Hyperhomocysteinemia Is Associated With the Presence of Retinopathy in Type 2 Diabetes Mellitus

The Hoorn Study

Ellen K. Hoogeveen, MD; Pieter J. Kostense, PhD; Petra E. D. Eysink, MD; Bettine C. P. Polak, MD; Pieter J. Beks, MD; Cornelis Jakobs, PhD; Jacqueline M. Dekker, PhD; Giel Nijpels, MD; Robert J. Heine, MD; Lex M. Bouter, PhD; Coen D. A. Stehouwer, MD

Background: Retinopathy is the leading cause of blindness among patients with type 2 diabetes mellitus (DM). Hyperhomocysteinemia is a recently recognized risk factor for cardiovascular disease, independent of established risk factors.

Objective: To study the association between the homocysteine level and retinopathy among subjects with and without DM.

Methods: We studied an age-, sex-, and glucose tolerance–stratified random sample of a 50- to 75-year-old general white population in the Hoorn Study (N=625). Retinal vascular changes (retinopathy) were assessed using ophthalmoscopy and/or fundus photography. Hyperhomocysteinemia was defined as a serum total homocysteine level greater than 16 µmol/L.

Results: The prevalence of retinopathy was 9.8% (28/285) in subjects with normal glucose tolerance, 11.8% (20/169) in those with impaired glucose tolerance, 9.4% (10/106) in those with newly diagnosed type 2 DM, and 32.3% (21/65) in those with known type 2 DM. The prevalence of retinopathy was 10.3% (39/380) in patients without hypertension and 16.3% (40/245) in subjects with hypertension; it was 12.0% (64/534) in subjects with a serum total homocysteine level of 16 µmol/L or less and 16.5% (15/91) in those with a serum total homocysteine level of more than 16 µmol/L. After stratification for DM and adjustment for age, sex, glycosylated hemoglobin, and hypertension, the odds ratio (95% confidence interval) for the relation between retinopathy and hyperhomocysteinemia was 0.97 (95% confidence interval, 0.42-2.82) in patients without DM and 3.44 (95% confidence interval, 1.13-10.42) in patients with DM (P=.08 for interaction).

Conclusion: The findings suggest that hyperhomocysteinemia may be a risk factor for retinopathy in patients with type 2 DM, but probably not in patients without DM.

Arch Intern Med. 2000;160:2984-2990
SUBJECTS AND METHODS

STUDY POPULATION

The Hoorn Study, conducted from 1989 to 1992, is a cross-sectional survey of glucose tolerance and other cardiovascular risk factors in a 50- to 75-year-old general white population. A random sample of all men and women aged 50 to 75 years was drawn from the municipal population registry office of Hoorn, the Netherlands; 2484 subjects participated (response rate, 71%). All subjects, except previously diagnosed diabetic subjects with DM who were treated with oral glucose-lowering agents or insulin, underwent a 75-g oral glucose tolerance test and were classified according to the World Health Organization criteria. A second oral glucose tolerance test (participation rate, 93%) was performed, for reasons of efficacy, in a random subsample (n = 1122), stratified by 2-hour glucose values of the first test, age, and sex. Finally, from this subsample another age-, sex-, and glucose tolerance– stratified random sample (n = 708) was drawn. The presence of retinal vasculopathy (as defined below) was investigated (n = 625; response rate, 88%) by 2 experienced ophthalmologists. The examination included both ophthalmoscopy and fundus photography (detailed below). Glucose tolerance was divided into 4 categories on the basis of the mean of the 2 oral glucose tolerance test results: subjects with normal glucose tolerance (n = 285), subjects with impaired glucose tolerance (n = 169), subjects with newly diagnosed DM (n = 100), and subjects with known DM (n = 65). The Hoorn Study was approved by the Ethical Review Committee of the University Hospital Vrije Universiteit, Amsterdam, the Netherlands. Informed consent was obtained from all participants.

