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Original Scientific Paper

SUPERFICIAL VESSEL DENSITY, RETINAL NERVE FIBER LAYER THICKNESS, AND VISUAL FIELD LOSS IN PRIMARY OPEN-ANGLE AND PSEUDOEXFOLIATIVE GLAUCOMA

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SUMMARY – The aim was to examine peripapillary and macular superficial vessel density, peripapillary thickness of the nerve fiber layer, and inner thickness of the macula in eyes with primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (PXG) depending on the visual field impairment. A total of 50 eyes were diagnosed as POAG and 50 as PXG. Both groups were divided into 3 subgroups according to HAP2 criteria of visual field impairment. All eyes underwent optical coherence tomography angiography (OCT-A). Radial peripapillary capillary (RPC) density, peripapillary retinal nerve fiber layer (pRNFL) thickness, superficial macular vessel density (SMVD), and macular thickness between the inner limiting membrane and inner plexiform layer (ILM-IPL) were compared. The overall mean measured values did not show a statistically significant difference between the groups. All measured parameters were statistically significantly lower with more extensive visual field damage within the group. The strongest positive correlation was found between the pRNFL thickness and RPC density values (p=0.893), and strongest negative correlation between the mean defect of visual field and ILM-IPL (p=-0.824). No statistically significant difference was found between POAG and PXG in all values measured on OCT-A. In conclusion, visual field loss is more strongly correlated with the loss of nerve fiber layer thickness than the superficial vessel density, which may suggest that the loss of RNFL precedes the loss of superficial vessel density.

Keywords: *Superficial vessel density; Retinal nerve fiber layer; Visual field; Optical coherence tomography angiography; Primary open angle glaucoma; Pseudoexfoliative glaucoma*

Introduction

To diagnose glaucoma as the most common cause of irreversible blindness worldwide, functional and structural tests are carried out using visual field and optical coherence tomography (OCT). The use of OCT has improved the diagnosis and monitoring of glaucoma over the last 20 years as it is noninvasive and offers simple safety. In recent years, the use of OCT angiography (OCT-A) has been intensively researched, not only in the diagnosis of glaucoma but also in diabetic retinopathy, senile macular degeneration, and

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retinal vascular occlusion[1]. OCT-A makes it possible to measure density of retinal blood vessels in the optic disc, peripapillary retina and macula. Together with increased intraocular pressure, changes in retinal microcirculation may play a role in the etiopathogenesis of glaucoma. Changes in retinal blood flow, together with retinal nerve fiber layer (RNFL) could therefore also be used for early diagnosis of the disease and monitoring of disease progression, especially if they occur before detectable visual field damage[2]. Previous studies have confirmed that in primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (PXG) there is a correlation between the degree of visual field damage and density of retinal blood vessels on OCT-A[2-5]. PXG is distinguished from the group of secondary open angle glaucoma by its aggressive clinical course, more frequent refractoriness to drug therapy, and more frequent need of surgical treatment [6,7]. Studies looking at differences in the progression of vascular loss between POAG and PXG have produced conflicting results. According to some authors, vascular perfusion parameters did not differ between the two patient groups[5,8-10]. Other authors found greater vascular damage in patients with PXG but some of these studies have been criticized for their poor design and thus unreliable results, making it important to conduct additional studies[11-13]. The aim of this study was to compare the aforementioned changes between POAG and PXG.

Patients and Methods

A total of 100 eyes of 59 patients were included in this longitudinal cross-sectional study between July 2022 and February 2023 at the Department of Ophthalmology, Sestre milosrdnice University Hospital Center in Zagreb, Croatia. We had previously diagnosed 50 eyes as POAG and 50 as PXG. Both investigated groups were divided into 3 subgroups according to HAP2 criteria of visual field impairment, as follows: early glaucomatous impairment (subgroup 1), moderate glaucomatous impairment (subgroup 2), and advanced glaucomatous impairment (subgroup 3)[14]. Each subgroup consisted of 21 eyes with early glaucomatous impairment, 12 eyes with moderate glaucomatous impairment, and 17 eyes with advanced glaucomatous impairment. Visual field tests were performed on an Octopus 900 perimeter (Haag Streit International, Koeniz, Switzerland). All eyes then underwent OCT-A scanning of the optic nerve head and macula on the RTVue XR Avanti device (Optovue, Inc., Fremont, CA, USA). We measured radial peripapillary capillary (RPC) density, peripapillary retinal nerve fiber layer (pRNFL) thickness, superficial macular vessel density (SMVD), and inner limiting membrane-inner plexiform layer (ILM-IPL) thickness. Exclusion criteria were unreliable visual field tests (fixation loss >20%, false positive errors >33%, false negative errors >33%), unreliable OCT-A scans (low signal strength or motion artifacts), any type of glaucoma other than POAG or PXG, history of intraocular surgery (except for uncomplicated cataract surgery or glaucoma surgery), and any other coexisting ophthalmologic pathology or trauma. All measurements were performed by the same two examiners on the same scanning machines. The study was conducted according to the principles of the Helsinki Declaration and good research practice, and all measurements were obtained in the same clinical conditions. Informed consent was obtained from patients during the study and was approved by the institutional Ethics Committee.

