Plasma Homocysteine Levels in Children and Adolescents with Type 1 Diabetes

Mehmet Emre Atabek, Ozgur Pirgon and Emrah Karagozoglu*

From the Section of Pediatric Endocrinology, Department of Pediatrics and Department of Biochemistry, School of Medicine, Selcuk University, Konya, Turkey.*

Correspondence to: Mehmet Emre Atabek, Selçuk Üniversitesi Meram Tip Fakültesi, Çocuk Sagligi ve Hastaliklari, 42080 Konya, Turkey.
e-mail: meatabek@hotmail.com

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Objective: *Hyperhomocysteinemia has been established as a risk factor for cardiovascular disease. The objective was to investigate total plasma homocysteine concentrations in children and adolescents with type 1 diabetes and a control group.* ***Method:*** *Twenty-seven children with type 1 diabetes and 27 subjects of an age- and sex-matched control group were recruited. Fasting samples were collected for plasma total homocysteine, serum vitamin B_{12}, folate, and creatinine.* ***Results:*** *Fasting total homocysteine concentrations showed no difference between patients and controls (5.6 ± 2.9 µmol/L* vs *5.7 ± 2.2 µmol/L; p >0.05). The diabetic patients had significantly higher serum folate than the healthy controls (11.4 ± 3.3 ng/mL vs 9.4 ± 4.1 ng/mL; P = 0.02 and higher serum B_{12} than the control group (282.8 ± 119 pg/mL* vs *228.5 ± 50.9 pg/mL; P = 0.03). Total plasma homocysteine concentration correlated with age (*r *= 0.44, P = 0.02), weight (*r *= 0.56, P = 0.002), body mass index (*r *= 0.57, P = 0.002), folate (*r *= –0.48, P = 0.01), and creatinine (*r *= 0.41, P = 0.03) in diabetic patients. In stepwise multivariate regression model for diabetics, the independent correlates for total plasma homocysteine concentration was folate (P = 0.002).* ***Conclusion:*** *We concluded that fasting plasma total homocysteine concentrations were within normal limits in children and adolescents with type 1 diabetes who were without any clinical evidence of microvascular and macrovascular complications.*

Key words: *Folate, Homocysteine, Type 1 diabetes, Vitamin B_{12}.*

RETROSPECTIVE and prospective studies have demonstrated that hyperhomocysteinemia is a risk factor for premature cardiovascular disease(1-3) independent of other classic risk factors, such as smoking, hypercholesterolemia, arterial hypertension, and diabetes(4). Diabetes is an important risk factor not only for premature atherosclerosis but also for its rapid progression, because the risk of cardiovascular and peripheral vascular disease is associated with the metabolic abnormalities involved in diabetes(5). Studies in adults with both type 1 and type 2 diabetes have shown higher values for total plasma homocysteine (tHcy) in those with microvascular(6) and macrovascular(7) complications. However, reduced tHcy concentrations have been found in some populations of adults with type 1 diabetes compared with control groups(8). Limited information exists regarding tHcy metabolism in normal children(9,10) or those with type 1 diabetes.

In the present study, we endeavored to evaluate a selected group of nonsmoking, normotensive, normolipidemic children and adolescent patients with type 1 diabetes who did not have any clinical evidence of microvascular and macrovascular complications.

