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RETINAL DISORDERS

Effects of intravitreal injection of bevacizumab with or without anterior chamber paracentesis on intraocular pressure and peripapillary retinal nerve fiber layer thickness: a prospective study

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Abstract
Purpose To investigate the effects of intravitreal injection of bevacizumab (IVB) with or without anterior chamber paracentesis on intraocular pressure (IOP) and peripapillary retinal nerve fiber layer (PRNFL) thickness.
Methods In this prospective randomized clinical trial, 90 eyes with center involving diabetic macular edema or wet type age-related macular degeneration (AMD) were randomly assigned to receive IVB either without (group A) or with (group B) anterior chamber paracentesis. IOP was measured before and within 2 min, 30 min, 24 hours and 3 months after injections. Peripapillary spectral-domain optical coherence tomography (SD-OCT) was performed before and 3 months after injections.
Results Mean IOP changes 2 minutes, 30 minutes, 24 hours, and 3 months after injections were 26.4 ± 5.7 mmHg ($P < 0.001$), 6.5 ± 6.3 mmHg ($P < 0.001$), 0.2 ± 2.9 mmHg ($P > 0.99$) and 0.5 ± 2.4 mmHg ($P > 0.99$) in group A and −1.3 ± 2.4 mmHg ($P < 0.001$), −3.2 ± 1.8 mmHg ($P < 0.001$), −3.1 ± 1.8 mmHg ($P < 0.001$) and −1.8 ± 2.2 mmHg ($P < 0.001$) in group B, respectively Mean baseline average PRNFL thickness was 85.3±5.6 μm and 85.6 ± 5 μm in groups A and B respectively. Mean PRNFL thickness changes after 3 month was −2 ± 2 μm ($P < 0.001$) in group A and 0 ± 2 μm ($P = 0.101$) in group B. Mean PRNFL thickness in group A decreased more than group B ($P < 0.001$).
Conclusion Conventional method of IVB injection was associated with acute IOP rise and significant PRNFL loss 3 months after injection. Anterior chamber paracentesis prevents acute IOP rise and PRNFL loss.

Keywords Peripapillary retinal nerve fiber layer thickness · Intravitreal injection · Intraocular pressure · Bevacizumab · Paracentesis

Introduction

Intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents such as bevacizumab is a new and effective treatment against diabetic macular edema (DME), wet-type age-related macular degeneration (AMD) and various other neovascular diseases of the eye [1, 2]. The duration of effectiveness of intravitreal bevacizumab (IVB) is limited. So, repeated injections are necessary to keep its anti-angiogenic effects. A transient and acute volume-related elevation of intraocular pressure (IOP) has been reported to happen following any kind of intravitreal injection [3]. Additionally, there are some reports of sustained elevation of IOP after intravitreal injection of anti-VEGF agents [4–7]. Acute elevation of IOP may increase the possibility of intraocular circulatory disorders, [8–14] which may cause peripapillary retinal nerve fiber layer (PRNFL) loss. Prophylactic anterior chamber paracentesis combined with intravitreal injection of anti-VEGF agents may reduce the risk of IOP elevation postoperatively [15–18]. Therefore, it is important to determine whether intravitreal injection of bevacizumab, transient IOP rises, and IOP fluctuations adversely affect PRNFL thickness. This

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prospective randomized clinical trial was designed to determine the effects of intravitreal injection of bevacizumab with or without anterior chamber paracentesis on IOP and PRNFL thickness.

Methods

This prospective randomized study was conducted between March 2013 and March 2015 at the department of ophthalmology, Labbafinejad medical center, Shahid Beheshti University of Medical Science, Tehran, Iran. The study was approved by the local ethical committee, and followed the tenets of the Declaration of Helsinki. A written consent was obtained from all participants. Eligible patients including those with non-proliferative diabetic retinopathy and center involving macular edema (macular edema within a radius of 500 μm from the center of fovea) and patients with wet-type age-related macular degeneration (AMD) who were candidates to receive intravitreal bevacizumab were enrolled. Each patient was randomly assigned into one of two study groups: in group A no paracentesis was performed, while in group B, prophylactic anterior chamber paracentesis was performed before the injection. Only one eye of each patient was included in the study. The exclusion criteria were as follows: age under 50 years, proliferative diabetic retinopathy, concomitant neovascular AMD and DME in the study eye, history of glaucoma, tilted disc and optic disc anomalies, dementia, nystagmus, one-eyed patients, usage of systemic or topical corticosteroids and history of intraocular injections in the past 6 months. In addition, patients with a history of intraocular surgery other than cataract surgery were excluded. A single IVB was injected in each eye, and all patients were followed for 3 months.

