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Association of plasma homocysteine and macular edema in type 2 diabetes mellitus

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PURPOSE. *The aim of this study was to assess the association of macular edema (ME) with plasma homocysteine, vitamin B6, vitamin B12, and folic acid levels in patients with Type 2 diabetes.*
METHODS. *Sixty-five diabetic subjects with no retinopathy and nonproliferative diabetic retinopathy (NPDR) (no DR, without ME, with ME: 16, 25, 24, respectively), 28 with proliferative diabetic retinopathy (PDR) (with and without ME: 14, 14, respectively), and 19 healthy subjects as control were recruited in this cross-sectional study. Plasma homocysteine, vitamin B12, vitamin B6, and folate levels were determined after 8-hour of fasting for all subjects. The levels of serum homocysteine and vitamin B6 were measured using high performance liquid chromatography (HPLC) with fluorescence detection, and the levels of serum vitamin B12 and folic acid were measured by electrochemiluminescence immunoassay.*
RESULTS. *When diabetic groups with ME were compared with diabetic groups without ME for homocysteine, vitamin B12, vitamin B6, and folic acid, the only significant difference was detected in homocysteine levels (p=0.001). There was no significant difference between NPDR with ME group compared with NPDR without ME group and no DR group for plasma homocysteine, vitamin B12, vitamin B6, and folic acid (p=0.200, p=0.660; p=0.999, p=0.678; p=1.0, p=0.248; p=1.0, p=0.982, respectively). On the other hand, when PDR with ME group was compared with PDR without ME group, there was only significant difference in homocysteine levels (p=0.023).*
CONCLUSIONS. *Mild to moderate elevation of homocysteine may explain the role of vascular dysregulation and endothelial dysfunction in patients with DR. The present study suggests hyperhomocysteinemia may be one of the crucial risk factors for development of ME. (Eur J Ophthalmol 2008; 18: 226-32)*

KEY WORDS. *Homocysteine, Vitamin B6, Vitamin B12, Folic acid, Macular edema, Type 2 diabetes mellitus*

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INTRODUCTION

Diabetic retinopathy (DR) is a major vascular complication of diabetes mellitus often leading to blindness. Poor glycemia control, vascular endothelial cell injury, hypercoagulability, ischemia and anoxia of retina, and genetic factors may contribute to formation of diabetic retinopathy (1). Macular edema (ME) is the crucial cause of visual impairment and may occur at any stage of DR (2-4). It occurs because of the breakdown of the tight blood–retinal barrier in the retinal capillaries and increased fluid accumulation in the outer layers of the retina. Recently, it has been shown that fasting plasma total homocysteine and postmethionine load plasma homocysteine concentrations are increased in the presence of micro and macrovascular complications in patients with Type 1 or Type 2 diabetes mellitus (DM) (5, 6).
Homocysteine is a by-product formed in the biologic

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transmethylation reactions and detoxified with the methionine synthetase, which is the enzyme depending on vitamin B12, B6, and folate as coenzymes for proper functioning S-adenosylmethionine and methylation (7). High plasma homocysteine level has been also reported as an independent risk factor for atherosclerosis, cardiovascular disease, and venous thrombosis (8-10). Homocysteine-stimulated vascular problems may be multifactorial, including direct toxic damage to the endothelium, stimulation of smooth muscle cells proliferation, enhanced low density lipoprotein peroxidation, increased platelet aggregation, and activation of coagulation system (11).

Because of this, in this study we aimed to explore whether there is an association between levels of homocysteine, vitamin B6, vitamin B12, and folic acid in patients with diabetes with or without ME and patients with Type 2 diabetes.

MATERIALS AND METHODS

Subjects

In this cross-sectional clinic study, we recruited 93 patients with Type 2 diabetes mellitus and 19 healthy controls admitted to the Ophthalmology and Endocrinology Clinics. Diabetes mellitus was diagnosed and classified according to 1985 WHO criteria. The study was designed and performed under the ethical standards of the Declaration of Helsinki. Informed consent was obtained from each patient prior to the study. Diabetic patients were divided into five groups and classified according to Early Treatment Diabetic Retinopathy Study (ETDRS) (12) and International Clinical Diabetic Retinopathy and Diabetic ME Severity Scale. Following standard protocol, fundus photography was performed for all patients and they were assigned to one of the following groups: Group 1: diabetic patients with no retinopathy (n=16, 6 males and 10 females with a mean age of 54.8±7.8, duration of diabetes [2–20] 8.1±5.8 years), Group 2: nonproliferative diabetic retinopathy (NPDR) without ME group (n=25, 12 males and 13 females with a mean age of 55±8.8 years, duration of diabetes [2–18] 8.5±5.1 years); Group 3: NPDR with ME group (n=24, 12 males and 12 females with a mean age of 59.7±10.9 years, duration of diabetes [6–25] 13.5±4.3 years), Group 4: PDR without ME group (n=14, 6 males and 8 females with a mean age of 61.0±9.4 years, duration [5–25] 13.6±7.3 years), Group 5: PDR with ME group (n=14, 8 males and 6 females with a mean age of 61.0±11.4 years, duration [7–30] 16.5±7.5 years), Group 6: control group (n=19, 8 males and 11 females with a mean age of 55.7±4.2 years).

