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Antithrombin III activity in subretinal fluid

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PURPOSE. *To evaluate antithrombin III (AT III) activity in subretinal fluid (SRF) and blood plasma.*
METHODS. *Prospective study of 32 patients, aged from 20 to 77 years (mean 53.8 years), undergoing retinal detachment surgery. Patients with vein or arterial disease or any other factors that could affect the parameters evaluated were excluded. Subretinal samples were obtained at the time of routine drainage during retinal detachment surgery. Venous blood samples were taken from the cubital vein into sodium citrate solution (9:1) immediately after induction of anesthesia but before surgery. AT III activity in citrated plasma and in SRF and its relations to patients' age, sex, duration and extent of retinal detachment and degenerative changes of the retina, were evaluated.*
RESULTS. *The median level of AT III activity in 32 SRF samples was 16.5% (lower quartile 8.5% and upper quartile 23%). The mean level of AT III activity in plasma was 105% (SD 24%). AT III levels in SRF were significantly lower than in plasma ($p < 0.0001$). We found no correlation between AT III activity in SRF and plasma, and age, sex, the degree of myopia, the duration and extent of retinal detachment, or retinal degenerative changes.*
CONCLUSIONS. *In this study we detected AT III activity in all 32 SRF samples. (Eur J Ophthalmol 2000; 10: 244-7)*

KEY WORDS. *Subretinal fluid, Coagulation, Antithrombin III*

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INTRODUCTION

It has now become apparent that the complex sequence of events resulting in a stable clot is balanced by a vanguard of circulating antagonists which are extremely efficient in limiting and localizing coagulation (1, 2). They are responsible for maintaining the fluidity of the blood, and are involved in tissue remodeling, cell migration, and the initiation of angiogenesis in areas of tissue damage or inflammation (1-4). Activated coagulation factors are serine proteases, and their activity is modulated and inhibited by several naturally occurring protease inhibitors, the most important of which is antithrombin III (3, 4). Antithrombin III (AT III) inhibits thrombin and factor Xa and, to a lesser degree, factors IXa, XIa and XIIa. Inhibition of these enzymes by AT III is markedly accelerated by heparin and by naturally occurring heparin sulfate (1, 2). Reduction of AT III levels to about 50% of normal predisposes to venous thrombosis (1).

Fibrinolytic activity is associated with a number of ocular structures (5, 6). However, not much information is available about coagulation in eyes (7). To our knowledge, the presence of AT III in subretinal fluid (SRF) has not been reported. The aim of this study was to evaluate the AT III content in SRF and the plasma levels of AT III in patients with rhegmatogenous retinal detachment and to establish whether this component of the coagulation cascade was related to age and sex, the degree of myopia, the duration and extent of the retinal detachment, and degenerative changes of the retina.

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PATIENTS AND METHODS

Subretinal fluid was collected from 32 eyes of 32 patients with rhegmatogenous retinal detachment undergoing retinal detachment surgery. There were 12 females and 20 males aged from 20 to 77 years. Their mean age was 53.8 years (SD 16.7). The operations included encircling combined with a segmental scleral buckling procedure, under general anesthesia. All patients gave their written informed consent before they were included in this study. The study had been approved by the Ethics Committee of the Faculty of Medicine, University School of Medical Sciences of Bydgoszcz.

Exclusion criteria comprised other illness or medical treatment besides the retinal detachment, surgical treatment in the past two years, tobacco smoking, pregnancy during the past two years, menopause (last menstruation not longer than a year ago), diabetes mellitus, previous or present venous thrombosis in the patient or in close relatives. None of the patients was receiving any medication that might affect the hemostatic mechanism. No perioperative thromboembolic prophylaxis was used.

The degree of myopia, duration and extent of detachment, changes in the fundus oculi such as degeneration of the retina (cystic, lattice and retinoschisis) and number of tears in the affected eye were analyzed (Tab. I). Eleven eyes were myopic, with refraction over -3D. Degenerative changes in the retina were present in 20 eyes.

SRF samples were obtained at the time of routine drainage during retinal detachment surgery, after cryopexy (22 cases) or before cryopexy (10 cases) and before the encircling band and the buckle had been placed. The area around the proposed site of scleral puncture was carefully dried. Scleral puncture was made at the point of the greatest projection of the detached retina. The SRF was slowly drawn from the perforation site into the dry syringe by using the plunger without penetrating the subretinal space. No clinical complications were observed.

The SRF was centrifuged within 15 min of sampling.

Venous blood samples were taken from the cubital vein into sodium citrate solution (9:1) immediately after induction of anesthesia but before surgery. All venepunctures were done using minimal venostasis, after a rest period of 15 min in the recumbent position.

AT III activity was determined using Berichrom Antithrombin III, produced by Behring Diagnostic GmbH. In this assay, the AT III in the sample is converted into an immediate inhibitor by heparin and inactivates thrombin in the receiver. The residual thrombin content is determined in a kinetic test by measuring the increase in absorbance at 405 nm. Normal reference values were 75-125%.

