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Serum Homocysteine Levels in Highway Toll Collectors and the Relationship with Intima-Media Thickness of the Carotid Artery

Authors
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Aim: A relationship between air pollution and increased intima–media thickness (IMT) has been recently reported, but its etiology is not yet well understood. We aimed to determine whether exposure to exhaust particles, which are important air pollutants, is associated with increased serum homocysteine (Hcy) levels and whether elevated Hcy levels are associated with increased IMT.

Materials and Methods: Serum Hcy levels were measured by fluorometric high performance liquid chromatography in 65 male highway toll collectors (HTCs) and 43 healthy male volunteers. IMT was measured with Doppler sonography by an experienced radiologist.

Results: Serum Hcy levels (14.5 ± 4.9 mmol/L, P < 0.005) and IMT (0.757 ± 0.18 mm, P < 0.001) were higher in the HTC group than in controls (12.0 ± 2.8 mmol/L and 0.612 ± 0.11 mm, respectively). A positive correlation was found between Hcy level and IMT both in the HTC group (r = 0.36; P < 0.005) and in the control group (r = 0.36; P < 0.05).

Conclusions: Exposure to exhaust particles may cause an increase in oxidative stress because of an increase in serum Hcy levels, which consequently may lead to an increase in IMT. Nevertheless, further studies on the subject are needed before drawing a firm conclusion.

Key Words: Homocysteine, exhaust particles, intima–media thickness, oxidative stress, highway toll collectors

Otoyol Gişe Memurlarında Serum Homosistein Düzeyleri ve Karotis Arterinde İntima-Media Kalınlığı ile İlişkisi

Amaç: Son zamanlarda hava kiriliği ve artışmış intima-media kalınlığı (IMT) arasında bir ilişki olduğu rapor edilmiş, ancak etyolojisi tam olarak anlamlaşılmıştır. Biz çalışmadada önemli hava kirleticiler olan egsoz partiküllerine maruziyetin artışmış homosistein (Hcy) düzeylerine iliskili olup olmadığını ve artışmış homosistein düzeylerinin artışmış intima-media kalınlığı ile ilişkili olup olmadığını araştırdık.

Yöntem ve Gereç: Altmışbeş erkek otoyol gİŞe memuru ve 43 sağlıklı erkek vücudunda yüksek performanslı svi kromatografi ile serum Hcy düzeyleri ölçüldü. IMT ise Doppler Sonografi kullanılarak deneyimli bir radyo-log tarafından ölçül dü.

Bulgular: Otoyol gİŞe memurlarında serum Hcy düzeyleri (14.5 ± 4.9 mmol/L, P < 0.005) ve IMT (0.757 ± 0.18 mm, P < 0.001) kontrollerden daha yüksek ti (srasıyla; 12.0 ± 2.8 mmol/L ve 0.612 ± 0.11 mm). Hem otoyol gİŞe memurları grubunda (r = 0.36; P < 0.005) ve hem de kontrol grubunda (r = 0.36; P < 0.05) Hcy ve IMT arasında pozitif bir korelasyon bulundu.

Sonuç: Egsoz partiküllerine maruziyet, sonuçta bir IMT artışına yol açabilen Hcy düzeyinde bir artıştan dolayı, oksidatif stres artışına neden olabilir. Ancak son karara varımdan önce hala daha ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Homosistein, Egsoz Partiküller, Intima-Media Kalınlığı, Oksidatif Stres, Otoyol Gişe Memurları

Introduction

An association between exposure to diesel exhaust and various negative health outcomes such as atherosclerosis and respiratory disease has been reported (1,2). Data have also suggested that exposure to exhaust particles might cause wall thickening of...
carotid arteries in highway toll collectors (HTCs), but the mechanism of this thickening is not well understood (3). Increased intima–media thickness (IMT) is generally considered to be an early marker of atherosclerosis (4). The IMT has been positively correlated with the plasma homocysteine (Hcy) concentration in the general population (5). Hcy is an intermediate of methionine metabolism that has been identified as a potential atherogenic agent and a risk factor for atherosclerosis (6–9). Smoking (10,11) and exposure to secondhand smoke (12) have been found to influence Hcy levels, and tobacco smoke is known to contain some molecules such as carbon monoxide and nitric oxides (3,13). Likewise, motor vehicles are known to generate air pollutants, and exhaust particles constitute a high percentage of the particles emitted in many towns and cities. Complete combustion of these particles produces water, liquids, solid particles, and various gases such as carbon monoxide, nitric oxides, sulfur dioxide, hydrocarbons, formaldehyde, transition metals, and carbon particles (1,2).