All patients underwent a complete ophthalmic examination, consisting of best-corrected visual acuity, slit-lamp biomicroscopy, dilated funduscopy, fundus photography, and fluorescein angiography if necessary.

Data regarding age, gender, detailed medical history of hyperhomocysteinemia, systemic hypertension, peripheral or coronary artery disease, cerebrovascular events, medication, and smoking habits were recorded. Patients with a history of coronary heart disease, cerebrovascular disease, neoplastic disease, renal and/or liver failure, vitamin supplementation, medications known to affect homocysteine levels, retinal vascular disease, and anterior ischemic optic neuropathy were excluded from the study to prevent false-positive increases in serum homocysteine levels due to these confabulating factors (13).

Measurement of plasma total homocysteine, vitamin B12, vitamin B6, and folic acid levels

Eight-hour fasting venous blood samples for serum homocysteine, vitamin B12, vitamin B6, and folic acid were collected in test tubes containing ethylenediamine tetraacetic acid. After the samples were obtained, they were centrifuged within 1 hour and stored at –40 °C. The levels of serum vitamin B6 and homocysteine were measured by high performance liquid chromatography (HPLC) with fluorescence detection (Chromsystem GmbH, Munchen, Germany) and vitamin B12 and folic acid were assayed using electrochemiluminescence immunoassay on the Roche Modular Analytics E 170 immunoassay analyzers (Cobas, Roche, Mannheim, Sweden). According to the manufacturer's guidelines, the normal values of the serum homocysteine are 5.5-17 µmol/L, vitamin B12 are 140-700 pg/mL, vitamin B6 are 3.6-18 µg/L, and serum folic acids are 1.8-9 ng/mL.

Statistical analysis

In this study, variables were determined to have normal distribution by Komogorov Simirnov test and so the comparisons of groups were done by one-way analysis of variance.

Differences of values in continuous variables were assessed by using Scheffé and Tamhane T^2 multiple comparison tests. The patients with diabetes with ME group and patients with diabetes without ME groups were compared by independent sample *t*-test. Continuous variables were expressed as mean and standard deviation. For categorical variable (gender), chi-square tests were used to find out differences between groups. A p value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS version 13, SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical data of diabetic patients and controls are summarized in Table I. No statistically significant differences among the groups with regard to sex and age distribution were noted. There was significant difference between the DM duration and HbA1c levels of no DR, NPDR, and PDR groups. Plasma homocysteine levels in control (8.2±2.5), no DR (13.3±3.3), NPDR without ME (13.6±3.3), NPDR with ME (15.9±4.4), PDR without ME (22.0±4.0), and PDR with ME (26.4±3.9) groups increased with a remarkable linear trend from the stage of no DR to

TABLE I - DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH DIABETES AND CONTROLS

	Control (n=19)	No DR (n=16)	NPDR (n=25)	NPDR+ME (n=24)	PDR (n=14)	PDR+ME (n=14)	F	p value*
Age, yr	55.7±4.2	54.8±7.8	55±8.8	59.7±10.9	61.0±9.4	61.0±11.4	1.869	0.106
Male/Female	8/11	6/10	12/13	12/12	6/8	8/6		0.911†
Duration, yr		8.1±5.8	8.5±5.2	13.5±4.3	13.6±7.3	16.5±7.5	6.694	<0.001
HbA1c		7.7±1.5	8.2±2.1	9.3±1.5	7.3±1.4	9.1±1.8	4.238	0.003
Hcy (µmol/L)	8.2±2.5	13.3±3.3	13.6±3.3	15.9±4.4	22.0±4.0	26.4±3.9	49.114	<0.001
Vitamin B12 (pg/mL)	317.3±99.8	314.5±116.1	456.4±176.5	399.3±169.3	345.1±124.9	429.3±116.0	3.288	0.009
Vitamin B6 (µg/mL)	8.2±2.0	11.1±3.1	10.8±3.4	11.9±6.1	9.0±4.9	8.2±3.9	2.644	0.027
Folic acid (ng/mL)	7.5±1.1	8.4±1.7	8.2±1.4	8.7±1.0	7.7±1.3	7.5±1.1	2.517	0.034

