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von Willebrand factor in iris vasculature of glaucoma patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AEF 1 **Wojciech Rokicki**
BG 2 **Małgorzata Żaba**
DE 1 **Ewa Mrukwa-Kominek**
BF 1 **Dorota Wyględowska-Promieńska**
BG 2 **Marlena Brzozowa**
CF 2 **Edyta Reichman-Warmusz**
ACE 2 **Romuald Wojnicz**

1 Department and Clinic of Ophthalmology, Medical University of Silesia, Katowice, Poland
2 Department of Histology and Embryology, Medical University of Silesia, Zabrze, Poland

Corresponding Author: Wojciech Rokicki, e-mail: wojtek.rokicki@gmail.com
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Background: Previous reports have indicated the role of endothelium disturbances, as expressed by von Willebrand factor (vWF) release, in pathophysiology of glaucoma. The objective of this study was to investigate the vWF expression in iris vasculature of patients with primary open-angle glaucoma (POAG).





Material/Methods: Immunohistochemistry of vWF expression was performed on cryostat sections of samples collected at the time of peripheral iridectomy and controls collected from dead donors.

Results: Twenty-seven Caucasians age 66.6 ± 3.7 with 5.8 ± 3.7 -year history of treated PAOG and 10 controls age 62.2 ± 1.92 with no history of glaucoma. The percentage of patients who presented normal and up-regulation of vWF phenotype expression differed statistically between examined and control groups: 48% versus 100% ($p=0.035$, *chi-square test with Yates' correction*). Sex, age, glaucoma duration, and visual field quantitative indices had no impact on vWF expression. A significant correlation between mean pre-surgery intraocular pressure and vWF expression was found (*Spearman* $r=0.42$, $p=0.03$).

Conclusions: Considering the results, it may be suggested that vWF is actively involved in the pathophysiology of glaucoma.

Keywords: **von Willebrand Factor • Glaucoma • Iris**

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Background

Glaucoma is a broad group of disturbances mainly causing progressive ganglion cell damage, visual field loss, and, finally, blindness. Despite well-developed diagnostic tools and relatively efficient treatment, glaucoma is still the world's leading cause of irreversible blindness among older people. Primary open-angle glaucoma may progress with elevated or normal (arbitrarily estimated) intraocular pressure (IOP). Therefore, the main, known glaucoma risk factor, elevated IOP, is neither sufficient nor necessary to trigger glaucoma neuropathy. Accordingly, previous reports indicated that many other factors may be involved in the pathogenesis of glaucoma.

If the mechanism of retinal ganglion death in glaucoma is assumed to be a type of necrosis, the vascular and ischemic mechanisms of glaucoma are less obvious [1] and need further research [2–5].

The endothelium plays a major role in maintaining the homeostasis of vascular tone. Imbalance between vasodilators and vasoconstrictors (produced by endothelial cells) results in disturbances in blood supply. This alteration is generally initiated by increased oxidative stress, and is also connected with glaucoma development [6]. Additionally, referring to pressure-related risk factors, endothelium in Schlemm's canal also can play role in the pathogenesis of glaucoma. It was reported that the endothelial cells of the inner wall of Schlemm's canal control the flow of aqueous humor from the spaces of the juxtacanalicular tissue (JCT) into Schlemm's canal [7]. Lip et al. [8] suggested that the pathogenesis of optic nerve damage in both NTG and POAG may be associated with abnormal vascular permeability and endothelial damage/dysfunction.

We aimed to investigate endothelial dysfunction measurement of biological markers. One of the most useful markers for vascular damage is von Willebrand factor (vWF). This glycoprotein is produced by endothelial cells and megakaryocytes throughout the body [9]. The vWF is stored in Weibel-Palade bodies and is also present in Schlemm's canal endothelium [10]. Levels of circulating vWF are increased following endothelial cell damage [11]. Serum vWF is increased in cardiovascular, metabolic, pulmonary, and other diseases [12]. Therefore, these pathological conditions make plasma vWF less reliable in elderly patients with glaucoma. Accordingly, the purpose of this study was to investigate the endothelial dysfunction in the iris determined by vWF expression in patients with diagnosed glaucoma.

