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Original Article

Homocysteine in Gestational Diabetes and Normal Pregnancy plus Effects of Folic Acid

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Abstract

Background: The aim of study was to assess serum homocysteine and its relation with serum folate, vit B12 and lipid profiles in gestational diabetes mellitus and comparison with normal pregnant women as well as effect of different doses of folic acid on Homocysteine (Hcy) and pregnancy outcome.

Methods: In a biphasic study first prospective controlled study 80 pregnant women were chosen at 24-28 weeks of gestation. In case group pregnant women with gestational diabetes mellitus and control group who had normal Oral Glucose Tolerance test (GTT) results. Levels of fasting glucose, homocysteine, vit B12, and folic acid, uric acid, total cholesterol, triglyceride, LDL, HDL, were measured. In phase II study a randomized clinical trial was done with diabetic women 15 with 1mg folic acid and 15 with 5 mg folic acid for six weeks and then above variables and pregnancy outcome was evaluated.

Results: The mean levels of homocysteine in Gestational Diabetes Mellitus (GDM) group were significantly higher but folic acid and vit B12 were significantly lower. Hcy levels were decreased in both groups after six weeks folic acid but decrease in Hcy for group 5mg was significantly more than 1mg group.

Conclusion: Homocysteine levels were higher in GDM than normal pregnancy. High dose folic acid can reduce Hcy levels more than low dose and it may be a safe, simple, inexpensive intervention that prevents major pregnancy complications.

Keywords: *Homocysteine, Gestational diabetes, Pregnancy outcome*

Introduction

Homocysteine is a naturally occurring amino acid that has recently become the subject of much research interest. Elevated plasma homocysteine, termed hyperhomocysteinemia, has been implicated in a number of pathologic processes in the venous and arterial vascular systems. Increased plasma homocysteine level is an independent risk factor for peripheral vascular disease and for coronary artery disease (1).

Data showed that the prevalence of the MTHFR C677→T polymorphism in pregnant women with type 1 diabetes mellitus and the associated morbidity with vascular dysfunction observed during pregnancy, i.e. preeclampsia, hypertension, varying degrees of albumin excretion rate and retinopathy, and preterm delivery (2). Studies have shown that folate deficiency is associated with increased homocysteine levels (3). In a normally functioning metabolic state, methionine produces homocysteine as an intermediate step before either trans-sulfuration via cystathionine into cysteine or remethylation to methionine (4). This remethylation may be folate-dependent, or may use betaine, a metabolite of choline (5). Methionine synthase, a vit B12-dependent enzyme, utilizes 5-methyltetrahydrofolate as the carbon donor for folate-dependent homocysteine remethylation; methionine synthase requires activation by methionine synthase reductase (MTRR). Betaine-homocysteine methyltransferase (BHMT) utilizes betaine as the carbon donor. Therefore, the improper function of remethylation enzymes, due to mutation or to insufficient intake of relevant nutrients, may result in elevated homocysteine levels and contribute to thrombophilias, and possibly, placental abruption (2, 6).

Homocysteine (Hcy) metabolism is also affected by the bioavailability of folic acid, methyl group donors, and B vits (7). Elevated Hcy is linked to

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pregnancy complications and increased pregnancy loss (8).Treatment in infertility in PCOS with Metformin, while improving insulin resistance, was shown in certain clinical situations to increase serum Hcy levels by reducing levels of folic acid and vit B12 (9).

Hyperhomocystenemia has emerged as a risk factor for arterial and venous thrombosis. Folic acid is an important factor in the metabolism of homocysteine. MTHFR is one of three enzymes that are responsible for the circulating form of folic acid. Recently, pregnancy complications such as RPL (recurrent pregnancy loss) and fetal neural tube defects have been reported in association with hyperhomocysteinemia. Folate deficiency is also a common cause of hyperhomocysteinemia (8, 9). Supplementation with folic acid and vit B6 has been applied with some beneficial effect in pregnancy with a history of intrauterine growth restriction and preeclampsia (10).

