definition of elevated Hcy, but Ronnenberg *et al* [27] defined it as a plasma concentration of Hcy above the 90th percentile among women. In this study we defined elevated Hcy as a plasma concentration of >12.61μ mole/l. A p value of <0.05 was considered as significant. All the statistical analysis were performed with the SPSS data (SPSS Inc, Chicago, IL, USA).

RESULTS

Table-1 depicts the levels of Plasma homocysteine, folate, vitamin B_{12} and creatinine of nonpregnant women and pregnant women in different trimesters. The median level of plasma homocysteine was found to be 9.52 μmol/L for nonpregnant women, while those for pregnant women in 1st, 2nd, and 3rd trimester were found to be 6.45, 5.19 and 5.22 μmol/L respectively. Plasma homocysteine values for pregnant women in all three trimesters were found to be significantly ($p<0.05$) lower than that of nonpregnant women. Amongst the pregnant women, values for 2nd and 3rd trimester were significantly lower than that for 1st trimester. However values for 2nd and 3rd trimester did not differ significantly. It is to be noted that plasma homocysteine concentration decreased in pregnancy, and also tended to be on a decrease with the progress of trimester or in other word with the progress of pregnancy.

The median level of plasma folate for nonpregnant women was found to be 15.07 nmol/L, while those for pregnant women in 1st, 2nd, and 3rd trimester were found to be 22.21, 24.02 and 36.94 nmol/L respectively. Plasma folate concentration for pregnant women in all three trimesters were found to be significantly ($p<0.05$) higher as compared to that for nonpregnan women. Amongst the pregnant women, plasma folate was found to increase with the progress of pregnancy, the value for

3rd trimester being significantly ($p<0.05$) higher than those of 1st and 2nd trimester group.

The median plasma vitamin B_{12} concentration for nonpregnant women was found to be 353.65 pmol/L, while the values for pregnant women in 1st, 2nd, and 3rd trimester were found to be 244.3, 200.3 and 159.7 pmol/L respectively. Values for pregnant women belonging to all trimesters were significantly ($p<0.05$) lower than that for nonpregnan women, indicating the fact that plasma vitamin B_{12} decreases during pregnancy. Amongst the pregnant women, there was found a decreases in plasma vitamin B_{12} concentration with the increase on trimester , the value for 3rd trimester being significantly ($p<0.05$) lower than those for 1st,and 3rd trimester. The median plasma creatinine concentration was found 1.04 mg/dl for nonpregnant women, and those for pregnant women in 1st, 2nd, and 3rd trimester were found to be 0.99, 0.92 and 0.85 mg/dl respectively. The values for pregnant women in all three trimesters were significantly ($p<0.05$) lower than those for nonpregnan women. Amongst the pregnant women, the values were found to be in a significantly decreasing ($p<0.05$) order with the increase of trimesters.

As depicted in table-2, Distribution of study subject to the cutoff value of homocysteine, folate and vitamin B_{12} indicate, only 3 subjects out of 30 (10%) in nonpregnant women and 1 subject out of 43 (2.3%) in 1st trimester group had plasma homocysteine level higher than 12.61 μmol/l.

No folate deficiency was found in pregnant women but 3.3% nonpregnant women had plasma folate concentration less than 6.8 nmol/l.

Pregnant women were more deficient in vitamin B_{12} than nonpregnant, and deficiency gradually increases with increasing gestational week. Only 26.8% (8 subject out of 30) in nonpregnant, 55.8% (24 out of 43) in 1st trimester group, 65.6% (28 out of 44) in 2nd trimester group and 83.7% (36 out of 43) in 3rd trimester group have plasma vitamin B_{12} level less than 258 pmol/l.