Material and Methods

The studied groups

Patients who were planned for deep sclerectomy with basal iridectomy were recruited to our study. The inclusion

criteria were (1) Caucasians with diagnosed and treated primary open-angle glaucoma (POAG), (2) age 65–75 years, (3) nonsmokers, and (4) normal or normalized blood pressure. The exclusion criteria included: (1) other than POAG type of glaucoma, (2) diabetes mellitus, (3) incidences of inflammation process in ocular tissue, (4) infective disorders, (5) antithrombotic or vasoactive cardiovascular therapy, (6) intra-bulbar surgery in the last 12 months, and (7) von Willebrand and/or Raynaud disease.

The type of glaucoma was confirmed by 2 experienced ophthalmologists based on gonioscopy, ophthalmoscopy, tonometry, visual field examination (Octopus 301 HS, Interzeag), and polyclinic history analysis. The average IOP was determined by 3 measurements using a Goldmann applanation tonometry (Haag-Streit, Bern, Switzerland) under topical anesthesia with 0.5% Alcaine (Alcon) eye drops. The first was measured 14–20±4.1 days before surgery, when the patient was present at our polyclinic. The third measurement was made on the day of surgery (before administration of any intravenous osmotic agents) and the second measurement was recorded during a control test 3–6±2.3 days after the first measurement. All IOP measurements were taken during the morning. The control sections were obtained at autopsy and processed within 8 hours after death. The methodology was similar, and, through corneal incision, a full-thickness piece of iris was sampled.

Following written consent, each patient underwent the surgical procedure, when the full-thickness piece of iris (approximately 1×1 mm) was removed using iridectomy scissors.

Sample collection

Each sample was gently irrigated with 10 ml of Ringer's solution, and then was placed into Eppendorf 2 ml Safe-Lock test tubes (Eppendorf Biopur®) containing 99.5% acetone for dehydration for 5 minutes at 4°C. Afterwards, the acetone was poured off and the test tube with sample inside was filled with Tissue-Freezing Medium (OCT Compound, Miles). The specimens were placed in a –70°C freezer until sectioning.

Immunohistochemistry study

For immunohistology, all specimens were cut serially into 5-µm thicknesses, air-dried at room temperature, and assayed. Frozen sections were incubated with murine monoclonal anti-human vWF from DAKO A/S (clone F8/86, the final dilution of 1:200). To suppress non-specific staining due to endogenous alkaline phosphatase activity, levamisole was used at a final concentration of 0.2 mM. The En-Vision method (DAKO En-Vision Kit/Alkaline Phosphatase detection system) was used according to the manufacturer's instructions.

Table 1. Characteristics of patients with POAG and control subjects.

	Glaucoma patients n=27				Controls n=10	
	♂ n=11	♀ n=16	♂ n=4	♀ n=6		
Age (years)	66.5±3.9		66.7±3.8		62.5±3.5	62.0±1.0
Median duration of known glaucoma (years)	1–15 5.8±4.6				∅	
Glaucoma drugs	B-b*	CAI*	α-m*	PG*	∅	
	12	10	9	1		

* B-b – β blockers; CAI – carbonic anhydrase inhibitors; α-m – α₂ agonists; PG – prostaglandin analogues.

