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Importance of Circulating Platelet Aggregates and Haemodynamic Changes in Ophthalmic Artery and Progression of Visual Field Loss at Pseudoexfoliation Glaucoma

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ABSTRACT

The aim of this work is to examine the role of circulating platelet aggregates (CPA) at pseudoexfoliation glaucoma (PXG), haemodynamic changes in the ophthalmic artery by ultrasonic color Doppler, searching for visual field progression. Vascular component at PXG and its role in VF progression dynamics has not been sufficiently explained, as well as CPA influence to ischaemic events related to optic nerve damage and VF progression. The examination included 80 patients, where of 35 (44%) men average age 68.3 ± 7.0 and 45 (56%) women average age 65.7 ± 7.0 (t=1.66; p=0.101). Forthy of them suffered from primary open angle glaucoma (POAG) as a control group (healthy), and 40 from pseudoexfoliative glaucoma (PXG) as an experimental group. All the examinees underwent complete ophthalmological examination: visual acuity, ocular fundus, intraocular pressure measured, anterior eye segment biomicroscopy with gonioscopy performed. Also VF examination was performed three times at 6 months intervals. Laboratory testing of CPA proportion values was performed by means of Wu an Hoak method and ultrasonic measurement of blood perfusion in the carotid tree, particularly concerning ophthalmic artery by means of color Doppler. Obtained decreased values of CPA proportion resulted in hypercoagulability of blood in PXG group. At PXG were also found increased blood flow resistivity indexes in ophthalmic artery (RI AO) and internal carotid artery (RI ACI), resulting with ischemia and hypoxia and finally progression of the visual filed damage. In conclusion, our study shows that examining CPA and ultrasonic monitoring of vascular parameters in ophthalmic artery with color Doppler may be the way of better understanding the vascular role in PXG prognosis.

Key words: pseudoexfoliative glaucoma (PXG), primary open-angle glaucoma (POAG), circulating platelet aggregates (CPA), ophtalmic artery (OA), color doppler sonography (CDI), visual field (VF), optic nerve (ON), ocular blood flow (OBF)

Introduction

Glaucoma is a syndrome, chronically progressive course, marked by characteristic damage to the optic nerve, assaults on VF and very often, but not always with increased IOP^{1,2}.

Etiology of this illness is multifactorial, and mechamism of optic nerve damage origin is still the subject of debates and investigations. Against earlier dominant mechanic theory, mechanism of optic nerve damage today explains more and more represented vascular theory^{3,4}.

According to vascular theory, damages of nerve fibers in the optic nerve and subsequent assaults on VF arise because of disturbances in blood vessels, in haemodynamics respectively, due to which changes in blood perfusion in the optic nerve head (ONH) arrive. Among the factors that affect blood perfusion disturbances in ONH, besides the changes in small blood vessels are also hematologic anomalies^{3–5}.

Platelet aggregation, as a physiological phenomenon, belongs to basic mechanisms of hemostasis. This mechanisms represent harmonious and balanced group of various reactions helping to maintain the blood within blood vessels in liquid state, or to stop bleeding after an injury^{6,7}.

Circulating platelet aggregates (CPA) are noticed in ischemic conditions of central nerve system (CNS), in ischemic heart illness and at peripheral arterial insuffiencies. Investigations showed that CPA are responsible for occlusion of small branches in brain and coronar blood vessels^{5,8,9}.

Until now investigations have not explained if there is any direct connection between the glaucoma and CPA^{10,11}.

Haematologic abnormalities play an important role in vascular genesis of glaucoma. CPA-s changes in ischemic CNS conditions are well known. Optic nerve is a part of the brain and is also particulary sensible to ischemic conditions. The question of the CPA role in glaucomic illness is imposed, especially at $PXG^{12,13}$.

PXG, as a part of the pseudoexfoliative syndrome (PXS), has not only ocular manifestations but also has accentuated systemic affections to other organs including also blood vessels $^{12-17}$. At PXG, the course of illness is more aggressive and optic nerve damage and VF deterioration is quicker in relation to primary open angle glaucoma $(POAG)^{12-17}$.

