CLINICAL STUDY

Hyperhomo-cysteinemia in patients with diabetes mellitus with and without diabetic retinopathy

M Goldstein[1], I Leibovitch[1], I Yeffimov[1], S Gavendo[2], B-A Sela[2] and A Loewenstein[1]

Abstract

Objective To evaluate the prevalence of hyperhomocysteinaemia in diabetic patients with no diabetic retinopathy (no DR), with non-proliferative diabetic retinopathy (NPDR) and with proliferative diabetic retinopathy (PDR).

Research design and methods This prospective, case-control study, included 179 diabetic patients and 156 age-matched controls with no diabetes and no history of ocular disease, who were undergoing routine physical checkups. Plasma homocysteine levels of all study participants were measured using high-performance liquid chromatography (HPLC). Hyperhomocysteinaemia was defined when homocysteine levels were higher than 15 μmol/l.

Results The mean plasma homocysteine level was 11.75 $\pm$ 0.24 in the control group, 13.46 $\pm$ 0.74 in the no DR group, 14.56 $\pm$ 0.64 in the NPDR group and 15.86 $\pm$ 1.34 in the PDR group. Mean homocysteine levels were significantly elevated in the NPDR and PDR groups compared to the control group ($P = 0.001$ and < 0.0001, respectively). The prevalence of hyperhomocysteinaemia was also higher in the NPDR and PDR groups compared to the control group ($P = 0.032$ and 0.011, respectively). No statistically significant difference was found between the no DR and the control group.

Conclusions Our findings suggest that hyperhomocysteinaemia may be associated with diabetic retinopathy and partially explain the increased risk of microvascular angiopathy occurring in these patients.

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[1]Department of Ophthalmology
Tel-Aviv Medical Center
Sackler Faculty of Medicine
Tel-Aviv University
Tel-Aviv, Israel

[2]Institute of Chemical Pathology
Sheba Medical Center
Sackler Faculty of Medicine
Tel-Aviv University
Tel-Aviv, Israel

Correspondence:
M Goldstein
Department of Ophthalmology
Tel-Aviv Medical Center
6 Weitzman Street
Tel-Aviv 64239, Israel
Tel: +972-3-6973408
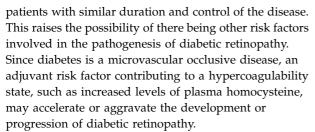Fax: +972-3-6973870
E-mail: michgold@netvision.net.il

Introduction

Hyperhomocysteinaemia is known to be a risk factor for vascular occlusive diseases.[1–3] Elevated levels of plasma homocysteine have been found in patients suffering from peripheral vascular occlusions, such as coronary artery disease,[4–7] cerebral vascular accidents,[8] and deep-vein thrombosis,[9] as well as from ocular vascular occlusions, such as retinal vein and retinal artery and anterior ischaemic optic neuropathy.[10–17]

High levels of plasma homocysteine are toxic to the vascular endothelium via the formation of free radicals. These free radicals cause direct injury to the endothelium by disrupting its integrity and exposing the underlying vascular matrix and smooth muscle, thus promoting a hypercoagulability state by the activation of platelets and thrombus formation.[18–22]

Increased plasma levels of homocysteine are found in any enzyme deficiency in the remethylation[23] or transsulphuration[24] process of methionine, and in a thermolabile variant of the enzyme MTHFR.[25,26] It was found to be associated with age (probably due to decreased intake of folic acid and vitamin B12[27,28]), male gender,[27] renal failure,[29–31] and medications.[32–36]

Diabetes is a microangiopathic atherosclerotic disease that affects the capillary bed in many body organs, mainly the kidneys, retina, and peripheral nervous system. Chronic hyperglycaemia is known to be the major determinant of diabetic retinopathy,[37–42] perhaps modified by genetic or acquired features that vary from one individual to another. Numerous factors were described as having an effect on the development and progression of diabetic retinopathy, such as puberty,[43,44] hypertension,[45] and pregnancy.[46] There is no explanation for the variation in the severity of diabetic retinopathy among diabetic

patients with similar duration and control of the disease. This raises the possibility of there being other risk factors involved in the pathogenesis of diabetic retinopathy. Since diabetes is a microvascular occlusive disease, an adjuvant risk factor contributing to a hypercoagulability state, such as increased levels of plasma homocysteine, may accelerate or aggravate the development or progression of diabetic retinopathy.

The aim of our study was to evaluate the mean levels of homocysteine and to estimate the prevalence of hyperhomocysteinaemia among three subgroups of diabetic patients: those with no diabetic retinopathy (no DR), those with nonproliferative diabetic retinopathy (NPDR) and those with proliferative diabetic retinopathy (PDR), compared to a control group.

Research design and methods

All diabetic patients seen at the retina unit of the Tel-Aviv Medical Center from September 2000 through December 2001 who agreed to participate in the study and signed an informed consent were included. The study was approved by the Institutional Review Board/Ethics Committee.

