Clinical research

Markers of inflammation in patients with coronary artery disease are also associated with glycosylated haemoglobin A\textsubscript{1c} within the normal range

Carl Gunnar Gustavsson\textsuperscript{a,*}, Carl-David Agardh\textsuperscript{b}

\textsuperscript{a} Department of Cardiology, University Hospital MAS, SE-20502 Malmö, Sweden
\textsuperscript{b} Department of Endocrinology, University Hospital MAS, SE-20502 Malmö, Sweden

Received 21 November 2003; revised 23 July 2004; accepted 3 September 2004
Available online 11 November 2004

Aims Diabetes is a risk factor for atherosclerosis and low-degree inflammation may play a central role in both diseases. Glycosylated haemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}) is an established measure of long-term glycaemic control but data on its correlation with markers of inflammation are limited, especially in patients with atherosclerotic manifestations. The aim of the present study was thus to investigate the associations between HbA\textsubscript{1c} and a panel of inflammation-sensitive parameters in patients with and without diabetes.

Methods and results This single centre cross-sectional study comprised 314 consecutive subjects who underwent coronary angioplasty for stable coronary artery disease. Sixty-six patients had diabetes mellitus. Haemoglobin A\textsubscript{1c} and markers of inflammation, i.e., plasma levels of CRP, fibrinogen, and albumin, erythrocyte sedimentation rate and white blood cell count were measured. All inflammation markers were altered in a more inflammatory direction in diabetic patients. Furthermore, when non-diabetic patients with HbA\textsubscript{1c} levels within the normal range were studied separately, all inflammation-sensitive parameters except albumin correlated significantly with HbA\textsubscript{1c}.

Conclusion In subjects with known coronary atherosclerosis, low-degree inflammatory activity is not only increased in diabetic patients, but also increased with increasing HbA\textsubscript{1c} in non-diabetic individuals with HbA\textsubscript{1c} within the normal range, i.e., at a pre-diabetic level of glucose metabolism derangement.

© 2004 The European Society of Cardiology. Published by Elsevier Ltd. All rights reserved.

Keywords
Inflammation; HbA\textsubscript{1c}; Atherosclerosis; Diabetes

Introduction

Inflammation has been suggested to play a central role in the development of atherosclerosis.\textsuperscript{1,2} Diabetes is not only a well known risk factor for atherosclerosis but is also associated with increased levels of sensitive markers of subclinical systemic inflammation.\textsuperscript{3–6} However, less data are available about the relationship between glycosylated haemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}), a measure of long term glycaemic control, and markers of inflammation. Wu et al.\textsuperscript{5} studied 5342 adult individuals who reported not having diabetes. In that study, elevated levels of C-reactive protein (CRP) were associated with higher HbA\textsubscript{1c} and insulin levels, and also with increased fasting glucose levels in women. Another study by Festa et al.\textsuperscript{6} found a
stronger association of CRP with post-challenge glycaemia than with fasting glucose but the study did not include HbA1c. Associations were also found between HbA1c and fibrinogen levels in patients with non-insulin-dependent diabetes and between HbA1c and white blood cell count. In addition, previous studies suggested that low-grade systemic inflammation is involved in the pathogenesis of type 2 diabetes and that a subclinical inflammatory reaction precedes the onset of type 2 diabetes.

The purpose of this study was therefore to investigate the associations between HbA1c and a panel of inflammation-sensitive parameters in patients with angiographically documented coronary artery disease with and without diabetes.

Methods

Patients

Patients considered for inclusion in the study were all 722 patients undergoing percutaneous transluminal coronary angioplasty (PTCA) for stable coronary artery disease at the University Hospital MAS, Malmö, Sweden from the start of the study 1st January 2000 until we had to close the study 3 1/2 years later for financial and practical reasons. To avoid a possible influence from inflammatory reactions due to previously performed coronary interventions, we excluded 398 patients with a history of PTCA or coronary artery bypass grafting surgery (CABG). Finally, additional ten patients were excluded due to missing HbA1c data. The study population then comprised 314 consecutive patients, out of whom 66 had diabetes. The diabetes diagnosis was based on previously known diabetes in 46 patients, and on HbA1c levels >5.3% in an additional 20 patients. The remaining 248 patients with HbA1c levels ≤5.3% were considered as non-diabetic. The proportions of patients with normal, impaired and diabetic fasting glucose levels in relation to case history and HbA1c is shown in Table 1. Patient characteristics are given in Table 2. Three patients had type 1 diabetes and the remaining 43 patients with previously diagnosed diabetes had type 2 diabetes (21 treated with insulin; eight in combination with oral antidiabetics, 16 with oral antidiabetics and the remaining six patients with diet only).