Furthermore, recent reports have hypothesized that elevated homocysteine plasma levels may predict, in the early second-trimester period, the subsequent development of preeclampsia (11).

Hyperhomocysteinemia has been associated with a number of pregnancy complications, such as neural tube defects, repeated miscarriages, abruptio placentae, fetal death, pre-eclampsia and IUGR. Hyperhomocysteinemia may be associated with a placental microvascular disease (12).

The metabolic challenge of pregnancy unmasks underlying abnormalities of glucose tolerance and blood pressure. Gestational diabetes mellitus (GDM, or diabetes diagnosed during pregnancy) develops in women who are susceptible, and typically resolves postpartum. This disorder is a risk factor for type 2 diabetes and hypertension in the long term (13). Women who have had GDM are 7 times more likely than controls to develop type 2 diabetes 22-28 yr later, and are also more likely to develop hypertension, hyperlipidemia, and show abnormal electrocardiograms. Thus, in addition to being a risk factor for type 2 diabetes and hypertension, GDM also increases a patient's risk of arteriosclerosis and coronary heart disease. Hyperhomocysteinemia has been detected in patients with type 1 and type 2 diabetes, and is associated with premature atherosclerosis (14).

The concentration of plasma Hcy is regulated by several factors, including genetically determined metabolic enzyme alterations, nutritional status, underlying disease, certain medication, age and pregnancy. Of these however, pregnancy is the only factor that specifically decrease concentration of plasma Hcy (15, 16). Levels of Hcy are generally lower during pregnancy, due to either a physiological response to the pregnancy, an increase in esterogen, hemodilution from an increase plasma volume, or increased demand for methionine by both mother and the fetus. Previously folic acid administration has been shown to reduce Hcy levels in healthy subjects and patients with renal and vascular disease (16, 17).

Folic acid, vit B12 and vit B6 are all cofactors in Hcy metabolism. Hence during gestational diabetes, Hcy levels might increase. Serum vit B12 and folic acid levels are known to decrease during GDM. Authors showed that serum Hcy was significantly increased in women with GDM, independently of other confounding variables, and was related to 2 h OGTT plasma glucose. (16, 18). Others showed that serum Hcy levels increased in women with GDM, were significantly related to 50 g glucose loads and seemed to be unrelated to insulin resistance in these women (19).

In view of these conditions first we studied serum levels of Hcy in normal pregnant women and comparison with GDM and second the effects of folic acid 1mg, 5mg on serum levels of Hcy in patients with GDM and follow-up the pregnancy outcome in both groups.

Materials and Methods

The biphasic study was conducted in the department of Obstetrics and Gynecology of School of Medicine, Tehran University of Medical Sciences, Mirza Kochak khan Hospital form March 2005 to February 2006. In phase I study, eighty women with uncomplicated pregnancies who were at 24-28 wk gestation, participated in this prospective case control study. The study was ap-

proved by the ethical committee of Tehran University. Informed consent was obtained from each patient just before entering the study.

