Homocysteine concentrations and vascular complications in patients with type 2 diabetes

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SUMMARY

Objective: Hyperhomocysteinemia is a well known risk factor for the diseases of the cardiovascular system, which seem to be the main cause of increased mortality in patients with type 2 diabetes. The aim of the study was to evaluate the levels of homocysteine in patients with type 2 diabetes in respect to the regimen of diabetes treatment as well as the presence of diabetic complications.

Methods: The investigation was carried out in the group of 64 patients with type 2 diabetes and in 18 healthy subjects from the control group. Clinical examination and measurements of homocysteine, folic acid, vitamin B12, glycosylated hemoglobin concentration and evaluation of parameters of the lipid metabolism, microalbuminuria and creatinine were done in both groups.

Results: Homocysteine concentration was significantly higher in the group of patients with diabetes in comparison to the control group (p = 0.0007). Diabetic patients had significantly lower concentrations of folic acid (p = 0.028) and HDL cholesterol (p = 0.025) together with higher levels of systolic blood pressure (p = 0.007). In the group of patients with diabetes no differences in homocysteine levels were found in respect to diabetes treatment. Diabetic patients with coronary artery disease had significantly higher homocysteine concentration in comparison to the group with diabetes without history of coronary artery disease (p = 0.0097). Homocysteine levels correlated significantly with incidence of ischaemic heart disease (r = 0.44, p = 0.001) and microalbuminuria (r = 0.26, p = 0.019). Negative correlation was noticed in HDL concentrations (r = -0.30, p = 0.013) and the levels of folic acid (r = -0.30, p = 0.008).

Conclusion: Our results suggest that hyperhomocysteinemia in diabetic patients may contribute to the development of chronic complications. The influence of diabetes treatment on Hcy levels requires further observations.

Key-words: Homocysteine · Diabetes · Diabetic Complications.

Homocystéine concentrations and vascular complications chez le diabétique de type 2

RÉSUMÉ

Concentrations d’homocystéine et complications vasculaires chez le diabétique de type 2

Objectif : L’hyperhomocysténémie est un facteur de risque cardio-vasculaire bien connu, les maladies cardiovasculaires étant la principale explication de l’augmentation de mortalité chez les diabétiques de type 2. Le but de cette étude était d’évaluer les niveaux d’homocystéine chez les diabétiques de type 2, en fonction du type de traitement et de la présence de complications du diabète.

Méthodes : L’exploration a été réalisée dans un groupes de 64 diabétiques de type 2 et 18 témoins sains. Elle a consisté en un examen clinique et une mesure des paramètres suivants : homocystéine, acide folique, vitamine B12, hémoglobine glyquée, marqueurs lipidiques, microalbuminurie et créatininémie.

Résultats : Les concentrations d’homocystéine étaient significativement plus élevées dans le groupe des diabétiques par rapport au groupe témoin (p = 0.0007). Les diabétiques avaient des concentrations plus basses d’acide folique (p = 0.028) et de cholestérol-HDL (p = 0.025) et des valeurs plus hautes de pression artérielle systolique (p = 0.007). Dans le groupe des diabétiques, les concentrations d’homocystéine n’ont pas paru influencées par le type de traitement du diabète. Les diabétiques insuffisants coronariens avaient des concentrations d’homocystéine plus élevées que les diabétiques sans coronaropathie (p = 0.0097). Les concentrations d’homocystéine étaient significativement corrélées avec l’incidence de cardiopathie ischémique (r = 0.44, p = 0.001) et la microalbuminurie (r = 0.26, p = 0.019). Une corrélation négative a été observée avec les concentrations de HDL (r = -0.30, p = 0.013) et celles d’acide folique (r = -0.30, p = 0.008).

Conclusion : Nos résultats suggèrent que l’hyperhomocysténémie peut contribuer au développement des complications chroniques chez les patients diabétiques. L’influence du traitement du diabète sur les niveaux d’homocystéine reste à explorer plus avant.