Blood sampling and analytical techniques

Venous blood samples were drawn on admission and were immediately sent to an accredited laboratory (Department of Clinical Chemistry at the University Hospital MAS, Malmö, Sweden) for analysis according to the clinical routine. High sensitive CRP was determined with a particle immunoassay rate methodology (Beckman Immage), albumin by means of a bichromatic methodology using brom cresol purple reagent (Beckman Synchron LX20), fibrinogen with a turbidimetric clotting rate method using

Table 1 Fasting glucose levels in relation to case history and HbA1c levels in 271 patients

<table>
<thead>
<tr>
<th></th>
<th>Without previously diagnosed diabetes</th>
<th>Known diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c ≤ 5.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal fasting glucose level &lt;5.6 mmol/L (n)</td>
<td>138</td>
<td>5</td>
</tr>
<tr>
<td>Impaired fasting glucose level 5.6–6.9 mmol/L (n)</td>
<td>71</td>
<td>6</td>
</tr>
<tr>
<td>Diabetic fasting glucose level ≥ 7.0 mmol/L (n)</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2 Demographic characteristics of patients with and without diabetes undergoing percutaneous transluminal coronary artery angioplasty

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (N = 66)</th>
<th>Non-diabetic patients (N = 248)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 (53, 71)</td>
<td>61 (53, 68)</td>
<td>0.9683</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>46/20</td>
<td>190/58</td>
<td>0.2478</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.8 (25.4, 30.9)</td>
<td>26.6 (24.3, 28.4)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Current smoking (yes/no; n)</td>
<td>14/52</td>
<td>55/193</td>
<td>0.8663</td>
</tr>
<tr>
<td>Coronary angiography (n)</td>
<td>3-vessel disease</td>
<td>15</td>
<td>0.2221</td>
</tr>
<tr>
<td></td>
<td>2-vessel disease</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-vessel disease</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>140 (120, 156)</td>
<td>140 (125, 150)</td>
<td>0.7815</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 (70, 82)</td>
<td>80 (70, 85)</td>
<td>0.7811</td>
</tr>
<tr>
<td>Anti-hypertensive drug therapy (yes/no; n)</td>
<td>37/29</td>
<td>87/161</td>
<td>0.0019</td>
</tr>
<tr>
<td>Acetylsalicylic acid therapy (yes/no; n)</td>
<td>50/16</td>
<td>233/15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Statin therapy (yes/no; n)</td>
<td>53/13</td>
<td>184/64</td>
<td>0.3052</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.300 (3.590, 5.335)</td>
<td>4.515 (3.945, 5.213)</td>
<td>0.3371</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.600 (1.900, 3.300)</td>
<td>2.600 (2.200, 3.200)</td>
<td>0.2860</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.090 (0.960, 1.220)</td>
<td>1.125 (0.930, 1.380)</td>
<td>0.2595</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.500 (0.975, 1.960)</td>
<td>1.300 (0.900, 1.853)</td>
<td>0.2595</td>
</tr>
</tbody>
</table>

Data are given as median (25th percentile, 75th percentile) and number of patients.
thrombin as initiator (Behring BCS) and HbA1c with an HPLC-method (Bio-Rad Variant II). White blood cells were counted on a Coulter Gen S counter.

The detection limits and interassay co-efficients of variation (mean concentrations) of these methods were: CRP 0.3 mg/L and 0.7 g/L and 3.6% (1.2 g/L) and 2.4% (4.8 g/L), respectively. The interassay co-efficients of variation (mean concentrations) for fibrinogen were 2.1 (0.9, 4.6) and 1.4% (27.0 g/L) and 1.4% (48.5 g/L), and for fibrinogen and HbA1c with an HPLC-reference method. HbA1c according to the Swedish standard was about 0.8% lower than HbA1c according to the US standard.

### Statistical analysis

The commercial program SPSS for Windows, Release 6.1 (SPSS Inc., Chicago, IL, USA) was used for the analyses. All tests were two-sided and p values < 0.05 were considered statistically sig-
Results