All pregnant women at our hospital are routinely screened for GDM between 24 and 28 wk gestation. Screening is performed with a 50-g oral glucose load, and a patient is considered negative when their blood glucose is 135 mg/dl. Women with blood glucose levels>135 mg/dl undergo a 3 h 100-g oral glucose tolerance test (OGTT). Blood samples are collected at 0700 h (after 12 h of fasting) and at 1, 2 and 3 h after glucose ingestion. Plasma glucose levels are measured using the glucose oxides method. The OGTT results are interpreted according to the present criteria (1). The cutoff levels for the fasting and the 1-, 2- and 3-h plasma glucose levels are 95, 180,155 and 140 mg/dl respectively. A patient is diagnosed with GDM if her glucose levels at two or more of the time points exceed these cutoffs. The women in the study were assigned to one of two groups according to the results of the OGTT, case group comprised 40 women with GDM according to the OGTT and 40 control group with normal screens and normal 3 h OGTT. A blood sample was drawn from each woman for plasma levels of glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were determined by calorimetric method using a Cobas Mira plus autoanalyzer. (Roche Diagnostics, Mannheim, Germany). Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol levels were calculated according to Friedwald formula (19). Each patient's plasma folic acid and vit B12 concentration was measured by electrochemiluminescence technique in a Roche Modular Analytics E170 immunoassay analyzer (Roche Diagnostics corporation, Indianapolis, IN, USA). Each woman's plasma homocysteine concentration was measured after at least 8 h of fasting on the same morning as the OGTT was performed. This was carried out in an Ax SYM hormone autoanalyzer with an assay based on ELISA. Data were expressed as mean±SD. Differences between two groups were analyzed using independent student's *t*-test and Regression analysis (logistic & binary). Correlations between groups were assessed by Pearson and Spearman correlation coefficient and multiple regression analyses. All statistical calculations were performed using the program SPSS for windows (version 13; SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant at levels of probability, 0.05.

At phase II (a clinical trial study) thirty GDM women were randomized to two groups by an allocation sequence generated from a random number table and assigned through consecutively numbered opaque, sealed envelopes. The first group (n=15) received folic acid 1mg daily; the second group (n=15) received folic acid 5 mg for 6 wk. Plasma level of glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides, uric acid, plasma folic acid, vit B12 concentration, plasma Hcy concentration was measured after 6 wk. Inclusion criteria in phase I were pregnant women with uncomplicated pregnancies who were at 24-28 wk, and Hb level≥ 11 mg/dl. Each woman gave their informed consent before they were enrolled. All 80 women were non-smoker. Women who had folic acid and vit B12 deficiency, hypertension, thyroid disease or a history of significant medical illness, PCOS, metabolic disorders, enzyme deficiency, cardiovascular disease, history of infertility and induction ovulation, any drug use except ferrous sulfate, history of recurrent abortion and age>35 yr old, altered renal function, treatment with antifolate drugs (antiepileptic, metotrexate) were excluded from the study.

In phase II (a randomized clinical trial study) beside above criteria pregnant women with GDM based on OGTT results were allocated in 2 groups. In one group 15 GDM women with 5mg/ day folic acid and in another group 15 GDM women with 1mg/day folic acid have participated. The paired-samples *t*-test was used to analyze changes in variables before and after treatment in groups

Results

Based on GTT results, 40 women were assigned to control group (normal blood glucose levels

after 100g OGTT) and 40 patients were assigned to case group (diagnosed with GDM based on 100g OGTT). Some clinical and demographic characteristics of two groups are shown in Table 1. The women in case group were significantly older than those in control ($P< 0.05$ for control group versus case group, 30.15±3.7 for GDM versus 26.4±4.5 for control group, $P= 0.0001$) but this difference was less than one decade and it had no confounding effect on homocysteine levels. The two groups were similar with respect to proportions at different levels of parity (1.3±1.01 in GDM group vs. 0.5±0.7 in normal group).

The mean level Hcy and TG, BMI in case group was significantly higher than the level in control group (Table-2, 3). Homocysteine= 7.8±1.6 µmol/L, TG= 259±44 mg/dl, BMI= 31.9±2.1 kg/m^2 versus Hcy= 5.05±1.1 µmol/L, TG= 217±95 mg/dl, BMI= 27±3 kg/m² respectively in diabetic and normal group (Table 4).

In the Pearson correlation test, no correlation was found between Hcy levels and age, BMI, LDL, HDL, TG, total cholesterol, uric acid, folate, and Vit B12 (Table 5).

The mean plasma levels of vit B12, folic acid, and HDL-C in case group was significantly lower than control. Vit B12= 235±52.9 pg/ml, serum folate= 6.72±1.3 ng/ml, HDL= 49.8±14.6 mg/dl versus vit B12= 329±191.8 pg/ml, serum folate= 7.9±2.7 ng/ml, HDL= 60.3±14 mg/dl respectively in diabetic and normal group. There were no significant differences among the groups with respect to plasma levels of total cholesterol, LDL-C.

