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Von Willebrand factor in subretinal fluid

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PURPOSE. *To measure the vWF antigen concentration (vWF Ag) in subretinal fluid (SRF) and blood plasma.*

METHODS. *Prospective study of 30 patients, aged from 15 to 78 years (mean 52.7 years), undergoing retinal detachment surgery. Excluded were patients with venous or arterial disease or any other factors that could affect the parameters evaluated. 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. VWF in plasma and in SRF and its relation to patients' age, sex, the duration and extent of retinal detachment, number of retinal tears, and cryopexy application were evaluated.*

RESULTS. *The median level of vWF Ag in 30 samples of SRF was 6.3%. The median level of vWF Ag in blood plasma was 70.34%. The levels of vWF Ag in SRF were significantly lower than in blood plasma ($p<0.00001$). We found no correlation between the vWF Ag concentration in plasma or in SRF and sex, the degree of myopia, the duration and extent of retinal detachment, number of retinal tears and the use of cryopexy.*

CONCLUSIONS. *Determination of vWF Ag showed that this factor in SRF is unrelated to patients', sex, the degree of myopia, the duration and extent of retinal detachment, number of retinal tears and use of cryopexy. (Eur J Ophthalmol 2001; 11: 361-5)*

KEY WORDS. *Subretinal fluid, Coagulation, Von Willebrand factor*

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INTRODUCTION

Von Willebrand factor (vWF) is a plasma glycoprotein synthetized in endothelial cells and megakaryocytes. It has been calculated that 75-85% of total circulating vWF is derived from the endothelium, and 15-25% resides in the unactivated circulating platelet, originating in the megakaryocytes (1, 2). Plasma levels of vWF are increased in certain physiological conditions inlcuding stress and pregnancy, and in diseases such as diabetes, hypertension, myocardial infarction, lupus erythematosus, rheumatoid arthritis and neoplastic disease, this is usually explained as an acute phase reactant or marker of endothelial injury (1, 3-6).

Biochemical research on the subretinal fluid (SRF) occupies an important place in the study of the pathogenesis of rhegmatogenous retinal detachment. Although many biochemical studies on SRF have been done, not much information is available about fibrinolytic and coagulation activity in eyes with retinal detachment (7, 8). We set out to characterize the hemostatic potential of SRF, as part of our work on assessment of clotting and fibrynolytic factors in SRF. Encouraged by the results presented in previous papers (7, 8) where we reported finding high levels of tPA Ag and PAI-1 activity, and the presence of AT III in SRF, we decided to search for vWF in SRF – the important component of primary hemostasis. To our knowledge there has been no report of vWF in the SRF in human eyes with rhegmatogenous retinal detachment.

The purpose of this study was therefore to measure the vWF Ag content in SRF and in the plasma of patients with rhegmatogenous retinal detachment and

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to establish whether this component of the coagulation cascade was related to sex and age, degree of myopia, duration and extent of the retinal detachment, number of retinal tears and whether cryopexy had been done.

PATIENTS AND METHODS

Subretinal fluid was collected from 30 eyes of 30 patients with rhegmatogenous retinal detachment undergoing retinal detachment surgery. There were 17 females and 13 males, aged from 15 to 78 years (mean ± SD 52.7 ± 19.2).

Exclusion criteria were other illness besides retinal detachment, medical or surgical treatment during the past two years, tobacco smoking, pregnancy during the past two years, menopause, with the last menstruation not longer than a year ago, diseases that could affect plasma levels of vWF Ag such as diabetes mellitus, myocardial infarction, lupus erythematosus, rheumatoid arthritis, neoplastic disease, and previous or present venous thrombosis in the patient or close relatives. None of the patients received any medication which might affect hemostatic mechanisms in the 30 days before entering the study, intraoperatively or in the postoperative period (e.g., aspirin, anticoagulants, steroids, non-steroid antinflammatory agents, oral contraceptives, vasoactive drugs, beta or calcium channel bockers). No perioperative thromboembolic prophylaxis was used.

The operations included encircling combined with a segmental scleral buckling procedure. SRF samples were obtained at the time of routine drainage during retinal detachment surgery, before cryopexy (10 cases) or after cryopexy (20 cases) and before the encircling band and 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 subretinal fluid was slowly drawn from the perforation site into the dry syringe 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): 24 h before surgery (day –1) and immediately after induction of anesthesia but before surgery (oper 0). All venepunctures were performed using minimal venostasis, after 15-min in the recumbent position.

Considering the possible influence of anesthesia or the stress before surgery on the levels of vWF Ag in blood we compared the vWF Ag values obtained 24 h before the operation with those immediately after induction of anesthesia, but before the operation. VWf concentration was determined by a commercially available enzyme-linked immunosorbent assay using antibodies of vWFIg (Dako, Glostrup, Denmark).