OPHTHALMOLOGIC INVESTIGATION

Retinopathy was assessed by ophthalmoscopy and/or fundus photography. In each participant, both eyes were dilated with 0.5% tropicamide and 5% phenylephrine hydrochloride eye drops. After an average period of 13 minutes, indirect and direct ophthalmoscopy (N = 625) was carried out by 2 ophthalmologists, and findings regarding the retinal status were reported on standard forms. Thereafter, two 45° standard field, 35-mm black-and-white fundus photographs (Kodak Tri-X 400 ASA; Eastman Kodak, Rochester, NY; Kowa Pro 1 fundus camera; Kowa Optical Industry, Tokyo, Japan) were taken of each eye. Photographs were taken with a green filter (to improve the contrast), centered on the macular area and the optic disc. Fundus photographs of 148 subjects were inadvertently lost. (The fundus photographs were randomly lost with regard to age, sex, hypertension, glucose tolerance category, and serum total homocysteine [tHcy] level of the subjects [data not shown].) Thus, for the present analysis fundus photographs of 477 subjects were available.

Both ophthalmoscopic and fundus photographic findings were graded according to the modified Airlie House classification. The fundus photographs were independently graded by 2 ophthalmologists. The independent judgment of a third ophthalmologist was taken to be decisive in case of disagreement about the grading of retinopathy on the fundus photograph. For the present analysis “the worst eye” of each subject according to ophthalmoscopy or fundus photography was used. Any retinopathy (yes/no) was defined as the presence of 1 or more hemorrhages, microaneurysms, soft or hard exudates, neovascularization, and/or laser coagulation scars in 1 or both eyes. Diabetic retinopathy was defined as presence of 1 or more microaneurysms and/or laser coagulation scars in 1 or both eyes (there were no subjects with neovascularization), regardless of other abnormalities.

MEASUREMENT OF SERUM tHcy LEVEL

Fasting blood samples were centrifuged within 1 hour following collection. Serum samples were stored at −20°C for 6 years. cThere is good evidence that serum tHcy levels in frozen samples are stable for 10 years or longer. The serum tHcy (free plus protein-bound) level was measured using tri-n-butylphosphine as the reducing agent and ammonium 7-fluorobenz-2-oxid-1.3-diazole-4-sulfonate as the thiol-specific fluorochromophore, followed by high-performance liquid chromatography with fluorescence detection. The intra-assay and interassay coefficients are 2.1% and 5.1%, respectively.

OTHER MEASUREMENTS

Subjects were classified as either a current cigarette smoker or nonsmoker. The body mass index was calculated as weight/height2. Results were expressed as mean ± SD. A 2-sided test was used to test for differences between subgroups. A P value of less than .05 was considered to indicate a statistically significant difference.

RESULTS

Of 5 right and 8 left eyes, ophthalmoscopic findings were missing. In addition to the photographs of 148 subjects that were lost, photographs of 3 right and 4 left eyes were missing. Moreover, because of poor quality, photographs of 18 right and 20 left eyes were ungradable for retinopathy. Thus, ophthalmologic data of 625 subjects were available for further analysis; 76% (477 of 625 subjects) were based on both ophthalmoscopic and fundus photographic findings.

With regard to the fundus photographs, the κ (95% CI) between the ophthalmologists was 0.87 (0.78-0.96) for the right eyes (n = 437) and 0.95 (0.89-1.00) for the left eyes (n = 428), indicating a good agreement; between ophthalmoscopic and fundus photographic findings, the κ was 0.39 (range, 0.22-0.56) for the right eyes (n = 450) and 0.39 (range, 0.21-0.58) for the left eyes (n = 443), indicating moderate agreement.

The baseline characteristics of the study population are presented in Table 1. The standardized prevalence of any retinopathy was 10.7%. The prevalence was 9.8% (28/285) in subjects with normal glucose tolerance, 11.8% (20/169) in subjects with impaired glucose tolerance, 9.4% (10/106) in subjects with newly diagnosed DM, and 32.3% (21/65) in subjects with known diabetes.

Continued on next page
DM; it was 10.3% (39/380) in subjects without hypertension and 16.3% (40/245) in those with hypertension; it was 12.0% (64/534) in those with serum tHcy levels higher than 16 µmol/L. The prevalence of diabetic retinopathy was 4.6% (13/285) in subjects with serum tHcy levels of 16 µmol/L or less and 16.5% (15/91) in those with serum tHcy levels higher than 16 µmol/L. Ophthalmologic investigations and laboratory measurements were carried out in a masked fashion for glucose tolerance status and other clinical data.