In phase II study all patients completed the study in 5 mg folic acid group (GDM with 5 mg folic acid) and 14 patients in 1mg folic acid group (GDM with 1mg folic acid), and were analyzed for the primary outcome after 6 wk of treatment mean level of Hcy, folic acid, vit B12, TG, cholesterol, HDL, LDL, and uric acid were measured. Baseline characteristics of the patients are given in Table -2 ,3, 4 but their follow-up continues after two months of delivery and secondary outcome was evaluated by neonatal weight, route of delivery, macrosomia (weight birth> 4000 g), IUFD, hypoglycemia (< 40 mg/dl at first hour of birth), and NICU admission rate.

In 5 mg group cesarean section (C/S) was done for 40% (6) of women, vaginal delivery for 60% (9) cases. One IUFD in 39 wk of pregnancy due to nuchal cord, indication of C/S in two was for history of previous C/S, one for breech presentation, one for CPD, one elective C/S, one for placenta previa with massive hemorrhage at 35 wk of gestation, this neonate admitted to NICU for 10 d, after 2 months follow-up all neonate were healthy.

In 1mg group 28/57% (4) C/S was done and 71.45% (10) vaginal delivery was done. C/S was done in two case with history of previous C/S, two case for macrosomia that neonatal weight were 4500 g, 4200 g at delivery. There were two preterm delivery (<37 wk gestation) at 35 wk of gestation that one of them was due to sever preeclampsia. Ten case of uncomplicated vaginal delivery was done. Two months after delivery all neonate were healthy. No case of hypoglycemia (Glu level< 40 mg/dl in first hour of birth) was observed in both groups.

Hcy levels were decreased from baseline in both groups after 6 wk folic acid but decrease in Hcy level in group 5 mg was significantly more than 1mg group. Serum folate had increased in group 5mg significantly ($P= 0/003$). There were no statistically significant changes recorded for Vit B12 and TG cholesterol, LDL, and Uric acid. There was statistically significant increase in HDL in group 5mg 49.2±17.4 mg/dl before treatment and 57±15.3 mg/dl after treatment ($P= 0.000$) and in group 1mg 54.07±11.7 mg/dl versus 57.6±0.2 mg/dl respectively before and after treatment ($P= 0.001$) but mean differences in group 1mg for HDL was 3.5±3.2 versus 8.31±5.5 in group 5 mg ($P= 0.009$). There was no statistically significant difference between two groups regard to age, BMI, parity, and neonatal weight.

Analysis of logistic regression in both groups was done for confounding effects and underlying factors like age and weight and level of homocysteine but it shows no correlation. Binary regression test for both diabetic groups showed the re-

sults like Table 1, even after analysis for 3 age groups(age< 20, 20< age< 30, age> 30), there was a significant relationship between homocysteine in diabetics and non diabetic.

Table 1: Demographic data and comparison of variables in diabetic and normal groups

Data	Diabetic Means± SD	Non diabetic Means± SD	*P* value
FBS	106±9.9	76.7±8.4	0.000
Age(year)	30.2±3.7.	26.4±4.5	0.0001
Parity	1.3±1.01	0.5±0.7	0.4
BMI(kg/m²)	31.9±2.1	27±3	0.000
Homocysteine (µmol/L)	7.8±1.6	5.05±1.1	0.000
Serum folate ng/ml	6.72±1.3	7.9±2.7	0.01
Vit B12(pg/ml)	235±52.9	329±191.8	0.004
LDL(mg/dl)	104±21	98±28	0.3
TG(mg/dl)	259±44	217±95	0.014
CHOL(mg/dl)	215.6±27	204±34	0.1
Uric acid	4.2±1.1	3.9±0.64	0.3
HDL(mg/dl)	49.8±14.6	60.3±14	0.0006

