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

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Aims Diabetes is a risk factor for atherosclerosis and low-degree inflammation may play a central role in both diseases. Glycosylated haemoglobin A_{1c} (HbA_{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_{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_{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_{1c} levels within the normal range were studied separately, all inflammation-sensitive parameters except albumin correlated significantly with HbA_{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_{1c} in non-diabetic individuals with HbA_{1c} within the normal range, i.e., at a pre-diabetic level of glucose metabolism derangement.

Introduction

Inflammation has been suggested to play a central role in the development of atherosclerosis.[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.[1–4] However, less data are available about the relationship between glycosylated haemoglobin A_{1c} (HbA_{1c}), a measure of long term glycaemic control, and markers of inflammation. Wu et al.[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_{1c} and insulin levels, and also with increased fasting glucose levels in women. Another study by Festa et al.[6] found a

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stronger association of CRP with post-challenge glycaemia than with fasting glucose but the study did not include HbA_{1c}. Associations were also found between HbA_{1c} and fibrinogen levels in patients with non-insulin-dependent diabetes[7,8] and between HbA_{1c} and white blood cell count.[9] 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.[10–13]

The purpose of this study was therefore to investigate the associations between HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} levels >5.3% in an additional 20 patients. The remaining 248 patients with HbA_{1c} levels $\leq$5.3% were considered as non-diabetic.[14] The proportions of patients with normal, impaired and diabetic fasting glucose levels[15] in relation to case history and HbA_{1c} 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 HbA_{1c} levels in 271 patients

	Without previously diagnosed diabetes		Known diabetes
	$HbA_{1c} \leqslant 5.3\%$	$HbA_{1c} > 5.3\%$	
Normal fasting glucose level <5.6 mmol/L (*n*)	138	5	3
Impaired fasting glucose level 5.6–6.9 mmol/L (*n*)	71	6	7
Diabetic fasting glucose level $\geqslant$ 7.0 mmol/L (*n*)	4	6	31

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

	Diabetic patients (*N* = 66)	Non-diabetic patients (*N* = 248)	*p* value
Age (years)	61 (53, 71)	61 (53, 68)	0.9683
Male/female (*n*)	46/20	190/58	0.2478
Body mass index (kg/m^2)	27.8 (25.4, 30.9)	26.6 (24.3, 28.4)	0.0007
Current smoking (yes/no; *n*)	14/52	55/193	0.8663
Coronary angiography (n)			
3-vessel disease	15	49	0.2221
2-vessel disease	30	91	
1-vessel disease	21	108	
Systolic blood pressure (mmHg)	140 (120, 156)	140 (125, 150)	0.7815
Diastolic blood pressure (mmHg)	78 (70, 82)	80 (70, 85)	0.7811
Anti-hypertensive drug therapy (yes/no; *n*)	37/29	87/161	0.0019
Acetylsalicylic acid therapy (yes/no; *n*)	50/16	233/15	<0.0001
Statin therapy (yes/no; *n*)	53/13	184/64	0.3052
Total cholesterol (mmol/L)	4.300 (3.590, 5.335)	4.515 (3.945, 5.213)	0.3371
LDL-cholesterol (mmol/L)	2.600 (1.900, 3.300)	2.600 (2.200, 3.200)	0.2860
HDL-cholesterol (mmol/L)	1.090 (0.960, 1.220)	1.125 (0.930, 1.380)	0.2595
Triglyceride (mmol/L)	1.500 (0.975, 1.960)	1.300 (0.900, 1.853)	0.2595

Data are given as median (25th percentile, 75th percentile) and number of patients.

Table 3 HbA_{1c} and markers of inflammation in diabetic and non-diabetic patients

	Non-diabetic patients with $HbA_{1c} \leqslant 5.3\%$ ($N = 248$)	Diabetic patients — known diabetes or $HbA_{1c} > 5.3\%$ ($N = 66$)	p value for the comparison to non-diabetic patients	Patients with previously known diabetes separately ($N = 46$)	p value for the comparison to non-diabetic patients
HbA_{1c} (%)	4.6 (4.4, 4.9)	6.1 (5.6, 7.1)	<0.0001	6.8 (6.0, 7.6)	<0.0001
P-C-reactive protein (mg/L)	2.1 (0.9, 4.6) ($N = 234$)	4.4 (2.3, 7.1) ($N = 63$)	<0.0001	4.7 (2.3, 7.2) ($N = 44$)	0.0001
P-fibrinogen (g/L)	3.3 (2.7, 3.8) ($N = 236$)	3.7 (2.8, 4.4) ($N = 64$)	0.0495	3.6 (3.0, 4.4) ($N = 45$)	0.0663
P-Albumin (g/L)	39 (37, 40) ($N = 239$)	38 (36, 40) ($N = 63$)	0.0206	38 (36, 40) ($N = 44$)	0.0311
Erythrocyte sedimentation rate (mm/hour)	8 (4, 14) ($N = 241$)	10 (8, 22) ($N = 63$)	0.0001	10 (8, 22) ($N = 43$)	0.0004
White blood cell count ($\times 10^9$/L)	7.0 (5.9, 8.4) ($N = 248$)	7.7 (6.6, 9.1) ($N = 66$)	0.0101	7.6 (6.5, 8.9) ($N = 46$)	0.0331

Values are given as median (25th percentile, 75th percentile) together with number of patients.

thrombin as initiator (Behring BCS) and HbA_{1c} 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 6.7% (0.76 mg/L) and 4.3% (2.03 mg/L), for albumin 10 g/L and 1.5% (27.0 g/L) and 1.4% (48.5 g/L), and for fibrinogen 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 HbA_{1c} are 2.4% (4.7%) and 1.8% (8.8%) and for white blood cell count 2.9% (3.41 times 10^9/L) and 1.2% (19.9 times 10^9/L).