Statistical analysis was performed using Medcalc v. 22 (Medcalc Software, Belgium). Comparisons of continuous numeric measurements between two groups were performed by use of Mann-Whitney test and among 3 subgroups with Kruskal Wallis test. Dunn post-hoc test was performed. Gender as categorical variable was assessed with χ^2-test. Correlations were assessed with Spearman rank correlation. The level of statistical significance was set at $p<0.05$.

Results

The study included 59 patients divided into POAG group (mean age 67.4±11.6 years) and PXG group (mean age 72.4±6.6 years). POAG patients tended to be younger than PXG patients, with a statistically significant difference (p=0.012). However, there was no statistically significant age difference among the subgroups of either group (p=0.195/p=0.785). The male-female ratio did not show a statistically significant difference (p=0.069) either. Different measurements

Table 1. Measurements across diagnosis and severity subgroups in PXG group

Variable	Severity			H value[1]	p-value[1]
	Median (IQR)	Median (IQR)	Median (IQR)		
	1 (n=21)	2 (n=12)	3 (n=17)		
MD/VF (dB)	1.9 (1.0-3.3)	7.6 (6.8-9.2)	16.8 (13.2-22.0)	42.7155	<0.0001[2]
Age (yrs)	70.0 (68.3-78.0)	73.5 (68.0-78.0)	74.0 (69.8-78.0)	0.4822	0.7846
ILM-IPL (μm)	94.8 (89.6-102.5)	77.9 (61.6-85.3)	54.2 (50.7-67.2)	27.7083	<0.0001[3]
pRNFL (μm)	99.3 (91.5-104.6)	79.0 (54.5-105.4)	54.8 (51.3-64.7)	22.8217	<0.0001[4]
RPC (%)	49.3 (45.8-51.6)	37.4 (29.0-49.8)	31.3 (24.3-37.9)	24.5266	<0.0001[4]
SMVD (%)	40.0 (37.0-43.1)	33.3 (28.4-37.3)	30.6 (26.8-33.4)	17.028	0.0002[3]

[1]Kruskal-Wallis test H and p values; [2]Dunn post-test indicated significant (p<0.05) pairwise differences among all 3 groups; [3]group 1 was different from groups 2 and 3; [4]group 1 was different only from group 3; PXG = pseudoexfoliative glaucoma; IQR = interquartile range; MD/VF = mean defect of the visual field; ILM-IPL = inner limiting membrane-inner plexiform layer thickness; pRNFL = peripapillary retinal nerve fiber layer; RPC = radial peripapillary capillary density; SMVD = superficial macular vessel density

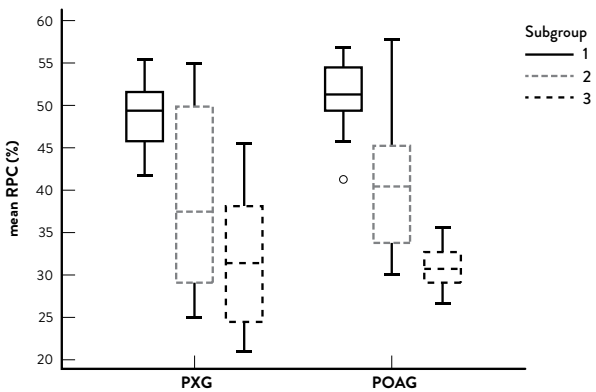

Fig. 1. Distribution of RPC density values in POAG and PXG groups and subgroups.