Table 2: Diabetic group with 1mg folic acid

Variable	Before treatment	After treatment	95% CI Lower upper	*P*
Homocysteine(µmol/L)	7.38±1.5	6.87±1.4	0.721 0.306	0.000
serum folate(ng/ml)	6.79±0.79	7.09±1.06	0.203 -0.803	0.22
Vit B12(pg/ml)	255.7±63	250.8±49	23.17 -13.46	0.5
Triglyceride(mg/dl)	256±43	244±30.7	21.99 0.293	0.04
Cholesterol(mg/dl)	206.8±24.3	201.3±17.2	8.1 -6.7	0.07
HDL(mg/dl)	54.07±11.7	57.6±0.2	-1.72 -5.42	0.001
LDL(mg/dl)	106.07±14	106.4±13.7	4.63 -5.35	0.88
Uric acid(mg/dl)	5.09±1.16	5.04±1.11	0.507 -0.403	0.8

Table 3: Data in diabetic group with 5mg folic acid

Variable	Before treatment	After treatment	95% CI,upper, Lower	*P*
Homocysteine(µmol/L)	7.99±1.6	6.7±1.2	1.63 0.08	0.000
Serum folate(ng/ml)	6.6±1.7	8.7±2	-1.71 -2.52	0.000
ViteB12(pg/ml)	233±39.5	235±34.1	16.27 -14.47	0.85
Triglyceride(mg/dl)	254.6±51	250.8±59.8	17.8 -1.8	0.86
Cholesterol(mg/dl)	221±31.3	202±24.7	22.6 15.9	0.03
HDL(mg/dl)	49.2±174	57±15.3	-5.25 -11.41	0.000
LDL(mg/dl)	106.5±23	102±20.6	14.4 -5.21	0.33
Uric acid(mg/dl)	4.5±1.17	4.9±1.13	0.11 -0.81	0.12

Table 4: Mean differences in diabetic women before and after treatment with two regiment of folic acid (1,5 mg)

Variable	Mean difference before, after1mg folic acid	Mean difference before, after5mg folic acid	*P*
age	29.5±4.6	29.9±4	0.1
BMI	30.6±2.6	31±1.6	0.8
parity	1.5±0.8	1.6±1.1	0.6
birth weight	3497±401	3290±283	0.1
Homocysteine	-0.5±0.3	-1.22±0.7	0.003
Serum folate	0.3±0.87	2.1±1.8	0.000
Vit B12	-4.85±31	1.6±32.2	0.7
Triglyceride	-11.1±18.7	-3.8±35	0.7
cholesterol	-5.5±10.6	-17.9±9.9	0.1
HDL	3.5±3.2	8.3±5.5	0.009
LDL	0.35±8.5	-4.4±17	0.3
Uric acid	-0.7±0.79	0.35±0.83	0.2

Table 5: Correlation between homocysteine and other variables

P	Variable	R
0.42	Age	0.58
0.84	BMI	0.03
0.18	LDL	-0.21
0.34	HDL	-0.15
0.50	TG	0.10
0.61	Cholesterol	-0.08
0.90	Uric acid	0.02
0.20	Vit B12	-0.19
0.10	Folate	0.24

Discussion

This study showed that the mean level of Hcy and TG, and BMI in diabetic group was higher than level in control group and the mean levels of vit B12, folic acid, and HDL were significantly lower. In phase II study showed that folic acid treatment in both group can reduce level of Hcy, but reduction was more significantly in 5 mg group, the same as truth for increase in HDL cholesterol. If there is an idea that says high Hcy is risk factor for many pregnancy complications like recurrent pregnancy loss, abruption, preterm labor, preeclampsia, vascular thrombotic events, gestational

diabetes, diabetes mellitus, and increase complication of diabetes and neonatal anomaly, it seems everything that reduces Hcy levels, it will have supportive effect against these complications. However, in this study Hcy levels were reduced with 5mg folic acid more than 1mg but the effect on pregnancy outcome was not significant. A reason may be small sample size, two macrosomic infant in group 1mg is marker of poor diabetic control in GDM but it is not statistically significant.