Mots-clés : Homocystéine · Diabète · Complications diabétiques.
Type 2 diabetes mellitus, recognised as a civilisation disease, leads to the development of various chronic complications which cause a significant epidemiological and therapeutical problem [1]. Cardiovascular system diseases (CVD) are the main cause of increased mortality in patients with diabetes. Damage of endothelial wall plays the key role in the development of diabetic complications [1, 2]. Hyperglycaemia, oxidative stress, genetic factors, disturbances of lipid metabolism and various growth factors are among major causes of chronic diabetic complications. In the recent years attention was shifted to other factors which may explain the phenomenon of increased atherogenesis in diabetes [3, 4]. Homocysteine (Hcy)- protein derived aminoacid, common in our everyday diet, is present in high concentrations in patients with atherosclerosis and its increased excretion in urine correlates with the decreased contractility of vessels in the patients with coronary heart disease (CHD) [4-7]. High levels of Hcy have been found in patients with diabetes and CHD and were considered as a strong and independent predictor of CHD event [8].

The role of high Hcy levels in the pathogenesis of atherosclerosis is not fully recognised, but may be related to the generation of free radicals, stimulation of smooth muscle cells proliferation and changes in the activity of platelets and hemostasis [9]. Hyperhomocysteinemia is a well known risk factor leading to endothelial dysfunction [9, 10]. Kidneys are most important in Hcy metabolism and their dysfunction leads to hyperhomocysteinemia [11, 12]. Moreover high protein intake in diet influences the levels of Hcy in the serum and so do folic acid, vit B6 and B12 deficiencies [13]. Those vitamins are co-factors in Hcy metabolism to methionine or cysteine [9].

The aim of the present study was to determine the differences in homocysteine concentrations in the serum of patients with type 2 diabetes treated with oral hypoglycaemic agents or insulin depending on the incidence of chronic vascular complications.

Materials and methods

The study was carried out in the group of consecutive 64 patients with type 2 diabetes hospitalized in the Department of Endocrinology, Diabetology and Internal Medicine of Medical University of Bialystok in the year 2002 in the period of five months (from February to June). The mean age of subjects was 64.14 ± 11.74 years, BMI — 30.50 ± 6.92 kg/m², mean duration of diabetes — 7.71 ± 6.07 years. Patients with diabetes and overt renal insufficiency and/or other serious medical problem were excluded from the study. Eighteen volunteers, recruited mainly from medical staff, with no history of serious diseases served as a control group (mean age 55.67 ± 13.45 years, BMI 27.80 ± 5.59 kg/m²). Both in the studied, and control group no person was administered any vitamin supplementation and drugs influencing Hcy metabolism. In the group of patients with diabetes 34 subjects were advised either diabetic diet or diabetic diet and oral hypoglycaemic agents. Mean diabetes duration in this group was 4.9 ± 3.93 years. The remaining 30 subjects were administered insulin and diabetes duration in this group was 10.63 ± 6.62 years.

In both groups clinical examination was performed. In all groups the assessment of antropometric parameters (body weight, hip and waist girths) was performed on the basis of which body mass index (BMI) and waist to hip ratio (WHR) were calculated. CHD was defined as history of myocardial infarction, coronary artery bypass grafting, changes in angiographic examination, or positive exercise test. CHD was diagnosed in 18 patients from the group receiving insulin and in 18 subjects from the group treated with oral agents. Diabetic retinopathy was diagnosed in all groups on the basis of ophthalmological examination and determined in 14 patients from the insulin group and in 10 from the oral agents group.

In the studied and control group blood samples were taken in order to measure the levels of Hcy (Imx Homocysteine Reagent Pack, FPIA, ABBOTT, Germany), folic acid (RIA, DPC,USA), vitamin B12 (RIA,DPC,USA), glycated hemoglobin (HPLC-Biorad), lipid parameters, creatinine, and urine sample to estimate microalbuminuria (immunoturbidymetric method, Clinitech 50). Total cholesterol (TC), HDL cholesterol (HDL-ch), triglycerides (TG) were measured by enzymatic method (Cormay, Poland). LDL-cholesterol fraction was calculated using Friedewald’s formula. Microalbuminuria was found in 14 patients treated with insulin and in 5 patients from the group treated with oral agents. Clinical characteristics of the studied groups is shown in Table I and Table II.

Statistical analysis

For statistical analysis statistical program Statistica 5.0 (StatSoft, Kraków) was used. Results are shown as mean ± SD. Mann-Whitney U test was used to compare clinical characteristics of the studied groups.