The main outcome measures were: best-corrected visual acuity (BCVA), IOP and PRNFL thickness changes. IOP was measured using a tonopen before and within 2 min, 30 min, 24 h and 3 months after injections. PRNFL thickness was measured using spectral-domain optical coherence tomography (OCT-1000) before and 3 months after injections by an expert technician.

All injections were performed by one surgeon in the operating room using an aseptic technique. First, topical tetracaine 0.5% (Sina Darou Laboratories Company, Tehran, Iran) and povidone–iodine 5% drops were installed. After prep by 5% povidone–iodine and draping the operating field, an eyelid speculum was inserted in order to stabilize the eyelids. Injection was performed 1 minute after instillation of the tetracaine drop. Bevacizumab (1.25 mg/0.05 ml; Genentech, South San Francisco, CA, USA) that was previously dispensed into a single-use 30-gauge needle syringe using an aseptic technique was injected into the vitreous cavity perpendicular to the sclera through the pars plana at the superotemporal quadrant 3.5 to 4.0 mm posterior to the limbus. Simultaneously, a cotton-tip applicator was placed on the entry site to minimize vitreal reflux as the injection needle was withdrawn. In group B, the paracentesis was performed prior to injection as a single slit temporally in the limbal region, with the tip of the 30-gauge needle syringe just penetrating the anterior chamber. Study participants used chloramphenicol eye drops four times daily for 3 days after injection.

Intraocular pressure was measured in sitting position just before and within 2 min, 30 min, 24 h and 3 months after IVB injections. The three first IOP measurements were made by the same ophthalmologist. In this study, all IOP measurements were done using a handheld tonometer (AVIA, Reichert, Inc., Buffalo, NY, USA). In this tonometer, ten applanations are needed to acquire an IOP value. After ten applanations are collected, the LCD displays the IOP along with a statistical confidence indicator. This final digitally displayed IOP was recorded for statistical analysis.

Peripapillary retinal nerve fiber layer (PRNFL) thickness measurements were performed with a 3D spectral-domain OCT-1000 device (Topcon Corporation, Tokyo, Japan). A 3D scan 512 × 128 protocol, covering 6 × 6 mm^2 centered on optic disk was used for all measurements. Using a 3.4 mm diameter circle around the ONH, the measurements were automatically averaged to yield 12 clock-hour thicknesses and a global average PNFL thickness measurement.

Good quality scans had to have focused images from the ocular fundus, adequate quality factor (>45), and the presence of a centered circular ring around the optic disk. Images with discontinuity, misalignment, involuntary saccade, or blinking artifacts were excluded.

Using a group of 43 eyes to detect a 15% difference between two study groups showed that the power of test was 0.90 when the level of significance was 0.05 (two-tailed). The available data was then entered into SPSS version 21.0. Double data entry was conducted to minimize missing data and entry error. All statistical analysis performed by SPSS (Version 21, IBM Corp, Chicago, IL, USA). Normal distribution of variables was assessed by Kolmogorov–Smirnov test and Q–Q plot. To compare the results between two groups we used *t*-test, Mann–Whitney and chi-square test. To compare the results in each group before and after the intervention we used paired *t*-test. IOP comparison during the follow-up period in each group was assessed by linear mixed model. In this evaluation, multiple comparisons were considered by Bonferroni method. *P*-value less than 0.05 were considered as statistically significant.

Results

A total of 90 eyes from 90 patients were recruited based on inclusion and exclusion criteria set at the beginning of the