Changes of blood perfusion velocities and resistivities in retrobulbar arteries, particularly in the ophthalmic artery, are monitored in substantial number of studies. That is also giving professional and scientific component also to this examination with tendency to find the way to prevent eye-sight loss at aggressive PSG¹³⁻¹⁷.

Examinees and Methods

Examination included 80 patients, 35 (44%) men average age 68.3 ± 7.0 and 45 (56%) women average age 65.7 ± 7 years (t=1.66; p=0.101). Forthy of them suffered from primary open angle glaucoma (POAG) as the control group (healthy), and 40 from pseudoexfoliative glaucoma (PXG) as the experimental group. In POAG group were 11 men (25%) and 29 women (75%). In PXG group were 24 (60%) men and 16 (40%) women (p≤0.001). Average age in PXG patients group was 70 ± 5.0 years, and average age in POAG patients group was 68 ± 6.6 years (p=0.062).

All the examinees were ophthalmologicaly controlled to quicksightness, eye fundus, estimation of optic nerve head excavation (CD>0.5) and intraocular pressure measured with Goldman aplanation tonometer (GAT). In both groups IOP values were <21 mmHg, compensated by means of local antiglaucomatous therapy. Anterior eye

segment biomicroscopy with gonioscopy was also peformed.

Laboratory – haematological testing of CPA values proportion by means of Wu and Hoak method and ultrasonic measurement of blood perfusion in the carotid artery was also performed.

CPA which arise in vivo in patient's circulation are determined as the ratio between number of platelets in plasma performed from blood sample with anticoagulant solution dinatrium-thylene-diamintetracetic acid (Na₂HEDTA) and formaldehyde and number of platelets in plasma anticoagulated only with Na₂HEDTA as the anticoagulant. In case where CPA are present in vivo they are fixed with formaldehyde solution and sedimented during plasma centrifugation, therefore such plasma contains less number of platelets than plasma not containing CPA. Reference values CPA are 0.75–1.1¹⁸.

Solutions are prepared, mixed well and stored into silicionized vials. In further proceedings one plastic vial for centrifugation is marked with the letter A and the other with the letter B. In the vial A is put 2 mL of solution 1, and in the vial B 2 mL of solution B. From the second examinee is taken 2 mL of veinous blood and in each vial is put 0.5 mL of blood. The vials remained 15 minutes at room temperature. After that both vials are centrifugated 8 minutes at 1000 revolutions to obtain plasma rich with platelets. In further proceedings in each plasma is determined number of the platelets. CPA determination test result is expressed with ratio of number of platelets obtained in the vial A and number of platelets obtained in the vial B. In the blood in which is more CPA we found the value of A/B ratio approximately 1, and in the blood in which is less CPA the ratio is less than 1.

Ultrasonic measurement of blood perfusion in the carotid artery was performed with particular attention to haemodynamic changes connected with ophthalmic artery by means of color Doppler.

The velocity of blood perfusion by CDI in joint carotid artery, internal carotid artery and ophthalmic artery was measured. Examiner did not know in which group the examinees belong. Velocity of blood perfusion was measured by standard color Doppler-duplex ultrasonic device. Viviol 3 (General Electric USA) was used and linear probe of 10 MHz in lying position of the body, after five minutes rest.

By color Doppler device, after blood perfusion in the artery was determined, velocity of blood perfusion was read: peak-systolic velocity – vps and end-diastolic velocity – ved. Pefusion velocities are expressed in centimeters per second (cm/s). Ultrasonic indexes were calculated, namely RI (resistivity index) and PI (pulsatility index).

Resistivity index (RI) is obtained by Pourcelot's equation RI = (vps-ved)/vps. Values from 0.55 to 0.75 are considered normal, but higher values tell us about increasing of circulating resistance and stenosing process distally.

Pulsatility index (PI) is obtained by Goslinger's equation PI=(vps-ved/TAP), (TAP is average perfusion through

several heart cycles). All measured data are recorded on polaroid photographs¹⁹.