Table I

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diabetic group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.1 ± 14</td>
<td>55.7 ± 13.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.5 ± 6.9</td>
<td>27.8 ± 5.6</td>
</tr>
<tr>
<td>WHR</td>
<td>1.14 ± 0.21</td>
<td>1.1 ± 0.18</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135.9 ± 17.5 *</td>
<td>123.3 ± 11.8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.0 ± 9.9</td>
<td>79.4 ± 8.2</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>7.7 ± 6.1</td>
<td>-</td>
</tr>
</tbody>
</table>

* p < 0.05 statistically significant difference in comparison to the control group.
values ± SD. Differences between groups were determined using non-parametric Mann-Whitney U test. Correlation between studied parameters was assessed using analysis of the Pearson’s coefficient and multiple regression analysis. The influence of confounding factors on Hcy concentration was tested in multiple regression analysis by means of analysis of residuals. Values of p < 0.05 were considered as statistically significant.

Results

Studied groups (diabetic subjects vs control group) did not differ significantly in respect to BMI and WHR (Tab I and II). However the group of diabetic patients was significantly older (p = 0.018). In the subgroup of diabetic patients significantly higher values of systolic blood pressure were noticed (p = 0.0052) (Tab I). Hcy concentrations were significantly higher in the group of patients with diabetes in comparison to the control group (p = 0.007) (Tab III). The difference in Hcy concentration was still significant after adjustment for age (the values of residuals in patients with type 2 diabetes — 0.70 ± 5.74 and in control group — -2.72 ± 3.70, p = 0.019). Moreover in the group of patients with diabetes significantly lower levels of folic acid (p = 0.028) and HDL cholesterol fraction (p = 0.025) (Tab III) were observed. In the group of patients with diabetes, taking into account diabetes treatment regimen (group administered insulin vs group administered oral agents), we found no significant differences in Hcy levels between the groups (Tab IV). The difference between the studied groups was noticeable only in HbA1c levels which were significantly higher in the group treated with insulin (p = 0.0001) (Tab IV). Both in the group administered oral agents and group treated with insulin statistically significant higher values of systolic blood pressure were observed and significantly lower levels of HDL cholesterol in comparison to the control group (Tab IV). Interestingly, Hcy levels were significantly higher in the group of diabetic patients with coronary heart disease (18.86 ± 6.78 μmol/l vs 14.48 ± 4.04 μmol/l, p = 0.0097) but after adjustment for age the difference in Hcy concentration was not statistically significant (the values of residuals in patients with type 2 diabetes mellitus and CHD — -1.59 ± 4.046, patients with type 2 diabetes mellitus without CHD — 1.224 ± 6.53, p = 0.11). There were no differences in the levels of homocysteine in the groups with and without retinopathy (17.16 ± 6.11 μmol/l vs 16.96 ± 6.26 μmol/l) and microalbuminuria: (18.81 ± 7.27 μmol/l vs 16.27 ± 5.52 μmol/l). Hcy concentration correlated significantly with age (r = 0.37, p = 0.001), microalbuminuria (r = 0.26, p = 0.019) and the incidence of ischaemic heart disease (r = 0.44, p = 0.001). Negative correlation was observed with serum HDL cholesterol fraction (r = -0.30, p = 0.013) and folic acid levels (r = -0.30, p = 0.008); levels of vitamin B12 correlated negatively with Hcy concentration on the border of statistical significance (r = -0.23, p = 0.056).

Multiple regression analysis model was constructed to best predict Hcy concentration in the studied group. Age, sex, BMI, smoking, blood pressure, incidence of coronary heart disease, retinopathy, microalbuminuria, creatinine, folic acid, vit.B12, and lipid parameters were included in the model as independent variables. We found that age (β = 0.48; p = 0.002) and folic acid (β = -0.25; p = 0.03) and microalbuminuria of borderline significance (β = 0.25; p = 0.060) were independent predictors of Hcy concentration.

