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Haemostatic factors, lipoproteins and long-term mortality in a multi-ethnic population of Gujarati, African-Caribbean and European origin

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ABSTRACT

Objectives: To examine the relations between haemostatic factors and lipoproteins with mortality in British Europeans, African-Caribbeans (AfC) and Gujarati Indians.
Methods: A prospective cohort study of 331 subjects (40–79 years), followed-up over 26 years for mortality. Apolipoprotein-A1 (Apo-A1), apolipoprotein-B (Apo-B), factor VII coagulant activity (FVIIc), fibrinogen and von Willebrand Factor (vWF) were measured at baseline in 118 Europeans, 100 AfC and 113 Gujaratis. Aortic pulse wave velocity (aPWV) was measured in 174 participants.
Results: 147 (44.4%) subjects died during a median of 24 years follow-up with 69 cardiovascular deaths. Women at baseline had higher, and AfC males the lowest FVIIc and Apo-A1 levels. Baseline age-sex and ethnicity adjusted FVIIc levels were higher in those who died (131.0 vs. 117.4%; $P = 0.048$). In similarly adjusted partial correlations, Apo-A1 was inversely related to arterial stiffness ($\rho = -0.23$, $P = 0.04$). Over the 26 years follow-up, participants below the median (i.e. with lower concentration) of FVIIc, Fibrinogen, Apo-B and vWF had better survival rates than those with higher concentrations; those with higher concentrations of Apo-A1 had better survival. In Cox multivariable regression analyses including sex, ethnicity and aPWV, independently increased risk of all-cause mortality came only from SBP (per 5 mmHg); $P = 0.011$), age (per year); $P < 0.0001$ and FVIIc at 7% (per 10-unit; HR 1.07 (1.02, 1.12); $P = 0.008$. Separately, Apo-A1 (HR 0.12 (0.02, 0.75; $P = 0.029$) was independently associated with a very significant 88% reduction in all-cause mortality.
Conclusions: Despite a relatively small sample size, long-term follow-up suggests an independent effect of the prothrombotic state (via FVIIc) and apo-A1 (a constituent of HDL) on mortality.

1. Introduction

Cardiovascular disease (CVD) is the single commonest cause of death in the western world. Despite this, mechanisms underlying the intricate relation of inflammatory and coagulation processes in the aetiology of coronary heart disease (CHD) are poorly understood. Significant reductions in CVD events for individuals at high risk may be achieved via control of 'conventional' risk factors including hypertension, hypercholesterolaemia, type 2 diabetes and smoking. These factors impair endothelial integrity and trigger atherosclerosis through processes including vascular inflammation, thrombosis and coagulation [1,2], smooth muscle cell hyperplasia, and elastin degradation [3].

Unfortunately, successfully identifying and subsequently intervening on high-risk individuals is made a complex task by the presence of additional unmeasured factors modifying CVD risk and their heterogeneity within the population. Amongst the additional factors identified as potential markers of CVD risk are plasma haemostatic factors, which influence an individual's propensity to thrombosis, the lipoproteins Apo-A1 and Apo-B, which are major constituents of HDL and LDL cholesterol respectively, and arterial stiffness [4].

Haemostatic factors with prothrombotic effects include fibrinogen, von Willebrand factor (vWF) and Factor VIIc. Fibrinogen is associated with CHD risk and severity of atherosclerosis [5,6]. vWF is a glycoprotein essential for normal haemostasis by mediating platelet adhesion and aggregation. It is involved in inflammation and has a novel link with angiogenesis [7]. An association between

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vWF with increased risk of CHD, peripheral vascular disease and inflammatory vascular disease has been reported [8,9]. Levels of vWF vary significantly in healthy subjects and may be linked to environmental factors including age, smoking and history of diabetes [10]. It remains unclear whether vWF is causally related to the occurrence of CHD or primarily mirrors endothelial dysfunction, which predisposes to atherosclerosis and subsequent CHD. The relation between FVIIc levels and CHD is less consistent [11–15], with differential associations with fatal and nonfatal cardiovascular events [10,16].

In addition to their roles in haemostasis and thrombosis in CVD, these factors have important associations with vascular inflammatory processes and their association with elevated blood pressure have also been described [17]. There are also strong associations between baseline levels of each of the lipoproteins Apo-AI, Apo-B, the ratio of ApoB/A and risk of CHD [18,19].

The aforementioned aberrant pathways of vascular inflammation, thrombosis and coagulation are also known to contribute to the pathogenesis of arterial stiffness. Arterial stiffness assessed non-invasively as aortic pulse wave velocity (aPWV) [20], is an independent marker of cardiovascular events and cardiovascular mortality [4] and has emerged as a potential tool for refining CHD risk, in addition to conventional risk factors [21–26]. The timing of the interplay between arterial stiffness and cardio-metabolic risk factors occurs from as early as in adolescence [27,28]. Changes in the lipid and lipoprotein composition of adolescents with type 2 diabetes correlate with early vascular stiffness [29]. Whether the above haemostatic factors and lipoproteins are associated with CHD risk through changes in the properties of the arterial wall, particularly arterial stiffening, has not been fully elucidated. In addition, although evidence of the association of these haemostatic and lipoprotein factors with CVD is accumulating, there remains uncertainty concerning their roles in the pathogenesis of the disease and the degree to which they occur independently of one another [30].

