The antibodies to beta-2-glycoprotein-I in blood serum and aqueous humor in patients with glaucoma

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Abstract
The recent studies tend to consider glaucoma, a leading cause of worldwide blindness, for autoimmunological disorder. The aim of the study was to evaluate frequency of β2-glycoprotein-I antibodies incidence in aqueous humor and serum of glaucoma patients and to estimate the relationship between serum and anterior chamber fluid antibodies in these patients. Samples of blood and anterior chamber fluid were obtained from 21 glaucoma and 27 non glaucoma patients. Incidence of β2-glycoprotein-I antibodies classes IgA and IgG was tested using ELISA method. In the group of glaucoma patients positive ratios were obtained in 8 cases (38%), most frequently in IgA class in aqueous humor (4 cases). The incidence of examined antibodies in anterior chamber fluid was observed in 6 persons (28.5%). In control group positive ratios were observed in 4 cases (15%), most frequently in IgA class in aqueous humor (2 cases). When the ratios were compared between glaucoma and cataract patients, statistically significant differences between β2-glycoprotein-I antibodies class IgA in aqueous humor (p=0.0031) and in serum (p= 0.0005) were observed. The data indicate possible participation of β2-glycoprotein-I antibodies in pathogenesis of glaucoma, yet to determine their pathological and clinical significance further studies are necessary.

Key words: glaucoma, antiphospholipid antibodies

Introduction
Glucomatous optic nerve degeneration is a leading cause of worldwide blindness. Precise cellular mechanisms underlying neurodegeneration in glaucoma and effective strategies for neuroprotection are not yet clear [1].

Glucoma and immunity are not traditionally perceived as being causally related. Recently, however, compelling observations have provided insight into a potential role for the immune system in the development of glaucomatous optic neuropathy.

It is proposed that the role of the immune system in glaucoma is twofold. In some patients, there is evidence that an autoimmune mechanism may be responsible for eliciting damage to the optic nerve, resulting in glaucomatous injury. Autoimmune damage to the optic nerve may occur directly by autoantibodies, or indirectly by way of a “mimicked” autoimmune response to a sensitizing antigen which, in turn, injures retinal ganglion cells. Autoimmune-mediated glaucoma injury occurs most often, but not exclusively, in patients in whom the intraocular pressure has never been found to be elevated.

A second role of the immune system in glaucoma is likely one of surveillance, in which signal pathways of the immune system regulate cell death in response to conditions that stress retinal neurons in glaucoma. These might include
mechanical stress from high intraocular pressure, ischemia, excessive excitatory amino acids, or toxic products resulting from excessive nitric oxide synthase production in either neurons or glial fibers that surround the optic nerve as it exits the eye. In these cases the immune system acts as an “arbiter” to help determine whether a neuronal cell will ultimately survive, or succumb to, those stressors that are perceived as injurious. It is conceivable that such surveillance and cell death regulation by the immune system is important in determining the fate of retinal neurons in both the more common “high-pressure” forms of glaucoma, such as primary open-angle glaucoma, and in cases in which the intraocular pressure appears within normal range [2].

As far as the potential immunological mechanism of glaucoma is concerned, antiphospholipid antibodies, including β2-glycoprotein-I antibodies, might be related to destruction of optic nerve according to ischemic theory of glaucoma pathogenesis.

The aim of the study was to evaluate the frequency of β2-glycoprotein-I antibodies incidence in aqueous humor and serum of glaucoma patients and to estimate the relationship between serum and anterior chamber fluid antibodies in these patients.

Materials and methods

Group of 21 persons (16 women and 5 men), aged 46-86 (mean age 70), suffering from glaucoma was examined. All patients have undergone trabeculectomy (operative creation of a new way of aqueous humor outflow) because of inefficient pharmacological treatment.

The group of 27 non glaucoma patients (16 men and 11 women), aged 47-82 (mean age 67), operated because of the age-related cataract (opaqueness of lens) constituted a control group.