study (45 for each group). However, only 86 patients (95.5%) completed the 3-month follow-up (44 for group A and 42 for group B). Two patients in group B defaulted from follow-up, one patient from group A developed high IOP (65 mmHg) 2 min after injection which needed anterior chamber paracentesis and was excluded from the study, and one patient in group B was subjected to cataract surgery. Mean age of the recruited patients was 66.4 ± 4.9 years. Thirty-nine patients (88.6%) in group A and 39 patients (92.9%) in group B were treatment-naïve. A few patients in group A and B had the history of previous injections, but there was no history of intraocular injections in the past 6 months (Table 1). Baseline characteristics including gender, best-corrected visual acuity (BCVA), IOP, and peripapillary RNFL thickness were not significantly different between study groups (Table 1). Patients in group B were slightly older than group A (67.8 ± 4.5 vs 64.9 ± 5, $P = 0.016$). At the 3-month follow-up, BCVA improved ($P < 0.001$) but PRNFL thickness decreased significantly ($P \leq 0.001$) compared with the baseline in group A. In this group IOP increased by 5% at the 3-month follow-up, but it was not statistically significant ($P = 0.16$). In group B, 3 months after injections, BCVA improved ($P < 0.001$) and IOP decreased (11%) significantly ($P < 0.001$), while PRNFL thickness did not change significantly ($P = 0.1$).

Between-groups changes were significant for variables PRNFL and IOP after 3 months, but changes in BCVA were comparable between two groups (Fig. 1 and Table 2). Table 3 and Fig. 2 show the mean IOP in group A and B at four IOP measurement points. The mean IOP at baseline was 14.2 ± 2 mmHg in group A and 13.8 ± 2.3 mmHg in group B. Mean IOP changes from baseline to 2 min, 30 min, 24 h and 3 months after injections were 26.4 ± 5.7 mmHg ($P < 0.001$), 6.5 ± 6.3 mmHg ($P < 0.001$), 0.2 ± 2.9 mmHg ($P > 0.99$), and 0.5 ± 2.4 mmHg ($P > 0.99$) in group A, and −1.3 ± 2.4 mmHg ($p < 0.001$), −3.2 ± 1.8 mmHg ($P < 0.001$), −3.1 ± 1.8 mmHg ($P < 0.001$) and −1.8 ± 2.2 mmHg ($P < 0.001$) in group B respectively. Mean IOPs in group A were higher than group B at all measurement points after injections. Thirty minutes after injection, IOP was under 30 mmHg in all eyes, and at 3 months post-injection all IOP readings were ≤20 mmHg in both study groups. In group B, IOPs were lower than baseline in all measurements after injections. Figure 1 shows the distribution of IOP in two study groups at the different points of measurement.

Mean baseline average PRNFL thickness was 85.3 ± 5.6 μm in group A and 85.6 ± 5 μm in group B. Compared to the baseline, 3 months after injections mean PRNFL changes in group A and B were −2 ± 2 ($p < 0.001$) and 0 ± 2 μm respectively ($P = 0.101$). Between group

Table 1 Baseline characteristics of patients

Parameter		Total	A	B	*P*
Age	Mean ± SD	66.4 ± 4.9	64.9 ± 5	67.8 ± 4.5	0.016†
	Median (range)	66 (56 to 76)	64 (56 to 76)	68 (59 to 76)	
Gender	F	47 (54.7%)	26 (59.0%)	21 (50.0%)	0.279*
	M	39 (45.3%)	18 (41.0%)	21 (50.0%)	
Diagnosis	CNV	35 (40.7%)	18 (41.0%)	17 (40.5%)	0.826*
	CSME	51 (59.3%)	26 (59.0%)	25 (59.5%)	
Eye	OD	39 (45.3%)	16 (36.4%)	23 (54.8%)	0.051*
	OS	47 (54.7%)	28 (63.6%)	19 (45.2%)	
History of injection	No	78 (90.7%)	39 (88.6%)	39 (92.9%)	0.714*
	Yes	8 (9.35)	5 (11.4%)	3 (7.15%)	
Baseline IOP	Mean ± SD	14 ± 2.2	14.2 ± 2	13.8 ± 2.3	0.456†
	Median (range)	14 (10 to 18)	14 (11 to 18)	14 (10 to 18)	
Baseline PRNFL Thickness	Mean ± SD	85.5 ± 5.3	85.3 ± 5.6	85.6 ± 5	0.785‡
	Median (range)	86 (72 to 95)	86 (72 to 95)	87 (74 to 93)	
Baseline VA (logMAR)	Mean ± SD	0.99 ± 0.21	0.99 ± 0.22	0.98 ± 0.21	0.787‡
	Median (range)	1 (0.54 to 1.3)	1 (0.6 to 1.3)	1 (0.54 to 1.3)	
Baseline VA (ETDRS)	Mean ± SD	36 ± 11	36 ± 11	36 ± 10	0.787‡
	Median (range)	35 (20 to 58)	35 (20 to 55)	35 (20 to 58)	

*Based on chi-square test.