Visual field (VF) examination was performed by Octopus 101/G2 programme. Beside initial visual field, control visual fields were made in 6 months intervals, three times during 1.5 year. Progressive visual field loss arises in case that for less than six months paracentral and nasal scotoma are increased in diameter, new scotomas arise and relative scotoma progresses into absolute scotoma²⁰.

All patients with cardiovascular conditions, arterial hypertension, diabetes, colagenous, vascular diseases, smokers and those having ocluar operations, patients taking drugs for changing aggregability of blood are excluded from the investigation. Damage in VP must not be caused by other ocluar or neurological diseases.

Obtained results are statistically elaborated in programmes Excel and Statistica 6.0. We used in statistical elaboration Kruskal-Wallis test, Mann-Whitney test, χ^2 test and T-test. We interpreted the results at significance level p<0.005 and showed it tabulary.

Results

Results of our investigation are shown as the results connected to VF progression, CPA values and ultrasonic parameters of carotid artery with particular regard to values of resistivity in the ophthalmic artery between two examined groups (POAG ang PXG).

Visual fields: Between left and right eyes we haven't proved statistically significant difference in damage degrees – visual fields progression, but clinically the difference is evident in both groups (POAG and PXG).

Comparing eyes in these two groups (POAG and PXG) statistically significant difference is found in sense of larger VF progression in the group PXG that in the group POAG (Table 1).

Haemotological examinations (CPA): Having analysed obtained values of circulating platelet aggregates between these two groups (POAG and PXG) we have obtained statistically significantly larger values of CPA ratio in the group POAG than in the group PXG for 1.4 times ($p \le 0.001$) (Table 2).

By Kruskal-Wallis test we have determined that CPA statistically significantly differs according to sex and groups (χ^2 =41.5; p≥0.001). Values of CPA ratio statisti-

	POAG	PXG	p*
Visual fields (VF)	1 (1–3)	2 (1–4)	< 0.001
Visual fields control (VFC)	2 (1–3)	3 (1–4)	< 0.001

^{*}Mann-Whitney test

TABLE 2
DISPLAY OF CPA VALUES RATIO AT GROUPS POAG AND PXG

Circulating platelet aggregates (CPA)	POAG	PXG	p*
Median	0.87	0.63	-0.001
(min-max)	0.63 – 1.01	0.41 – 0.89	< 0.001

^{*}Mann-Whitney test

cally significantly did not differ between men and women in PXG group (Mann-Whitney test: z=1.05; p=0.294) as well as between women and men in POAG group (Mann-Whitney test: z=0.545; p=0.586).

CPA ratio values at women in PXG group are significantly smaller than at women in POAG group (Mann-Whitney test: z=3.46, $p\le0.001$). Statistically is significantly smaller CPA ratio value at men in PXG than in POAG group (Mann-Whitney test: z=5.03; $p\le0.001$).

We have analyzed obtained CPA ratio values between the groups POAG and PXG in a manner that we have divided patients with PXG in 3 groups according to pseudoexfoliation localization (glaucoma): right eye (n=11), left eye (n=16), both eyes (n=13) (Table 3.).

By Kruskal-Wallis variance analysis we have obtained statistically significant difference of CPA ratio values between 4 mentioned groups (POAG and PXG) (χ^2 =41.3; p<00.1)

The difference makes larger CPA ratio value in POAG group toward PXG group with signs of illness on both eyes (Mann-Whitney test. z=3.85; p<0.001) and in PXG group with signs of illness only at the left eye (z=4.4; p<0.001).

CPA at patients with PXG in both eyes statistically does not significantly differ according to CPA ratio value in PXG group with the right eye illness (z=1.23; p=0.154), neither towards PXG group with signs of illness only in the left eye (z=1.36; p=0.113).

Between the groups of patients with PXG signs only in right eye and those having signs of illness only in left eye there is statistically no significant difference in CPA ratio value (z=0.31; p=0.804).