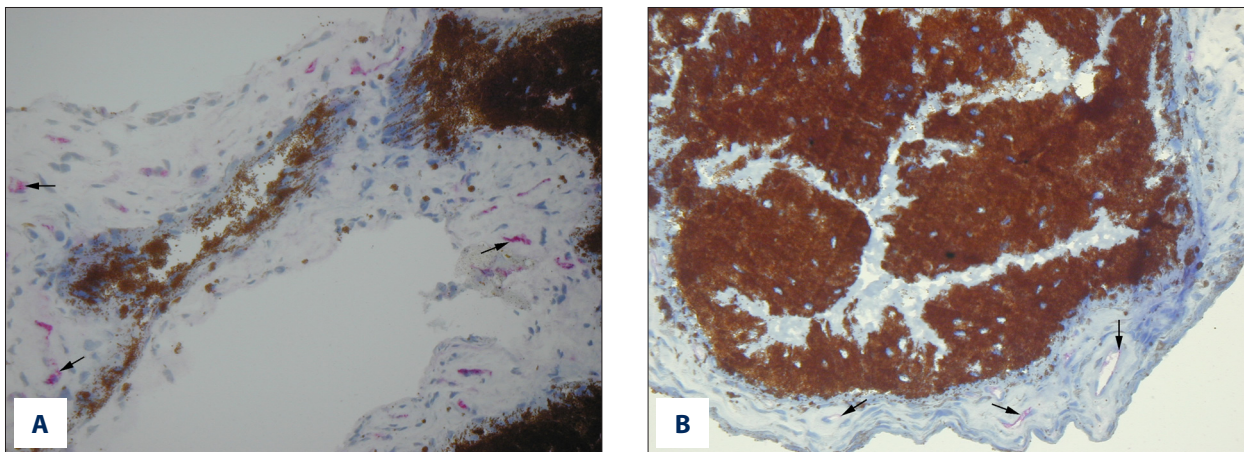


Figure 1. (A) Iris cryostat section from the studied group. Intensive vWF staining on capillary vessels (arrows) (final magnification, ×200). (B) Iris cryostat section from the control group. The scattered endothelial cells are weakly stained with vWF (arrows) (final magnification, ×200).

The bound primary antibody was detected using New Fuchsin Substrate System (DAKO A/S). The primary antibody was omitted from negative control slides. As a positive control we used cryostat sections from the heart. The sections were counterstained with Mayer's hematoxylin. Each specimen was evaluated qualitatively and semi quantitatively (score index from 0 to 3+). The semi quantitative score index was: (0) no staining, (1+) – lack of or weak staining; (2+), moderate staining; and (3+), severe staining.

Ethics statement

The study protocol was approved by the Ethics Committee of the Medical University of Silesia, Katowice (permission number: KNW/0022/KB1/123/10) and adhered to the tenets of the Declaration of Helsinki. Permission was obtained for experiments involving human tissue and samples and for collection of donor iris tissue.

Statistics

Due to the discrete distribution of von Willebrand factor, non-parametric tests were used. Spearman rank correlation was performed, and Mann-Whitney or Kruskal-Wallis test for comparison of selected variables between groups.

The p-value of <0.05 was considered statistically significant.

Results

Baseline patient characteristics are summarized in Table 1.

The perimetric quantitative indices mean defect (MD) was 10.4±6.6 (1.9–22.3) and loss variance (LV) was 23.5±16.1 (2.9–68.4). No significant correlation between

MD, LV, and vWF expression was found ($r=0.06$, $p=0.78$ and $r=0.04$, and $p=0.84$, respectively).

The average intraocular pressure from the preoperative period, which ranged from 18 mmHg to 51 mmHg, was 31.6 ± 9.4 mmHg. This factor was significantly correlated with vWF expression in the iris vasculature (*Spearman* $r=0.42$, $p=0.03$).

Immunoreactivity of vWF from patients with glaucoma differed significantly from the control subjects ($p=0.035$, *chi-square test with Yates' correction*). In the studied group, vWF immunoreactivity was: $\leq 1+$ in 13 patients (48%), $2+$ in 10 (37%), and $3+$ in 4 patients (15%) (Figure 1A). In contrast, all the control specimens presented $\leq 1+$ vWF expression (Figure 1B). The differences were significant when comparing the examined and control groups ($p=0.035$, *chi-square test with Yates' correction*).

No significant differences between the male and female patient subgroups were found in vWF expression ($p=0.44$).