As depicted in table-3, a spearmans nonparametic correlation coefficient (r) analysis of plasma homocysteine with plasma folate, vitamin B_{12} and creatinine of nonpregnant and pregnmant women (all trimester combined) revealed a significant negative correlation between plasma homocysteine and folate in both pregnant women ($r= -0.327$, $p<0.001$) and nonpregnant women ($r = -0.453$, $p<0.012$). There was also found a Significant positive correlation ($r= 0.276$, $p<0.001$), between plasma homocysteine and creatinine in pregnant women, but no such correlation was found in nonpregnant women. It is to be noted that plasma homocysteine was not found to be correlated significantly with plasma vitamin B_{12} either in case of pregnant or nonpregnant women.

Table-4 depicts the spearmans nonparametic correlation coefficient (r) of plasma homocysteine with plasma folate, vitamin B_{12} and creatinine of nonpregnant women and pregnmant women in different trimester. There was found a significant negative correlation ($r=-0.453$, $p<0.012$) between homocysteine and folate in nonpregnant women. Similar correlation ($r= -0.681$, $p<0.001$) was found to exist between homocysteine and folate in pregnant women belonging to 2nd trimester. A

significant negative correlation was found to exist between plasma homocysteine and vitamin B_{12} in pregnant women at 1st trimester ($r= -0.322$, $p<0.035$) and 2nd trimester ($r= -0.394$, $p< 0.044$). A significant positive correlation ($r= 0.37$, $p<0.015$) was found to exist between plasma homocysteine and creatinine in pregnant women at 1st trimester.

Table- 5 depicts the effect of folate and B vitanmins supplementation on plasma homocysteine, folate, vitamin B_{12} and creatinine levels of pregnant women in different trimesters. Plasma homocysteine level was found to be significantly ($p<0.002$) lower in pregnant women at 2nd trimester who received vitamin supplementation as compared to those who remained unsupplemented. However homocysteine level was found to be in a gradual decline from 1st trimester group to 3rd trimester in unspplemented pregnant women. Plasma folate concentration was found to be significantly ($p<0.001$) higher for pregnant women who received vitamin supplementation at all three trimester (1st, 2nd and 3rd) as compared to those who did not. Plasma vitamin B_{12} levels tended to be higher for vitamin supplemented pregnant women as compared to the unsupplemented ones, though the values did not differ significantly. Plasma creatinine concentrations were not found to differ significantly between the vitamin supplemented and unsupplemented pregnant women. The finding was same for all three trimesters under consideration.

Table 1: Plasma homocysteine, folate, vitamin B_{12} and creatinine concentration of nonpregnant women and pregnant women in different trimester

Parameter	Group			
	Nonpregnant (n=30)	1st trimester (n=43)	2nd trimester (n=44)	3rd trimester (n=43)
Homocysteine (μmol/l)	9.52[a] (5.82-16.2)	6.45[b] (2.96-14.31)	5.19[c] (2.38-9.82)	5.22[cd] (2.77-11.87)
Plasma folate (nmol/l)	15.07[a] (5.44-45.09)	22.21[b] (9.29-54.38)	24.02[bc] (6.8-80.22)	36.94[d] (13.37-81.8)
Vitamin B_{12} (pmol/l)	353.65[a] (57.1-766.6)	244.3[b] (100.7-03.2)	200.3[bc] (109.6-77.3)	159.7[d] (60.3-470.9)
Plasma creatinine (mg/dl)	1.04[a] (0.83-1.2)	0.99[b] (0.76-1.22)	0.92[c] (0.78-1.18)	0.85[d] (0.67-1.16)

Data are presented as median (range) for nonparametric value; n= Number of subject; Values in the same lining not sharing common superscript letter are significantly different ($p<0.05$ and less).

Table 2: Distribution of study subject to the cutoff value of homocysteine, folate and vitamin B_{12}

Parameter	Nonpregnant (n=30)	Pregnant Women		
		1st Trimester (n=43)	2nd Trimester (n=44)	3rd Trimester (n=43)
Homocysteine < 12.61μ mol/l	27 (90.0%)	42 (97.7%)	44 (100%)	43 (100%)
Homocysteine ≥ 12.61μ mol/l	3 (10%)	1 (2.3%)	-	-
Plasma Folate <6.8 nmol/l	1 (3.3%)	-	-	-
Plasma Folate ≥6.8 nmol/l	29 (96.4%)	43 (100%)	44 (100.0%)	43 (100%)
Plasma Vitamin B_{12} <258 pmol/l	8 (26.7%)	24 (55.8%)	28 (63.6%)	36 (83.7%)
Plasma Vitamin B_{12} ≥ 258 pmol/l	22 (73.3%)	19 (44.2%)	16 (36.4%)	7 (16.3%)

Data are presented as number (percentage); n= Number of subjects.