All patients agreed to collection of the samples. None of the patients revealed clinical symptoms of any autoimmunological disorders; neither suffered from any infectious diseases nor used the drugs associated with the incidence of the antiphospholipid antibodies.

About 100 µl of anterior chamber fluid (aqueous humor) was obtained by paracentesis from patients and controls just before the operation. The day before the operation samples of venous blood (5 ml) were collected.

The samples of blood were centrifuged for 10 min. and the serum was separated. Samples of anterior chamber fluid and samples of serum were then portioned out and kept in -20°C to await analysis.

The levels of antibodies to β2-glycoprotein-I classes IgA and IgG were measured in plasma and anterior chamber fluid of patients and controls using ELISA method (commercial kits produced by Euroimmun) according to the producer’s instruction. Briefly, in the first step, diluted patients samples were incubated with the wells. In case of positive samples, specific antibodies (class IgA or IgG) bound to the antigen. To detect bound antibodies, a second incubation was carried out using an enzyme-labelled anti-human IgA or IgG, which was capable of promoting a colour reaction. The intensity of the formed colour in photometric measurement was proportional to the concentration of antibodies against β2-glycoprotein-I.

After the measurements the calculation of the results was performed. The calculation of the ratio was carried out according to the following formula:

\[
\text{RATIO} = \frac{\text{Extinction of the serum sample}}{\text{Extinction of the calibration serum 2}}
\]

The ratios ≥1.0 were considered as positive, the ratios <1.0 as negative.

Statistical analysis was performed using Statistica programme. U Mann-Whitney test was used to confirm the statistical significance (p<0.05 was considered as statistically significant) and Spearman rank correlation test to specify the statistical correlations between the studied antibodies.

Results

In the group of glaucoma patients positive ratios were obtained in 8 cases (38%): 2 for IgG class in aqueous humor (ratios 1.090 and 1.269), 1 for IgG class in serum (ratio 1.141), 4 for IgA class in aqueous humor (ratios: 1.212, 1.080, 1.115, 1.118) and 1 for IgA class in serum (ratio: 1.055). The highest ratio amounted to 1.269 for IgG class in aqueous humor. None of patients had positive ratios in more than one group of the studied antibodies (Table 1.).

In the control group positive ratios were observed in 4 cases (15%): 1 for IgG class in aqueous humor (ratio: 1.010), 2 for IgA class in aqueous humor (ratios: 1.076 and 1.133) and 1 for IgA in serum (ratio: 1.296). None of the patients was detected antibodies against β2-glycoprotein-I IgG in serum. The highest ratio amounted to 1.296 for IgA in serum. Similarly to the examined group none of the cataract patients showed elevated ratio in more than one group (Table 2.).

When the ratios were compared between the glaucoma and cataract patients, significant differences were observed between β2-glycoprotein-I antibodies class IgA in aqueous humor (p=0.0031) and in serum (p= 0.0005). Mean values of β2-glycoprotein-I antibodies titers in both studied group are shown in Table 3.
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In the group of glaucoma patients almost statistically significant correlations were found between the ratios of antibodies against beta-2-glycoprotein-1 IgA in serum and anterior chamber fluid (p=0.059, R=0.61) and between the ratios of antibodies against beta-2-glycoprotein-1 IgA in serum and class IgG in aqueous humor (p=0.0075, R=0.78).

In the group of non-glaucoma patients statistically significant correlations of the ratios of IgG in serum and in aqueous humor (p=0.01, R=0.49) and of IgA in serum and in aqueous humor (p=0.053, R=0.39) of the cataract patients were not observed in the glaucoma patients.

Similarly to the patients with glaucoma, there was almost statistically significant correlation between the ratios of the antibodies against beta-2-glycoprotein-1 IgA in aqueous humor and class IgG in serum (p=0.053; R=0.39) of the cataract patients.

