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β-Thromboglobulin and platelet factor 4 levels in retinal vein occlusion

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SUMMARY Fifty-six patients with retinal vein occlusion—35 with central and 21 with branch vein occlusion—were investigated for comparison with an age and sex matched control group. Mean levels of β-thromboglobulin and platelet factor 4 were significantly higher ($p<0{\cdot}001$) in both the group with central and the group with branch retinal vein occlusion than in the control group. A significant increase of β-thromboglobulin ($p<0{\cdot}001$) was also found in the retinal vein occlusion group in those patients who were not hyperlipidaemic or diabetic ($n=39$). Weak correlations were found between levels of lipoprotein cholesterol and plasma β-thromboglobulin. Increased platelet aggregation may contribute to the aetiology of retinal vein occlusion.

Although many previous studies have suggested a role for platelet aggregation in the pathogenesis of arterial thrombosis, its role in venous occlusion is less well understood.[1,2] A useful clinical condition to assess in this regard is retinal vein occlusion, because its occurrence is easy to establish by fluorescein angiography. The pathogenesis of retinal vein occlusion is not well understood, but primary thrombosis does appear to occur histologically.[3] Evidence that platelet activity may be important is shown by the report of increased platelet aggregability in impending central retinal vein occlusion, and in one study 5 of 21 eyes with retinal vein occlusion were found to have histological evidence of primary thrombosis.[4,5] Histological evidence does, however, suggest that thrombosis secondary to other local factors also occurs.[1]

No simple and reliable method exists for measuring platelet function in vivo. However, it has recently become possible to measure plasma levels of β-thromboglobulin and platelet factor 4, which are specific platelet proteins.[6] They are stored in platelet α-granules and are released to the surrounding plasma during platelet aggregation.[7] β-Thromboglobulin, the most abundant platelet protein, is stored in platelets at concentrations 3×10^5 times higher than in other tissues.[8] Since it is cleared from plasma with a half time of 100 min at 37°C,[9] an increase in β-thromboglobulin levels may be regarded as a useful indicator of enhanced in-vivo platelet activation and release reaction.

In this study we have measured levels of β-thromboglobulin in 56 patients with angiographically proved retinal vein occlusion and platelet factor 4 serially in the first 39 patients for comparison with an age and sex matched control group in order to assess whether patients with retinal vein occlusion have enhanced in-vivo platelet activity.

Patients and methods

Fifty-six patients with retinal vein occlusion—35 with central retinal vein, 21 with branch retinal vein occlusion—were investigated serially with no prior selection at Moorfields Eye Hospital. Diagnosis of retinal vein occlusion, apart from suggestive clinical history and funduscopy, was confirmed by fluorescein angiography. Further clinical assessment included a search for the presence of macroangiopathy, smoking habits, and associated drug therapy. After full clinical examination (by P.M.D.) the following investigations were performed: oral glucose tolerance test with a 75 g load, liver function tests, full haematological profile, platelet count, electrocardiogram, and chest *x*-ray.

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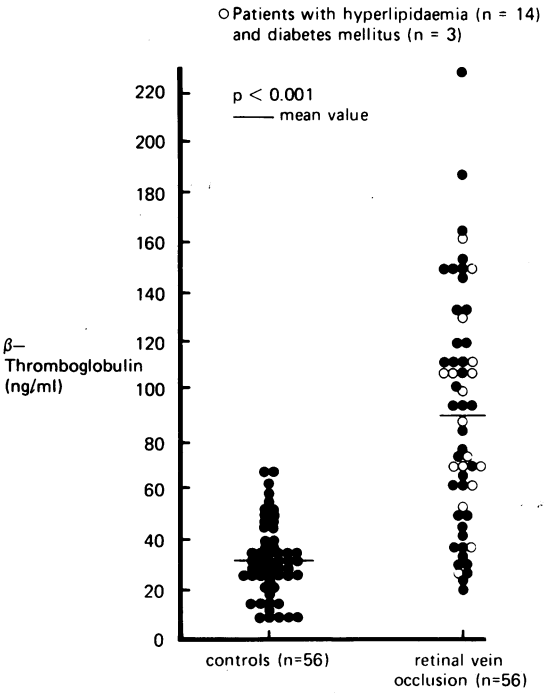

Fig. 1 *β-Thromboglobulin levels in patients with retinal vein occlusion and in age and sex matched controls.*

The patients with retinal vein occlusion were compared with a group of apparently healthy controls, with no history or clinical evidence of vascular disease, drawn from laboratory and hospital staff. Both patients and controls had not taken platelet suppressive drugs for at least 2 weeks prior to blood sampling.