The level of Hcy and the relationship with IMT in HTCs have not been reported in the literature. We investigated the levels of Hcy in HTCs exposed to diesel exhaust and the relationship between the Hcy level and IMT of the carotid artery as an indicator of early atherosclerotic changes and risk for cardiovascular disease.

Materials and Methods

Subjects

A total of 65 male HTCs (mean age ± SD: 35.8 ± 7.1 years) and 43 male healthy volunteers (mean age ± SD: 36.4 ± 8.8 years) were included in the study. The HTCs were employed in manual tollbooths in the Kaynaşlı, Gölâyaka-Düzce region, located on the busiest line of the trans-European motorway in Turkey. Employees were working eight-hour days. Exclusion criteria included systolic or diastolic hypertension, diabetes mellitus, hypercholesterolemia, use of any systemic medication plus folate and vitamins B₆ or B₁₂, and a state of serious acute or chronic inflammatory diseases. Control cases were randomly selected among completely healthy age-matched hospital personnel. Subjects were instructed to refrain from drinking beverages containing caffeine for 6 h before the examination. The HTCs in the study group and the controls had body mass index (BMI) values under 30.

To account for exposure duration, the HTCs were divided into three subgroups based on the duration of employment as a tollbooth operator as: <10, 10–20, and >20 years.

Biochemical Assays

Serum samples were taken within 30 min of blood sample collection. The serum samples were stored at –20°C until they were biochemically analyzed; all assays were conducted in one batch.

Fasting serum total Hcy levels, equal to the total amount of free and protein-bound Hcy, were measured by fluorometric high performance liquid chromatography (Hewlett Packard 1100) using Chromosystems® calibrators and kits (Chromosystems Instruments and Chemicals GmbH, Munich, Germany).

Serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels were measured with a chemistry analyzer (Olympus AU 640 Analyzer, Olympus Corporation, Tokyo, Japan) using specific commercial calibrators and original commercial kits.

Carotid Ultrasonography

The Doppler sonographic examinations were performed by the same radiologist using the same duplex Doppler system (EUB 6500, Hitachi, Japan) with a 7-14 MHz linear probe. The examinations were conducted in a dark room with the subject in a supine position on the ultrasound examination table with their head held in the midline position or slightly tilted to either side. Measurements were performed at the areas proximal to the common carotid artery bulb of the right internal and external carotid arteries. For the evaluation of IMT of the common carotid artery, the transducer was manipulated so that the near and far walls were parallel to the transducer footprint, and the lumen diameter was maximized in the longitudinal plane. IMT was measured between the initial bright echo along the far wall that defines the lumen–intima interface and the second echogenic line that represents the media–adventitia interface. This measurement was performed at three adjacent sites, and the mean of these three measurements was recorded as the intima–media complex of the carotid artery.
### Statistical Analysis

Data were expressed as the mean ± SD. Values of P < 0.05 were considered statistically significant. Significant differences between the HTC and control group were analyzed using Student’s t test. The significance of the difference among all subgroups was analyzed using analysis of variance (ANOVA); if the F value was found to be significant, differences between the means were then analyzed using the post-ANOVA (Tukey’s) test. Pearson’s correlation test was used to evaluate the correlation analyses of all parameters.

### Results

The results of biochemical assays of the study participants are summarized in Table 1. In the HTC group, serum Hcy levels (14.5 ± 4.9 mmol/L, P < 0.005) and IMT (0.757 ± 0.18 mm, P < 0.001) were higher than those of the controls (12.0 ± 2.8 mmol/L and 0.612 ± 0.11 mm, respectively). There were no statistically significant differences between the HTCs and controls regarding serum total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, and glucose levels, tobacco smoke exposure, and age (P > 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Highway toll collectors (n = 65)</th>
<th>Controls (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (mmol/L)</td>
<td>14.5 ± 4.9*</td>
<td>12.0 ± 2.8</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.757 ± 0.18**</td>
<td>0.612 ± 0.11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.8 ± 7.1</td>
<td>36.4 ± 8.8</td>
</tr>
<tr>
<td>Duration of employment (years)</td>
<td>12.5 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Smoking (pack-years)</td>
<td>11.2 ± 9.2</td>
<td>11.6 ± 6.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>172.4 ± 17.9</td>
<td>172.7 ± 19.7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>111.2 ± 29.5</td>
<td>111.3 ± 31.0</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>43.5 ± 6.6</td>
<td>41.5 ± 5.8</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>87.7 ± 8.1</td>
<td>87.3 ± 8.4</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>133.4 ± 47.8</td>
<td>141.0 ± 38.2</td>
</tr>
</tbody>
</table>

*: P < 0.005 when compared with controls
**: P < 0.001 when compared with controls

Hcy: Homocysteine. IMT: Intima-media thickness. LDL: Low-density lipoprotein. HDL: High-density lipoprotein.

