Carotid imaging in cardiovascular risk assessment

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CHAPTER 7

Vascular Phenotype and Subclinical Inflammation in Diabetic Asian Indians Without Overt Cardiovascular Disease

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ABSTRACT

Although Asian Indian (AI) patients with diabetes mellitus type 2 (DM2) are at high risk for cardiovascular disease (CVD), not all patients develop CVD. The vascular phenotype of AI-DM2 without CVD has not been elucidated and may point to protective features. Using baseline data from a clinical trial we provide an initial description of vascular parameters in AI-DM2 compared to European Caucasian controls (ECs) matched for age and gender. Endpoints of the study were endothelial function, low-grade systemic inflammation (CRP), and carotid intima-media thickness (cIMT).

AIs had longer duration of diabetes, worse glycemic control and more microangiopathy. Both groups demonstrated marked endothelial dysfunction. CRP levels were similar: 1.7 (4.9) mg/L in AIs and 2.8 (3.6) mg/L in ECs. cIMT values were significantly lower in AI-DM2 than EC-DM2 (0.655mm (0.12) vs. 0.711mm (0.15), p=0.03). Multiple regression analysis showed that variability in CRP was mainly determined by waist circumference, not by ethnicity. In contrast, ethnicity was a significantly explanatory variable for cIMT.

Vascular phenotype of AI-DM2 without CVD was characterized by endothelial dysfunction and relatively low levels of CRP, comparable to EC-DM2 controls. In contrast, lower cIMT values were observed in AI-DM2 despite longer duration of diabetes and worse metabolic control. We propose that mechanisms slowing its progression may have atheroprotective potential in AI-DM2.

INTRODUCTION

In the Netherlands a large community from the former Dutch colony of Surinam, originally of Asian Indian (AI) descent, has settled. Epidemiologic data suggest that the excess of type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD) noted in AI populations across the world [1] [2] is also present in AIs in the Netherlands [3] [4]. An important pathogenic factor is the high prevalence of insulin resistance and DM2 in AIs. However, traditional risk factors do not fully explain the excess of CVD [5]. Several other risk factors such as low grade systemic inflammation [6] [7] and endothelial dysfunction [8] [9] have been proposed to contribute to initiation and progression of atherosclerosis in AIs.

Despite the well-established high cardiovascular risk, not all AI-DM2 develop CVD. The vascular phenotype of AI-DM2 without CVD has not been elucidated and may point to protective features regarding the development of CVD. We aimed to provide a first evaluation of vascular parameters in AIs and matched EC controls with DM2 but without CVD.

MATERIALS AND METHODS

Subjects

This study is a substudy of a previously reported randomized clinical trial. The study design and results of which have been described elsewhere[10-12]. Using this database, we were able to identified 48 subjects of AI descent and 48 EC subjects from the same cohort matched for age and gender. There were no differences in demographics between the two groups, both living in an urban area in the Netherlands. The predecessors of the AI population migrated from India to Surinam starting 1873. Most of our study subjects were first generation immigrants in this country and third or fourth generation out of India. Patients were eligible for the study if they had been diagnosed with DM2 for at least 1 year, aged 30-80 years and without CVD. CVD was defined as angina pectoris, clinically manifest coronary artery disease, ECG criteria for a past myocardial infarction, ischemic stroke, peripheral artery bypass surgery, percutaneous transluminal angioplasty, or amputation because of atherosclerotic disease. Patients with marked dyslipidemia (fasting total cholesterol >6.9 mmol/l or triglycerides >6.0

mmol/l) were excluded from the original population, as prior statin therapy was an exclusion criterion in the clinical trial. Eligible patients gave their written informed consent. The study was approved by the hospital's Medical Ethics Committee.

Endpoints

The endpoints of this study were differences in inflammatory markers (serum C-reactive protein (CRP) and fibrinogen levels), endothelial function (as estimated using measurement of flow mediated dilatation (FMD)) and cIMT as a non-invasive measure of atherosclerosis. Furthermore, presence and risk of coronary atherosclerosis was assessed using measurement of silent coronary ischemia (ambulatory ECG) and UKPDS risk scores for CVD.