Table 3: Spearman's nonparametric correlation coefficient's (r) of plasma level of homocysteine with folate,vitamin B_{12} and creatinine of nonpregnant and pregnant women

Parameter	Nonpregnant (n=30)		Pregnant women (n=130)	
	r	p	r	p
Plasma Folate	-0.453	0.012	-0.327	0.001
Vitamin B_{12}	-0.183	0.334	-0.128	0.146
Plasma Creatinine	0.054	0.777	0.276	0.001

Spearman's nonparametric correlation coefficient are performed for the analysis; $p<0.05$ are considered as statistically significant; n= Number of subjects.

Table 4: Spearman's nonparametric correlation coefficient's (r) of homocysteine with plasma folate, vitamin B_{12} and plasma creatinine in nonpregnant women and pregnant women in different trimester

Parameter	Nonpregnant (n=30)		1st Trimester (n=43)		2nd Trimester (n=44)		3rd Trimester (n=43)	
	r	p	r	*p*	r	p	r	p
Plasma Folate	-0.453	0.012	-0.062	0.695	-0.681	0.001	-0.161	0.303
Vitamin B_{12}	-0.183	0.334	-0.322	0.035	-0.394	0.008	-0.046	0.768
Plasma Creatinine	0.054	0.777	0.370	0.015	0.173	0.261	0.177	0.257

Spearman's nonparametric correlation coefficient are performed for the analysis; $p<0.05$ are considered as statistically significant; n= Number of subjects;.

Table 5: Plasma Homocysteine, folate, vitamin B_{12} and creatinine levels for supplemented and unsupplemented pregnant women in different trimesters

Parameters		Group		P value
		Supplemented	Unsupplemented	
Homocysteine (μmol/l);	1st trimester	6.45 (2.96-9.28)	6.36 (3.62-14.31)	0.842
	2nd trimester	4.68 (2.38-6.4)	6.0 (2.87-9.82)	0.002
	3rd trimester	5.41 (2.77-11.87)	4.97 (4.08-10.76)	0.581
Plasma folate (nmol/l);	1st trimester	44.64 (30.82-54.38)	15.86 (9.29-29.23)	0.001
	2nd trimester	44.64 (28.33-80.22	16.1 (6.8-25.61)	0.001
	3rd trimester	42.83 (19.03-81.8)	25.38 (13.37-29.68)	0.001
Vitamin B12 (pmol/l);	1st trimester	244.3 (140.4-469.0)	239.1 (100.7-603.2)	0.709
	2nd trimester	205.8 (114.1-474.3)	200.1 (109.6-421.1	0.823
	3rd trimester	159.8 (60.3-470.9)	148.75 (113.4-283)	0.788
Plasma creatinine (mg/dl);	1st trimester	1.02 (0.88-1.22)	0.96 (0.76-1.14	0.071
	2nd trimester	0.92 (0.79-1.18)	0.92 (0.78-1.1)	0.962
	3rd trimester	0.85 (0.67-1.16)	0.87 (0.8-0.94)	0.702

Data are presented as median (range) for nonparametric value. n= Number of subject; $p<0.05$ are considered statistically significant at 95% confident interval; test are done by Mann-Whitney U test.