The degree of myopia, duration and extent of detachment and number of retinal tears of the affected eye and influence of cryopexy were analyzed. The main ocular details of the group, such as extent and duration of detachment, number of tears, are summarized in Table I. Seven eyes were myopic, with refraction over –3.0 D. In 19 eyes the duration of retinal detachment before surgery was less than 20 days.

All patients gave their written informed consent before they were included in this study, which had been approved by the Ethics Committee of the Faculty of Medicine, University School of Medical Sciences of Bydgoszcz.

TABLE I - EXTENT AND DURATION OF DETACHMENT, NUMBER OF TEARS

Extent of detachment		Duration of detachment		No. of tears	
No. of quadrants	**No. of eyes**	**Days**	**No. of eyes**	**No. of tears**	**No. of eyes**
1-1.5	4	<20	19	1	18
>1.5-2	9	21-40	6	2-3	9
>2	17	>40	5	>3	3

Statistical analysis

Results of normally distributed data are expressed as mean and standard deviation. Parameters with non-Gaussian distribution are presented as median, range and interquartile range. The Mann-Whitney U test was used for independent and ordinal data and the Spearman rank correlation test to determine the association for ordinal variables. The Wilcoxon matched sign rank test was used for paired data (day-to-day variations). A p-value below 0.05 was considered statistically significant.

RESULTS

Table II shows the median levels of vWF Ag in 30 samples of SRF. In blood plasma 24 h before surgery (day –1) the median was 165.6% (interquartile range 169.8%) and after induction of anesthesia but before operation (oper 0) it was 70.34% (interquartile range 190.5%). No significant difference was seen between vWF Ag in blood 24 h before surgery and at time oper 0 (Wilcoxon test, $p=0.13$). vWF Ag in SRF was significantly lower than in blood plasma (Mann-Whitney U test, $p<0.00001$) (Fig. 1).

TABLE II - vWF Ag IN BLOOD AND SUBRETINAL FLUID (SRF), DEGREE OF MYOPIA, DURATION AND EXTENT OF DETACHMENT AND NUMBER OF RETINAL TEARS IN CONSECUTIVE PATIENTS

Case	vWf Ag in blood (%)	vWf Ag in SRF (%)	Degree of myopia	Duration of detachment	Extent of detachment	No. of tears
1	122.820	18.820	-6.0	7.0	1.0	5.0
2	159.720	4.070	-3.5	7.0	1.0	1.0
3	224.340	6.800	0.0	14.0	1.0	1.0
4	395.71	36.710	-10.0	21.0	3.0	1.0
5	82.76	6.320	0.0	7.0	2.0	1.0
6	186.180	31.840	0.0	180.0	4.0	2.0
7	178.160	27.110	0.0	14.0	3.0	1.0
8	238.090	0.0	-20.0	2.0	1.5	1.0
9	314.750	0.0	-3.5	21.0	3.0	2.0
10	437.310	0.0	0.0	60.0	3.0	1.0
11	427.410	0.0	0.0	1.0	2.0	1.0
12	87.110	0.0	-10.0	60.0	4.0	6.0
13	362.500	10.970	0.0	30.0	4.0	1.0
14	71.61	543.670	0.0	7.0	4.0	1.0
15	468.48	0.0	0.0	30.0	4.0	3.0
16	68.000	26.000	0.0	14.0	2.0	1.0
17	514.95	27.640	0.0	14.0	2.0	1.0
18	422.55	45.450	0.0	21.0	2.5	1.0
19	365.49	118.480	0.0	3.0	2.0	2.0
20	302.23	0.0	-2.0	14.0	2.0	1.0
21	346.19	19.090	0.0	300.0	4.0	2.0
22	157.13	6.770	0.0	2.0	3.0	1.0
23	46.820	4.260	0.0	7.0	4.0	3.0
24	81.310	0.23	0.0	14.0	2.0	1.0
25	69.070	14.720	0.0	7.0	4.0	2.0
26	300.100	2.48	0.0	2.0	4.0	4.0
27	208.100	3.170	0.0	28.0	2.0	2.0
28	317.120	0.0	0.0	11.0	2.0	1.0
29	177.660	4.140	0.0	120.0	4.0	2.0
30	507.730	22.730	-8.0	14.0	4.0	1.0
Median (interquartile range)	70.34 (190.5)	6.3 (26.0)				