Statistical analysis

Results of normally distributed data are expressed as mean and standard deviation. Parameters with non-Gaussian distribution are presented as median and the values of the 25th and 75th percentile [lower quartile (Q_1) and upper quartile (Q_u)]. In such cases the Spearman rank correlation test was used to determine the association of ordinal variables. For AT III activity in plasma (normally distributed data) and in SRF (non-Gaussian distribution) the t-test was used to compare the two samples and the Pearson correlation test to describe the strength of the relationship between variables - all after logarithmic (log. 10) transformation. A p-value below 0.05 was considered statistically significant.

TABLE I - MAIN OCULAR FEATURES OF THE 32 PATIENTS STUDIED

Myopia		**Extent of detachment**		**Duration of detachment**		**No. of tears**	
D	No. of eyes	Quadrants	No. of eyes	Days	No. of eyes	No. of tears	No. of eyes
-3/-4	5	1-1.5	7	<20	12	1	14
4.1/-7	3	>1.5-2	13	21-40	11	2-3	10
Over-7	3	>2	12	>40	9	>4	8

RESULTS

Table I summarizes the patients as regards refractive error, extent and duration of detachment and number of tears. The median AT III activity in 32 samples of SRF was 16.5% (Q_1 8.5%, Q_u 23%) and mean activity in blood plasma was 105% (SD 24%). AT III activity in SRF was significantly lower than in plasma (t-test, $p < 0.0001$) (Figs. 1, 2).

We found no correlation between the AT III activity in SRF or plasma (Pearson correlation), and age, sex, degree of myopia the duration and extent of retinal detachment, retinal degenerative changes, number of tears and application of cryopexy before or after the SRF drainage (Spearman rank correlation).

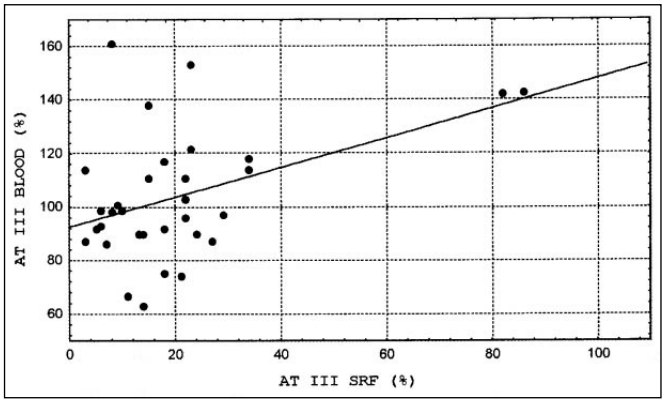

Fig. 1 - *Antithrombin III activity in subretinal fluid (AT III SRF) and in plasma (AT III blood) from patients with rhegmatogenous retinal detachment.*

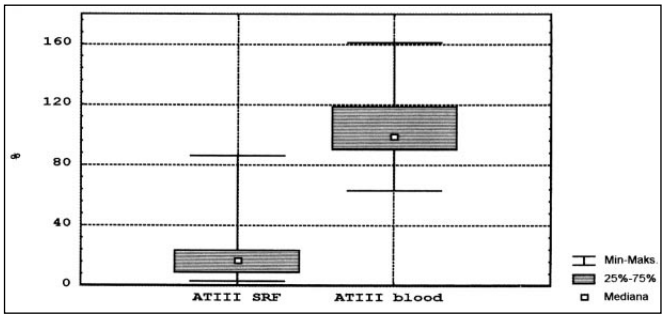

Fig. 2 - *Antithrombin III activity (median, range) in subretinal fluid (AT III SRF) and in plasma (AT III blood) from patients with rhegmatogenous retinal detachment.*

DISCUSSION

The constituents of subretinal fluid may be derived from vitreous, blood and surrounding ocular tissues (7). Most biochemical analyses of subretinal fluids have dealt with proteins, carbohydrates and lipids. SRF contains components of serum protein (8, 9). Normal human vitreous body may contain a large amount of serum protein, so its presence does not indicate that it is derived solely from blood (10). The total protein concentration of the SRF tends to rise with the duration of the detachment, which may indicate an increasing permeability of the choroidal capillaries (7). Other studies, however, found no correlation between the level of protein in the SRF and the duration of the detachment (8). There are few investigations of other components in the SRF (7-9).

AT III may inhibit activated coagulation factors in a very wide spectrum of physiological and pathological situations. Thrombi may form in any part of the vascular system including the microvasculature (1). The complications of thrombosis are caused either by the effects of local obstruction of the vessel or distant embolization of thrombotic material or, less often, consumption of hemostatic elements (1, 2).