Subjects and Methods

Study population

The baseline study population consisted of

27 patients (15 female, 12 male, mean age: 11.3 ± 0.7 years, age range: 4-17 years) with type 1 DM diagnosed according to the World Health Organization (WHO) definition and a control group of 27 age-, sex- and body mass index -matched healthy children (12 female, 15 male, mean age: 10.9 ± 2.8 years, age range: 6-16 years) (staff member's children). One hundred fourteen children with type 1 diabetes had been treated in our clinic during the study period. Inclusion criteria was >1 year period from diagnosis of type 1 diabetes which was detected <18 years of age and requiring insulin treatment. None of the individuals studied had diseases known to affect tHcy concentrations such as hypertension, hyperlipidemia and other cardiovascular diseases, and no one was under any medication. In history of our patients; differences between subjects in the diabetes group and the control group were largely related to higher intakes foods containing complex carbohydrates, particularly fruits, vegetables, and cereals in the diabetes group and lower intake of foods containing refined sugar. The patients were seen for period of 3 months. Anthropometric data were obtained by trained research personnel. Height was measured using a wall-mounted stadiometer, and weight was determined using a balance scale. Body mass index was calculated as the ratio of weight (kg) to the square of height (m^2). Arterial blood pressure was monitored under standard conditions after at least 5 min at rest in triplicate at 5-min intervals. Ophthalmoscopy through dilated pupils was carried out in all diabetic patients to assess the presence of retinopathy, by the same ophthalmologist. In our center, twenty four-hour urine samples were obtained and estimated for albuminuria after excluding proteinuria due to urinary tract infection. Microalbuminuria (MA) was defined as urinary albumin excretaion (UAE) above 30 mg/24 h, and overt clinical nephropathy was recorded when UAE exceeded 300 mg/ 24 h in at least two urine samples evaluated within a 12 weeks interval. None of the patients had a diagnosis of renal disease unrelated to diabetes during the follow up. Our institutional review board approved the study. Before the study, written informed consent was obtained from the older children and from both parents of all children.

Laboratory determinations

Fasting blood glucose, HBA1c, total cholesterol, HDL-cholesterol, riglycerides, LDL-cholesterol, and UAE were determined. Fasting tHcy concentrations were determined in EDTA plasma with competitive immunoassay. Samples were separated from the cells, and matched samples were spiked with tHcy and then assayed by IMMULITE 2000 Analyzer (Dignostic Products Corporation, Los Angeles, USA). Results were expressed μmol/L. Normal concentrations were 5 and 15 μmol/L(11).

Serum folate and vitamin B_{12} were measure by competitive immunoassay and IMMULITE 2000 Analyzer, and the normal range for folate was 3-17 ng/mL, and that for vitamin B_{12} was 193-982 pg/mL. Serum creatinine and lipids were measured by an automated enzymatic method using Olympus 2700 Analyzer (Olympus Diagnostica GmbH, Ireland). HbA1c was determined by ion-exchange chromatography at least twice in those with less than a year's duration of diabetes and at least three times per year for the rest (reference rate <6.05 %).

Statistical methods

Data were expressed as mean ± SD. The Kolmogorov-Smirnov test was applied separately for patients and controls to check the normality of the variables. Differences between data were studied using the Student's t test. Statistical correlation was assessed using

the Pearson test (*r*). Multiple linear regression analysis was performed in forward stepwise selection to identify independent factors affecting tHcy and to estimate the final predictors of its variability. Statistical significance was taken as P <0.05. All statistical analysis was performed using the Statistical Package for Social Sciences (SPSS/Windows version 11·0, SPSS inc, Chicago, IL, USA).

Results

The characteristics of the study population are shown in *Table I*. The groups were matched for age, sex, and body size. No significant differences were observed in the values of weight, height, body mass index, systolic and diastolic blood pressure between the both groups. In addition; total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol levels were not significant between the groups. None of the patients with diabetes had evidence of the microvascular complications, such as diabetic retionopathy, clinical neuropathy and overt nephropathy.