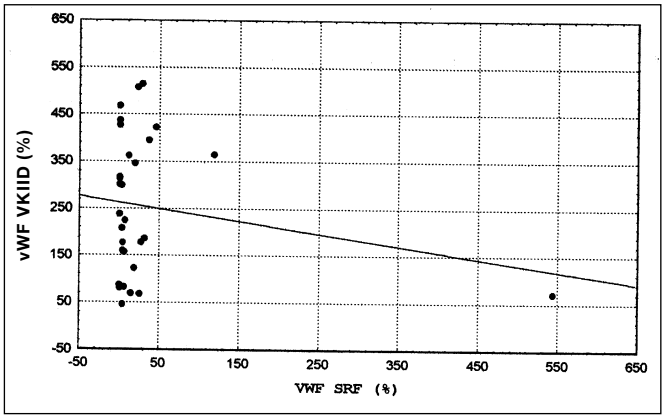

Fig. 1 - *Individual blood plasma (vWF blood) and subretinal fluid (vWF Ag SRF) concentrations in patients with rhegmatogenous retinal detachment.*

We found no correlation between the vWF Ag concentrations in SRF and in plasma and sex, degree of myopia, the duration and extent of retinal detachment, number of tears (Spearman rank correlation) (Tab. III). Although there was no correlation between vWF Ag levels in blood and age (p=0.61), vWF Ag in SRF and age were correlated (p=0.006). The median vWF Ag concentration in SRF before cryopexy was 4.2% (interquartile range 12.24%) and after cryopexy it was 10.9% (interquartile range 27.1%). There was no correlation between the vWF Ag concentration in SRF sampled before and after cryopexy (Spearman rank correlation).

DISCUSSION

In the past, most biochemical analyses of SRF have dealt with proteins, carbohydrate compounds or the lipid content (9-14). It is established that coagulation factors play an important role in a very wide spectrum of physiological and pathological situations but we are not aware of any reports on vWf Ag in SRF.

The VIII/vWF complex consists of two circulating plasma proteins bound non-covalently. Both components are of great importance in hemostasis. Factor VIII participates in the middle part of the intrinsic coagulation cascade in which it serves in conjunction with factor IX in the activation of coagulation factor X and, after further steps, accelerates thrombinogenesis and the convertion of fibrinogen to fibrin (1).

The vWF factor contributes to primary hemostasis by facilitating platelet attachment to subendothelial substances in an injured vessel wall, stimulating the formation of a primary platelet plug. In the circulation, vWF protects factor VIII from proteolysis, and also serves to direct F VIII to sites in need of hemostasis (1, 2, 15, 16). With its multi-adhesive properties vWF is able to bind a number of different proteins (1). All these functions explain why vWF is so important in hemostasis.

The origin of vWF in SRF is not known. Many mechanisms and factors trigger the acute release of vWF from endothelial cells: α-thrombin, plasminogen activator, plasmin, adrenalin, bradykinin, interleukin-1 and calcium ionophore all increase its release (1, 15, 16). Usually the mechanism of increase in the vWF level involves an increase of acute phase proteins, or is a result of endothelial injury (1, 6, 15, 16). In our study vWF release was unrelated to the endothelial cell trauma induced by cryopexy, but there was a tendency to lower values of this factor in SRF before cryopexy.

Disruption of the blood-eye barrier after retinal detachment may allow access to the vitreous and the subretinal space. The total protein concentration of the SRF tends to rise with the duration of the detachment, which may indicate increasing permeability of the choroid capillaries (10, 11, 14). Other studies, however, found

TABLE III - SPEARMAN RANK CORRELATION BETWEEN vWF Ag IN SRF AND SEX, AGE, DEGREE OF MYOPIA, DURATION AND EXTENT OF DETACHMENT, NUMBER OF RETINAL TEARS AND CRYOAPPLICATION

		vWF Ag in blood	Sex	Age	Degree of myopia	Duration of detachment	Extent of detachment	No. of tears	Cryopexy
vWF Ag in	R	0.13	0.06	0.49	0.17	0.02	-0.11	0.14	0.19
SRF	P	0.49	0.75	0.006	0.38	0.99	0.59	0.47	0.33

R = Spearman rank correlation value; P = p value

no correlation between the level of protein in the SFR and the duration of the detachment (9). In our study there was no correlation between the vWF Ag concentration and the duration of the disease but we noted a positive correlation between the patients' age and vWF Ag levels in SRF.

The significantly lower concentration of vWF Ag in SRF than in plasma may indicate that it is not exuded from the vascular bed or that the permeability of the capillary endothelium of these vessels and Bruch's membrane is limited.

Disturbances of the retinal pigment epithelium may also play a role in regulating vWF Ag levels in SRF.

The physiological significance of vWF in SRF is unclear. Being an important component of the coagulation cascade vWF certainly plays a significant role after hemorrhage and clot formation and acts in many ways to maintain hemostasis, and the right balance of tissue remodeling and recovery (7-10).

The clinical relevance of our findings is still not clear but measurements of vWF may provide some insights into the nature of rhegmatogenous retinal detachment.

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