The upper limit of normal for HbA_{1c} was considered to be 5.3%.[14,16] This apparently rather low level is in accordance with the Swedish national standard. In a recent publication, HbA_{1c} data from our hospital, which is representative of this standard, were compared with the IFCC Reference Method.[17] Hospitals representative of the US and the Japanese national standards also participated in that study. All three national standards correlated well with the IFCC Reference Method ($r^2 = 0.998$—0.996) but they all produced values at different levels above the reference method. HbA_{1c} according to the Swedish standard was about 0.8% lower than HbA_{1c} 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-

Table 4 Spearman's rank correlation's (r) between HbA_{1c}, age, body mass index and inflammation sensitive parameters in the total material

	HbA_{1c}	Age	Body mass index	P-C-reactive protein	P-fibrinogen	P-albumin	Erythrocyte sedimentation rate
Age	0.1278 $P = 0.024$ ($N = 314$)						
Body mass index	0.1563 $p = 0.006$ ($N = 314$)	−0.1349 $p = 0.017$ ($N = 314$)					
P-C-reactive protein	0.2804 $p < 0.001$ ($N = 297$)	0.0250 $p = 0.668$ ($N = 297$)	0.1908 $p = 0.001$ ($N = 297$)				
P-fibrinogen	0.1983 $p = 0.001$ ($N = 300$)	0.1043 $p = 0.071$ ($N = 300$)	0.1014 $p = 0.080$ ($N = 300$)	0.5805 $p < 0.001$ ($N = 285$)			
P-albumin	−0.2082 $p < 0.001$ ($N = 302$)	−0.3403 $p < 0.001$ ($N = 302$)	−0.0573 $p = 0.321$ ($N = 302$)	−0.2689 $p < 0.001$ ($N = 288$)	−0.1561 $p = 0.008$ ($N = 288$)		
Erythrocyte sedimentation rate	0.3146 $p < 0.001$ ($N = 304$)	0.2208 $p < 0.001$ ($N = 304$)	0.0517 $p = 0.369$ ($N = 304$)	0.4279 $p < 0.001$ ($N = 290$)	0.6144 $p < 0.001$ ($N = 290$)	−0.2783 $p < 0.001$ ($N = 293$)	
White blood cell count	0.1817 $p = 0.001$ ($N = 314$)	−0.1612 $p = 0.004$ ($N = 314$)	0.1220 $p = 0.031$ ($N = 314$)	0.3535 $p < 0.001$ ($N = 297$)	0.3530 $p < 0.001$ ($N = 300$)	−0.0928 $p = 0.108$ ($N = 302$)	0.1823 $p = 0.001$ ($N = 304$)

Table 5 Relationship between HbA_{1c} and the inflammation sensitive study parameters

	Diabetic patients	Non-diabetic patients
P-C-reactive peptide	0.1558	0.1621
	$p = 0.251$	$p = 0.014$
P-fibrinogen	0.1174	0.1480
	$p = 0.384$	$p = 0.025$
P-albumin	0.0740	−0.0904
	$p = 0.588$	$p = 0.170$
Erythrocyte sedimentation rate	0.0900	0.1853
	$p = 0.510$	$p = 0.004$
White blood cell count	0.0335	0.1632
	$p = 0.801$	$p = 0.011$

Partial Spearman correlation co-efficients controlling for age, body mass index, smoking, anti-hypertensive treatment, treatment with acetylsalicylic acid and statins and number of stenotic coronary arteries.

nificant. Comparisons between groups were performed using the χ^2 test for categorical parameters and the Mann—Whitney *U* test for continuous parameters, as several study parameters (especially CRP and erythrocyte sedimentation rate) were not normally distributed. Spearman rank correlation and partial Spearman rank correlation tests were used for correlation analyses.

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 HbA_{1c} and all inflammation-sensitive parameters (Table 4). However, HbA_{1c} 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 potentional 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 HbA_{1c} 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 with than without diabetes. This is in accordance with several previous studies[1–4] 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.[3]

Few studies have addressed the possible relationships between HbA_{1c} 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 HbA_{1c}. There was also a non-significant tendency towards lower albumin concentrations with increasing HbA_{1c}-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 HbA_{1c} 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[1,2,18] and fibrinogen,[1,18] lower plasma albumin concentrations, [18] and higher white blood cell counts.[18] 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 HbA_{1c}. This is in contrast to a study by Bruno et al., who reported a correlation between HbA_{1c} and fibrinogen levels in 1574 patients with non-insulin-dependent diabetes.[7] 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.,[19] the likelihood of elevated CRP increased with increasing HbA_{1c}, but only in patients with an HbA_{1c} level above 9%. Thus, the different results could also be due to different levels of glycaemia, with a median HbA_{1c} 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.[20] This is also supported by the results from the Women's Health Study, in which CRP was found to be independently associated with fasting hyperinsulinaemia in non-diabetic women.[21] 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 HbA_{1c} of 6% or less.[10] 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.[16] showed good correlation between HbA_{1c} at hospitalisation for myocardial infarction and OGTT three months later using the same core laboratory and the same upper limit of normal for HbA_{1c} as our study.

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 HbA_{1c} within the normal range, indicating an early association between degree of glycaemia, inflammation and atherosclerosis prior to the development of diabetes.

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