Clinical Examination

Anthropometric measurements were performed by two observers using standardized methods. Waist circumference (WC) was measured midway between the iliac crest and the lowest costal margin at the end of normal expiration; hip circumference (HC) was measured at the maximal circumference at the level of the femoral trochanters. Blood pressure was measured using a standard sphygmomanometer after a 10 minute resting period in supine position. Hypertension was defined as systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg. The presence or absence of retinopathy was determined from the subject's medical files, wherein reports from ophthalmologists were retrieved.

Laboratory Investigations

Lipid and safety measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to ISO 15189 standard procedures. Blood samples were collected after an overnight fast. A urine sample was collected for the determination of the albumin over creatinine ratio. Serum or plasma was isolated by centrifugation at 2900 rpm for 5 minutes. Levels of total cholesterol and triglycerides were measured by enzymatic methods on a Synchron LX20-analyzer (Beckman Coulter, Brea, USA). LDL-cholesterol was calculated according to the Friedewald formula[13]. If triglycerides were above 4.5 mmol/l, LDL-cholesterol was measured directly with the use of a reagent kit (Genzyme Diagnostics). HDL-cholesterol levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins. Creatinine kinase and alaninaminotransferase were measured by an enzymatic rate method on a Synchron LX20 multichannel chemistry analyzer, according to IFCC-methods. HbA1c was measured by HPLC on a Variant II (BioRad, USA). For the urine sample, a Jaffé rate method was used for the measurement of creatinine on a Synchron LX20-analyzer, while albumin was measured by rate nephelometry. Presence of microalbuminuria was defined as >2.5 gram albunim/mol creatinine for men and >3.5 gram albumin/mol creatinine for women.

The high-sensitivity CRP assay was performed in the Leiden University Medical Center with the Tinaquant CRP (latex) high-sensitive assay from Roche. This particle enhanced immunoturbidimetric assay was carried out on a Roche Module P using serum.

CVD risk scores, aECGs and Metabolic Syndrome criteria

Absolute 10-year risk scores for developing a cardiovascular event were calculated using the UKPDS risk engine version 2.0 [14]. For patients using anti-hypertensive medication the systolic blood pressure was arbitrarily set at 160 mmHg. The aECG registration and analysis were conducted as previously described [15]. Criteria for the presence of the metabolic syndrome (MS) were according to the European Group for the Study of Insulin Resistance modification of the WHO guidelines[16,17]: presence of DM2 (per definition in our population), and 2 or more of the following characteristics: waist circumference ≥ 94cm in males and ≥ 80cm in females; triglycerides > 1.7 mmol/L; HDL-cholesterol < 0.9 mmol/L in males and < 1.0 mmol/L in females; blood pressure ≥140/≥90 mmHg.

Ultrasound protocol

Ultrasound imaging was performed with an Acuson Aspen scanner with a linear array 7.5 MHz probe. For FMD, an optimal longitudinal image of the brachial artery at, or just above the elbow, was established and kept stable using a specially designed fixative. The exact FMD protocol was described earlier [12]. For cIMT, all images were recorded digitally for off line, blinded, analysis by an independent core laboratory, Heartcore, Leiden, the Netherlands as described previously [10]. Briefly, the left and right distal 1.0 cm of the common carotid arteries, near and far walls, was examined longitudinally in the angle resulting in an optimal and maximal cIMT (while avoiding plaques). For each segment, three

R-wave triggered images were stored. Mean cIMT was measured, when possible, over the entire 1 cm of the vessel segment. Mean common cIMT was obtained by averaging the mean IMTs of far and near wall, left and right.

STATISTICAL ANALYSIS

All binary data were analyzed using the Pearson chi-square test. All continuous outcome data were significantly skewed and therefore analyzed using the non-parametric Mann-Whitney test or log transformed (hsCRP, lp(a)) before being analyzed using the student t-test. Values are reported as medians (IQR). P-values <0.05 were considered statistically significant. Correlations were calculated with the Spearman's rank test. To test the impact of correlated parameters on the variability of the outcome variables a stepwise regression analysis was performed.