Despite a growing understanding of the factors driving the pathogenesis of CHD, there are important ongoing questions concerning how circulating levels of haemostatic factors and lipoproteins relate to ethnic and geographic differences in cardiovascular risk and mortality. For example, African-Caribbean people in Britain have a high prevalence of diabetes and yet up to 50% lower mortality from coronary heart disease (CHD) compared to the national average, in the presence or absence of diabetes [31,32]. This experience contrasts with that of British peoples of Indian sub-continent origin of all denominations for whom both diabetes and CHD are in excess of national rate [33]. The association between arterial stiffness and type 2 diabetes is well documented [34–36] but there have been few comparative data on arterial function of these populations despite evidence that arterial stiffness vary by ethnicity [22,37] independent of cardiovascular risk factors [36]. Whether and how these haemostatic risk factors are related to eventual mortality, arterial stiffness or account for their ethnic variation is unclear [38].

We previously reported an association between fatty acid profiles and mortality in this population [39]. Here we present analyses of the association between mortality and baseline anthropometric, coagulation factors, glucose intolerance as well as arterial stiffness in representative samples of African-Caribbean, local Europeans and Gujarati Indian origin populations after some 26 years follow-up. We tested the hypothesis that: a) circulating baseline haemostatic factors and lipoproteins would predict cardiovascular and total mortality, b) ethnic differences exist in haemostatic factors and lipoproteins which could contribute to known ethnic differences in mortality and c) the association between circulating baseline haemostatic factors and lipoproteins with mortality is independent of arterial stiffness, glucose intolerance, BMI and smoking history.

2. Methods

A random sample of people aged 45–74 years was drawn from population registers held in two North West London health centres, stratified by age and sex in 1987–88. The study included the three main local ethnic groups – people of European, African-Caribbean, or Gujarati Indian origin. Ethnic group defined by self-reported grandparental origin (at least 3 of 4 grandparents in a particular group), anthropometric and other details were described earlier [40]. Of 1055 invited by recorded-delivery letter, 553 (52%) were no longer at the listed address (398) or were away for long periods, usually abroad (155). Of the remaining 502, 385 (77%), evenly spread by ethnic group and sex, agreed to participate. Of the 385 subjects who attended, 54 were excluded because they did not meet the ethnic criteria or did not have full 2 h glucose tolerance tests or had known type 2 diabetes. The decision to exclude known diabetes was made *a priori* because of the potential for diabetes treatment to modulate the parameters measured in this study. A total of 331 subjects without known diabetes were eligible for the study (118 Europeans, 100 African Caribbean and 113 Gujarati Indian origin subjects). All measurements and blood samples were collected on the same day. A sub-set of 174 participants had PWV measurements.

Participants fasted for a 75-g glucose tolerance test (GTT), classified by 1985 and 2006 World Health Organization criteria. For this study, those with impaired fasting glucose, impaired glucose tolerance, or new diabetes were termed glucose intolerant. Individuals with known diabetes were not included. The methodology for the measurement of the anthropometric indices presented here has been described previously [40]. The Northwick Park and Central Middlesex Hospital committees granted ethical permission for all baseline measurements and mortality follow-up. Follow-up was via tagging of death certificates at the Office of National Statistics, United Kingdom. Their 3-monthly reports recorded dates of death or emigration, with censoring at June 30, 2013. Some subjects have emigrated and were right censored at the date of embarkation.

2.1. Laboratory assays

Blood was collected, centrifuged within 2 h of test completion separated and plasma frozen at −70 °C until analysis. Factor VII coagulant activity (FVIIc) was measured by a one-stage biological assay [11,41,42] and fibrinogen by the Clauss method [43] on citrated plasma samples. The Apo-A1 and B were measured by immunonephelometry (Beckman Auto Immuno-Chemical System, Beckman Instruments, High Wycombe, UK). Plasma concentrations of von Willebrand factor were estimated by the ELISA method.

2.2. Arterial stiffness

Arterial stiffness was determined by measuring aortic pulse wave velocity (aPWV) using Doppler probes as described previously [22,39]. aPWV measurement used 2 continuous-wave Doppler probes. One probe was clamped at the base of the left side of the neck to insonate the root of the left subclavian artery and the other insonated the abdominal aorta, above its bifurcation, to obtain stable aortic arch and distal aortic waveforms. Participants were supine for >5 min before recording. Signals from the foot of the proximal to the foot of the distal waveform generated transit times over the measured cutaneous distance