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INCREASED FIBRIN TURNOVER IN PERIPHERAL ARTERIAL DISEASE: COMPARISON WITH A POPULATION STUDY

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ABSTRACT Copley hypothesised the existence of an endoendothelial fibrin lining of blood vessels; while the Rokitansky-Duguid hypothesis suggests that ongoing fibrin formation on the arterial wall contributes to atherosclerosis. We assessed in vivo fibrin turnover by measurement of plasma levels of cross-linked fibrin degradation products (D-dimer antigen) using a sensitive immunoassay, in 68 patients with extensive atherosclerosis (chronic peripheral arterial disease) compared to 239 controls (a random population sample in the same area). Plasma D-dimer levels were significantly higher in patients than controls ($p<0.01$) and correlated with clinical severity. We suggest that the plasma D-dimer level may be a useful index of intravascular fibrin turnover and of the ongoing contribution of thrombosis to arterial disease.

INTRODUCTION

Copley (1) proposed that a thin layer of fibrin(ogen) lines the inner surface of the endothelium of blood vessels: the endoendothelial fibrin lining (EEFL), which may play a role in thrombus formation. In 1852, Rokitansky (2) suggested that focal deposition of fibrin on the arterial wall contributed to atherosclerosis. Almost a century later, Duguid (3,4) showed the presence of fibrin both upon and within arterial plaques. Small encrustations of fibrin are frequently observed on apparently normal arterial intima at necropsy (5). Such fibrin deposits may promote atherogenesis by several mechanisms (6).

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Keen and Smith (7) have recently shown that plasmin digests of aortic thrombi and early gelatinous aortic lesions contain relatively high amounts of D-dimer (the characteristic degradation product of cross-linked fibrin) relative to D-monomer (the characteristic degradation product of fibrinogen and non-cross-linked fibrin). These findings support the hypothesis that ongoing deposition of cross-linked fibrin on arterial intima contributes to atherogenesis (6,7).

The recent production of specific monoclonal antibodies against D-dimer has allowed the development of sensitive ELISA assays for cross-linked fibrin degradation products in plasma (8,9). Such assays provide an index of ongoing fibrin turnover (formation and degradation) in vivo, and high levels are clinically useful in diagnosis of acute venous thromboembolism and disseminated intravascular coagulation (9). Elevated levels have also been described in acute coronary artery thrombosis (myocardial infarction or unstable angina)(10), but no studies have been reported in chronic arterial disease, nor in random population samples. We therefore studied (a) the distribution of plasma levels of D-dimer in a middle-aged population sample, and their relationships to risk factors for arterial disease (including fibrinogen and blood viscosity levels); and (b) plasma D-dimer levels in patients from the same geographical area with symptomatic peripheral arterial disease, who have widespread atherosclerosis in the lower aorta and its branches to the lower limbs, as well as a high risk of coronary and cerebral atherosclerosis and thrombosis.

SUBJECTS AND METHODS

Population Study 239 men and women aged 40-59 years (mean 50 years), randomly sampled from two districts in the Glasgow area during the Scottish Heart Health Study (11) were studied. The methods for sampling and clinical and biochemical measurements have been described (11). These subjects were also part of a population study of blood viscosity, plasma viscosity, haematocrit and fibrinogen levels (12).

Patients with peripheral arterial disease 68 men and women aged 40-79 years (mean 64 years) referred from the Glasgow area to the Peripheral Vascular Unit, Glasgow Royal Infirmary for investigation of peripheral arterial disease, were studied. All had peripheral arterial disease confirmed by arteriography and/or ankle-brachial pressure index (ABPI) less than 0.9; none had limb necrosis or infection at time of study.

Laboratory methods Arm vein blood samples were anticoagulated with dry dipotassium EDTA (1.5 mg/ml) and centrifuged to obtain platelet-poor plasma which was stored at -70°C prior to measurement of D-dimer antigen by an enzyme-linked immunoassay (8) (Dimertest ELISA, AGEN Co., Parsippany, New Jersey) with a sensitivity of 30 ng/ml. In the population study, the remainder of the sample was used for measurement of whole blood viscosity, plasma viscosity, microhaematocrit and fibrinogen as previously described (12). In patients with peripheral arterial disease, serum C-reactive protein was measured as a marker of the acute-phase response.

Statistical analysis Between-group comparisons were performed by Mann-Whitney tests, and correlations determined by Spearman rank tests.

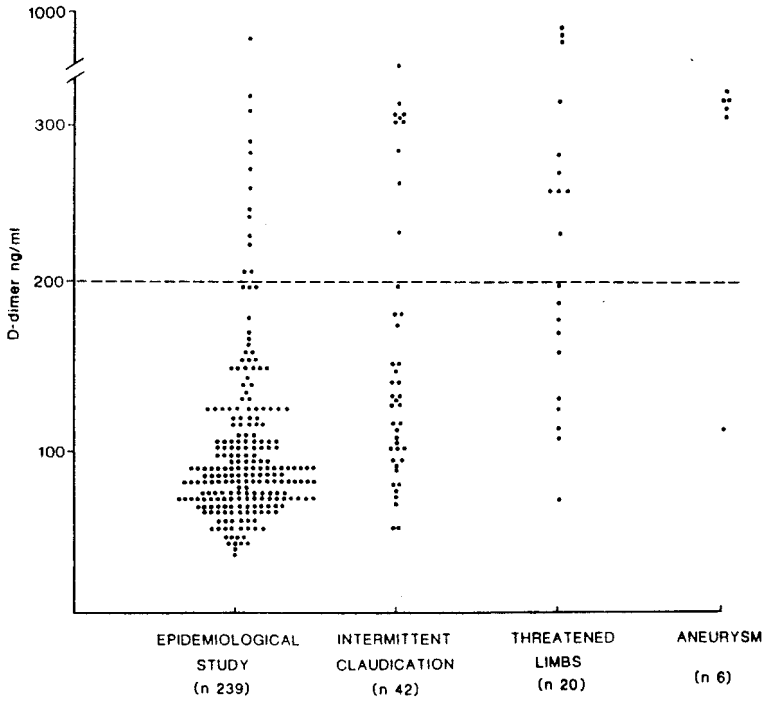

FIGURE 1

Plasma D-dimer levels in subject groups. The broken line indicates the upper 95% confidence interval for the population sample in the epidemiological study.