RESULTS

Patient characteristics are given in table 1. Despite similar age distribution AIs had a significantly longer duration of diabetes (12.4yrs vs. 6.3yrs; p < 0.001) and worse glycemic control as shown by higher median HbA1c levels (7.85% vs. 7,20%; p=0.006). Microangiopathy was observed more frequently in AIs, as shown by elevated prevalence of retinopathy (29% vs. 6%; p=0.003) and higher level of microalbuminuria (1.3mg/L vs. 0.6mg/L; p=0.009).

Cardiovascular risk and anthropometry

Smoking was less prevalent in AIs compared to ECs, both at present and ex-smokers. No significant differences were observed in hypertension (defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or the use of antihypertensive medication) and family history of CVD in first degree relatives. Lipid parameters including plasma HDL-C levels were comparable in both ethnic groups. Lp(a) was significantly different between the groups (215.5mg/dL (410) in AIs vs. 95.0mg/dL (316) in ECs (p=0.02)). The UKPDS risk scores for myocardial infarction were found to be 14.9% /10years in AIs vs. 10.5% /10 years in ECs (p=NS).

The anthropometric data are summarized in table 2. AIs were significantly

Table 1 | Patient characteristics and Laboratory findings

	Asian Indians (n=48)	European Caucasians (n=48)	p-values
male gender	20 (42%)	20 (42%)	1.0
age (yrs)*	50.7 (8.6)	50.9 (7.6)	0.89
diabetes duration (yrs)*	12.4 (8.2)	6.3 (5.4)	<0.001
HbA1c (%)	7.85 (1.9)	7.20 (1.7)	0.006
retinopathy	14 (29%)	3 (6%)	0.003
microalbunimuria	14 (29%)	8 (17%)	0.15
microalbuminuria† (mg/l)	1.3 (7.7)	0.6 (1.1)	0.009
family history of CVD	16 (33%)	13 (27%)	0.51
hypertension	23 (48%)	19 (40%)	0.41
Smokers	20 (42%)	31 (65%)	0.024
UKPDS (%/10yrs)	14.9 (14.1)	10.5 (13.7)	0.29
creatinine (μmol/l)	80.0 (30)	76.0 (19)	0.32
Clearance (ml/min)	81.2 (34.1)	101.9 (29.7)	<0.001
total cholesterol (mmol/l)	5.2 (1.1)	5.5 (1.1)	0.26
HDL-cholesterol	1.1 (0.4)	1.2 (0.5)	0.26
LDL-cholesterol	3.3 (1.4)	3.5 (1.3)	0.83
triglycerides (mmol/l)	1.6 (1.1)	1.7 (1.2)	0.51
lipoprotein(a)† (mg/dL)	215.5 (410)	95.0 (316)	0.02
fibrinogen (g/L)	3.6 (1.9)	3.2 (1.3)	0.88
hsCRP† (mg/L)	1.7 (4.9)	2.8 (3.6)	0.83
hsCRP≥3.0mg/L	14 (29%)	18 (38%)	0.39

All continuous data are expressed in medians (IQR) and compared using non-parametric test (Mann-Whitney) except:
*Data were normally distributed and expressed in means (sd), compared using student t-test
†Data were compared after log transformation using student t-test

smaller and lighter. EC women had higher values for WC and HC, as well as higher average BMI as compared to AI women. In men no differences were observed regarding these parameters. The MS score was fully comparable in the two groups, and did not change using ethnicity-specific cut-off values as recently proposed by the International Diabetes Federation (data not shown).

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Inflammation, endothelial function and vascular parameters

No differences were observed between the groups for low-grade chronic inflammation as assessed by CRP. The median value was 1.7 mg/L (4.9) in AIs vs. 2.8 mg/L (3.6) in ECs (p=0.83). In addition the number of subjects with evidence of low-grade inflammation (defined as CRP levels ≥ 3 mg/L and < 15mg/L) did not significantly differ between the groups and was 14 (29%) in AIs and 18 (38%) in ECs (p=0.39). Median serum levels of fibrinogen were comparable (3.6 g/L (1.9) in AIs and 3.2 g/L (1.3) in ECs; p=0.88).