Low HDL-C level is a risk factor for cardiovascular disease and diabetic are in increased risk for CAD and treatment with 5mg folic acid significantly increases HDL-C level that it has supportive effect against CAD.

Plasma tHcy has been studied in adult insulin-dependent and non-insulin-dependent diabetic patients (15, 16), and moderate hyperhomocysteinemia has generally been found. In other experience, the most interesting data about the relationship between abnormal glucose tolerance and tHcy values has been observed in the glucose-intolerant group, which showed significantly lower tHcy levels than the control pregnant. In the gestational diabetes class, either tHcy or other AA concentrations did not show significant differences with controls, probably because the low-glycaemic diet of these patients implying lower insulin release might determine higher plasma AA concentrations (7, 20). In our study plasma Hcy in GDM group was higher than normal pregnant group. In normal pregnancy, the inhibition of the urea cycle function (5, 21). Seems to be reflected in lower ucAA concentrations. Authors found a significant decrease in ucAA values during pregnancy when comparing subjects with non-pregnant controls (8, 22).

In another study, diabetic pregnant women showed a significant increase in ucAA concentrations compared with the glucose-intolerant pregnant women and controls (4). Nevertheless, the differences in ucAA values observed in gestational diabetes in the second trimester disappeared in the third trimester, when insulin resistance became more evident (12, 13). This observation suggests that the mild association between ucAA and gestational diabetes found in that study group cannot easily be explained by the influence of insulin resistance in pregnancy. Numerous studies have associated major malformations with diabetes during pregnancy, and mechanisms by which the diabetic environment might alter normal embryonic development (including nutrients, hormones, growth factors and cytokines) have been examined (23). Recently, other authors have tried to determine whether folate metabolism in pregnant diabetic women was significantly different from that in their non-diabetic counterparts, thus predisposing them to having offspring with major congenital anomalies, but no differences were found (20).

Elevated homocysteine is found frequently in women with RPL. This is probably an underestimate because virtually all of these women were taking prenatal vits with folate, vit B6, and vit B12 at the time of the assay. Fasting homocysteine is easy to test for and is easily treated with supplemental B vits (folate, B6, B12). Data showed that folic acid can reduce Hcy levels and this effect probably is dose dependent it means when high dose of folic acid was used reduction of Hcy was more significant (19, 21). Our results do however confirm the reduction in homocysteine levels with folic acid supplementation recently reported by Murphy et al. (9). This is like our result that folic acid can reduce Hcy levels. Out with pregnancy there is evidence that intervention with folic acid may be effective in reducing plasma homocysteine levels and vascular events (10, 24).

Further large-scale studies are required, however, to determine whether this modest alteration in homocysteine concentration induced by folic acid supplementation for the whole of pregnancy would be effective in reducing adverse pregnancy outcome. However in our study Hcy level decreased but its effect of these two regimens (1, 5 mg) on pregnancy outcome was not statistically significant. (However our study has limitation due to low sample size)

High circulating homocysteine concentrations in pregnancy are associated with adverse pregnancy

outcomes. Authors have demonstrated that folic acid supplementation throughout pregnancy suppresses the change in plasma homocysteine concentration which occurred in their study group (21, 22).

This simple, safe and inexpensive intervention may therefore play a preventative role. Further large-scale studies are required to determine the effectiveness of folic acid supplementation in the prevention of poor obstetric outcomes.

Conclusively, in our best knowledge we can not find study that dose 5 mg folic acid is prescribed to gestational diabetic women at 24-28 wk for 6 wk and comparison of its effect with 1 mg folic acid and for this reason we could not comparison results of our study with others about pregnancy outcome and Hcy levels in two regimens. Our study limitation was low sample size. We recommend a large clinical trial with different dose of folic acid and even different dose of Vit B groups and evaluation of their effects on Hcy, HDL, LDL, pregnancy outcome.

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The authors declare that they have no conflict of interests.

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