N = number of examinees	n = number of exa- minees with signs of illness at eyes	Circulating platelet aggregates (CPA) Median (min-max)
POAG	Both eyes (n=40)	0.87 (0.63-1.01)
(N=40)	Both eyes (n=13)	0.58 (0.41-0.89)
PXG	Right eye (n=11)	0.68 (0.54-0.86)
(N=40)	Left eye (n=16)	0.62 (0.43-0.85)
		p<0.001*

^{*}Kruskal-Wallis test

Ultrasonic examination of blood vessels: We have also performed ultrasonic (CDI) analysis of examined parameters, velocity and resistivity of left and right side in the ophthalmic artery and internal carotid artery in both glaucoma groups (PXG and POAG).

Increased resistivity index was found in the ophthalmic artery in PXG group in relation to POAG group, where found values were lower (p=0.029). Statistically were significant larger ultrasonic resistivity index were values found in internal carotid artery (RI ACI) (p=0.031) and pulsatility index in internal carotid artery (PI ACI) (p=0.013) in PXG group than in POAG group.

There is also 90% probability for difference between PXG and POAG groups regarding pefusion velocity in diastola in ophthalmic artery (VD AOF) (p=0.106) namely in the sense of bigger values in POAG group than in PXG group. Left and right eyes in POAG group statistically differ significantly only in systolic perfusion velocity in internal carotid artery (VS ACI) (p>0.031), but towards all other variables there is no statistically significant difference.

Between left and right eyes in PXG group exists statistically significant difference regarding systolic perfusion velocity in internal carotid artery (VS ACI) (p=0.025) in sense of larger values found in left eye (Table 4).

Discussion

Our results corelate with Galassi's and associates' investigation who show increased pefusion resistivities at glaucoma in the ophthalmic artery¹⁶.

Earlier examination which we have performed studying circulating platelet aggregates in PXG showed its

connection, but with some larger CPA values, which we consider is the result of remarkably lesser number of examinees included in the study, their difference in age and choosing CPA examination method according to Born²¹.

Our earlier PXG investigation and monitoring haemodynamic ultrasonic parameters of heart showed that there is difference in heart diastola function pointing at discrete asymptomatic miocardic diastolic difunction and stressed systemic connection with PXG²².

Similar haemodynamic disturbances based on the same consecutive patophysiological prinicples of ischemia and hypoxia are also confirmed at visual field damage progression. With decreased values of CPA ratio, i.e. increased hypercoagubile status of the blood, increased perfusion resistivities in the ophthalmic artery, dynamic of progression noticed at PXG does not surprise. Therefore we consider that PXG in relation to POAG is remarkably more aggressive type of glaucoma with worse response to present treatments.

Although controversal and sometimes »incomprehensible« results exist, our study speaks in behalf of vascular changes in PXG which are not connected only to local circulation in the ophthalmic artery, but in one part also to the circulation in internal carotid artery.

Nemeth and associates confirm reliability of perfusions measured by color Doppler in orbital blood vessels and prove reproductibility of results¹⁹.

Our measurements also confirm mentioned findings and speak about relative accuracy at repeated insonations and measurements of velocities and resistivities of blood perfusion at the same patients.

Coagulation disturbances, i.e. increased blood viscosity (hypercagubile status) are noticed at glaucomatous groups,

Average valuex±SD	POAG (n=80)	PXG (n=53)	p*
Perfusion velocity in systola in ophthalmic artery (cm/s) (VS)	364.7 (124.7–570.2)	340.3 (179.5–617.8)	0.283
Perfusion velocity in diastola in ophthalmic artery (cm/s) (VD)	$68.8 \\ (23.2 - 188.4)$	$58.8 \\ (23.2 - 171.9)$	0.106
Resistivity index in ophthalmic artery (RI)	0.79 $(0.28-0.98)$	0.81 $(0.63-0.92)$	0.029
Pulsatility index in ophthalmic artery (PI)	0.331 (0.177–0.44)	0.340 (0.197–0.42)	0.266
Perfusion velocity in systola in internal carotid artery (cm/s) (VS)	341 (185.5–659.8)	362 (170.2–567.9)	0.531
Perfusion velocity in diastola in internal carotid artery (cm/s) (VD)	102.7 (37.7–208)	$95.8\\ (40.6–209.3)$	0.208
Resistivity index in internal carotid artery (cm/s) (RI)	$0.69 \\ (0.37-0.87)$	$0.72 \\ (0.55-0.85)$	0.031
Pulsatility index in internal carotid artery (cm/s) (PI)	0.26 $(0.112-0.386)$	0.29 $(0.134-0.373)$	0.013

^{*}Mann-Whitney test

which probably leads to decreased perfusion, i.e. ischemia and hypoxia of optic nerve forming microvascular disturbance and appertaiming to VF progression $^{23-25}$.