STATISTICAL ANALYSIS

Variables are presented as mean (SD), number (percentage of the total), or, in case of skewed distribution, median and interquartile range. For age, sex, and glucose tolerance category, standardized prevalence of any retinopathy and hyperhomocysteinemia was calculated, as described previously in detail. Briefly, the frequency was determined in 24 strata (age, 3; sex, 2; and glucose tolerance, 4) of the original population-based sample (standard, N = 2,484). The prevalence was calculated from the magnitude of each variable. For age, sex, and glucose tolerance category stratum, k coefficients were calculated to assess agreement with regard to the prevalence of retinopathy on fundus photographs between 2 ophthalmologists, and of the presence of retinopathy between fundus photographic and ophthalmoscopic examinations. Differences between subjects with and without any retinopathy were tested using the t test or Wilcoxon rank sum test for continuous variables and Pearson χ² test for frequency measures. Associations of risk factors for retinopathy with serum tHcy level (logarithmically transformed) were studied by calculation of the Pearson product-moment correlation coefficients.

We performed logistic regression analyses to study the relation between the serum tHcy level and the presence of any retinopathy. The number of cases in the separate classes of retinopathy was insufficient to allow a more detailed analysis. We chose 2 different approaches to investigate the nature of the relation between the serum tHcy level and retinopathy, because it is unclear whether this relation, if any, is linear or has a certain threshold. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) both for the serum tHcy level as a categorical (to allow for a nonlinear dose-response relation and to reduce the influence of outliers) and as a continuous variable, the latter expressed per 5-µmol/L (about 1 SD) increment of serum tHcy level (restricted to the range from the minimum tHcy value to 35 µmol/L). For analysis with the serum tHcy level as a categorical variable, we calculated ORs for the tHcy level divided in 2 (>16 µmol/L vs ≤16 µmol/L) and in 3 categories (<9.0 µmol/L, 9.1-16.0 µmol/L, and ≥16.0 µmol/L). To test for trend, the 3 categories were entered in the model as an ordinal variable. We used multiple logistic regression analysis to control for potential confounders, ie, age, sex, glucose tolerance category, known duration of DM of more than 10 years (in the present study a duration effect was seen after 10 years, which is in agreement with other studies), HbA₁c level, and hypertension. To exclude the possibility of residual confounding as thoroughly as possible, we also tested models that, in addition, included serum creatinine level, creatinine clearance, hypercholesterolemia, current smoking, body mass index, and/or fasting insulin level. Possible interaction between the tHcy level and DM for risk of retinopathy was assessed in a stratified analysis and with an interaction term. All analyses were performed using SPSS for Windows 93 version 7.5.2 (SPSS, Chicago, Ill). A 95% CI not including 1.0 was considered to indicate statistical significance.
(0.89-2.39) for hypercholesterolemia (yes/no), 1.09 (0.64-1.86) for current smoking (yes/no), and (among subjects with known DM) 7.31 (2.69-19.85) for a DM duration of more than 10 years.

After stratification by DM (yes/no) and adjustment for age, sex, HbA1c level, and hypertension, we observed a substantial difference between the 2 strata with regard to relative risk of retinopathy. The OR of retinopathy associated with hyperhomocysteinemia (>16 µmol/L) was 0.97 (95% CI, 0.42-2.82) in subjects without DM and 3.44 (95% CI, 1.13-10.42) in subjects with DM (P = .08 for interaction); after additional adjustment for serum creatinine clearance it was 1.01 (95% CI, 0.44-2.33) in subjects without DM and 3.33 (95% CI, 0.99-11.19) in subjects with DM, respectively. The results per category increment of serum tHcy level are shown in Table 2 and Figure 2 (P = .03 for interaction). This indicates that hyperhomocysteinemia is associated with retinopathy in subjects with DM but not in subjects without DM. After adjustment for age, sex, HbA1c level, and hypertension, the OR per 5-µmol/L increment of serum tHcy level was 0.89 (95% CI, 0.60-1.34) in subjects without DM and 1.50 (95% CI, 0.93-2.41) in subjects with DM. Additional adjustment for serum creatinine level, serum creatinine clearance, hypercholesterolemia, current smoking, body mass index, and/or fasting insulin level did not markedly change the results (data not shown).