In order to characterize the coagulation potential of subretinal fluid, we investigated AT III which is one of the major components of the coagulation cascade. As far as we are aware, our results show for the first time that AT III is present in SRF. We found AT III in every sample.

The presence of AT III in the SRF implies its release either by diffusion (passive or facilitated) or by active transport. It would be difficult – even impossible – to distinguish whether AT III is synthesized and secreted by ocular tissues or simply originates from plasma or from vitreous humor or anterior chamber fluid, for instance.

We found AT III levels in SRF were significantly lower than in plasma and did not correlate with the duration of retinal detachment. The lower AT III concentration in SRF may mean that it is not exuded from the vascular bed or that the capillary endothelium of these vessels and Bruch's membrane have limited permeability. The latter suggestion is in accordance with the opinion of previous investigators who reported that one of the fractions always present in normal serum (IgM) was not found in any of their cases (8). Some authors, however, who link an increase in the amount

of total protein with the duration of detachment consider transudation from the vascular bed as the most important feature in the formation of SRF. This was not apparent in our study.

Other possible sources of AT III in SRF should not be excluded. The retinal pigment epithelium or the cells in the SRF itself may well be sources.

Our data suggest that the AT III level does not correlate with the extension of the detachment and does not reflect the degree of myopia or the degenerative processes in the retina. AT III activity in SRF is thus not an indicator of the duration of the disease or of the condition of the ocular tissues. The physiological significance of AT III in SRF is therefore unclear. This activity certainly plays an important role after hemorrhage and clot formation, being one of the components of the coagulation cascade. Thrombin holds a central role in the process of hemostatic plug formation, influencing its form, rate of formation, and limitation (1, 11, 12). Thrombin acts on many substrates, including fibrinogen, converting it to fibrin. Fibrin formation accompanies tissue degeneration and regeneration. AT III is the main inhibitor of thrombin and plays a major regulatory role in the entire coagulation process (1, 11-13). It promotes the right balance of hemostasis, tissue remodeling and recovery (1, 12, 13).

The clinical relevance of our findings is at present unknown but they may help give some clue to the role of the local coagulation system in retinal detachment.

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REFERENCES

1. Colman W, Hirsh J, Marder VJ, Salzman EW, Hirsh J. Hemostasis and thrombosis: basic principles and clinical practice. Philadelphia: JB Lippincott Co 1994; 3-16.
2. Conrad J, Horellou MH, Van Dreden P, et al. Coagulation and fibrinolysis in 215 patients with history of idiopathic venous thrombosis. Thromb Hemost 1985; 54: 43-9.
3. Pandolfi M, Coccheri S, Astrup T. Thromboplastic and fibrinolytic activities in tissues of the eye. Proc Soc Exp Biol Med 1962; 109: 159-61.
4. Carson SD, Haire WD, Broze GJ, Novotny WF, Pirrucello SJ, Duggan MJ. Lipoprotein associated coagulation inhibitory coagulation factor, factor VII, antithrombin III, and monocyte tissue factor following surgery. Thromb Hemost 1991; 66: 534-9.
5. Geanon JD, Tripathi BJ, Tripathi RC, Barlow G. Tissue plasminogen activator in avascular tissues of the eye: a quantitative study of its activity in the cornea, lens, and aqueous and vitreous humors of dog, calf and monkey. Exp Eye Res 1987; 44: 55-63.
6. Immonen I, Salonen EM, Vaheri AI, Laatikainene LT. Plasmin in subretinal fluid. Acta Ophthalmol Copenh 1988; 6: 647-51.
7. Chignell AH, Carruthers M, Rahi AHS. Clinical biochemical, and immunoelectrophoretic study of subretinal fluid. Br J Ophthalmol 1971; 55: 525-32.
8. Akhmeteli LM, Kasavina BS, Petropavlovskaja GA. Biochemical investigation of the subretinal fluid. Br J Ophthalmol 1975; 59: 70-7.
9. Heath H, Beck TC, Foulds WS. Chemical composition of subretinal fluid. Br J Ophthalmol 1962; 46: 385-96.
10. Kaluzny J. Pochodzenie bialek plynu podsiatkówkowego w odwarstwieniu samoistnym. Klin Oczna 1973; 43: 997-1000.
11. Okwusidi J, Falcone M, Van Ryn Mckena J, et al. *In vivo* catalysis of thrombin inhibition by antithrombin III, or heparin cofactor II and antithrombotic effect: differential effects of unfractionated heparin and dermatan sulphate. Thromb Haemorrh Disorders 1990; 1/2: 77-80.
12. Hirsh J, Piovella F, Pini M. Congenital antithrombin III deficiency: Incidence and clinical features. Am J Med 1989; 87: 34-41.
13. Cohen JR, Sarfati I, Birnbaum E, Benacquista T, Wise L. The inactivation of antithrombin by serum elastase in patients with surgical infections. Am Surg 1990; 56: 665-71.