The difference in mean tHcy concentrations was not significant between patients and controls (5.6 ±. 2.9 μmol/L; and

TABLE 1–*Characteristics of the Study Groups*

	Diabetes group	Control group	P value
No (Male/Female)	27 (12/15)	27 (15/12)	
Age (years)	11.3 ± 4	10.9 ± 2.8	NS
Height (cm)	140.7 ± 19.7	139.3 ± 12.6	NS
Weight (kg)	38 ± 15.7	34.3 ± 5.9	NS
Body mass index kg/boy(m^2)	18.8 ± 3.5	17.4 ± 2.0	NS
Systolic BP (mmHg)	113.1 ± 12.4	117.2 ± 7.1	NS
Diastolic BP (mmHg)	72.7 ± 10.3	74.2 ± 6.4	NS
Total cholesterol (mg/dL)	169.1 ± 31.4	166.3 ± 26.6	NS
Triglyceride (mg/dL)	169.4 ± 29.0	156.7 ± 31.3	NS
LDL-cholesterol (mg/dL)	148 ± 27.7	134.1 ± 25.6	NS
HDL-cholesterol (mg/dL)	44.8 ± 9.8	46.3 ± 8.3	NS
Plasma total homocystein (μmol/L)	5.6 ± 2.9	5.7 ± 2.2	NS
Serum folate (ng/mL)	11.4 ± 3.3	9 ± 4.1	0.02
Serum B_{12} (pg/mL)	282.9 ± 119.0	228.5 ± 50.9	0.03
Creatinine (mg/dL)	0.5 ± 0.2	0.6 ± 0.1	NS
Diabetes duration (years)	2.6 ± 2.2	-	
HbA1c (%)	6.9 ± 2.3	-	
Microalbumin (mg/24h)	22.7 ± 27.2	-	
Daily insulin dose (U/kg/24h)	0.82 ± 0.3	-	

Data were expressed as mean ± SD. NS: not significant.

5.7 ± 5.2.2 μmol/L, respectively, P > 0.05). The diabetic patients had significantly higher serum folate (11.4 ± 3.3 ng/mL vs 9.0 ± 4.1 ng/mL; P = 0.02) and vitamin B_{12} than the healthy controls (282.9 ± 119 pg/mL vs 228.5 ± 50.9 pg/mL; P = 0.03).

Fasting tHcy concentration correlated with age (r = 0.44, P= 0.02), weight (r = 0.56, P = 0.002), body mass index (r = 0.57, P= 0.002), folate (r = –0.48, P = 0.01), and creatinine (r = 0.41, P = 0.03) in diabetic patients. In stepwise multivariate regression model for diabetics, the independent correlates for tHcy was folate (β = –0.48, P = 0.002), with the total variance explained only being 23%. No significant correlations were determined between tHcy and other parameters in controls (*Table II*).

Discussion

In the present report, we found that children and adolescent patients with diabetes, who have normal weight, normal blood pressure, excellent diabetes control, and normal lipids, when studied soon after onset of disease (mean duration 2.6 years), have normal levels of plasma homocysteine. There is little and somewhat conflicting information regarding the impact of the diabetic state per se on tHcy levels, particularly in children and adolescents with type 1 diabetes. One study included some children with diabetes but

TABLE II–*Pearson Correlation Coefficients and Statistical Significance between Plasma Total Homocysteine Level and other Variables*

	Diabetes group		Control group	
	r	P	r	P
Age (years)	0.44	0.02	0.19	NS
Height (cm)	0.32	NS	0.14	NS
Weight (kg)	0.56	0.002	0.22	NS
Body mass index kg/boy(m^2)	0.57	0.002	-0.20	NS
Systolic BP (mmHg)	0.17	NS	0.15	NS
Diastolic BP (mmHg)	0.09	NS	0.34	NS
Total cholesterol (mg/dL)	0.11	NS	0.09	NS
Triglyceride (mg/dL)	0.25	NS	-0.34	NS
LDL-cholesterol (mg/dL)	–0.19	NS	–0.28	NS
HDL-cholesterol (mg/dL)	–0.15	NS	0.24	NS
Serum folate (ng/mL)	0.13	NS	–0.33	NS
Serum B12 (pg/mL)	-0.005	NS	–0.04	NS
Creatinine (mg/dL)	0.41	0.03	–0.09	NS
Diabetes duration (years)	–0.11	NS	–	–
HbA1c (%)	0.08	NS	–	–
Microalbumin (mg/24h)	–0.12	NS	–	–
Daily insulin dose (U/24h)	–0.21	NS	–	–

