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A Pilot, Randomized Sham Control Trial of Autologous Bone Marrow Derived Mononuclear Cells in Acute Ischemic Central Retinal Vein Occlusion

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this pilot, sham controlled randomized control trial (RCT) in patients with ischemic central retinal vein occlusion (CRVO), we studied the safety and efficacy of intravitreal injection of autologous bone marrow derived mononuclear cells and found that both patients who received stem cell injections did not develop anterior segment neovascularization at 1 year follow up. Except for some sterile inflammatory reaction in the initial follow up, no long term injection related serious adverse events (SAEs) were observed. Based on our observations we recommend a larger, multicentric study to further establish the safety and efficacy of this treatment in patients with ischemic CRVO.

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Purpose: To study the safety and efficacy of autologous bone marrow derived mononuclear cells injected intravitreally in patients with ischemic CRVO.
Study Design: Randomized sham controlled trial.
Methods: 4 cases with ischemic CRVO were recruited into the study. 2 cases were randomized into intervention group and 2 into control group. Baseline investigations included best corrected visual acuity (BCVA), intra ocular pressure (IOP), fundus fluorescein angiography (FFA), gonioscopy and optical coherence tomography (OCT). Patients in the intervention group received intravitreal injection of autologous bone marrow derived mononuclear cells (MNCs) and those in control group received sham injection. Patients were followed up over a 12-month period.
Main Outcome Measures: Development of anterior segment neovascularization.
Results: Both patients in the intervention group did not develop anterior segment neovascularization over a follow up period of 12 months. 1 patient in control group developed neovascularization of iris and elevated intra ocular pressure over a follow up period of 6 weeks and required trabeculectomy for control of IOP. The other patient in control group was lost follow up after 2 weeks.
Conclusions: Our initial observations suggest that intravitreal injection of mononuclear cells may reduce the risk of developing anterior segment neovascularization in patients with ischemic central retinal vein occlusion. A larger, multicentric study would be valuable to gain further evidence to our preliminary observations.

Keywords: Mononuclear cells; vascular occlusion; retina; intravitreal injection; neovascular glaucoma.

1. INTRODUCTION

Retinal vein occlusion is the most common vascular cause of visual loss after diabetic retinopathy [1]. Central retinal vein occlusion (CRVO) is the occlusion of central retinal vein at or just behind the lamina cribrosa [2-4]. Based on the studies of May and colleagues, CRVO is classified as ischemic CRVO and non-ischemic CRVO. Ischemic CRVO is defined as central retinal vein occlusion characterized by more than 10 disc areas of retinal non perfusion or more than 50% of capillary non perfusion areas in a 30 degree fundus photograph by fundus fluorescein angiography [5]. Major complications of ischemic CRVO are macular edema and anterior segment neovascularization with subsequent development of neovascular glaucoma. Treatment options available for the management of ischemic CRVO include pan retinal photocoagulation, intravitreal anti-VEGF (vascular endothelial growth factor) agents and corticosteroids. CRVO study has shown that pan retinal photocoagulation causes regression of neovascularisation in 56% of cases if instituted after early evidence of neovascularization of iris (NVI). But neither pan retinal photocoagulation nor macular grid can help in improvement of vision [6]. RAVE trial (Rubeosis Anti-VEGF trial) has shown that after intravitreal injection of ranibizumab monthly for 9 months, at 6 months of follow up 90% of cases had resolution of macular edema, 60% of cases had improvement in visual acuity by four lines and none of the patients developed neovascularization of iris. At 3 years of follow up however, patients had deterioration of visual acuity and 30% of cases developed neovascular glaucoma [7]. So far there is no established treatment algorithm for ischemic CRVO.

Studies have shown that injection of autologous bone marrow derived mononuclear stem cells in ischemic stroke results in axonal plasticity and functional recovery in both experimental models and patients [8-10]. The neuro-protective effect of stem cells is presumably due to expression of neurotrophic factors like insulin like growth factor, basic fibroblast growth factor and epidermal growth factor which rescue the injured neuron. Similarly, intravitreal injection of mononuclear bone marrow derived stem cells in animal model of retinal ischemia have shown reduction in development of pre-retinal neovascular tufts [11].