Discussion:

In the present study pregnant women have been found to have lower levels of plasma tHcy, and the lowest values was found in 2nd trimester of pregnancy which is consistent with previous findings [9,10] which reported that tHcy concentrations were 29–60% lower in pregnant women than in nonpregnant women and reached their lowest values during the second trimester of pregnancy. However, Murphy et al. (2002) [11] has shown that tHcy concentration decreased gradually up to 32 weeks of pregnancy with no further decrease onwards. The present data, however, are in partial conformity with the longitudinal data provided by Cikot et al[13] and also with that of Rolf *et al.*[28], which reported only a slight reduction in tHcy concentration during early pregnancy and no further decrease throughout mid-to-late pregnancy.

It is interesting to note that tHcy concentration in plasma actually decreases in normal pregnancy. Various hypotheses have been proposed to explain the decrease in tHcy concentration during pregnancy. Among these are hormonal influences on tHcy metabolism [12,13], maternal dietary protein intake during pregnancy[12], pregnancy-associated hemodilution [9,10,13], or fetal utilization [9,13,14].

In the present study plasma creatinine concentration is significantly lower in different groups of pregnant women than in nonpregnant women. This finding is similar to that of Murphy *et al.*[11], who reported that plasma tHcy and creatinine concentrations decrease in parallel from preconception to pregnancy in the same group of women. The result suggested that the magnitude of the corresponding reductions in tHcy during pregnancy are considerably greater than the changes in serum creatinine, a marker of renal function. Similar reduction is also found in the present study where plasma creatinine concentration decreased to 4.8%, 11.5% and 18.25% at 1st, 2nd and 3rd trimester groups respectively, and the magnitude of the corresponding reduction in tHcy are 32.23%, 45.48% and 45.16%, which are considerably greater than the change in plasma creatinine. Lower tHcy concentrations observed during pregnancy due to dilution resulting from the expansion in blood volume. The increase in gestational blood volume begins at 6 to 10 weeks, proceeds rapidly during the second trimester, and peaks at 30 to 34 weeks—the average total increase is 1.2 to 1.5 L [29,30].

In the present study plasma folate is significantly higher in pregnant women in all trimester (1st, 2nd and 3rd trimester) as compared to nonpregnant women. Several previous studies reported that serum folate concentration slightly decreases during pregnancy, with recovery after delivery [28,31,32]. A significant negative correlation between plasma tHcy and folate levels were found in nonpregnant ($r=-0.453$, $p<0.012$) as well as in pregnant groups ($r= -0.327$, $p<0.001$) and this is in agreement with previous findings [10,33]. There may be two reasons for the observed increase of folate concentration in the present study: one may be dietary intake, another may be folate supplementation in pregnancy.

When folate supplementation is considered, significantly ($p<0.001$) higher concentration of folate is found in supplemented women at different trimesters of pregnant women as compared to unsupplemented women. Plasma tHcy

concentration is significantly lower in vitamin-supplemented group compared to unsupplemented group in the 2nd trimester ($p < 0.002$). In unsupplemented group tHcy concentration is lower throughout pregnancy. Murphy et al. [11] also shown that tHcy concentration gradually decline through out pregnancy from 1st trimester to 3rd trimester in both supplemented and unsupplemented group . Thus it can be hypothesized that lower tHcy concentration in unsupplemented group due to physiologically induced reduction in pregnancy, which is independent to folate supplementation. Alternately, the observed value of plasma folate in the unsupplemented group may be sufficient to have a physiological response of tHcy in pregnancy.

In the present study plasma vitamin B_{12} level is lower in all trimesters of pregnancy compared to nonpregnant group and the data are supported by a previous study[32]. Although significant negative correlation between tHcy and vitamin B_{12} is found in 1st trimester group ($r= -0.322$, $p<0.035$) and 2nd trimester group($r= -0.394$, $p<0.008$), but no significant difference is found in whole pregnancy ($r = -0.128$, $p<0.146$) and it also supported the previous study by Park *et al.* [33], where marginal negative correlation ($r= -0.26$, $p<0.06$) between tHcy and vitamin B_{12} levels have been found among pregnant women.

In conclusion present study indicate, the reduction of plasma total homocysteine is a physiological response in pregnancy and the level of plasma folate and vitamin B_{12}, but can not be explained by renal hemoidynamic changes.

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