RPC = radial peripapillary capillary density; PXG = pseudoexfoliative glaucoma; POAG = primary open-angle glaucoma

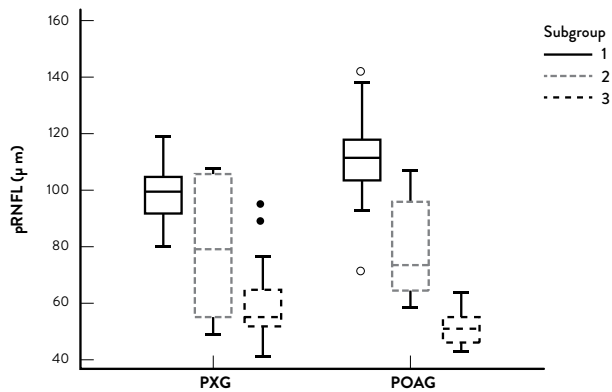

Fig. 2. Distribution of pRNFL thickness values in POAG and PXG groups and subgroups.

pRNFL = peripapillary retinal nerve fiber layer; PXG = pseudoexfoliative glaucoma; POAG = primary open-angle glaucoma

in the two patient groups and subgroups are shown in Tables 1 and 2. Figure 1 shows RPC density values in the two groups and their three subgroups. No statistically significant difference was found between the POAG and PXG groups (p=0.484), while there was a highly statistically significant difference among the subgroups (p<0.001) within each diagnosis, as expected. The same pattern of results held true for pRNFL thickness values (Fig. 2), SMVD values (Fig. 3), and ILM-IPL values (Fig. 4). No statistically significant difference was found between POAG and PXG for the pRNFL thickness values (p=0.730), SMVD values (p=0.302) and ILM-IPL values (p=0.724). On the other hand, for most of the examined variables

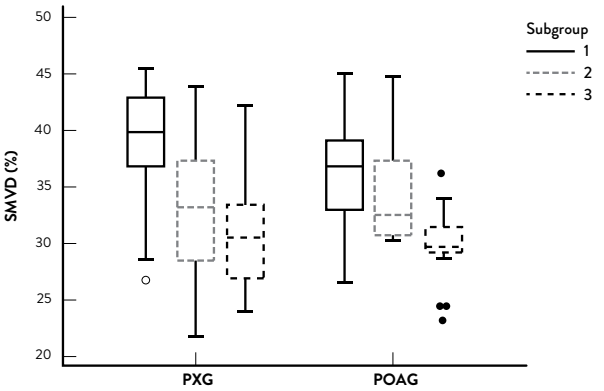

Fig. 3. Distribution of SMVD values in POAG and PXG groups and subgroups.

SMVD = superficial macular vessel density; PXG = pseudoexfoliative glaucoma; POAG = primary open-angle glaucoma

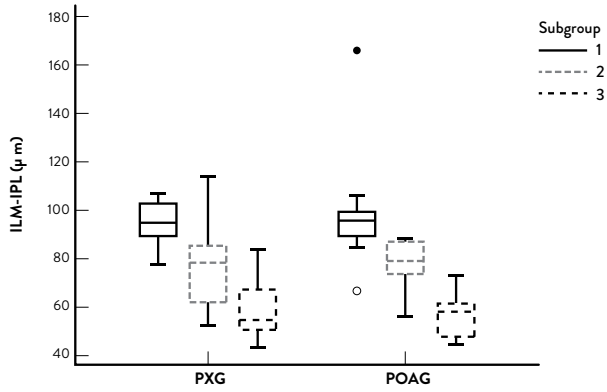

Fig. 4. Distribution of ILM-IPL values in PEX and POAG groups and subgroups.

ILM-IPL = inner limiting membrane-inner plexiform layer thickness; PXG = pseudoexfoliative glaucoma; POAG = primary open-angle glaucoma

Table 2. Measurements across diagnosis and severity subgroups in POAG group

Variable	Severity			H value[1]	p-value[1]
	1 (n=21)	2 (n=12)	3 (n=17)		
	Median (IQR)	Median (IQR)	Median (IQR)		
MD/VF (dB)	4.4 (1.6-5.1)	8.6 (7.4-11.2)	19.5 (16.2-22.7)	42.4465	<0.0001[2]
Age (yrs)	70.0 (59.0-76.0)	71.0 (64.5-81.0)	67.0 (57.0-70.0)	3.2541	0.1948
ILM-IPL (μm)	95.2 (90.0-99.3)	79.0 (73.5-86.9)	57.4 (47.7-61.3)	37.5749	<0.0001[2]
pRNFL (μm)	111.0 (102.6-117.7)	72.9 (64.5-95.6)	50.5 (46.3-54.0)	38.5775	<0.0001[2]
RPC (%)	51.3 (49.3-54.5)	40.3 (33.8-45.1)	30.8 (29.0-32.6)	35.3827	<0.0001[3]
SMVD (%)	37.0 (33.2-39.3)	32.6 (30.7-37.4)	29.8 (29.3-31.5)	17.6134	0.0002[3]