† Based on *t*-test.

‡ Based on Mann–Whitney test.

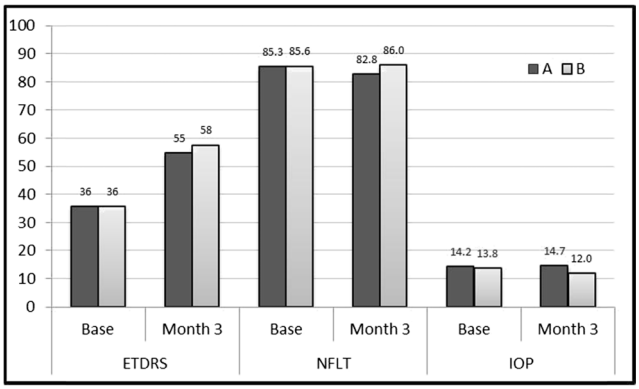

Fig. 1 Mean BCVA, IOP, and PRNFL thickness in groups A and B at baseline and 3 months after injections. PRNFL thickness in group A decreased significantly 3 months after injections

analysis showed that mean PRNFL thickness in group A decreased significantly more than group B. ($P < 0.001$, Table 2).

No complication related to the intravitreal injections or performing anterior chamber paracentesis was observed. We did not have any case of hypotonia or inflammation. There was no case of endophthalmitis during the 3-month period of follow-up after injections.

Discussion

In this prospective randomized study, all post-injection IOP measurements showed significantly higher values in group A (with no paracentesis) than in group B (with paracentesis). Although in both groups and in all eyes, mean IOP values were under 30 mmHg 30 minutes after injections, and IOP readings were ≤20 mmHg in both study groups 3 months after injections, the mean peripapillary RNFL thickness in group A decreased significantly 3 months after injections. Eyes in group A experienced an acute IOP rise and mean IOP of 40.6 mmHg 2 minutes after injections, but there was no acute IOP rise in group B. In group B, IOPs were lower than baseline in all measurements after injections, and the mean peripapillary RNFL thickness did not change 3 months after injections. So, it seems that prophylactic anterior chamber

Table 2 Comparison between preoperative and postoperative BCVA, IOP, and nerve fiber layer thickness

Time		Total		Group				Diff	95% CI	P
				A		B				
		Mean ± SD	Median (range)	Mean ± SD	Median (range)	Mean ± SD	Median (range)			
IOP	Baseline	14 ± 2.2	14 (10 to 18)	14.2 ± 2	14 (11 to 18)	13.8 ± 2.3	14 (10 to 18)	0.3	−0.6 to 1.3	0.456†
	Last	13.4 ± 2.1	13.5 (9 to 18)	14.7 ± 1.6	15 (11 to 18)	12 ± 1.6	12 (9 to 16)	2.7	2 to 3.3	0†
	Change	−0.6 ± 2.6	−1 (−7 to 5)	0.5 ± 2.4	1 (−5 to 5)	−1.8 ± 2.2	−2 (−7 to 2)	2.3	1.3 to 3.3	0†
	Change (%)	−3 ± 18	−7 (−39 to 45)	5 ± 18	7 (−31 to 45)	−11 ± 15	−13 (−39 to 20)	17	10 to 24	<0.001‡
	P-within ξ			.168		.000				
NFLT	Baseline	85.5 ± 5.3	86 (72 to 95)	85.3 ± 5.6	86 (72 to 95)	85.6 ± 5	87 (74 to 93)	0	−3 to 2	0.824†
	Last	84.4 ± 5.8	85 (70 to 94)	82.8 ± 6	84 (70 to 93)	86 ± 5.3	87 (73 to 94)	−3	−6 to −1	0.012†
	Change	−1 ± 2	−1 (−5 to 3)	−2 ± 2	−2 (−5 to 2)	0 ± 2	1 (−3 to 3)	−3	−4 to −2	0†
	Change (%)	−1 ± 3	−1 (−7 to 3)	−3 ± 2	−2 (−7 to 2)	0 ± 2	1 (−4 to 3)	−3	−4 to −3	<0.001‡
	P-within ξ			.000		.101				
BCVA	Baseline	0.99 ± 0.21	1 (0.54 to 1.3)	0.99 ± 0.22	1 (0.6 to 1.3)	0.98 ± 0.21	1 (0.54 to 1.3)	0.00	−0.09 to 0.09	0.935†
	Last	0.58 ± 0.21	0.6 (0.18 to 1.1)	0.6 ± 0.2	0.6 (0.18 to 1.1)	0.55 ± 0.21	0.6 (0.18 to 1.1)	0.06	−0.03 to 0.14	0.212†
	Change	−0.41 ± 0.21	−0.4 (−0.92 to 0.3)	−0.38 ± 0.22	−0.4 (−0.7 to 0.3)	−0.44 ± 0.21	−0.4 (−0.92 to 0.1)	0.05	−0.04 to 0.14	0.263†
	Change (%)	−41 ± 20	−45 (−84 to 43)	−38 ± 21	−45 (−74 to 43)	−44 ± 18	−45 (−84 to 10)	6	−3 to 15	0.237‡
	P-within ξ			.000		.000				
ETDRS	Baseline	36 ± 11	35 (20 to 58)	36 ± 11	35 (20 to 55)	36 ± 10	35 (20 to 58)	0	−5 to 4	0.935†
	Last	56 ± 10	55 (31 to 76)	55 ± 10	55 (31 to 76)	58 ± 10	55 (31 to 76)	−3	−7 to 2	0.207†
	Change	21 ± 11	20 (−15 to 46)	19 ± 11	20 (−15 to 35)	22 ± 11	20 (−4 to 46)	−3	−7 to 2	0.26†
	Change (%)	68 ± 46	62 (−30 to 175)	65 ± 46	67 (−30 to 175)	71 ± 47	57 (−11 to 175)	−6	−26 to 14	0.719‡
	P-within ξ			0.000		0.000				