The diabetic patients had a higher BMI than non-diabetic patients whereas the age distribution was rather similar in both groups and there were no differences regarding gender, smoking, extent of coronary artery disease or systolic and diastolic blood pressure (Table 2). Anti-hypertensive drug therapy was however more common in diabetic patients and acetylsalicylic acid therapy more common in non-diabetic individuals. There were no differences regarding statin therapy or lipid concentrations. All five inflammation-sensitive parameters were altered in an inflammatory direction in the diabetic patients (Table 3), but the fibrinogen concentration difference did not reach statistically significance when only the subgroup with previously diagnosed diabetes was compared to non-diabetic patients. When all patients were taken together we found highly significant correlations between HbA1c and all inflammation-sensitive parameters (Table 4). However, HbA1c also correlated with age and BMI which in turn both correlated with some of the inflammation-sensitive parameters. We thus performed a partial correlation analysis controlling for age and BMI. Additional potential confounders included in that analysis were smoking, number of stenotic coronary arteries and drug treatment with acetylsalicylic acid, statins and anti-hypertensive drugs. In the non-diabetic patients, this analysis again showed significant correlations between HbA1c and the inflammation-sensitive parameters CRP, fibrinogen, erythrocyte sedimentation rate and white blood cell count, which were all altered in an inflammatory direction (Table 5). In contrast, no correlations were found in the diabetic patients and the calculated correlation co-efficients were generally lower in this portion of the patient material. We also analysed the material for possible influence from drugs for diabetes but could not find any such associations (data not shown).

Discussion

In this study of patients with well documented coronary artery disease, markers of inflammation were higher in patients than without diabetes. This is in accordance with several previous studies reporting increased inflammatory activity in diabetic patients. All inflammation-sensitive proteins were changed in an inflammatory direction, i.e., plasma levels of CRP and fibrinogen were increased and albumin reduced. Erythrocyte sedimentation rate, which largely depends on fibrinogen and albumin concentrations, was also increased in the diabetic patients and, similar to previous studies, white blood cell count was higher.

Few studies have addressed the possible relationships between HbA1c and markers of inflammation, especially in patients with coronary artery disease. In our patients without diabetes, all markers of inflammation, except for plasma albumin were associated with HbA1c. There was also a non-significant tendency towards lower albumin concentrations with increasing HbA1c-levels, i.e., an alteration of this parameter also in an inflammatory direction. Our interpretation is that even a lower degree of derangement in glucose metabolism, but still with HbA1c within the normal range, is associated with increased inflammatory activity in these patients.

All our patients had documented coronary artery disease, which in several studies has been associated with increased concentrations of CRP and fibrinogen, lower plasma albumin concentrations, and higher white blood cell counts. Thus, in patients traditionally considered prone to have an increased inflammatory activity, a superimposition by a subclinical derangement in glucose metabolism might be of importance.

In the diabetic patients, no inflammatory marker correlated with HbA1c. This is in contrast to a study by Bruno et al., who reported a correlation between HbA1c and fibrinogen levels in 1574 patients with non-insulin-dependent diabetes. Our material was possibly underpowered in this respect but the generally lower correlation co-efficients in the diabetic patients indicate...
that probably also pathophysiological differences between diabetic and non-diabetic patients are involved. In a study by King et al., the likelihood of elevated CRP increased with increasing HbA1c, but only in patients with an HbA1c level above 9%. Thus, the different results could also be due to different levels of glycaemia, with a median HbA1c of 6.1% in the diabetic patients in our study. Also, in the study of King et al., a more crude method for measurement of CRP was used in contrast to the highly sensitive method used in this study.

Further support for the present hypothesis is the finding that insulin resistance (IRS) is associated with chronic subclinical inflammation, and both conditions are linked with increased risk for type 2 diabetes and atherosclerotic vascular disease. In 1008 non-diabetic individuals with no clinical coronary artery disease, Festa et al. found CRP, fibrinogen and white blood cell counts to be associated with several components of the IRS. This is also supported by the results from the Women’s Health Study, in which CRP was found to be independently associated with fasting hyperinsulinæmia in non-diabetic women. In a previous study of apparently healthy middle-aged women, CRP and IL-6 were found to be determinants of risk for type 2 diabetes, especially CRP after adjustment for obesity. Similar results were found in women with a baseline HbA1c of 6% or less. However, in that study, the four-year cardiovascular event rate was low. Thus, it seems that the relation between development of atherosclerosis and markers of inflammation, and the diabetic state may differ in its development.

For practical reasons, it was not possible to include oral glucose tolerance testing (OGTT) in the present study. However, a previous study by Norhammar et al. showed good correlation between HbA1c at hospitalisation for myocardial infarction and OGTT three months later using the same core laboratory and the same upper limit of normal for HbA1c as Arch Int Med.

In summary, the present study confirms that diabetic patients with macrovascular disease have increased levels of markers of inflammation, but also adds new information on a relationship between these markers and HbA1c within the normal range, indicating an early association between degree of glycaemia, inflammation and atherosclerosis prior to the development of diabetes.

Acknowledgements

We are indebted to Dr. Johan Malm for detailed descriptions of the analytical methods and to Mr. Jan-Åke Nilsson for statistical advice.

References