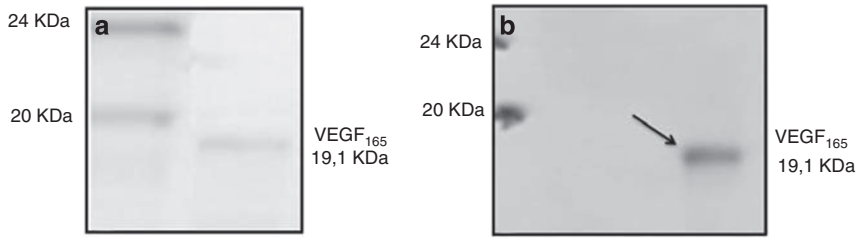

Figure 1 Western blot analysis of $VEGF_{165}$. (a) Right lane: $VEGF_{165}$ is shown on electrophoresis gel stain by Coomassie Brilliant Blue (Sigma-Aldrich) at 19 kDa. Left lane: Trypsinogen (24 kDa) and trypsin inhibitor (20 kDa) were used as controls. (b) Positive immunoreaction of bevacizumab with $VEGF_{165}$ transferred to nitrocellulose membranes was revealed by a chemiluminescent reaction from peroxidase-conjugated goat anti-human secondary antibody (arrow). The corresponding positions of the controls were marked on the left side of the membrane.

evaluated the effect of the lyophilization process of bevacizumab solution on the *in vitro* binding activity of bevacizumab to $VEGF_{165}$.

The commercial solution of bevacizumab (Avastin 100 mg/4 ml; Genentech Inc., South San Francisco, CA, USA) was frozen at −40 °C for 24 h and then lyophilized (E-C Modulyo; E-C Apparatus Inc., New York, NY, USA). It was then stored in dry bottles in a low moist environment. The lyophilized bevacizumab was diluted in Tris-buffered saline at 1 mg/ml and used in western blot analysis.

The $VEGF_{165}$ (Sigma-Aldrich, St Louis, MO, USA) was transferred to nitrocellulose membranes (Transblot 0.45 μM; Bio-Rad Laboratories, Richmond, CA, USA) after denaturation. The blot was blocked by 5% skim milk in Tris-buffered saline with 0.05% Tween (TTBS) for 1 h at room temperature and incubated overnight at 4 °C with primary antibody against VEGF or bevacizumab (Avastin lyophilized) at 1 : 2500 dilution. After washing with TTBS, we incubated blots with peroxidase-conjugated goat anti-human secondary antibody for 30 min at room temperature. After the final wash, we incubated the blot with chemiluminescence reagent (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and the signal was detected with Amersham film (Hyperfilm; GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Gel electrophoresis confirmed the migration pattern of $VEGF_{165}$ (Figure 1a). Subsequently, western blot analysis showed that bevacizumab could still bind to $VEGF_{165}$ *in vitro*. A positive immunoreaction of bevacizumab with $VEGF_{165}$ was revealed by a chemiluminescent reaction (Figure 1b).

This study shows that bevacizumab is still active after freezing and lyophilization, which are essential steps in the development of a biodegradable intravitreal implant. Even deep freezing did not alter the binding activity of bevacizumab to $VEGF_{165}$, which was a concern.[5] Upcoming *in vivo* studies on chick embryos will allow us to definitely test the bioactivity of bevacizumab biodegradable intravitreal implants.

Conflict of interest
The authors declare no conflict of interest.

Acknowledgements

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Sir,

Acute-angle closure glaucoma as the presenting sign of hypertensive crisis

Severe elevations in blood pressure (BP) are classified as hypertensive emergencies in the presence of acute end-organ damage or as hypertensive urgencies in the absence of acute target-organ involvement.[1,2] We report a

patient with high BP and unilateral acute-angle closure glaucoma without any other end-organ damage.

Case report

A 22-year-old man presented with severe pain in the left eye without any significant past ocular history. The right eye examination was within normal limits and the left eye had visual acuity and intraocular pressure (IOP) of 20/400 and 55 mm Hg, respectively. The refractive error was plano in the right and −3.00 in the left eyes. Anterior chamber of the left eye was significantly shallower and gonioscopy revealed Shaffer grade 4 (OD) and 0 (OS). Fundoscopy showed optic nerve head swelling, soft exudates, generalized narrowing of arteries, and venous engorgement (Figure 1). On the basis of these findings, we checked BP and found it to be 250/160 mm Hg. There were no signs or symptoms of hypertensive encephalopathy and the brain MRI was normal. The patient was admitted for intravenous anti-hypertensive medications. Because of the high BP, mannitol could not be administered and the IOP was lowered to 28 mm Hg using intravenous lidocaine (0.8 mg/kg) and ocular massage.[3] Although fundoscopy showed no choroidal detachment, ocular sonography revealed choroidal thickening and choroidal effusion (Figure 2). Peripheral iridoplasty was carried out and systemic and topical steroid, topical anti-glaucoma, and cycloplegic medications were started.

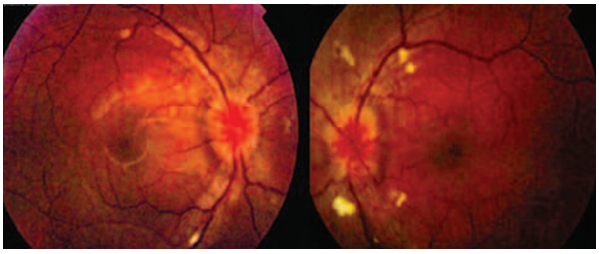

Figure 1 Fundoscopy showing optic nerve head swelling, soft exudates, generalized narrowing of arteries, A-V nicking, and venous engorgement in both eyes.

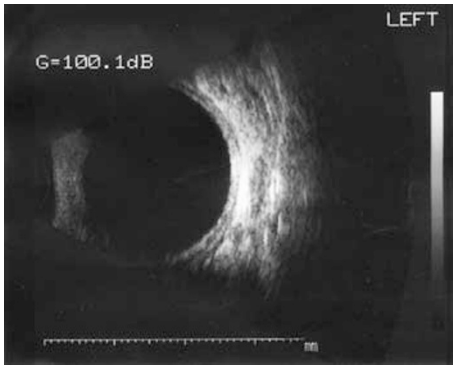

Figure 2 Left eye sonography showing choroidal thickening and choroidal effusion.

After 2 months, the patient's visual acuity returned 20/20 (uncorrected) and IOP was 18 mm Hg without medication. A complete laboratory work-up for possible causes of hypertension was negative.

Comment

The increased BP creates mechanical stress and endothelial injury that can lead to leakage from choroidal vessels and accumulation of fluid in the sub-retinal space.[4] The leaked fluid may result in choroidal effusion and anterior rotation of ciliary body and myopia, as happened in this case.

A PubMed search for 'acute angle-closure glaucoma', 'hypertension' yielded no relevant publications. We cannot attribute with absolute certainty the acute angle-closure glaucoma as end-organ damage of hypertensive crisis. Our aim was rather to sensitize the scientific community to this possibility.

Conflict of interest

The authors declare no conflict of interest.

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Sir,

An unusual case of recurrent endogenous *Klebsiella* endophthalmitis

We report an unusual case of recurrent endogenous *Klebsiella* endophthalmitis.

Case report

A 58-year-old HIV-negative male with diabetes mellitus, intravenous drug use, and *Klebsiella pneumoniae* sepsis complained of floaters of the right eye following 6 days of intravenous ceftriazone. Visual acuity of the right eye was 6/120 with 3+ anterior chamber cells, posterior