To reduce the effect of possible misclassification, we repeated the analysis after classifying subjects with only hemorrhages in one or both eyes as having no retinopathy in an additional analysis. After adjustment for age, sex, and HbA1c level, the OR for hyperhomocysteinemia was 1.11 (95% CI, 0.46-2.68) in subjects without DM and 5.28 (95% CI, 1.67-16.67) in subjects with DM, and per 5-µmol/L increment of serum tHcy level it was 0.96 (95% CI, 0.62-1.48) and 1.64 (95% CI, 1.00-2.71), respectively. If diabetic retinopathy (see “Subjects and Methods” section) was taken as the dependent variable, the OR for hyperhomocysteinemia was 1.11 (95% CI, 0.36-3.41) in subjects without DM and 4.45 (95% CI, 1.21-16.37) in subjects with DM, and per 5-µmol/L increment of serum tHcy level it was 0.96 (95% CI, 0.55-1.66) and 1.45 (95% CI, 0.84-2.53), respectively. Additional adjustment of the previous analyses for hypertension or BP revealed similar results (data not shown). Among subjects with DM, after stratification for hypertension and adjustment for age and sex, the OR for hyperhomocysteinemia was 6.00 (95% CI, 0.37-96.80) among subjects with normotension and 4.11 (95% CI, 0.95-17.79) among subjects with hypertension. These ORs did not differ significantly from each other, which suggests that the association between hyperhomocysteinemia and retinopathy is independent of the presence of hypertension.

This population-based study indicates that hyperhomocysteinemia is associated with retinopathy in subjects with type 2 DM, independent of known determinants, ie, DM duration, glycemic level, and hypertension. We found a dose-response relation between the serum tHcy level and retinopathy among subjects with type 2 DM (Table 2). For each 5-µmol/L (about 1 SD) increment in serum tHcy level, the risk of retinopathy rose by about 50% (95% CI, −7 to 141) in the subjects with DM. Hyperhomocystein-

Figure 1. Prevalence of retinopathy according to the absence or presence of hyperhomocysteinemia (>16 µmol/L) in subjects with and without diabetes mellitus (DM). Error bars represent the upper half of the 95% confidence intervals.

Table 1. Characteristics of the Subjects*

<table>
<thead>
<tr>
<th></th>
<th>Subject With No Diabetic Retinopathy (n = 546)</th>
<th>Subjects With Diabetic Retinopathy (n = 79)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men, %</strong></td>
<td>49.1</td>
<td>41.8</td>
<td>.3</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>64.1 (7.3)</td>
<td>65.7 (6.7)</td>
<td>.06</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>27.1 (3.9)</td>
<td>28.0 (4.5)</td>
<td>.07</td>
</tr>
<tr>
<td><strong>Current smoker, %</strong></td>
<td>28.7</td>
<td>28.2</td>
<td>.9</td>
</tr>
<tr>
<td><strong>Blood pressure, mm Hg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>138 (19)</td>
<td>147 (22)</td>
<td>.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>82 (10)</td>
<td>85 (11)</td>
<td>.04</td>
</tr>
<tr>
<td><strong>Hypertension, %</strong></td>
<td>37.5</td>
<td>50.6</td>
<td>.03</td>
</tr>
<tr>
<td><strong>Glucose tolerance, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>47.1</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td>Impaired</td>
<td>27.3</td>
<td>25.3</td>
<td></td>
</tr>
<tr>
<td><strong>Newly diagnosed diabetes mellitus, %</strong></td>
<td>17.6</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td><strong>Known diabetes mellitus, %</strong></td>
<td>8.1</td>
<td>26.6</td>
<td>&lt;.001‡</td>
</tr>
<tr>
<td><strong>Duration of diabetes mellitus, y</strong></td>
<td>5.9 (2.0-9.5)</td>
<td>9.4 (4.3-16.2)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>Fasting glucose level, mMol/L</strong></td>
<td>6.5 (2.3)</td>
<td>7.8 (3.6)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Glycosylated hemoglobin, % hemoglobin</strong></td>
<td>5.8 (1.2)</td>
<td>6.6 (1.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Fasting insulin level, µMol/L</strong></td>
<td>83 (62-116)</td>
<td>93 (72-143)</td>
<td>.01</td>
</tr>
<tr>
<td><strong>Total serum cholesterol level, µMol/L</strong></td>
<td>6.6 (1.2)</td>
<td>6.8 (1.2)</td>
<td>.08</td>
</tr>
<tr>
<td><strong>Hypercholesterolemia, %</strong></td>
<td>53.5</td>
<td>63.3</td>
<td>.1</td>
</tr>
<tr>
<td><strong>Total serum homocysteine level, µMol/L</strong></td>
<td>11.5 (9.3-14.1)</td>
<td>11.1 (8.4-14.0)</td>
<td>.99</td>
</tr>
<tr>
<td><strong>Serum creatinine level, µMol/L</strong></td>
<td>91 (17)</td>
<td>94 (30)</td>
<td>.5</td>
</tr>
<tr>
<td><strong>Serum creatinine clearance, mL/min</strong></td>
<td>75 (17)</td>
<td>74 (19)</td>
<td>.6</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD), percentage of the total or median (interquartile range). Ellipsis indicates not applicable.
†Tested with t test or Wilcoxon rank sum test for continuous variables and Pearson χ² test for frequencies.
‡Test for trend.
§Diabetes duration since diagnosis of those subjects with known diabetes mellitus.
∥Estimated creatinine clearance.
zyme activity, leading to an exaggerated hyperhomocysteinemia, defined as a serum tHcy level of 16 µmol/L or higher, was also related to retinopathy among subjects with type 2 DM (OR, 3.4 [95% CI, 1.1-10.6]). This mutation in the methylenetetrahydrofolate reductase gene was found in 5% to 15% of the general population. Persons who are homozygous for this mutation appear to have impaired enzyme activity, leading to an exaggerated hyperhomocysteinemic response to a low intake of folic acid. In contrast, Agardh et al,32 Araki et al,33 and Smulders et al34 found no association between hyperhomocysteinemia and diabetic retinopathy in types 1 and 2 DM, respectively. However, none of these studies adjusted for the known determinants of retinal vasculopathy and one32 was rather small.