Venous blood without stasis was taken on 3 separate occasions after 6 weeks following the diagnosis of retinal vein occlusion for measurement of β-thromboglobulin and platelet factor 4 into plastic tubes containing anticoagulant (150 μl of a solution containing 7·5 mg EDTA and 0·4 mg of theophylline) and kept on ice. The tubes were inverted 3 times and centrifuged at 2300 *g* for 30 min at 4°C to obtain platelet-poor plasma. This was pipetted off and kept at −20°C prior to assay of β-thromboglobulin and platelet factor 4 levels by radioimmunoassay within 2 weeks by means of Amersham Kits (Radiochemical Centre, Amersham) and Abbott Kits (Abbott Laboratories, North Chicago, USA) respectively. Results are expressed as nanograms per millilitre of platelet-poor plasma. (SI conversion: ng/ml=μg/l.)

Blood was also taken for lipid and lipoprotein analysis, since it is known that there is an increased prevalence of hyperlipidaemia in patients with retinal vein occlusion.[10] Very low-density lipoprotein (VLDL) was precipitated from serum by the addition of sodium dodecyl sulphate, and VLDL-cholesterol and VLDL-triglyceride were measured in the redissolved pellet.[11] Chylomicrons, VLDL, and low-density lipoprotein (LDL) were precipitated from serum with heparin and manganous chloride and high-density lipoprotein (HDL)-cholesterol was measured in the supernatant. LDL-cholesterol was obtained by subtracting the sum of HDL and VLDL from the total serum cholesterol. Cholesterol and triglyceride concentrations were measured by semi-automated fluorometric techniques, Technicon method N77 and Lieberman-Burchard's reagent being used for cholesterol, Cramp and Robertson's method being used for triglyceride.[12] Lipoprotein-cholesterol and lipoprotein-triglyceride values obtained by precipitation techniques have been shown to correlate closely with values obtained by ultracentrifugation.

Statistical analysis was performed by the unpaired *t* test, and correlations were made by the Pearson linear correlation coefficient. Statistical significance was also verified by nonparametric tests (chi-squared and Spearman test).

Results

Sixteen patients in the group with retinal vein occlusion were found to be hypertensive (diastolic>95 or systolic blood pressure>160 mmHg). Four patients had evidence of ischaemic heart disease on electrocardiography, and 5 patients were cigarette smokers. However, there was no evidence of peripheral vascular disease in the group of patients with retinal vein occlusion studied. Platelet counts were in the normal range in all patients studied with retinal vein occlusion.

Plasma β-thromboglobulin levels in 56 patients with retinal vein occlusion compared with those of age and sex matched controls are presented in Fig. 1. Considerable overlap was seen between the 2 groups but the difference between the means was highly significant (Table 1). Fourteen of the patients with retinal vein occlusion were found to be hyperlipidaemic (serum cholesterol>6·8 mmol/l or serum triglyceride levels>2·1 mmol/l); 3 patients were also found to have diabetes mellitus. However, when the plasma β-thromboglobulin values of these 17 patients were subtracted from the total retinal vein occlusion group, the mean value compared with controls was still significantly elevated (Table 1).

Similarly the mean plasma levels of platelet factor 4 were elevated (Fig. 2), though considerable overlap with the values of the controls was observed.

If patients are considered with either central or branch retinal vein occlusion, significantly elevated

Table 1 *Plasma β-thromboglobulin and platelet factor 4 levels in patients with retinal vein occlusion and in an age and sex matched control group*

Subjects	*Number*	*Sex*	*Mean age (yr)*	*Plasma β-thromboglobulin (ng/ml)*	*Plasma platelet factor 4 (ng/ml)*
Control	56	18F 38M	55·1±1·78	32·8±1·9	12·4±0·95 (n=39)
All retinal vein occlusion patients studied	56	18F 38M	54·9±1·71	†91·6±6·3	†32·1±4·2 (n=39)
Retinal vein occlusion with hyperlipidaemia (n=14) or diabetes mellitus (n=3)	17	4F 13M	53·5±3·4	†90·95±9·1	*33·6±7·7 (n=13)
Retinal vein occlusion without hyperlipidaemia or diabetes mellitus	39	14F 25M	55·3±1·9	†92·3±8·1	†31·1±5·1 (n=26)

Figures are mean ± SEM. Statistics by unpaired *t* test. *p<0·01, †p<0·001 compared with control.
SI conversion: ng/ml=μg/l.

mean β-thromboglobulin levels compared with those of controls were still shown (central, 90·7±6·3 and branch 86·1±8·52 ng/ml).