† Based on *t*-test.

‡ Based on Mann–Whitney test.

ξ Based on paired *t*-test.

Table 3 Mean IOP and change from baseline at different time points

Time		Total		Group				*P*†
				A		B		
		Mean ± SD	Median (range)	Mean ± SD	Median (range)	Mean ± SD	Median (range)	
Baseline		14 ± 2.2	14 (10 to 18)	14.2 ± 2	14 (11 to 18)	13.8 ± 2.3	14 (10 to 18)	0.456†
2 min		26.6 ± 14.6	25.5 (8 to 55)	40.6 ± 5.1	41 (33 to 55)	12.5 ± 2.2	13 (8 to 18)	<0.001†
	Change from baseline (mmHg)	12.6 ± 14.6	10 (−7 to 41)	26.4 ± 5.7	26 (17 to 41)	−1.3 ± 2.4	−1 (−7 to 3)	<0.001†
	Change from baseline (%)	92 ± 109	68 (−44 to 318)	192 ± 57	192 (106 to 318)	−8 ± 17	−7 (−44 to 30)	<0.001‡
	P-within§			<0.001		<0.001		
30 min		15.7 ± 6.6	14.5 (8 to 45)	20.7 ± 5.8	19 (14 to 45)	10.6 ± 1.7	10 (8 to 15)	<0.001†
	Change from baseline (mmHg)	1.7 ± 6.7	−1 (−8 to 34)	6.5 ± 6.3	5 (−1 to 34)	−3.2 ± 1.8	−3 (−8 to 0)	<0.001†
	Change from baseline (%)	14 ± 53	−6 (−44 to 309)	50 ± 53	38 (−6 to 309)	−22 ± 10	−22 (−44 to 0)	<0.001‡
	P-within§			<0.001		<0.001		
24 hours		12.6 ± 2.7	12 (8 to 18)	14.4 ± 2.2	14 (11 to 18)	10.7 ± 1.6	10 (8 to 16)	<0.001†
	Change from baseline (mmHg)	−1.5 ± 3	−2 (−8 to 7)	0.2 ± 2.9	0 (−6 to 7)	−3.1 ± 1.8	−2 (−8 to 0)	<0.001†
	Change from baseline (%)	−9 ± 21	−15 (−47 to 64)	4 ± 22	0 (−33 to 64)	−22 ± 10	−18 (−47 to 0)	<0.001‡
	P-within§			>0.99		<0.001		
3 months		13.4 ± 2.1	13.5 (9 to 18)	14.7 ± 1.6	15 (11 to 18)	12 ± 1.6	12 (9 to 16)	<0.001†
	Change from baseline (mmHg)	−0.6 ± 2.6	−1 (−7 to 5)	0.5 ± 2.4	1 (−5 to 5)	−1.8 ± 2.2	−2 (−7 to 2)	<0.001†
	Change from baseline (%)	−3 ± 18	−7 (−39 to 45)	5 ± 18	7 (−31 to 45)	−11 ± 15	−13 (−39 to 20)	<0.001‡
	P-within§			>0.99		<0.001		

† Based on *t*-test.

