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High Serum Homocysteine Levels Correlate with a Decrease in the Blood Flow Velocity of the Ophthalmic Artery in Highway Toll Collectors

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Memişoğulları, R., Yüksel, H., Coskun, A., Yüksel, H.K., Yazgan, O. and Bilgin, C. *High Serum Homocysteine Levels Correlate with a Decrease in the Blood Flow Velocity of the Ophthalmic Artery in Highway Toll Collectors*. Tohoku J. Exp. Med., 2007, **212** (3), 247-252 ── Highway workers, such as policemen, automotive service companies, and toll collectors, are placed at risk of the accelerated atherosclerotic process, since recent studies have suggested that exposure to exhaust particles and ambient air pollution increases carotid intima-media thickness and reduces ocular blood flow velocity. Therefore, we assessed the relationship between serum homocysteine, a potential parameter for atherosclerosis, and the ocular blood flow velocity and the resistivity index in highway toll collectors. The peak systolic and end diastolic flow velocities and the resistivity index were measured in 22 toll collectors and 24 control subjects by color Doppler ultrasonography. The resistivity index, which is an indirect measure of the atherosclerotic process, was calculated: resistivity index = (peak systolic velocity – end diastolic velocity)/peak systolic velocity. Serum homocysteine levels were determined by fluorometric high-performance liquid chromatography. In the highway toll collectors, the serum homocysteine level (14.4 ± 4.8 μmol/l; $p < 0.005$) and the resistivity index of the ophthalmic artery (0.741 ± 0.015; $p < 0.05$) were higher and the ophthalmic blood flow velocity (33.0 ± 3.0 cm/s; $p < 0.001$) was lower than those in the controls (10.6 ± 3.1 μmol/l; 0.728 ± 0.023; 36.8 ± 2.2 cm/s; respectively). There were significant correlations between the serum homocysteine level and ophthalmic artery resistivity index in both highway toll collectors ($p < 0.001$) and controls ($p < 0.005$). Exposure to exhaust particles might increase the serum homocysteine level, which in turn could lead to the decreased ocular blood flow and the increased resistivity index. ──── homocysteine; ocular blood flow; highway toll collectors; color Doppler ultrasonography

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Many studies have suggested that exposure to exhaust particles, smoking, and ambient air pollution causes wall thickening in the arteries and decreases the blood flow velocity, but the mechanisms of these alterations are not well understood (Kunzli et al. 2005; Erdogmus et al. 2006; Park et al. 2006). Studies of the general population have suggested associations between mild elevations of plasma homocysteine (Hcy) levels and coronary, cerebral, and peripheral atherosclerosis such as orbital occlusive disease (Taylor et al. 1991; Wilcken 1998; Southern et al. 1998; Uysal et al. 2005). Increased intima media thickness (IMT) is generally considered an early marker of atherosclerosis (Erdogmus et al. 2006) and causes a decrease in blood flow velocity. Exposure to exhaust particles was also shown to reduce ocular blood flow velocity as measured using color duplex Doppler ultrasonography (Erdogmus et al. 2007). The relationship between the Hcy level and orbital blood flow has not been studied. Therefore, this study investigated the effect of the Hcy level on the orbital blood flow velocity in the ophthalmic artery (OA), using color Doppler ultrasonography (CDS). The correlation between the Hcy level and ocular blood flow indices was also assessed in highway toll collectors (HTCs) who were exposed to diesel exhaust.

Materials and Methods

Patients

Sixty-five toll collectors working in manual tollbooths on the trans-European motorway in the Kaynasli, Golyaka, and Duzce regions of Turkey were evaluated. Smokers were excluded from the study. Twenty-two non-smoking workers (all male; mean age, 38 years) and 24 non-smoking healthy age- and gender-matched control subjects were included in the study. All the subjects were examined thoroughly by an ophthalmology fellow. Intraocular pressure, fundus examination, and visual acuity were all normal. The systolic and diastolic blood pressures and heart rates of all cases were within normal limits. None of the workers or control subjects had diabetes mellitus or hypercholesterolemia. Subjects with a fasting cholesterol and glucose levels greater than 200 and 110 mg/dl, respectively, were excluded from the study. The fasting glucose level was less than 100 mg/dl in all but two workers. These two workers had fasting glucose levels between 100 and 110 but showed normal regulation of glucose metabolism on oral glucose tolerance testing. No subject used any drugs. The subjects were instructed to strictly refrain from drinking beverages containing caffeine for 6 hr before the examination. The body mass index (BMI) was less than 30 in the both study and control groups. Subjects with symptoms of vitamin B_{12} or folate deficiency, elevated creatinine, or serious acute or chronic inflammatory diseases, as well as those taking drugs, including vasoconstrictors, vasodilators, anticoagulants, and vitamins, were also excluded. Color Doppler imaging of the carotid arteries was performed in all HTCs and controls to screen for stenotic vascular disease and revealed normal findings. All subjects gave written informed consent. The Ethics Committee of Duzce Medical Faculty approved the study.

Biochemical assays

Blood samples were collected and centrifuged within 30 min of collection. Samples were stored at –20°C until biochemical analysis, and all assays were conducted in one batch.