Endothelial dysfunction was observed in both groups with FMD levels under 2% (table 2) but comparable between AIs and ECs. In both groups 9 subjects (19.1%) had abnormal findings on their aECG suggesting silent ischemia. These 18 subjects had comparable values of IMT (0.730mm (0.18) vs. 0.680mm (0.15) in subjects with normal aECGs, p=0.472). Further analysis of these subjects revealed no ethnic difference for the number of ischemic episodes, the duration of ischemia or the ischemic burden (data not shown).

AIs were found to have significantly lower median cIMT values of 0.655mm (0.12) compared to ECs (0.711mm (0.15); p=0.03). Luminal diameter was not a predetermined endpoint, but was assessed in 66 cases (35 ECs and 31 AIs). In this subset AIs had smaller lumina (7.294mm (1,12) vs 7.770mm (1.05) in ECs; p=0.02).

In a stepwise regression analysis logCRP, IMT, FMD and logLp(a) were entered as dependent variables (table 3). LogLp(a) was included because it has consistently been found to be high in AI patients. Co-variables that were taken into account were: age, race, duration of diabetes, HbA1c, smoking status, WC, LDL- and HDL-cholesterol, triglycerides and systolic BP. FMD was impacted by age only (r^2=0.05; β=-0.01; p=0.003). The strongest determinant of variance in Lp(a) levels was race (r^2=0.11; β=0.37; p=0.003). WC had the greatest impact on variance in CRP levels (r^2=0.24; β=1.24; p=0.001) and race did not contribute significantly. Finally, age and race explained variance in cIMT (r^2=0.13; β=0.004; p=0.005 for age and β=-0.47; p=0.047 for race).

CONCLUSIONS

In this study we observed, for the first time, a low cIMT in AI-DM2 patients without CVD, compared to matched EC counterparts. Low cIMT was present despite longer duration of diabetes and worse glycemic control, the significance of the

Table 2 | Anthropometry and Vascular parameters.

	Asian Indians (n=48)	European Caucasians (n=48)	p-values
height (cm)			
♂	167.5 (12.0)	180.0 (11.0)	<0.001
♀	156.5 (8.0)	164.5 (9.0)	<0.001
weight (kg)			
♂	77.0 (10.0)	87.0 (21.0)	0.003
♀	76.0 (18.0)	96.0 (28.0)	0.001
body mass index (kg/m^2)			
♂	27.1 (5.2)	27.2 (4.9)	0.98
♀	30.8 (7.0)	34.3 (8.9)	0.016
waist circumference (cm)			
♂	97.5 (13.0)	98.0 (15.0)	0.83
♀	100.0 (19.0)	108.5 (20.0)	0.045
hip circumference (cm)			
♂	97.5 (8.0)	102.5 (8.0)	0.42
♀	101.5 (11.0)	109.5 (19.0)	0.001
waist/hip ratio			
♂	1.0 (0.09)	0.99 (0.10)	0.33
♀	0.98 (0.13)	0.99 (0.12)	0.87
metabolic syndrome	33 (69%)	37 (77%)	0.36
MS score*	2.10 (1.1)	2.13 (0.87)	0.92
FMD (%)	1.56 (2.5)	1.88 (2.8)	0.44
cIMT (mm)	0.655 (0.12)	0.711 (0.15)	0.03
abnormal aECG	9/47 (19.1%)	9/47 (19.1%)	1.0

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Table 3 | Multiregression analysis

	age	race	waist circumference	model p-value
cIMT (r^2=0.15)	β=0.004 *(p=0.005)*	B=-0.47 *(p=0.047)*	-	0.003
FMD (r^2=0.05)	β=-0.01 *(p=0.045)*	-	-	0.045
log CRP (r^2=0.16)	β=-0.009 *(p=0.136)*	B=0.055 *(p=0.589)*	β=1.24 *(p=0.001)*	0.004
Log Lp(a) (r^2=0.11)	β=0.002 *(p=0.799)*	B=0.371 *(p=0.003)*	β=0.552 *(p=0.137)*	0.035

latter being illustrated by increased measures of microangiopathy in AI-DM2. Longer duration of diabetes and increased prevalence of microalbuminuria are in line with previous publications on AIs in Netherlands, and elsewhere [18] [19]. In addition, the high Lp(a) levels observed in AIs have been previously reported [19]. Thus, the low cIMT is a new and intriguing finding and it was found in a population with very similar characteristics to these earlier reports with one exception: absence of overt CVD, despite presence of DM2 in our study population.