There was no significant correlation between the age of patients with glaucoma and vWF expression in the iris specimens ($r=-0.18$, $p=0.35$). Similarly, no relationship was found between both glaucoma duration (Table 1) and immunohistological outcomes ($r=0.11$, $p=0.57$).

We found no association between type and number of anti-glaucoma ophthalmic agents used and vWF expression.

Discussion

The main reason to involve PAOG patients in our study was opportunities to sample intraocular tissue for examination.

The von Willebrand factor levels increase with age [13], thus, age-matched participants were involved in this study.

The current study is, to the best of our knowledge, the first one evaluating vWF immunoreactivity in the human iris. We revealed increased vWF expression in iris microcirculation of patients with PAOG. In addition, the current study demonstrated a relationship between pre-surgery intraocular pressure and vWF expression in the iris. When viewed together, the above observations confirm that endothelial activation may play a significant role in glaucoma pathogenesis.

Studies by Lip et al. have shown increased serum levels of vWF in glaucoma patients [8]. However, the serum level of vWF may be increased in response to different stimuli even without endothelial injury [14,15]. Accordingly, peripheral vascular disease, cardiovascular diseases, diabetic vasculopathy, hypertension, and other disturbances affect vWF release [16].

Thus, the relevance of plasma vWF up-regulation and its association with glaucoma remains doubtful.

In contrast, our results confirmed the presence of up-regulation of vWF in iris microcirculation and may suggest that endothelial activation is an important event for glaucoma.

The examined patients were referred to our hospital in the phase of attempting IOP normalization. As recorded, each patient presented raised/fluctuating IOP approximately 2-3 weeks before surgery. Our data demonstrated a strong association between mean pre-surgery IOP and vWF expression. In an animal model, Reidy et al. [17] observed 2.7 times increased vWF release from endothelial cells 14 days after mechanically injury. Therefore, we suggest that up-regulation of vWF in the iris microcirculation may be the result of endothelial pressure-related injury.

It could be supposed that pressure factor, as much as vessel endothelial cells, denudes endothelial cells that line the channels of the trabecular meshwork and Schlemm's canal, additionally increasing intraocular pressure. In contrast to previous reports in which POAG and NTG patients were examined [8], endothelial dysfunction in POAG seems to be secondary to glaucoma pathogenesis. In primary open-angle glaucoma, and even more in normal-tension glaucoma, endothelial dysfunction and ischemic disturbances seem to play a more important, primary, role in glaucomatous neuropathy development [4].

The present study showed no correlation between visual field outcome, duration of glaucoma, and vWF expression. It should be remember that perimetric indices are only a summary of glaucomatous pathological processes, which fluctuate over stable and progressive states [18].

Finally, our data demonstrated statistically significant differences in vWF release between the examined group and controls. On one hand, these results provide preliminary evidence in support of our hypothesis, but on the other hand, further investigation is need to estimate prognostic and diagnostic properties of vWF in glaucomas.

Conclusions

The results of the current study confirmed the presence of up-regulation of vWF in iris microcirculation and may suggest that endothelial activation is an important event in glaucoma.

Study limitations

- The current study is limited by the relatively small numbers of patients studied. However, the results are encouraging enough to warrant designing a larger study.

- Glaucoma is a disease with several rates of progression [1,18] and should not be treated as a typical chronic disorder. A prospective follow-up study is needed.
- The time of diagnosis glaucoma was self-reported by patients and should be considered as a subjective, tentative factor, which also applies when considering the connection with vWF determinations.
- The ABO blood groups were not considered. Nevertheless, the influence of ABO blood groups on vWF levels is controversial [19,20].
- The axial length of the examined eyes, which reflect collagen disturbances and determining glaucoma susceptibility, was not considered [21].

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Disclosure statement

There are no conflicting interests or relationships among authors of the manuscript.

Conflict of interest

Costs of patient's recruitment were covered by local government. Local authority provided all materials necessary for the study such as copies of the questionnaire, and prepared space for conduct of the study. The authors have no proprietary or commercial interest in any materials discussed in this article.