NS: not significant.

measured non-fasting tHcy concentrations and did not examine determinants (vitamin levels) of tHcy(12). Another recent study examined adolescents with type 1 diabetes and found no difference from a control group(13). The other study reported that tHcy values are lower in children and adolescents with type 1 diabetes(14). In the most studies, no information was available on nutritional status and parameters, especially serum folate and vitamin B_{12} levels, which factor are known to influence plasma tHcy levels(15-17).

Homocysteine levels in the blood are inversely related to serum levels of folate, vitamin B_{12} and pyriodoxal-5-phosphate, as well as to intake of these vitamins(18-19). The most consistent associations are with lower folate intake and lower levels of serum folate, with homocysteine levels starting to rise below plasma folate levels of 20-25 nmol/L(20). The correlation of tHcy values with folate levels found in our patients and in most other studies(5,13) confirms the relationship between the two parameters. We found a direct inverse relation between the blood levels of folate and tHcy concentrations. Reduced intake of nutrients and vitamins and lower levels of serum folate can elevate the homocysteine levels in early stage in diabetic children and adolescents. However, serum levels of folic acid and vitamin B_{12} in diabetic patients were significantly higher than in controls. Although vitamin levels were within the normal range in patients, perhaps, because of the greater attention paid to their food and the likelihood of having less junk, appear to have better vitamin levels than controls. These findings imply that normal mean plasma tHcy level in our diabetic patients can be result of high serum folate concentrations.

Although tHcy values seem still to be normal in diabetic adolescents, they may become higher in adults in association with renal impairment and low blood folate levels, especially in people carrying the C677T mutation in MTHFR gene(21-22). Two common polymorphisms, 667 C-T and 1298 A-C, occur in the gene coding for MTHFR. Homozygosity for the 677T allele and compound hetwrozygosity for the 677T and 1298C alleles are associated with reduced enzyme function and elevated tHcy, particularly with lower folate levels(23). Folic acid and vitamin B_{12} are required for remethylation of hococysteine, and even subclinical deficiency of these vitamins can increase plasma hococysteine levels. Folates are widely distributed in leafy green vegetables, fruits (particularly orange juice) and cereals (especially fortified)(24). Children with diabetes may require additional folate intake to avoid the risk for cardiovascular disease.

Homocysteine metabolism is especially important in renal parenchyma(25) and is altered in early stages of impaired renal function, depending on individual genetic and nutritional factors(26). In our patients, no alteration in renal function was observed, so this possibility seems an unlikely explanation. None of our patients had microalbuminuria. No association was found between tHcy levels and anthropometric risk factors for cardiovascular disease except for body mass index. In addition, creatinine values showed an association with tHcy concentrations. This finding might reflect the increase of creatinine synthesis during puberty, secondary to the development of muscle mass(27). Our results found in this study should be interpreted with caution because of its method limitations (cross-sectional design, heterogeneity of study population, and small number of studied patients). Longitudinal studies will be needed to determine whether it becomes more important at older ages.

Key Messages

- Hyperhomocysteinemia is a risk factor for premature cardiovascular disease.
- A direct inverse relation between the blood levels of folate and tHcy concentrations.
- Reduced intake of nutrients and vitamins and lower levels of serum folate can elevate the homocysteine levels in early stages in diabetic children and adolescents.

In conclusion, the plasma tHcy concentrations in our diabetic patients were within normal limits as in our population of normal control children with the same age range and sex ratio.

Contributors: MEA conceptualisation of study, critical appraisal of protocol, final manuscript; OP preparation of the protocol, clinical data collection, draft of the manuscript; EK helped in analysis and final draft of the manuscript.

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