In spite of contradictions on the question of origin of glaucomatous excavation and attack into VF, there is no doubt that ischemia is the consequence of various factors acting independently or combined because of decreased blood perfusion in the optic nerve. Decreased perfusion is the leading cause of characteristic damage and functional deficiency²⁶.

Examining circulating platelet aggregates and monitoring vascular parameters by color Doppler could contribute to a better understanding of early development of

visual field damage at PXG and according to clinical findings also to intervene therapeuticaly.

We think that because of mentioned facts PXG is local manifestation of systemic disturbance.

This seems accetable having in mind similar effects in healing of ischemic tissue at infarctus miocardi and temporary cerebrovascular attacks. There is also noticed influence of platelet aggregation in origin of theese illness^{27–34}.

In conclusion, because of all mentioned, there is no wonder at all about recently renewed interest for studying PXG. In the future we could therefore expect many answers to still unexplained questions and solve at least one part of existing controversies connected to PXG.

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ULOGA CIRKULIRAJUĆIH AGREGATA TROMBOCITA I HEMODINAMSKIH PROMJENA U ARTERIJI OFTALMICI U PROPADANJU VIDNOGA POLJA TIJEKOM PSEUDOEKSFOLIJATIVNOG GLAUKOMA

SAŽETAK

Cilj rada je ispitati ulogu cirkulirajućih agregata trombocita (CAT) kod pseudoeksfolijativnoga glaukoma (PXG) i hemodinamske promjene u arteriji oftalmici (AO) ultrazvučnim obojenim doplerom (CDI) te tražiti progresiju u vidnom polju (VP). Vaskularna komponenta kod PXG i njezina uloga u dinamici progresije VP nije dovoljno objašnjena pa tako i utjecaj CAT na ishemijska događanja vezana za oštećenje vidnog živca i progresiju VP. Ispitivanjem je obuhvaćeno 80 bolesnika, od kojih 35 (44%) muškaraca prosječne životne dobi 68,3±7,0 godina i 45 (56%) žena prosječne životne dobi 65,7±7 godina (t=1,66; p=0,101). Njih 40 bolovalo je od primarnog glaukoma otvorenoga kuta (POAG) kao kontrolna skupina (zdravi), a 40 od pseudoeksfolijativnoga glaukoma (PXG) kao eksperimentalna skupina. Svim ispitanicima učinjen je kompletni oftalmološki pregled: vidna oštrina, pregled očne pozadine, mjerenje očnog tlaka, biomikroskopija prednjeg segmenta oka s gonioskopijom. Također je učinjeno i ispitivanje VP u intervalima od 6 mjeseci u tri

navrata. Izvršeno je i laboratorijsko ispitivanje vrijednosti omjera CAT-a pomoću metode Wu i Hoak i ultrazvučno mjerenje protoka krvi karotidnog stabla, s posebnim osvrtom na arteriju oftalmiku pomoću obojenoga dopplera. Nađene snižene vrijednosti omjera CAT-a su rezultirale hiperkoagubilnošću krvi u skupini PXG. Kod PXG su također nađeni povećani indeksi otpora toka krvi u arteriji oftalmici (RI AO) i unutrašnjoj karotidnoj arteriji (RI ACI) što je u konačnici rezultiralo ishemijom i hipoksijom i tako pripomoglo oštećenu vidnoga polja. U zaključku naša studija pokazuje se da ispitivanjem CAT-a i ultrazvučnim praćenjem vaskularnih parametara obojenim doplerom u arteriji oftalmici može bolje spoznati vaskularna uloga i prognoza PXG.