**Table II**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic patients administered insulin n = 30</th>
<th>Diabetic patients administered oral agents n = 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 6.2</td>
<td>30.4 ± 7.5</td>
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<tr>
<td>Age (years)</td>
<td>66.1 ± 10.9</td>
<td>62.4 ± 12.4</td>
</tr>
<tr>
<td>WHR</td>
<td>1.13 ± 0.21</td>
<td>1.15 ± 0.22</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>10.6 ± 6.6</td>
<td>4.9 ± 3.9</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.5 ± 17.4</td>
<td>134.6 ± 17.7</td>
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<tr>
<td>DBP (mmHg)</td>
<td>84.3 ± 10.3</td>
<td>82.0 ± 9.7</td>
</tr>
<tr>
<td>Retinopathy (n)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Microalbuminuria (n)</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>CHD (n)</td>
<td>18</td>
<td>18</td>
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</table>

**Table III**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic group n = 64</th>
<th>Control group n = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (μmol/l)</td>
<td>17.03 ± 6.11*</td>
<td>11.8 ± 2.9</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 ± 1.9*</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>196.1 ± 41.5</td>
<td>194.8 ± 62.50</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.9 ± 10.4*</td>
<td>53.9 ± 21.6</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>118.2 ± 35.7</td>
<td>120.1 ± 39.5</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>148.5 ± 69.2</td>
<td>133.6 ± 81.0</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>402.7 ± 238.2</td>
<td>310.6 ± 148.4</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>7.1 ± 4.8*</td>
<td>10.6 ± 8.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.10 ± 0.36</td>
<td>0.92 ± 0.34</td>
</tr>
</tbody>
</table>

* p < 0.05 statistically significant difference in comparison to the control group.
levels. In the stepwise forward regression analysis incidence of coronary artery disease, age, folic acid were responsible for 40% of Hcy variability, with microalbuminuria, total cholesterol, HDL-cholesterol, vit. B12 and sex slightly increasing the R² value to 0.59.

### Discussion

In recent prospective studies on large populations, significantly higher concentrations of homocysteine were found in the groups of patients with diabetes [13-15]. Hoogeveen et al. have reported on increased mortality due to CVD in the population of diabetic patients with high Hcy levels [16, 17] in the prospective Hoorn study. Similar results come from research done by Soinio et al. [8] and Coppola et al. [18]. Also Meigs et al. have found that hyperhomocysteinemia is an independent risk factor of CVD incidence in diabetic patients [19]. Strong association between atherosclerosis, hyperhomocysteinemia and diabetes type 2 has been found in the Japanese population [20]. There have been reports of lower than in general population Hcy levels in the group of patients with diabetes contributing to a higher rate CVD mortality [21]. In the present study higher levels of Hcy in the whole group of diabetic subjects were noticed. Similarly to other authors we found a relation between the concentration of Hcy and incidence of CHD in the groups of diabetic patients in our study. One observation coming from recent studies seems particularly interesting and it concerns statistically significant correlation between systolic blood pressure and Hcy concentration [16, 17]. Similar observations were made in a large population study of 2 484 patients where a strong correlation between Hcy concentrations and systolic blood pressure [16, 17] was noted. However some authors have found no major relation between high Hcy levels and the incidence of hypertension [22]. Buysschaert et al. have found a higher incidence of complications such as macroangiopathy and nephropathy in the population of 122 diabetics in the group with hyperhomocysteinemia in comparison to the patients with normal Hcy levels [23]. No such dependence was observed in relation to microalbuminuria, retinopathy and diabetic neuropathy. On the contrary, in our study Hcy concentrations strongly correlated with microalbuminuria and diabetic macroangiopathy. Furthermore, no dependence was observed between Hcy concentrations and the incidence of diabetic retinopathy. Higher Hcy levels were determined in the Hoorn study in diabetic patients with renal damage and retinopathy [17]. Our results confirmed a positive correlation between Hcy levels and microalbuminuria. In Framingham study a relation between hyperinsulinaemia, Hcy levels and increased risk of ischaemic heart disease incidence was determined [19]. Similarly to findings from the Hoorn study Davies et al. have proved that Hcy significantly correlates with albumin excretion and creatinine clearance in diabetic patients [24]. A significant dependence