The fasting serum total Hcy level was measured as the total amount of free and protein-bound Hcy by using fluorometric high-performance liquid chromatography (Hewlett Packard 1100, Palo Alto, CA, USA) with Chromosystems® calibrators and kits (Chromosystems instruments and Chemicals GmbH, Munich, Germany).

Serum cholesterol, triglyceride, and glucose levels were measured with a chemistry analyzer using specific commercial calibrators and kits (Olympus AU 640 Analyzer, Olympus, Tokyo). The characteristics of the study subjects are given in Table 1.

Ultrasonic examination

Color Doppler sonographic examinations were performed with a Hitachi EUB 6500 system (Tokyo), which has specific software for measuring the peak systolic velocity (OA PSV), end diastolic flow velocity (OA EDV), and resistivity index (OA RI) of the ophthalmic artery. A single experienced radiologist performed the examinations in the left eye in all cases, using a high-resolution 7-14 MHz wideband transducer. The resistivity index (RI) was calculated according to the formula:

RI = (peak systolic velocity – end diastolic velocity)/peak systolic velocity.

The resistivity index is an indirect measure of arterial resistance and is the ratio of the peak systolic and end diastolic velocities of the spectral Doppler vascular flow waveform. The ultrasonographic examinations were repeated by a second blinded radiologist in a randomized sample of 10 subjects to assess inter-observer variability. The intra- and inter-observer variabilities (coefficient of variation) for repeated measures were both < 5%. The subjects lay briefly in the supine position on an ultrasonography examination table in a dark room, and the examinations were performed with the head held in the midline position. The proper technique for obtaining flow velocity measurement has been reported (Erickson et al. 1989; Steigewalt et al. 1995).

Statistical analysis

Data are expressed as the mean ± S.D. Values of $p < 0.05$ were considered statistically significant. Significant differences between the HTC and control groups were analyzed using Student's *t*-test. Pearson's correlation test was used to evaluate the correlation analyses of all parameters.

Results

The results of the biochemical assays of the study participants are summarized in Table 1. As shown in the table, the demographic, fasting glucose, and lipid variables were similar between the two groups.

In the HTC group, the serum Hcy level (14.4 ± 4.8 μmol/l; $p < 0.005$) and OA RI (0.741 ± 0.015; $p < 0.05$) were higher and the OA PSV (33.0 ± 3.0 cm/s; $p < 0.001$) and OA EDV (8.4 ± 0.8 cm/s; $p < 0.001$) were lower than in the controls (10.6 ± 3.1 μmol/l; 0.728 ± 0.023; 36.8 ± 2.2 cm/s; 9.8 ± 1.1 cm/s; respectively).

Positive correlations were obtained between the Hcy level and OA RI ($p < 0.001$) or working years ($p = 0.047$) in the HTC group, and between the Hcy level and OA RI ($p = 0.002$) in the control group (Fig. 1). Negative correlations were present between the Hcy level and OA EDV ($p = 0.003$) in the HTCs, and between the Hcy level and OA PSV ($p = 0.02$) or OA EDV ($p < 0.001$) in the control group (Table 2).

Discussion

In this study, the HTCs had a higher serum Hcy level and OA RI and lower ocular blood velocity than the controls. Correlations were demonstrated between ocular blood flow and the serum Hcy level and between OA RI and the serum Hcy level. Exposure to exhaust particles might increase serum Hcy levels, which in turn could lead to decreased ocular blood flow and an increased resistivity index.

Table 1. Comparison of ocular blood flow and the biochemical variables between toll collectors and control subjects.

	Toll collectors	Controls	*p*
OA RI	0.741 ± 0.015	0.728 ± 0.023	< 0.05*
OA PSV (cm/s)	33.0 ± 3.0	36.8 ± 2.2	< 0.001*
OA EDV (cm/s)	8.4 ± 0.8	9.8 ± 1.1	< 0.001*
Hcy (μmol/l)	14.4 ± 4.8	10.6 ± 3.1	< 0.005*
Glucose (mg/dl)	85.5 ± 9.6	88.4 ± 5.3	NS
Triglyceride (mg/dl)	130.0 ± 48.5	130.7 ± 50.0	NS
Total Cholesterol (mg/dl)	171.3 ± 33.6	175.0 ± 27.5	NS
HDL-C (mg/dl)	42.6 ± 6.6	46.4 ± 7.2	NS
LDL-C (mg/dl)	134.1 ± 38.0	135.6 ± 25.7	NS
Age (years)	39.7 ± 7.1	38.0 ± 6.7	NS

OA RI, resistivity index of the ophthalmic artery; OA PSV, peak systolic velocity of the ophthalmic artery; OA EDV, end diastolic flow velocity of the ophthalmic artery; Hcy, homocysteine; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; NS, Non-significant.

*Statistically significant.