*One-way analysis of variance.
†Pearson chi-square test.
DR = Diabetic retinopathy; NPDR = Nonproliferative DR; ME = Macular edema; PDR = Proliferative DR

TABLE II - DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH DIABETES WITH AND WITHOUT MACULAR EDEMA (ME)

	Patients with diabetes with ME (n=38)	Patients with diabetes without ME (n=55)	p value*
Age, yr	60.2±11.0	56.5±8.9	0.81
Male/Female	20 (52.6%)/18 (47.4%)	24 (43.6%)/31 (56.4%)	0.393†
Duration, yr	14.6±5.8	9.7±6.2	<0.0001
HbA1c	9.2±1.6	7.8±1.8	<0.0001
Hcy (µmol/L)	19.8±6.6	16.6±5.4	0.001*
Vitamin B12 (pg/mL)	410.3±150.8	386.7±160.0	0.476
Vitamin B6 (µg/mL)	10.5±5.6	10.5±5.6	0.969
Folic acid (ng/mL)	8.2±1.2	8.3±1.5	0.736

*Two independent sample t-test.
†Pearson chi-square test

the stage of PDR (µmol/L, respectively).

When diabetic groups with ME together were evaluated to compare with diabetic groups without ME for homocysteine, vitamin B12, vitamin B6, and folic acid, the only significant difference was detected in homocysteine levels (p=0.001) (Tab. II).

The comparisons of plasma homocysteine, vitamin B12, vitamin B6, and folic acid between diabetic and control groups are summarized in Table III. When we assessed diabetic subgroups, there was no significant difference between NPDR with ME group compared with NPDR without ME group and no DR group for plasma homocysteine, vitamin B12, vitamin B6, and folic acid. On the other hand, when PDR with ME group was compared with PDR without ME group, there was only significant difference in homocysteine levels (Tab. III, Fig. 1).

We also found a significant difference when plasma homocysteine levels in diabetic groups were compared with control group (Tab. III) (p=0.002, p<0.001, p<0.001, p<0.001, p<0.001).

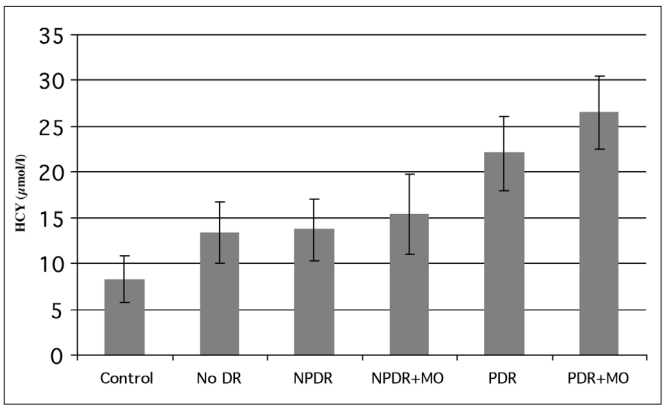

Fig. 1 - *Mean levels of homocysteine in diabetic and control groups.*

DISCUSSION

Homocysteine is involved in a system of vascular injury and repair although whether hyperhomocysteinemia may contribute to the development of diabetic microangiopathy is debated. More recent studies have been conducted on showing a relation among hyperhomocysteinemia, diabetic nephropathy, and retinopathy; however, it remains obscure whether this association is causal (14-18). The relationship between plasma homocysteine and DM has been researched in various studies. To our knowledge, this is the first report that explores the relationship of plasma homocysteine and the severity of DR and ME in patients with Type 2 diabetes.

The pathogenesis of micro and macrovascular complications of DM is complex and probably involves the interaction of multiple factors including hyperglycemia (19, 20),

TABLE III - COMPARISON OF HCY, VITAMIN B12, VITAMIN B6, AND FOLIC ACID LEVELS IN DIABETIC SUBGROUPS AND CONTROL GROUP

Comparisons	**p values of Turkey**					
	Hcy	**Vitamin B12**	**Vitamin B6**	**Folic acid**	**DM duration**	**Hba1c**
I–II	1.0	0.031*	1.0	0999	0.999	0.884
I–III	1.0	0.248	1.0	0.982	0.04	0.04
II–III	0.200	0.660	0.999	0.678	0.03	0.19
III–V	0.0001	0.989	0.356	0.088	0.54	0.997
IV–V	0.023*	0.633	1.0	0.996	0.677	0.064
I–VI	0.002*	1.0	0.063	0.454		
II–VI	<0.001*	0.029*	0.028*	0.425		
III–VI	<0.001*	0.409	0.098	0.037*		
IV–VI	<0.001*	0.985	1.000	0.993		
V–VI	<0.001*	0.229	1.000	1.000		

*Statistically significant.