### Table IV

Mean values of the studied parameters depending on diabetes treatment regimen.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic patients administered insulin</th>
<th>Diabetic patients administered oral agents</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (µmol/l)</td>
<td>16.56 ± 6.03*</td>
<td>17.44 ± 6.24*</td>
<td>11.78 ± 2.92</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.6 ± 1.8*#</td>
<td>7.0 ± 1.5*</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194.8 ± 42.9</td>
<td>197.3 ± 40.7</td>
<td>197.8 ± 62.5</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.3 ± 9.7*</td>
<td>39.6 ± 11.1*</td>
<td>53.9 ± 21.6</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>162.1 ± 83.9</td>
<td>136.2 ± 50.7</td>
<td>133.6 ± 81.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>119.3 ± 39.1</td>
<td>117.2 ± 33.0</td>
<td>120.1 ± 39.5</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>385.4 ± 238.6</td>
<td>420.1 ± 241.2</td>
<td>310.6 ± 148.4</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>6.4 ± 3.3*</td>
<td>7.8 ± 5.9</td>
<td>10.6 ± 8.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.25 ± 0.39*</td>
<td>0.96 ± 0.28</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

*p < 0.05 statistically significant difference in comparison to the control group.

#p < 0.05 — statistically significant difference between groups of subjects with diabetes.
was noticed between Hcy levels and microalbuminuria [16, 17, 24]. On the other hand Smuldiers et al. have found no correlation between Hcy levels, microalbuminuria and diabetic retinopathy [25].

Interestingly, we found that HDL-cholesterol levels proportionally negatively correlated with Hcy levels. Similar results were obtained in Framingham and Hoorn studies [16, 17, 19]. This may partly explain the tendency to premature atherosclerosis in patients with hyperhomocysteinemia.

One of the main causes for elevated Hcy levels are vitamin B6, B12 and folic acid deficiencies which are cofactors in Hcy metabolism. Also vitamin supplementation is shown to reduce the levels of Hcy in diabetic subjects [26]. We observed significantly lower levels of folic acid in the group of diabetics as well as a strong negative correlation between folic acid, vit B12 and Hcy levels. Lower folic acid concentrations observed in diabetic patients population may be explained in terms of hyperglycaemia increasing soluble vit B secretion [27]. Some studies have shown influence of lifestyle on Hcy levels, in our study however no correlation between BMI or smoking and Hcy levels was noticed. We did not however assess alcohol and coffee intake [28].

The influence of diabetes control on Hcy levels needs further explanation. Our results do not confirm the dependence between diabetes control (expressed by HbA1c levels) and Hcy concentrations. In the subgroup of patients with diabetes who were administered insulin, HbA1c levels were statistically significantly higher in comparison to the group treated with oral agents, however Hcy levels were comparable in both groups. Scientific data are contradictory. Results achieved by Drzewoski and Czupryniak and colleagues indicate that metabolic control of diabetes may influence Hcy levels. Those authors have shown that patients with bad metabolic control of diabetes (HbA1c 9.8%) had significantly higher Hcy levels in comparison to diabetics with normal HbA1c levels (6.6%). Statistically significant correlation between Hcy concentration and HbA1c was also noticed in some studies [29, 30]. However Hoogeven et al. did not note such dependence [16, 17].

Treatment of diabetes in relation to Hcy levels has also been taken into account in our study, but we found no significant differences between the groups (group administered insulin vs group administered oral agents). It is in agreement with results obtained by other authors. In the Hoorn study no dependence between diabetes treatment and Hcy levels was found [16]. However, some authors have proved that glimepiride had a lowering effect on Hcy levels, whereas metformin does not [31]. Other reports show no significant relation between metformin treatment and Hcy concentrations [31, 32]. However, in experimental studies troglitazone treatment has influenced Hcy levels and the enzymes involved in its metabolism [33]. Vitamin supplementation seems to significantly influence Hcy level with most data supporting the use of folic acid [34, 35]. There are reports of Vit B12, B6 and antioxidants treatments significantly reducing Hcy levels [36]. However, the patients recruited to the presented study did not use any additional vitamin medication.

One of the limitations of our study, apart from a small number of patients, was the statistically significant age difference between diabetic and control group which was on average 10.2 years of age. On the other hand some authors suggest that age difference of over 20 years was associated with a significant increase in Hcy levels of above 1.3 µmol/l [7] while others speculate that the increase of Hcy levels over 5 µmol/l can have noticeable harmful effects [36].

In conclusion it seems that hyperhomocysteinemia in diabetic patients may contribute to the development of chronic complications. The influence of diabetes treatment on Hcy levels requires further observations.

References