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*The level of alpha-antitrypsin and ceruloplasmin in the subretinal
fluid of patients with rhegmatogenous retinal detachment*

Some details of the retinal detachment pathology have not been clarified so far. Today inflammatory process of immune character is believed to take part in the mechanisms of retinal detachment (1). Hence, considerable attention is given to the composition and origin of the subretinal fluid. Proteins belong to the essential and best known components of the subretinal fluid. Their level and composition depend, among others, on the duration and extent of retinal detachment (2, 9).

Alpha 1-antitrypsin (AAT) and ceruloplasmin (CER) are glycoproteins of blood serum belonging to the group of acute phase proteins. They are mainly produced in the liver and accomplish very important functions in homeostasis regulating a normal course of inflammatory process, both locally and systematically (10). AAT with the molecular mass of 52,400u inhibits the activity of proteolytic enzymes – among others trypsin, thrombin, prothrombin, collagenase and cathepsin (4, 11).

Ceruloplasmin with the molecular mass of 132,000u neutralizes toxic free oxygen radicals thus inhibiting lipid oxygenation and therefore, is considered one of the main antioxidants of blood serum (4, 5). The aim of the study was to determine AAT and CER level in the subretinal fluid of patients with rhegmatogenous retinal detachment (RRD).

MATERIAL AND METHODS

Investigation was performed in 41 patients (22 women, 19 men) operated on for RRD at the age from 24 to 81 years. The group of patients was divided with regard to the extent and duration of RRD. In 24 patients detachment involved 1 or 2 quadrants and in 17 patients – 3 or 4 quadrants. In 16 subjects RRD lasted shorter than 2 weeks, in

6 patients about one month and in 19 patients – from 1 to 3 months. The subretinal fluid was collected during operation after ocular puncture. Next, the fluid was stored frozen at -20°C . The levels of the examined substances were determined with the radial immunodiffusion method on Nor-Partigen plates by Behring containing specific antibodies directing against the examined proteins. The statistical analysis was performed by means of t-Student test. The informed consent of all patients was obtained as well as an approval of the Ethical Committee of the Medical University of Lublin.

RESULTS

The results are presented in Tables 1–3. Table 1 shows mean values of antitrypsin (AAT) level and ceruloplasmin (CER) in the subretinal fluid from 41 patients with RRD. Table 2 and 3 show the level of the examined proteins with regard to the extent of retinal detachment and duration of the pathologic process.

Table 1. Mean level of alpha-1-antitrypsin and ceruloplasmin in the subretinal fluid of all the patients with RRD

Examined protein	Level in g/l number of patients =41
alpha-1-antitrypsin	0.739±0.475
ceruloplasmin	0.186±0.122

Table 2. Mean level of alpha-1-antitrypsin and ceruloplasmin in the subretinal fluid of patients with RRD with regard to the extent of retinal detachment

Examined protein	Extent of detachment		
	2 quadrants n=24	4 quadrants n=17	significance level
alpha-1-antitrypsin	0.850±0.610 g/l	0.661±0.336 g/l	NS
ceruloplasmin	0.142±0.062 g/l	0.542±0.407 g/l	P<0.005

n – the number of patients in the study group, NS – no statistical significance, P – the level of statistical significance.

Table 3. Mean level of alpha-1-antitrypsin and ceruloplasmin in the subretinal fluid of patients with RRD with regard to the duration of retinal detachment

Examined protein	Duration of detachment		
	2 weeks	1 month	1-3 months
	n=16	n=6	n=19
alpha-1-antitrypsin	0.636±0.330 g/l ^A	0.615±0.244 g/l ^B	1.130±0.639 g/l ^C
ceruloplasmin	0.142±0.062 g/l ^D	0.154±0.045 g/l ^E	0.301±0.161 g/l ^F

Comparison of mean values: C>B, C>A P<0.01; F>D, F>E P<0.01; n – number of patients in study group, P – level of statistical significance, A – mean value of AAT in group of patients with RRD lasting 2 weeks, B – mean value of AAT in group of patients with RRD lasting 1 month, C – mean value of AAT in group of patients with RRD lasting 1-3 months, D – mean value of CER in group of patients with RRD lasting 2 weeks, E – mean value of CER in group of patients with RRD lasting 1 month, F – mean value of CER in group of patients with RRD lasting 1–3 months.