‡ Based on Mann–Whitney test.

§ Based on linear mixed model analysis, adjusted for multiple comparison by Bonferroni method.

paracentesis is a good method for preventing acute IOP rise and peripapillary RNFL loss.

VEGF is known to have neurotrophic properties [19]. Theoretically, suppression of a neurotrophic cytokine by intravitreal injection of anti-VEGF agents may result in peripapillary RNFL loss. In our study, intravitreal injection of bevacizumab (an anti-VEGF agent) was performed in both groups, but peripapillary RNFL loss occurred only in group A, and there was no RNFL loss in group B. It seems that peripapillary RNFL loss is mostly due to IOP elevation rather than suppression of neurotrophic cytokines. On the other hand, suppression of neurotrophic properties of VEGF may happen after multiple IVB injections. In the current trial, we studied the effects of a single IVB on peripapillary RNFL thickness.

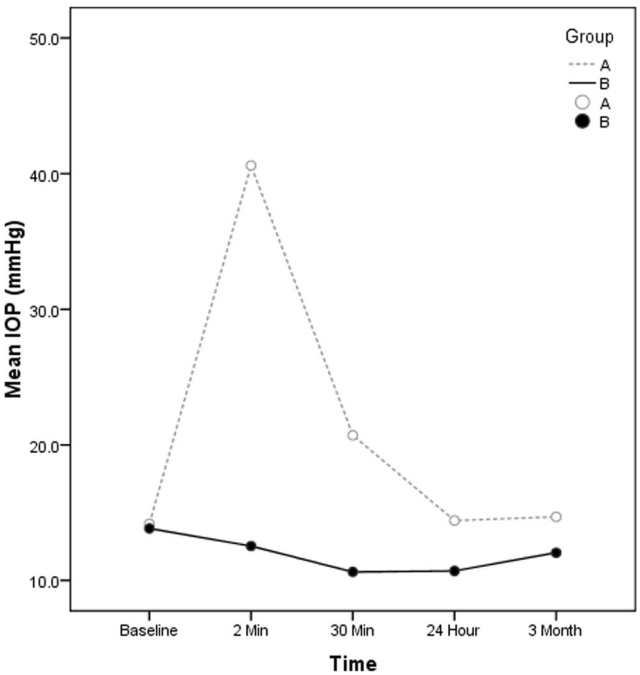

Fig. 2 Comparison of mean IOP at follow-up visits according to treatment groups. There is an IOP spike 2 minutes after injections in group A. There is no IOP spike in group B

The study of Fortune et al. on nine non-human primates using SD-OCT demonstrated that acute IOP elevation adversely affects RNFL thickness [20]. Liew et al. showed that acute IOP elevation in patients with acute angle-closure attack was associated with swelling and thickening of peripapillary RNFL right after the acute attack and then progressed to thinning and atrophy of RNFL over time [21].

Similarly to our study, Martinez-de-la-Casa et al. showed significant reduction in RNFL thickness in patients receiving intravitreal injection of ranibizumab for wet-type AMD [22]. In one study conducted by Fenkel et al. in 2010, it was concluded that intraocular pressure spikes after intravitreal injection of anti-VEGF agents are common and in most cases, prophylactic use of IOP-lowering medications is essentially ineffective in preventing these IOP spikes [23]. In a prospective study on 41 eyes of 41 patients receiving intravitreal pegaptanib injections with or without anterior chamber paracentesis, Knip et al. found that prophylactic anterior

chamber paracentesis helps to prevent high postoperative IOP spikes without causing any additional pain [15].

Our study showed that acute IOP rise and IOP fluctuations following a single intravitreal injection of bevacizumab is associated with significant peripapillary RNFL loss. When looking at the mean peripapillary RNFL thickness values reported in Table 2 and Fig. 1, it seems that in some patients in group B, peripapillary RNFL thickness has increased after injection. This is most likely caused by the SD of the method, and we think that intravitreally injected eyes with anterior chamber paracentesis had nearly stable peripapillary RNFL thickness.