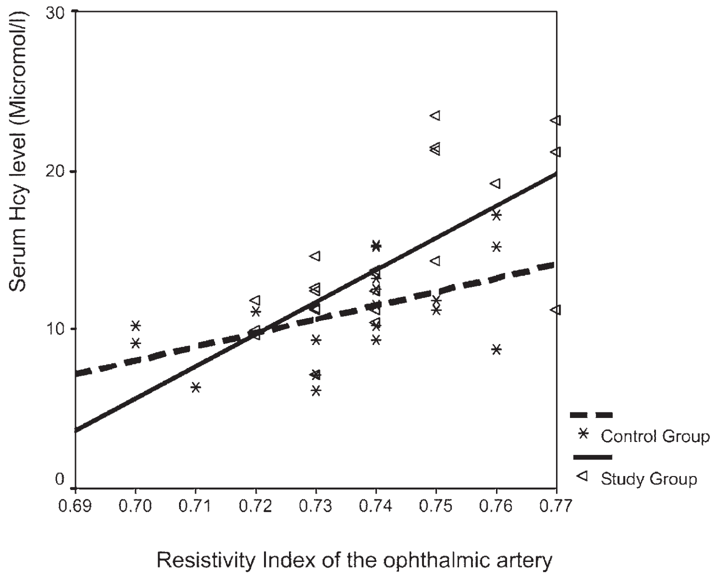

Fig. 1. The scatter graph and regression lines for the resistivity index of the ophthalmic artery and serum homocysteine level.

TABLE 2. Correlation of the ocular blood flow indices with serum Hcy levels.

	HTC group (n = 22)		CONTROL group (n = 24)	
	r	p	r	p
OA RI	0.684	< 0.001*	0.606	0.002*
OA PSV	–0.265	0.233	–0.471	0.02*
OA EDV	–0.602	0.003*	–0.658	< 0.001*
Working years	0.427	0.047*	-	-

HTC, highway toll collector; OA RI, resistivity index of the ophthalmic artery; OA PSV, peak systolic velocity of the ophthalmic artery; OA EDV, end diastolic flow velocity of the ophthalmic artery.

*Statistically significant.

Although the mechanism is unknown, exposure to exhaust particles has been suggested to cause coronary, cerebral, and peripheral atherosclerosis (Kunzli et al. 2005; Erdogmus et al. 2006). Greater formation of reactive oxygen species has been shown to produce changes in the plasma thiol redox status in smokers (Bergmark et al. 1997; Blom 1998; Wilcken 1998; Targher et al. 2000). Exhaust particles contain many constituents, some of which are cytotoxic and have the potential to cause tissue injury and oxidative stress (Sydbom et al. 2001). During exposure to oxidative stress, the uptake and utilization of sulfur-containing amino acids such as cysteine and methionine are increased in cells and are essential for survival. Consequently, the oxidative stress produced by exhaust particles increases serum Hcy levels (Targher et al. 2000; Memisogullari and Akçay 2004). Tanriverdi et al. (2006) also found that patients with slow coronary flow have a significantly higher serum Hcy level and increased carotid IMT compared with patients with normal coronary flow.

As an intermediary amino acid, Hcy is formed during the conversion of methionine to cysteine. It may decrease the level of nitric oxide by reducing its synthesis or by increasing its degradation *via* the generation of oxygen-derived free radicals such as superoxide anion and hydrogen peroxide. Furthermore, Hcy has a direct toxic effect on vascular endothelial cells, resulting in exposure of the subendothelium to platelets and coagulation factors, and Hcy inhibits protein C activation (Loscalzo 1996; Bostom and Lathrop 1997; Blom 1998). Therefore, an elevated serum Hcy level might be partially responsible for the impaired ocular blood flow in HTCs.

Southern et al. (1998) reported that a Hcy-supplemented diet produced elevated serum Hcy levels and subsequently increased intimal hyperplasia, with a resultant increase in luminal stenosis in a rat model. In another rat model, smoking significantly increased serum Hcy levels and luminal stenosis, and folic acid supplementation attenuated this effect (Davis et al. 2004); however, none of the cases in our study was taking folic acid or smoking cigarettes. Sex, estrogen, pregnancy, diet, and vitamin intake have also been reported to be related to the serum Hcy level. All of the subjects in our study group were male, and we controlled for folate and vitamin B_{12} intake. Therefore, we believe that the higher serum Hcy level in HTCs was related to exhaust particles and that the lower ocular blood flow was related to a higher serum Hcy level. One criticism of our study is that we did not measure B_{12} and folate levels, which previous studies have found to be related to Hcy levels (Cantu et al. 2004; Kocer et al. 2004); the present study was not originally designed to investigate B_{12} and folate levels.

Recently, we showed that the carotid intima-media thickness was increased and was related to the serum Hcy level in HTCs (unpublished data). In addition, the RI has been shown to be an indirect measure of the atherosclerotic process (Frauchiger et al. 2001). This study is the first to find a possible link between an indirect index of increased atherosclerotic processes (RI) and increased Hcy levels in subjects exposed to high levels of exhaust particles.

In conclusion, exposure to exhaust particles places highway workers such as policemen, automotive service companies, and toll collectors at risk for increased serum Hcy levels, which in turn can lead to abnormal ocular blood flow and an increased resistivity index. These manifestations may be related to the acceleration of premature atherosclerosis in these patients. This issue needs to be examined in larger randomized studies.

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