DM = Diabetes mellitus; I = No diabetic retinopathy (DR) group; II = Nonproliferative DR (NPDR) without macular edema (ME) group; III = NPDR with ME group; IV = Proliferative DR (PDR) without ME group; V = PDR with ME group; VI = Control group

oxidative stress (21, 22), advanced glycation end products (AGEs) (23, 24), and hyperhomocysteinemia (6). In vitro studies showed that homocysteine induces thrombomodulin release in endothelial cells prestimulated with AGE-albumin, confirming synergism between divergent factors (AGE and homocysteine) mediating endothelial cell damage (17).

An increase in homocysteine plasma level is considered an independent risk factor for the development of vascular damage (25). There are numerous studies that point to homocysteine, a mediator of oxidative stress in endothelial cells, as inducing intimal damage and activating a serine elastase in arterial smooth muscle cells. Then activation of matrix metalloproteinase 2 causes elastolysis of elastin and fibrillar collagen in arterial media (26, 27). Oxidative stress may explain the effect of homocysteine in systemic vascular diseases.

Endothelial-cell–derived nitric oxide (eNOS) relaxes vascular smooth muscle cells, causes vasodilatation, and inhibits platelet aggregation (28). Homocysteine demonstrates an indirect inhibitory effect on receptor-mediated, nonreceptor-mediated, and L-arginine stimulated nitric oxide (NO) release by endothelial cells; however, it does not suppress the enzyme directly (29). Awata et al have also reported that eNOS gene polymorphisms may contribute to the development of ME by impairing basal eNOS expression and resulting in the breakdown of the blood–retina barrier (14).

Looker et al reported that vitamin B12 and folate were negatively correlated with homocysteine concentrations in patients with Type 2 diabetes (30). However, in our study, we did not detect any significant correlation among homocysteine and vitamin B12, vitamin B6, or folic acid.

The results of the current study demonstrated that the plasma homocysteine concentration in PDR with or without ME groups was significantly higher than NPDR with or without ME groups. When diabetic groups with ME together were compared with diabetic groups without ME, a statistically significant rise of homocysteine levels was detected in diabetic groups with ME. These findings reflected the association between hyperhomocysteinemia and ME.

Goldstein et al (31) reported that no significant difference was found between diabetic (NPDR, PDR) and the control groups for homocysteine levels. However, in their study, subjects with Type 1 and Type 2 diabetes were evaluated together and vitamin B12, B6, and folic acid levels were not included.

Even though Albdella et al (32) determined that there was no association between plasma homocysteine levels and diabetic retinopathy severity in patients with Type 2 diabetes, which was similar to the study of Agardh et al (33), in our study, we detected significantly increased homocysteine levels in PDR groups rather than NPDR groups, but no significant difference among no DR group, NPDR without ME group, and NPDR with ME group. This result might depend on the similarity of disease severity grading and longer real disease duration than informed between NPDR with ME and NPDR without ME groups. In the NPDR without ME group, 6 of 25 patients and in no DR group 5 of 16 patients reported the duration of their disease less than 5 years in their anamnesis, but the real duration of diabetes in those patients might be longer than informed. The situation presumably affected the real disease duration of NPDR without ME and no DR groups.

Huang et al found no significant difference between plasma homocysteine levels of PDR and NPDR groups since both of these groups had patients with similar mean duration of diabetes (34). This finding was contrary to our study; we found significant difference between PDR with ME and NPDR with ME groups.

In the present study, we investigated an association between hyperhomocysteinemia levels in patients with diabetes with ME as compared to patients with diabetes without ME. It is well known that many factors contribute to the development and severity of diabetic retinopathy and some of them are unknown. It seems as if high homocysteine levels are among the contributing factors in formation of ME. Therefore, homocysteine levels should be evaluated in all patients with diabetes.

Further controlled prospective studies with a higher number of participants (with NPDR and PDR) should be constituted to evaluate the possible role of homocysteine and NO in microvascular angiopathy and maculopathy of patients with Type 2 diabetes and the effect of vitamin B12, B6, and folic acid supplementation on disease progression.

None of the authors has any proprietary interest in any technique or product described.

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