Discussion
Beta-2-glycoprotein-1 antibodies, directed against epitopes on oxidized phospholipids complexed with a beta-2-glycoprotein-I, belong to a heterogeneous group of antiphospholipid antibodies, which are the serological markers for the antiphospholipid syndrome, a disorder of hypercoagulability with highly variable symptomatology (including ocular manifestation), characterized by recurrent venous and arterial thrombosis, fetal loss and thrombocytopenia.

Antiphospholipid antibodies were first reported in patients affected by systemic lupus erythematosus and subsequently in association with other collagen vascular diseases, infectious conditions and use of certain drugs. However, these antibodies were also detected in clinically healthy patients, leading to differentiation primary as the opposite to secondary antiphospholipid syndrome [3].

Table 1. Frequency of beta-2-glycoprotein-I antibodies incidence in glaucoma patients

<table>
<thead>
<tr>
<th></th>
<th>Positive ratios</th>
<th>Negative ratios</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>8 patients (38%)</td>
<td>13 patients (62%)</td>
</tr>
<tr>
<td><strong>Aqueous humor</strong></td>
<td></td>
<td></td>
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<tr>
<td>IgA</td>
<td>1.212</td>
<td>17 patients (81%)</td>
</tr>
<tr>
<td>IgG</td>
<td>1.090</td>
<td>19 patients (95%)</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>1.055</td>
<td>20 patients (95%)</td>
</tr>
<tr>
<td>IgG</td>
<td>1.141</td>
<td>20 patients (95%)</td>
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</tbody>
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Table 2. Frequency of beta-2-glycoprotein-I antibodies incidence in non-glaucoma patients

<table>
<thead>
<tr>
<th></th>
<th>Positive ratios</th>
<th>Negative ratios</th>
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<tbody>
<tr>
<td></td>
<td>4 patients (15%)</td>
<td>23 patients (85%)</td>
</tr>
<tr>
<td><strong>Aqueous humor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>1.076</td>
<td>25 patients (92%)</td>
</tr>
<tr>
<td>IgG</td>
<td>1.010</td>
<td>26 patients (96%)</td>
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<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>1.296</td>
<td>26 patients (96%)</td>
</tr>
<tr>
<td>IgG</td>
<td>1.010</td>
<td>27 patients (100%)</td>
</tr>
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Table 3. Mean values of beta-2-glycoprotein-I antibodies titers in studied groups

<table>
<thead>
<tr>
<th>beta-2-glycoprotein-I antibodies</th>
<th>Glaucoma</th>
<th>Cataract</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA in aqueous humor</td>
<td>0.68 ± 0.3</td>
<td>0.47 ± 0.29</td>
<td>0.0031</td>
</tr>
<tr>
<td>IgA in serum</td>
<td>0.77 ± 0.22</td>
<td>0.48 ± 0.29</td>
<td>0.0005</td>
</tr>
<tr>
<td>IgG in aqueous humor</td>
<td>0.39 ± 0.23</td>
<td>0.36 ± 0.18</td>
<td>0.89</td>
</tr>
<tr>
<td>IgG in serum</td>
<td>0.42 ± 0.19</td>
<td>0.42 ± 0.19</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Antibodies to β2-glycoprotein-I are reported to be more strongly associated with clinical antiphospholipid syndrome (history of thrombosis) than anticardiolipin antibodies [10].

Ocular features associated with antiphospholipid syndrome are anterior chamber abnormalities (including iritis, scleritis and filamentary keratitis), whereas the most represented feature of posterior involvement is retinal vasculitis, followed by vitritis, retinal detachment, posterior scleritis, and central retinal artery occlusion [3]. The recent studies tend to consider glaucoma for autoimmune disorders, because of incidence of different autoimmune antibodies (to phospholipids – APL, cardiolipin – ACL, phosphatidylinerine – APS and beta 2-glycoprotein-I – beta-2GP) [4-6].