Previous studies have suggested that endothelial function may be more vulnerable in AIs than in ECs and thus contributes to the development of atherosclerosis [20]. In our population of DM2 patients without CVD both ethnic groups exhibited endothelial dysfunction and we could not demonstrate ethnic differences.

CRP is a cardiovascular risk indicator with additional predictive power to the Framingham risk scores [21]. CRP levels were found to be higher in AI migrants compared to native populations in several [7,22] but not all [23] studies. We observed intermediate values of CRP in AI-DM2 and EC-DM2 with medians of respectively 1.7 and 2.8 mg/L. It could be hypothesized that an attenuated individual inflammatory response could be part of a protective phenotype, thus being in line with epidemiologic data relating CRP to CVD. The intermediate CRP levels were observed in AI men and EC men with similar WCs. Using the recent ethnicity specific cut-off values for WC, AI-DM2 patients had a more outspoken abdominal obesity compared to EC-DM2. As several reports in literature link central and overall adiposity to CRP levels in AIs [7,22] [24-27], we expected higher CRP levels in AI-DM2. These relatively low levels of CRP were therefore compatible with the hypothesis of an attenuated inflammatory response in these patients. Further studies should be performed to explore the possible abrogation of inflammation in high-risk subjects without overt CVD.

The most interesting observation was the relatively low cIMT values in AIs, despite longer duration of diabetes and worse glycemic control. This could be a race-related phenomenon, which is in line with the observation of smaller luminal diameters in AIs. To date, IMT studies on predictive power[28,29] have not taken diameter into account. In our subjects a significant variability of IMT for a given diameter was observed (data not shown), indicating the need for further explorative studies. There are no firm data on ethnicity-specific IMT values. We observed a median cIMT of 0.66mm (0.12). Previous IMT studies in AIs have reported cIMT values of 0.59mm (+/-0.17) in non-diabetics and 0.63mm (+/-0.22) in DM2 patients living in South India [30]; and cIMT values of 0.93 +/- 0.36mm vs. 0.85 +/- 0.21 mm in diabetics with and without retinopathy, respectively have been reported in same population [31]. Based on our and other studies we calculated that future prospective comparative studies in different ethnic groups would require a sample size of at least 115 AIs vs 115 ECs to detect a 0.05mm difference in cIMT with a power of 0.80 and a two-sided significance of 0.05

The low cIMT observed may also have been due to pathophysiologic differences between AI-DM2 and EC-DM2 leading to slower progression of cIMT. Pathophysiologic changes directly related to diabetes seem unlikely as DM2 was milder in EC than in AI in this study. Thus, low cIMT is for instance not readily explained by decreased glycosylation of the extracellular matrix. Other candidate pathophysiologic mechanisms influencing cIMT progression in AI-DM2 could be endothelium dependent, i.e. intrinsic or environmental acquired resistance against oxidative stress. In this regard the lower smoking rates in AI-DM2 may be of relevance. Mechanisms could also be endothelium-independent and more related to the pathophysiology of the intima. An attenuated intimal inflammatory response, in line with the intermediate levels of low-grade inflammation observed, would be such a mechanism.

In summary, the data presented provide a first description of vascular parameters in AI-DM2 from Surinam *without* CVD. In these patients, we observed ethnicity-defined, significantly lower cIMT than EC-DM2, despite presence of a number of robust cardiovascular risk factors. Following this interesting observation, reported for the first time, we propose that atheroprotective mechanisms are in play, slowing progression of cIMT and CVD. This and other similar AI cohorts should be intensivly researched to unravel he protectiv factor(s).

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