RESULTS

Population study

The distribution of plasma D-dimer antigen levels in the population sample was positively skewed (Figure 1) with a median of 80 ng/ml (inter-quartile range 65-115) and upper 95% confidence interval of 200 ng/ml: this accords well with the manufacturer's reference range. No significant differences were observed between men and women, smokers and non-smokers, or the 8 subjects with clinical or electrocardiographic evidence of coronary heart disease compared to subjects without such evidence. There were no significant correlations with serum cholesterol, body mass index (weight/height^2), diastolic blood pressure, whole blood viscosity, plasma viscosity or haematocrit; and only weak correlations with age, systolic blood pressure and fibrinogen ($r=0.12$, $p<0.05$).

Patients with chronic peripheral arterial disease

Whereas plasma D-dimer levels greater than 200 ng/ml occurred in 5% of the population sample, 35% (25/68) of patients with chronic peripheral arterial disease had levels above this value ($p<0.01$; Figure 1). When patients were grouped by clinical severity, significant differences ($p<0.05$) were observed between patients with uncomplicated intermittent claudication (median 130 ng/ml, interquartile range 110-200; raised levels in 24%); critical leg ischaemia with threatened limbs (rest pain and/or pregangrene) (median 215 ng/ml, interquartile

range 150-270; raised levels in 50%); and abdominal aortic aneurysm (median 320 ng/ml; raised levels in 5 of 6 patients) (Figure 1). No significant correlatations were observed between D-dimer levels and age, sex, smoking habit, ABPI, or C-reactive protein level.

Intra-individual variation

Repeat samples were taken after 6 weeks in ten patients with stable intermittent claudication, and from ten healthy hospital staff: little intra-individual variation over time was observed in either group.

DISCUSSION

Patients with symptomatic chronic peripheral arterial disease usually have widespread atherosclerosis, and consequently a high risk of coronary and cerebral ischaemic thrombotic events (13). Previous studies have shown that such patients have increased levels of plasma fibrinogen and blood viscosity (14) and also activation of platelets and coagulation, as measured by plasma levels of beta-thromboglobulin and fibrinopeptide A (15). While these changes might promote arterial fibrin deposition, the present study is to our knowledge the first to report that patients with chronic arterial disease have increased fibrin turnover (ongoing fibrin formation and degradation) in vivo, as measured by a sensitive assay for D-dimer in plasma. This study is therefore the first in vivo test of the hypotheses of Copley (1) and Rokitansky/Duguid (2-4): its findings support a role for intravascular fibrin deposition in the progression of arterial disease.

Plasma D-dimer levels showed minimal correlation with plasma fibrinogen levels in the population, confirming the specificity of the monoclonal antibody (8,9). The raised levels in patients with chronic peripheral arterial disease correlated with its clinical severity (Figure 1), suggesting an intravascular origin of the fibrin turnover (patients with gangrene or infection, which can cause extravascular fibrin formation, were excluded from the study). Patients with critical limb ischaemia threatening limb viability had significantly higher D-dimer levels than patients with ischaemia only on exercise (intermittent claudication). The onset of critical limb ischaemia is often acute or subacute, consistent with thrombotic occlusion at atherosclerotic stenoses, which is frequently confirmed by successful thrombolysis, angioplasty or surgery. Furthermore, high D-dimer levels were observed in 5 of 6 patients with abdominal aortic aneurysms, within which fibrin thrombi are frequently observed during surgery.

In the population study, plasma D-dimer levels showed no strong correlation with age, sex or other cardiovascular risk factors (smoking habit, blood pressure, serum cholesterol, body mass index, fibrinogen, haematocrit, blood viscosity or plasma viscosity). Hence plasma D-dimer level may be a useful index of the thrombotic contribution to arterial disease, which is not confounded by associations with conventional risk factors: it might account for part of inter-individual variations in cardiovascular risk which are unexplained by known risk factors. Serial measurement over 6 weeks in claudicants and in healthy volunteers suggested that an individual's plasma D-dimer level is relatively stable: hence high levels may reflect a chronic process rather than acute episodes. This is supported by the lack of correlation with the acute-phase marker, serum C-reactive protein, in patients with peripheral arterial disease.

Normal D-dimer levels were observed in the 8 subjects with chronic coronary artery disease in the population sample; possibly reflecting the small sample size or the lesser extent of generalised arterial disease in such persons compared to persons with chronic peripheral arterial disease. Further case-control studies are in progress to examine the associations of plasma D-dimer levels with peripheral and coronary artery disease and their relationships to risk factors and prognosis. The effects of interventions such as long-term anticoagulation, which has recently been shown to reduce mortality in persons with chronic peripheral arterial disease (16), also merit study. Plasma D-dimer levels may also be useful markers of the thrombogenicity of peripheral arterial grafts (17).

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