Evidence that these platelet-specific proteins are released together in vivo was further shown in this study by the strong linear correlation between plasma β- thromboglobulin and platelet factor 4 levels (r= 0·65, n=39, p<0·001).

Since plasma lipids are known to influence platelet function, we examined relationships between plasma lipoproteins and levels of β-thromboglobulin. Weak positive correlations were found between plasma β-thromboglobulin and total cholesterol: HDL-cholesterol ratio (r=+0·28, n=54, p<0·05); and a weak negative linear correlation with HDL-cholesterol (r=−0·319, n=54, p<0·05).

No relationship was found between the severity of the retinal vein occlusion, assessed by a scoring method described in a previous communication,[10] and the levels of platelet-specific proteins.

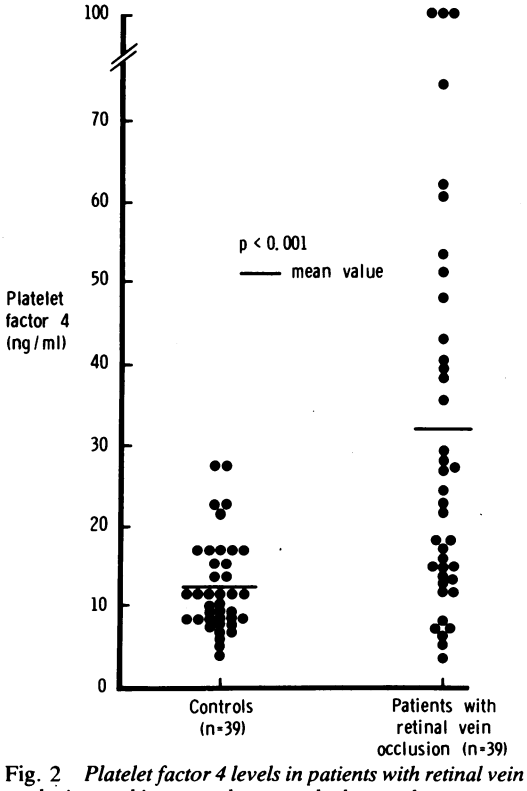

Fig. 2 *Platelet factor 4 levels in patients with retinal vein occlusion and in age and sex matched controls.*

Discussion

No previous reports have appeared on levels of β-thromboglobulin or platelet factor 4 in patients with retinal vein occlusion compared with those of an age matched control group. Our results provide evidence that in-vivo platelet activation and release of platelet-specific proteins is enhanced in many patients with retinal vein occlusion and this may possibly relate to a prethrombrotic state. Levels of β-thromboglobulin and platelet factor 4 were significantly correlated in both control and patient groups, suggesting that these 2 proteins may be released from the same platelet pool and presumably at the same rate.

The enhanced platelet release reaction in patients with retinal vein occlusion may be playing a primary role in its pathogenesis or may be secondary to other factors. It is already established that the platelet release reaction is increased in hyperlipidaemic patients, and we have confirmed that there are weak positive correlations between β-thromboglobulin and levels of lipoprotein cholesterol. Hyperlipidaemia and diabetes mellitus have also been shown to be associated with increased levels of plasma β-thromboglobulin.[13 14]

However, when the subjects with hyperlipidaemia and diabetes mellitus are removed from the patient group with retinal vein occlusion, the remainder still

show augmented levels of plasma β-thromboglobulin and platelet factor 4 levels compared with controls. This suggests that the enhanced platelet release reaction may be playing a more direct role in the pathogenesis of retinal vein occlusion. Other possible factors, such as endothelial swelling and venous constriction or alterations in whole blood viscosity, may also contribute to the final occlusive event.[15]

However, if platelet aggregation is a major event in the formation of a retinal vein occlusion, as our results would suggest, and, since retinal vein occlusion can lead to severe visual loss, the use of anti-platelet drug therapy in this condition might be indicated in 3 types of case: firstly, in patients with retinal vein occlusion and evidence of an enhanced platelet release reaction to prevent recurrence; secondly, to prevent development or progression of capillary closure following vein occlusion; and, thirdly, as described in one case report, prevention of a complete occlusion developing from an incipient central retinal vein occlusion.[4]

We conclude that an in-vivo prethrombrotic state may be present in patients with retinal vein occlusion, and this is independent of the increased prevalence rate of hyperlipidaemia found in this condition. This abnormality may contribute to the aetiology of retinal vein occlusion. Treating associated factors such as hypertension or hyperlipidaemia would also be necessary, and the effects of this treatment and anti-platelet drug therapy in retinal vein occlusion are at present under investigation in our laboratory.

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