Diabetic retinopathy involves both morphologic and functional changes of the retinal capillaries.35,36 Hyperhomocysteinemia may induce endothelial dysfunction and injury followed by platelet activation and thrombus formation, possibly by increasing oxidative stress.37 Therefore, it is conceivable that hyperhomocysteinemia is causally related to retinal vasculopathy through changes of the retinal vasculature and formation of microthrombi. Since oxidative stress is thought to be increased in type 2 DM,38 this may make them more susceptible to hyperhomocysteinemia-induced oxidative damage.

We can think of 3 sources of disease misclassification that may have resulted in bias of the relation between hyperhomocysteinemia and retinopathy. Of 24% of all subjects, the fundus photographs were missing, and therefore, in these subjects the diagnosis of retinopathy was solely dependent on the ophthalmoscopic examination findings. There is evidence that the sensitivity to detect retinopathy by ophthalmoscopy, even in the hands of an experienced ophthalmoscopist, is lower than that of fundus photography using color or black-and-white transparencies.40-42 In addition, the method of detecting any retinopathy with 2 stereoscopic standard fields compared with 7 is slightly less sensitive (sensitivity about 0.85).42 Finally, a number of early small lesions may be missed on 45° fundus photographs compared with photographs taken with a smaller angle. All 3 limitations of the present study may have introduced false-negative disease misclassification, which was, in all likelihood, non-differential for the serum tHcy level. This would tend to underestimate the strength of the reported relation between hyperhomocysteinemia and retinopathy.

In an additional analysis we showed, independent of hypertension, a positive association between hyperhomocysteinemia and diabetic retinopathy, with the presence of
We are indebted to Wendy Guérard, Monique Meijers-Kuperus, and Petra van de Weg-Raaphorst for their excellent laboratory assistance.

Corresponding author: Ellen K. Hoogeveen, MD, Institute for Research in Extramural Medicine, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands (e-mail: ellenhv@casema.net).

REFERENCES


CONCLUSIONS

Hyperhomocysteinemia is associated with retinopathy among subjects with type 2 DM, but probably not in subjects without DM. If the finding of the present study can be confirmed in a prospective study, this may have implications for the clinical management of subjects with type 2 DM since the serum tHcy level can be lowered substantially with folic acid supplementation.47

Accepted for publication April 28, 2000.

This investigation was supported by a Clinical Research Fellowship from the Dutch Diabetes Research Foundation, Amersfoort, the Netherlands, and the Netherlands Organization for Scientific Research, The Hague, the Netherlands (Dr Stohouwer).

We are indebted to Wendy Guérard, Monique Meijers-Kuperus, and Petra van de Weg-Raaphorst for their excellent laboratory assistance.

Corresponding author: Ellen K. Hoogeveen, MD, Institute for Research in Extramural Medicine, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands (e-mail: ellenhv@casema.net).

REFERENCES