[1]Kruskal-Wallis test H and p values; [2]Dunn post-test indicated significant (p<0.05) pairwise differences among all 3 groups; [3]group 3 was different from groups 1 and 2; POAG = primary open-angle glaucoma; IQR = interquartile range; MD/VF = mean defect of the visual field; ILM-IPL = inner limiting membrane-inner plexiform layer thickness; pRNFL = peripapillary retinal nerve fiber layer; RPC = radial peripapillary capillary density; SMVD = superficial macular vessel density

there was a statistically significant difference among the subgroups in both groups. We found higher results in all studied parameters in subgroup 1 of eyes with early glaucomatous impairment and lower values in subgroup 3 of eyes with advanced glaucomatous impairment. The p values within the subgroups between the measured parameters were as follows: RPC density, pRNFL thickness, SMVD and ILM-IPL, p<0.001 all. The highest positive Spearman rank correlation (Table 3) was found between RPC density and pRNFL thickness (rho=0.893), pRNFL thickness and ILM-IPL (rho=0.861) and RPC density and ILM-IPL (rho=0.843), while the highest negative correlation was found between ILM-IPL and the mean defect of the visual field (MD/VF) (rho=-0.824) and pRNFL thickness and MD/VF (rho=-0.773). All listed correlations were highly statistically significant (p<0.001).

Table 3. Correlation among measured variables

		Severity	MD/VF	ILM-IPL	pRNFL	RPC	SMVD
Severity	Rho p value	1	0.931 <0.0001	-0.823 <0.0001	-0.797 <0.0001	-0.767 <0.0001	-0.563 <0.0001
MD/VF	Rho p value	0.931 <0.0001	1	-0.824 <0.0001	-0.773 <0.0001	-0.763 <0.0001	-0.560 <0.0001
ILM-IPL	Rho t p value	-0.823 <0.0001	-0.824 <0.0001	1	0.861 <0.0001	0.843 <0.0001	0.727 <0.0001
pRNFL	Rho p value	-0.797 <0.0001	-0.773 <0.0001	0.861 <0.0001	1	0.893 <0.0001	0.626 <0.0001
RPC	Rho p value	-0.767 <0.0001	-0.763 <0.0001	0.843 <0.0001	0.893 <0.0001	1	0.726 <0.0001
SMVD	Rho p value	-0.563 <0.0001	-0.560 <0.0001	0.727 <0.0001	0.626 <0.0001	0.726 <0.0001	1

Rho = Spearman rank correlation coefficient; MD/VF = mean defect of the visual field; ILM-IPL = inner limiting membrane-inner plexiform layer thickness; pRNFL = peripapillary retinal nerve fiber layer; RPC = radial peripapillary capillary density; SMVD = superficial macular vessel density

Discussion

Many studies on this topic have compared superficial peripapillary and macular vessel density between POAG and PXG but to our knowledge, no study to date has divided the groups into subgroups based on HAP2 criteria of visual field impairment. A few studies found that there was no significant difference in superficial peripapillary or macular vessel density between POAG and PXG[8-10], whereas others report that the measured parameters of vessel density were statistically significantly lower in the PXG group[11-13,15-17]. However, Duzova *et al.* report that peripapillary vessel density was significantly lower in the PXG group than in the POAG group but there was no significant difference between the PXG and POAG groups in macular vessel density, except for nasal parafoveal vessel density[18]. In our study, there were no statistically significant differences between the groups but we found statistically significant differences among subgroups, as all measured values were lower with progression of the visual field impairment. In some studies, correlation analysis revealed a significant correlation between RNFL and peripapillary vessel density[8,9,12,18]. Subasi *et al.* found that vessel density and the corresponding thicknesses had a significant positive correlation in both the peripapillary and macular regions. In addition, macular vascular parameters were found to correlate with peripapillary vascular parameters[11]. Studies comparing MD of VF found a strong correlation between peripapillary vessel density[12] and macular vessel density[18]. Zloto *et al.* found a significant positive correlation between peripapillary vessel density and central macular thickness in PXG patients[15]. In our study, the strongest positive correlation was found between peripapillary vessel density and peripapillary RNFL and central thickness of the inner macula. We also found that MD of VF correlated more negatively with central thickness of the inner macula and peripapillary RNFL than did peripapillary and macular vessel density. Even though our correlation results do not support this theory, some studies indicate that microvascular damage can be the mechanism underlying changes in PXG and that it can precede significant structural damage[19]. Güngör *et al.* report that structural test results were similar between the healthy group and exfoliation syndrome group, while macular vessel density values were lower in exfoliation syndrome eyes[19]. OCT-A can be one of the first quantitative pieces of evidence of the microvascular disturbance that accompanies exfoliation syndrome and PXG[17].