In present studies elevated incidence of antibodies to β2-glycoprotein-I in glaucoma patients was observed in comparison to non glaucoma subjects. The most frequently present antibodies were IgA class in aqueous humor (4 persons - 19%). Especially significant is the incidence of examined antibodies in anterior chamber fluid (in 6 persons - 28.5%), which may indicate the disorder strictly limited to the eye and/or changes of blood-eye barriers permeability. The positive ratios obtained in our studies were close to the cut-off value of the positive ratio contrary to the results in the patients with active autoimmune disorders (own unpublished data).

The optic neuropathy of glaucoma appears to be multifactorial in etiology. Both mechanical and vasogenic theories remain viable explanations for the observed nerve damage and the destructive effect of trophic withdrawal could be espoused by either theory. Each theory feeds into the final common pathway of cell death; even the immune system may kill cells in glaucoma by apoptosis [7].

Glaucomatous optic neuropathy often occurs in the absence of elevated intraocular pressure and, conversely, elevated intraocular pressure may occur without associated damage of the optic nerve. These findings challenge the simple explanation of intraocular pressure being the sole cause of neural loss and have led to theories of ischemic causes of the morbidity.

The posterior ciliary artery circulation is the main source of the blood supply to the optic nerve head with additional lesser supply via the central retinal artery and the choroidal circulation. There is considerable individual variation in the distribution of this circulation and complex regulatory systems govern its function. It is likely that microcirculatory changes in the vascular supply of the optic disc play a role in glaucoma, either as the primary abnormality or as a cofactor that increases susceptibility to damage from increased intraocular pressure through impaired auto-regulation [8].

Biochemical changes in the chamber humor play an important role in the pathogenesis of glaucoma and are largely determined by the permeability of the blood-eye barrier, which is formed by two main barriers: the blood-aqueous barrier and the blood-retinal barrier.

Comparative study of total protein content in the chamber humor in mature and far advanced glaucoma showed that the disease involves a progressive increase in the permeability of the blood-aqueous humor barrier, which may be due to dystrophic changes in ocular tissues in the course of glaucoma progress and accumulation of metabolites damaging [9].

The blood-aqueous barrier is formed by an epithelial barrier located in the nonpigmented layer of the ciliary epithelium and in the posterior iridial epithelium, and by the endothelium of the iridial vessels. Both these layers have tight junctions of the “leaky” type. The permeability of the blood-aqueous barrier shows a significant degree of pressure-dependent diffusion associated with transport activity, resembling the standing gradient osmotic flow model.

The blood-retinal barrier (BRB) is located at two levels, forming an outer barrier in the retinal pigment epithelium and an inner barrier in the endothelial membrane of the retinal vessels. Both these membranes have tight junctions of the “non-leaky” type and cellular transport processes predominate.

Modification of blood-eye barrier permeability produced by antibodies or drugs seemed to play a role in glaucomatous neurodegeneration. Increase in antibodies to beta2GP concentration in serum and aqueous humor of the glaucoma patients may lead to local thrombosis, microcirculation disturbances and retinal ganglion cell apoptosis.

An improved understanding of the site of primary injury to optic nerve, the mediator pathways of apoptotic cell death and intrinsic protection mechanisms in retinal ganglion cells, the role of glial activation on the survival and death of retinal ganglion cell bodies and their axons, and the protective and destructive consequences of immune system involvement can facilitate development of effective neuroprotective strategies in glaucoma.

**Conclusions**

Higher incidence of β2-glycoprotein-I antibodies in glaucoma patients supports autoimmune theory of glaucoma, yet to determine the clinical potential of serum and aqueous humor antibodies estimation in glaucoma patients a randomized clinical trial would be necessary.
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The site of production of the antibodies remains unclear (systemic or topical antibody production). The differences in relations between serum and aqueous humor antibodies in glaucoma patients and controls might also suggest changes in the permeability of the blood-aqueous humor barrier.

Knowledge of the immunological pathogenesis in the glaucomatous nerve damage can be curtailed or even prevented with the use of proper agents. Indeed, there are many intriguing new therapeutic possibilities on the horizon.

The study is the preliminary communication.

References