DISCUSSION

Mean AAT concentration in subretinal fluid of all patients operated on for RRD was 0.739±0.475 g/l and CER was 0.186±0.122 g/l. Mean AAT concentrations did not differ statistically significantly between the group of patients with detachments involving not more than 2 quadrants and the group with detachment in 3 or 4 quadrants. However, CER level was significantly higher in the group with higher RRD. Considering the duration of detachment mean AAT and CER concentration was significantly higher in the group with RRD lasting from 1 to 3 months than in the group with RRD lasting one month or shorter.

Theories pertaining to the origin of the subretinal fluid say that some of its components come from vitreous humor, others from the blood, still others result from a damage to retinal cells. The longer RRD lasts, the higher level of permeability of the blood-retina barrier which may be evidenced by the presence of macromolecular compounds such as some proteins with big molecular mass, e.g. IgM, in the subretinal fluid (13). The presence in the subretinal fluid of proteolytic enzymes as well as various mediators of the inflammatory process may account for inflammatory process of immune character taking part in retinal detachment (1). Obuchowska found the growth of proteolytic activity in the subretinal fluid caused by increased concentration of cathepsin A, which reached the highest values about the fourth week from the onset of the disease and then was gradually decreasing (12). The growth of proteolytic activity can affect the degree of structural degradation and the function of detached retina. Proteolytic enzymes contained in the subretinal fluid cause digestion of proteins in external photoreceptors fragments and de-

grade the mucopolysaccharide matrix lying between photoreceptors (3). Katsnelson has shown the presence of trypsin-like proteolytic enzymes and their inhibitors in the subretinal fluid. Their concentration increased with the advancement of inflammatory and proliferative processes at subsequent proliferative vitreoretinopathy (PVR) stages. At the same time he found a slight increase in concentration of proteinases inhibitors in peripheral blood of the degree characteristic of a small intensity inflammation (9).

Bosch discovered in the subretinal fluid products of lipid peroxidation partly coming from external photoreceptor fragments. According to this author the process of lipid peroxidation may play some role in the pathogenesis of RRD (3). Retinal damage during its detachment is thus caused by inadequate diet, increase of lysosomal enzymes concentration in the subretinal fluid as well as by lipid peroxidation of external photoreceptor fragments (7, 8, 9, 14). Hence, antiproteolytic functions of alpha-1-antitrypsin and antioxidative functions of ceruloplasmin may have a special role in controlling the course of local inflammatory process and in protecting retinal cells against the damage.

In our investigations AAT level in the subretinal fluid was a few times lower and CER concentration was comparable with the concentration of these proteins of blood serum (according to those assumed as normal concentrations in blood serum: AAT from 1.23–2.43 g/l; CER from 0.171–0.651 g/l; acc. to Behring's standards). It seems interesting that ceruloplasmin concentration with a bigger molecular mass (132,000u) is similar to the concentration in the blood while alpha-1-antitrypsin concentration with a smaller molecular mass (52,400u) is lower than in blood serum (4, 11). A lower AAT level in the subretinal fluid can be explained by the way AAT inactivates proteases forming complexes consisting of one AAT molecule and one molecule of the proteolytic enzyme. The complexes formed in this way are then metabolized inside the cells. Thus, a lower AAT level may result from "wear". Berrod found in the subretinal fluid of 48% of 25 patients examined by him the presence of alpha-1-antitrypsin with the concentration of 0.86 g/l, but did not show any correlation between the duration of RRD and AAT level (2). In our study the increase in protein concentration in the subretinal fluid is connected with the duration of detachment. Differences in the levels of examined proteins did not occur if detachment lasted not longer than 1 month, i.e. up to 2 weeks or 3–4 weeks. Higher AAT and CER concentrations, however, were observed if detachment lasted longer than 1 month. It is difficult to explain why the extent of RRD did not affect the changes of AAT level in the subretinal fluid while in the case of CER its concentration was significantly statistically higher than in the case when detachment involved 3 or 4 quadrants.