The limitations of this study were unequal distribution of patients by age and unequal number of eyes in all subgroups, and our study did not include healthy control eyes. Future studies should include a larger number of patients in all groups and subgroups.

It would also be useful to include new variables in the correlation, such as intraocular pressure. Although our study results suggested that there were no significant differences between POAG and PXG patients in all measured parameters, there were significant correlations between peripapillary vessel density, peripapillary RNFL, and central inner macular thickness. Since thickness of the inner macula and peripapillary RNFL also correlated more strongly with MD in VF, we can conclude that the loss of retinal nerve fibers precedes the loss of superficial vascularity, although some other authors reached different results[19]. This calls for further studies in this field because the vascular pathogenesis of glaucoma is still being explored. Overall, our study confirmed that glaucoma disease progression, in this case expressed by peripapillary RNFL and inner macular thickness, as well as peripapillary and macular superficial vessel density, could be detected with OCT-A used in standard ophthalmic practice. Further research is also needed to determine the relevance of vascular diagnostics in glaucoma for early diagnosis and follow-up.

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Sažetak

GUSTOĆA POVRŠINSKIH KRVNIH ŽILA, DEBLJINA SLOJA ŽIVČANIH VLAKANA RETINE I GUBITAK VIDNOG POLJA KOD PRIMARNOG GLAUKOMA OTVORENOG KUTA I PSEUDOEKSFOLIJATIVNOG GLAUKOMA

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Cilj je bio ispitati gustoću peripapilarnih i makularnih površinskih žila, peripapilarnu debljinu sloja živčanih vlakana i unutarnju debljinu makule u očima s primarnim glaukomom otvorenog kuta (POAG) i pseudoeksfolijativnim glaukomom (PXG) ovisno o oštećenju vidnog polja. Ukupno je 50 očiju dijagnosticirano kao POAG, a 50 kao PXG. Obje ispitivane skupine podijeljene su u 3 podskupine prema kriterijima oštećenja vidnog polja HAP2. Sve su oči podvrgnute angiografiji optičke koherentne tomografije (OCT-A). Uspoređene su radijalna gustoća peripapilarnih kapilara (RPC), debljina sloja peripapilarnih živčanih vlakana (pRNFL), gustoća površinskih makularnih žila (SMVD) i debljina makule između unutarnje granične membrane i unutarnjeg zrnatog sloja (ILM-IPL). Ukupne srednje vrijednosti gustoće RPC, debljine pRNFL, SMVD i ILM-IPL nisu pokazale statistički značajnu razliku između skupina. Što je oštećenje vidnog polja unutar skupine bilo veće, to su sve izmjerene vrijednosti bile statistički značajno niže. Najjača pozitivna korelacija utvrđena je između debljine pRNFL i vrijednosti gustoće RPC (p=0,893), a najjača negativna korelacija između prosječnog defekta vidnog polja i ILM-IPL (p=-0,824). Nije utvrđena statistički značajna razlika između POAG i PXG u svim ispitivanim parametrima na OCT-A. Što je oštećenje vidnog polja veće, to su svi ispitivani parametri niži. Gubitak vidnog polja jače je povezan s gubitkom debljine sloja živčanih vlakana nego s gustoćom površinskih žila, što može ukazivati na to da gubitak RNFL-a prethodi gubitku gustoće površinskih žila.

Ključne riječi: *Gustoća površinskih krvnih žila; Sloj živčanih vlakana retine; Vidno polje; Angiografija optičkom koherentnom tomografijom; Primarni glaukom otvorenog kuta; Pseudoeksfolijativni glaukom*