Thinning and loss of peripapillary RNFL has been found to precede measurable visual field defects [24]. It has been shown that up to 40% of peripapillary nerve fibers may be lost without any detectable visual field defect [25]. OCT-derived RNFL thickness has been revealed to have an acceptable correlation with histological measurement [26]. Measurement of peripapillary RNFL thickness with SD-OCT has shown good intra and inter-observer reproducibility [27].

It is proposed that the injury to the peripapillary nerve fibers occurs immediately after IOP rise. Elevation of IOP can cause apoptosis of ganglion cells and peripapillary RNFL loss [28]. Post-injection IOP spikes are short-lived, and IOP usually returns to baseline within 30 to 60 min after injections [29]. Therefore, it is important to measure the IOP within 30 min after injections to be successful in recording these IOP spikes. The rapid elevation of IOP compresses the vessels in the prelaminar region and decreases the blood flow to the optic nerve head [30]. Therefore, ischemic and hypoxic damage occurs in retinal nerve fibers. Since the IOP spikes are transient, when the IOP returns to the baseline, blood flow increases and the second damage (reperfusion injury) occurs in optic disc and retinal nerve axons [31, 32]. Ischemic and reperfusion injury interferes with normal axonal physiology and axonal transport. The ganglion cells cannot tolerate these mechanical compression and vascular insufficiency, so severe blockade of axonal transport and axon death occur [33].

Based on the findings of the present study, even a single intravitreal injection of bevacizumab without anterior chamber paracentesis is associated with significant peripapillary RNFL loss (3% of peripapillary RNFL was lost in group A) 3 months after injection. Patients with retinal vascular diseases such as wet-type AMD, diabetic macular edema and retinal vein occlusion need multiple intravitreal injections. Therefore, these patients are at increased risk of developing glaucomatous optic nerve injury. On the other hand, in our daily practice, there are a lot of patients who received more than 20 intravitreal injections without anterior chamber paracentesis, but they have not lost 60% of their peripapillary nerve fibers. So, it may not be true to say that each intravitreal injection is associated with 3% of peripapillary nerve fiber layer loss. It seems that the injected eye behaves differently in multiple injections, and unknown defense mechanisms may alter the amount of damage to the optic nerve. Earlier reports have shown that intravitreal injection of the same amount of drug without anterior chamber paracentesis causes a different amount of IOP rise in different patients [34]. This may be related to the different amount of reflux from the injection site and scleral rigidity [35]. No relationship has been found between IOP elevation and pharmacological properties of anti-VEGF agents [36]. As mentioned earlier, IOP rise can cause hypoperfusion in optic nerve head. When the perfusion drops below the auto-regulatory range of retina, damage to the optic nerve occurs, and this auto-regulatory range may change in different situations. As candidates for intravitreal injection are mainly elderly and most of them have concomitant cardiovascular diseases and compromised blood flow, even a transient IOP spike might be harmful for the optic nerve and retinal nerve fibers. We recommend anterior chamber paracentesis to prevent these transient IOP spikes. Some rare complications of anterior chamber paracentesis such as patient discomfort, hyphema, lens damage, and infection have been reported in literature [37]. We had no complications associated with anterior chamber paracentesis in our study.

The present study is limited by the short period of follow-up and small number of patients. We also studied a single IVB injection and analyzed mean peripapillary RNFL thickness, and did not compare four peripapillary quadrants. Also, we did not consider phakic or pseudophakic status of the eyes as a variable in our study groups. Additionally, DME and AMD patients have different diseases with different pathogenesis and course, and it was better to use only one group of either DME or AMD patients. Future studies with larger sample size and multiple injections with longer follow-up period and measurement of four peripapillary quadrants and multiple injections are recommended.

In conclusion, this study showed that the conventional method of intravitreal injection of bevacizumab without anterior chamber paracentesis was associated with acute IOP rise and statistically significant nerve fiber layer loss after 3 months. We suggest combined intravitreal injection of bevacizumab and anterior chamber paracentesis as the preferred method of intravitreal injection to prevent acute IOP spikes and peripapillary RNFL loss.

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Compliance with ethical standards

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Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or

other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval The study was carried out after receiving the approval of the research ethics committee of Shahid Beheshti University of Medical Sciences, and was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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