There was a positive linear relation between Hcy levels and duration of work. Analysis of ANOVA showed a positive but non-significant increase in conjunction with the duration of work (Table 2). Positive correlations were obtained between Hcy level and IMT (P < 0.005; r = 0.36; Figure) and Hcy level and age (P < 0.005; r = 0.36) in the HTC group, and between Hcy level and IMT (P < 0.05; r = 0.36) in the control group.

### Discussion

This is the first study in the English literature showing a relation between IMT and Hcy levels in HTCs. Furthermore, a likely positive correlation was found between the duration of work and an increase in serum Hcy. A possible link between premature atherosclerosis due to long-term exhaust exposure was speculated.

Atherosclerosis is a slow, progressive disease that can begin in childhood and not become manifest until an advanced age (6). IMT and carotid plaque have been associated with the levels of atherogenic lipids, hemostatic factors, diabetes, hypertension, smoking, and male sex (14,15). Hcy is an intermediary amino acid formed during the conversion of methionine to cysteine. High levels of Hcy cause lipid peroxidation, vascular...
endothelial injury, impaired vasomotor regulation, and increases in prothrombotic surfaces, and consequently lead to athero-thrombogenesis (6–9). We found that both the serum Hcy level and IMT were higher in HTCs than in controls.

Recently, Erdogmus et al. (3) reported that there was a relationship between IMT and years of employment in HTCs, confirming our results. We further analyzed the potential association of IMT with serum Hcy levels due to exhaust exposure. When HTC workers were divided into subgroups according to years of employment, serum Hcy levels increased in conjunction with the increase in working years.

Among the well-known covariates of serum Hcy are sex, smoking, estrogen, pregnancy, diet and folic acid, vitamin B₆ and vitamin B₁₂ supplements. In the present study, all of the study participants were male and none was using vitamin supplement. Dietary habits were also similar in our study group, and mean smoking duration measured as pack-years was not statistically different. None of the subjects had renal failure, diabetes, or symptoms of vitamin deficiency. Therefore, we believe that increased IMT and serum Hcy levels in HTCs may be related to exposure to exhaust particles.

Exhaust particles contain many constituents, some of which are cytotoxic and have the potential to cause tissue injury and oxidative stress (2). 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 needed for survival. The transsulfuration and transmethylation pathways of methionine metabolism provide the cysteine and methionine needed for the synthesis of antioxidants such as glutathione. Methionine is converted to S-adenosylmethionine (SAM), and SAM is converted to S-adenosylhomocysteine (SAH); ultimately, SAH is also converted to Hcy (8,11). Consequently, the oxidative stress produced by exhaust particles increases serum Hcy levels. The present study supports these data. Furthermore, changes are present in the plasma thiol redox status because of the increased formation of reactive oxygen species (10,16–18). Therefore, the exhaust particles contribute to increased oxidative stress. Arbak et al. (1) reported that oxidative stress is related to diesel exposure.

Southern et al. (19) reported that an Hcy-supplemented diet produced elevated serum Hcy levels and subsequently increased intima hyperplasia, resulting in an increase in luminal stenosis in a rat model. Till et al. (5) reported that the IMT positively correlates with the plasma Hcy concentration in the general population and also that supplementation with vitamins B₆, B₁₂, and folic acid significantly reduces IMT in at-risk patients. This suggests that increased IMT may be caused by increases in the Hcy level. In accordance with these data, we also found a correlation between serum Hcy levels and IMT. In the present study, we found a stronger correlation between the serum Hcy levels and IMT in the HTC group compared with the control group.

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>n</th>
<th>Serum Hcy level (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 working years</td>
<td>22</td>
<td>13.1 ± 4.6</td>
</tr>
<tr>
<td>10–20 working years</td>
<td>36</td>
<td>14.8 ± 5.2</td>
</tr>
<tr>
<td>&gt;20 working years</td>
<td>7</td>
<td>17.2 ± 2.8</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>14.5 ± 4.9</td>
</tr>
</tbody>
</table>

P value: non-significant.

Figure. The correlation between serum Hcy level and IMT.
A minor limitation of the present study was that we could not quantitatively measure exhaust exposure, or vitamin B12 and folate levels.

In conclusion, in our country, exposure to exhaust particles is still a risk for highway workers (i.e. policemen, automotive service companies) and for the general population living around the motorways.

Exposure to exhaust particles may cause an increase in oxidative stress because of an increase in the serum Hcy level, which consequently may lead to an increase in the IMT, a marker of atherosclerosis. Nevertheless, the subject requires further studies before drawing any firm conclusions.

References