CONCLUSIONS

1. Concentration of the examined AAT and CER in the subretinal fluid was higher in patients in whom RRD lasted longer than 1 month compared with patients with RRD lasting shorter.
2. CER concentration in the subretinal fluid was higher in patients with more extensive RRD.
3. The detection of acute phase proteins in the subretinal fluid suggests that they may play a part in the pathogenesis of RRD by affecting the intensity of local inflammatory reaction.

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SUMMARY

The aim of the study was to determine alpha-antitrypsin (AAT) and ceruloplasmin (CER) level in the subretinal fluid of patients with rhegmatogenous retinal detachment (RRD).

Investigation was performed in 41 patients (22 women, 19 men) operated on for RRD at the age from 24 to 81. The group of patients was divided with regard to the extent and duration of RRD. In 24 patients detachment involved 1 or 2 quadrants and in 17 patients – 3 or 4 quadrants. In 16 subjects RRD lasted shorter than 2 weeks, in 6 patients – about one month and in 19 patients – from 1 to 3 months. The subretinal fluid was collected during operation. The levels of the examined substances were determined with the radial diffusion method on Nor-Partigen plates by Behring. The results were as follows: mean AAT concentration in the subretinal fluid of all the patients operated on for RRD was 0.739 ± 0.475 g/l and CER was 0.186 ± 0.122 g/l. Mean AAT concentrations did not differ statistically significantly between the group with bigger and smaller retinal detachments while CER level was higher in the group with a bigger RRD. Mean AAT and the CER concentration was statistically significantly higher in the group with RRD lasting from 1 to 3 months compared with the group with RRD lasting shorter than one month. Conclusions: the fact of finding acute phase proteins in subretinal fluid suggests that they may play a part in the pathogenesis of RRD by affecting the intensity of local inflammatory reaction.

Poziom alfa-antytrypsyny i ceruloplazminy w płynie podsiatkówkowym pacjentów z otworopochodnym odwarstwieniem siatkówki

Celem pracy było oznaczenie poziomu AAT i CER w płynie podsiatkówkowym u chorych z otworopochodnym odwarstwieniem siatkówki (OOS). Badanie przeprowadzono u 41 chorych (22K, 19M), operowanych z powodu OOS w wieku od 24 do 81 lat. Grupa chorych została podzielona ze względu na rozległość i czas trwania OOS. U 24 chorych odwarstwienie obejmowało 1 lub 2 kwadranty, a u 17 chorych dotyczyło 3 lub 4 kwadran-

tów. U 16 chorych OOS trwało krócej niż 2 tyg., u 6 chorych ok. 1 miesiąca, a u 19 chorych czas trwania OOS wynosił od 1 do 3 miesięcy. Płyn podsiatkówkowy pobierano podczas zabiegu operacyjnego. Poziomy badanych substancji oznaczano metodą immunodyfuzji radialnej na płytkach NorPartigen firmy Behring.

Średnie stężenie AAT w płynie podsiatkówkowym u wszystkich chorych operowanych z powodu OOS wynosiło $0,739 \pm 0,475$ g/l, a CER= $0,186 \pm 0,122$ g/l. Średnie stężenia AAT nie różniły się w sposób istotny statystycznie pomiędzy grupą z większymi i mniejszymi odwarstwieniami siatkówki, natomiast poziom CER był wyższy w grupie z większym OOS. Średnie stężenie AAT było w sposób istotny statystycznie wyższe w grupie z OOS trwającym od 1 do 3 miesięcy niż w grupie z OOS trwającym 2 tygodnie. Stężenie CER było w sposób istotny statystycznie wyższe u chorych z OOS trwającym od 1 do 3 miesięcy niż u chorych z OOS trwającym jeden miesiąc lub krócej.

Stwierdzenie obecności białek ostrej fazy w płynie podsiatkówkowym sugeruje, że mogą one odgrywać pewną rolę w patogenezie OOS, wpływając na nasilenie miejscowej reakcji zapalnej.