A detailed medical history was obtained from each patient, including the duration of diabetes, the presence of renal disease, hypertension, cardiovascular disease, cerebrovascular disease, previous thromboembolic events, and current medications. Creatinine levels were assessed in all candidates in an attempt to identify those with renal failure. Patients with renal dysfunction known to be associated with high homocysteine levels[29–31] were excluded from the study. Those treated with drugs like methotrexate,[32,33] fibrates,[34–35] or vitamin supplements[28] (including ascorbic acid) in the previous 6 months, or patients consuming moderate amounts of alcohol were also excluded.[47] All patients who participated in the study were treated with oral hypoglycaemic drugs, subcutaneous insulin injections or a combination of both. None of these conventionally used hypoglycaemic drugs are known to influence plasma levels of homocysteine.

The control group consisted of subjects of similar gender and age with no ocular disease who were undergoing routine physical examinations.

All subjects in the study and control group underwent a complete eye examination, including stereoscopic colour fundus photographs to all diabetic patients. All diabetic patients included in the study were subdivided into three categories (no DR, NPDR, and PDR) based on their funduscopic findings. The degree of diabetic retinopathy was determined in all patients by a clinical examination of one retina specialist (MG). The stereoscopic colour fundus photographs were assessed independently by a second retina specialist (AL) to confirm the clinical classification. Both assessments were based on the grading diabetic retinopathy system as used in the ETDRS.[48]

The intra-individual coefficient of variation of plasma homocysteine was recently shown to be relatively low;[49] therefore, one single plasma sample for evaluating homocysteine level was sufficient.

A 2-ml fasting blood sample was obtained from each participant. The blood was centrifuged within 30 min after collection at 3000 g for 6 min. The plasma was removed and analysed for homocysteine by the HPLC method with fluorescent detection.[50] Hyperhomocysteinaemia was defined when homocysteine levels were higher than 15 μmol/l.

Statistical analysis

The Fisher exact test was used for comparing differences in proportions among the three groups. The two-sample t-test was used to examine differences in mean values between the groups. The Gabriel test was used for multiple comparisons. A P value of ≤ 0.05 was considered as being statistically significant.

Results

In total, 179 patients (92 males, 87 females) diagnosed with diabetes mellitus type II were included in the study. Patients were divided into three groups according to their ophthalmological findings as described previously. There were 62 patients with no DR (mean age 72.06 ± 1.24 years), 71 patients with NPDR (mean age 68.1 ± 1.04 years) and 46 patients with PDR (mean age 69.2 ± 1.36 years). The control group consisted of 156 patients (80 males, 76 females, mean age 69 ± 0.4 years), with no diabetes and a normal funduscopic examination, who underwent routine ophthalmological examinations in our clinic.

There was no significant difference in age among the three groups of diabetic patients and the control group. The mean diabetes duration of 17.62 ± 9.29 years for the PDR patients was significantly longer than that for the NPDR and the no DR patients (14.58 ± 8.04 and 11.96 ± 8.13, respectively; $P = 0.001$). The mean plasma glucose level (normal value < 110 mg/dl) of 146.32 ± 43.48 for the no DR patients was significantly lower than for the NPDR and the PDR groups (181.04 ± 58.03 and 189.61 ± 70.85, respectively; $P < 0.0001$). No significant differences were found in the prevalence of hypertension, atherosclerotic cardiovascular disease, cerebrovascular accident, or thromboembolic events among the three groups and compared to the control group (Table 1). Table 2 summarizes the mean plasma homocysteine levels and the prevalence of

Table 1 Prevalence of systemic diseases in diabetic patients and controls

	No DR (n = 62)		NPDR (n = 71)		PDR (n = 46)		Control (n = 156)	
	n'	%	n'	%	n'	%	n'	%
HTN	15	24.2	14	19.7	10	21.7	34	21.8
CVA	3	4.8	4	5.6	3	6.5	7	4.5
IHD	10	16.1	12	16.9	8	17.4	20	12.8
Thrombo	1	1.61	1	1.4	0	0	0	0

HTN = hypertension, CVA = cerebrovascular accident, IHD = ischaemic heart disease, Thrombo = thromboembolic events, no DR = no diabetic retinopathy, NPDR = nonproliferative diabetic retinopathy, PDR = proliferative diabetic retinopathy.

Table 2 Plasma homocysteine levels and hyperhomocysteinaemia prevalence

	Subjects (n)	Homocysteine level ($\mu mol/l \pm SE$)	Hyperhomocysteinaemia ($>15 \mu mol/l$)
No DR	62	13.46 ± 0.74 ($P = 0.152$)	22.42% ($P = 0.40$)
NPDR	71	14.56 ± 0.64 ($P = 0.001$)	36.6% ($P = 0.032$)
PDR	46	15.86 ± 1.34 ($P < 0.0001$)	39.1% ($P = 0.011$)
Control	156	11.75 ± 0.24	16.82%

No DR = no diabetic retinopathy, NPDR = nonproliferative diabetic retinopathy, PDR = proliferative diabetic retinopathy.

hyperhomocysteinaemia in each group. Mean homocysteine levels were significantly elevated in the NPDR and PDR groups compared to the control group ($P = 0.001$ and <0.0001, respectively), but no significant difference was found between the no DR group and the control group ($P = 0.152$). The prevalence of hyperhomocysteinaemia was also higher in the NPDR and PDR groups compared to the control group ($P = 0.032$ and 0.011, respectively), but no significant difference was found between the no DR group and the control group ($P = 0.40$).

Conclusions

The results of our study revealed a significantly higher prevalence of hyperhomocysteinaemia and higher levels of mean plasma homocysteine in diabetic patients with PDR and NPDR compared to a matched control group.

Numerous studies dealing with a possible correlation between hyperhomocysteinaemia and vascular complications in subjects with diabetes mellitus have appeared over the past few years. In analysing the Hoorn study, Hoogeveen *et al*[51] concluded that hyperhomocysteinaemia is a stronger risk factor for overall mortality in diabetic patients than among nondiabetic subjects. Ambrosch *et al*[52] examined 65 patients with type II diabetes; 43 were found to have diabetic neuropathy and this subgroup had elevated levels of homocysteine and a higher prevalence of hyperhomocysteinaemia. Vaccaro *et al*[53] studied 66

patients with type I diabetes and found similar plasma homocysteine levels in patients and healthy controls. In a subgroup of patients with proliferative retinopathy; however, homocysteine was significantly higher when compared to patients without retinopathy due to the genetic homozygote C677T mutation which was at least twice as frequent in the former. In another report of the Hoorn study, Hoogeveen *et al*[54] looked for an association between homocysteine level and retinopathy among subjects with and without diabetes. They found a 12% prevalence of retinopathy in subjects with normal serum homocysteine level ($<16 \mu mol/l$) compared to 16.5% in individuals with higher serum levels of homocysteine ($>16 \mu mol/l$). The odds ratio between retinopathy and hyperhomocysteinaemia was 0.97 in patients without diabetes and 3.44 in patients with diabetes.

It is known that proliferative diabetic retinopathy is augmented by retinal hypoxia.[55–57] Homocysteine is toxic to the vascular endothelium and therefore induces thrombosis, and thus may play a role in aggravating the hypoxic state such as that seen in diabetic retinopathy by further closure of the capillary bed. Many of the above-quoted studies have shown that higher levels of plasma homocysteine were found in patients suffering from known microvascular complications of more severe or advanced stages of diabetes, among them diabetic neuropathy, nephropathy, and retinopathy. In addition, it had been suggested that hyperhomocysteinaemia in these advanced cases might be secondary to diabetic

renal dysfunction.[29] All participants included in our study had normal renal functions.

It is our contention that higher plasma levels of homocysteine in diabetic patients may play a role in accelerating the microvascular retinal changes and may, therefore, contribute to the severity of diabetic retinopathy. The prevalence of hyperhomocysteinaemia and mean plasma homocysteine levels in our diabetic retinopathy subgroups of patients were higher than in the control group, but there was no significant difference between the no DR subgroup and the control group. These findings may in part explain the possible progression of diabetic retinopathy in diabetic patients with higher homocysteine levels compared to diabetic patients with lower homocysteine levels who have no DR, although the shorter diabetes duration and lower plasma glucose levels in the no DR group compared to the NPDR and PDR groups are probably the most important contributing factors. Therefore, a longer follow-up period is needed to evaluate the long-term effects of homocysteine levels on the progression of diabetic retinopathy.

Since there are numerous factors influencing the development and progression of diabetic retinopathy, some of them yet unknown, we believe that hyperhomocysteinaemia is another contributing factor to microvascular angiopathy via thrombus formation in the capillaries and further impairment in blood supply to the affected tissue. We believe that plasma homocysteine should be assessed in all diabetic patients and that any existing hyperhomocysteinaemia should be treated with the aim of reducing the toxic effect of homocysteine and preventing further capillary closure and hypoxia. Treatment of hyperhomocysteinaemia is easy, safe, and well tolerated. Folic acid supplement in a dose of 250 μg/day in addition to the usual dietary intake was shown to be effective in reducing plasma homocysteine in young women,[58] and 400 μg/day together with vitamin B6 and B12 was effective in the elderly population.[59]

Our lab is currently running a series of studies on the natural progression of diabetic retinopathy in diabetic patients with hyperhomocysteinaemia compared to patients with normal homocysteine levels. Validation of the possible role of homocysteine in the pathogenesis and progression of a microangiopathic vascular disease such as diabetic retinopathy, and the determination of a possible supplementary treatment policy warrant further investigation.

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