2. METHODS

Approval was obtained from institutional committee for stem cell research and therapy (letter enclosed) and institute ethics committee (IESC/T-448/30.11.2012), AIIMS. Patients were recruited from the Retina clinic services at Dr. Rajendra Prasad Centre for Ophthalmic Sciences, A.I.I.M.S (All India Institute of Medical Sciences). They were evaluated by both retina (PV) and glaucoma specialists (RS). Bone marrow aspiration was carried out by specialists in the department of hematology (TS). Mononuclear cell layer separation was performed

in the Department of Stem cell facility. Four cases with ischemic CRVO confirmed by fundus fluorescein angiography without evidence of anterior segment neovascularization or glaucoma or other concurrent ocular pathology such as cataract or diabetic retinopathy were recruited into the study. All four patients were adults; two of them had hypertension as a risk factor for the development of CRVO. Two patients were of the male gender. By simple randomization using enclosed chits, two of the four cases were enrolled into intervention group and two others into study group. All patients underwent thorough ocular examination including measurement of best corrected Snellen visual acuity and intra ocular pressure (by Goldmann applanation tonometry), slit lamp biomicroscopy for anterior segment evaluation. Goldmann single mirror gonioscopy was performed to rule out anterior chamber neovascularization. All patients also underwent fundus fluorescein angiography (Carl Zeiss Meditec AG, FF450 plus Fundus Camera), to note the retinal perfusion status and SD-OCT (Carl Zeiss Meditec AG, Cirrus 500 HD OCT) to note central macular thickness. Retinal ischemia was more than 70% in all four cases.

Patients in intervention group underwent bone marrow aspiration by a standard technique. In lateral decubitus position, skin over the iliac bone was cleaned with antiseptic solution and draped. Skin and soft tissue down to periosteum was infiltrated with local anaesthetic 1% lignocaine with 1:1000 adrenaline. Approximately 40 ml of bone marrow was aspirated with 15G bone marrow aspiration needle from posterior superior iliac spine. Patients in control group underwent sham bone marrow aspiration in which patients were similarly positioned, parts cleaned and draped, and the skin over the posterior superior iliac spine was pressed with hub of syringe (and no needle) to produce sensation of pain.

Bone marrow aspirate was immediately transferred to the Department of stem cell facility. Bone marrow mononuclear cell layer was separated by Ficoll density separation method. Bone marrow sample was layered over lymphocyte separation medium (Bio Whittaker) and centrifuged at a speed of 1500 rpm for 25 min. Mononuclear cells were aspirated and washed thrice in heparinized normal saline to remove the traces of Ficoll. All the procedures were done under strict aseptic condition. The harvested mononuclear cells were evaluated for viability, total count, morphology and Giemsa staining. Total cell count was calculated by counting the cells in the Neubaur counter under the microscope. Cell viability was identified by using trypan blue dye exclusion test. More than 90% viability was considered as acceptable. Giemsa stain was used to assess cell morphology.

Using flow cytometry, the mononuclear cells were characterized using the following antibodies- CD-34, CD-45 (all antibodies from BD PharMingen). During flow cytometry, approximately 0.5 million mononuclear cells were stained with the above antibodies at 4 degree Celsius for 30 minutes. Isotope controls were also stained in parallel. Analysis was done using FACS LSR-II and FACS DIVA (BD Biosciences) software [12].

Intravitreal injection was given within 2 hours of mononuclear cell isolation. Standard procedure was followed for intravitreal injection. In brief, pupil was dilated with Tropicamide 1% eye drops and ocular surface was anaesthetized using Proparacaine0.5% drops. Eye was cleaned with povidone iodine and draped. Bone marrow derived cell preparation of 0.09 ml suspended in normal saline (containing 6-8 million mononuclear cells) and 0.01 ml of triamcinolone acetonide (containing 0.04 mg of the drug) was drawn into a tuberculin syringe. The mixture was injected at a distance of 3.5-4.0 mm from limbus with a 26G needle in the inferotemporal quadrant. Anterior chamber paracentesis was done to normalize intraocular pressure intraoperatively. For patients in control group, a sham injection was performed; eye was cleaned, draped and the globe was pressed with the syringe hub to produce sensation of discomfort. Postoperatively all patients were prescribed Moxifloxacin 0.5% eye drops to be used four times daily for one week. Patients in the intervention group received only one injection of autologous bone marrow stem cells. None of the patients received any additional intravitreal injections like bevacizumab, ranibizumab or triamcinolone. Also, no periocular injection of corticosteroids was used in the follow up period.

Patients were followed up over a period of 12 months at 1 week, 2 weeks, 4 weeks, 8 weeks, 12 weeks, 6 months and 12 months. At each visit we recorded best corrected visual acuity, and intra ocular pressure. Slit lamp biomicroscopy for evidence of intra ocular inflammation, and iris neovascularization along with gonioscopy for angle neovascularization was also performed. SD-OCT was done in every follow up

while fundus fluorescein angiography was undertaken only at week 4 and week 24. For detailed summary of patient baseline and follow up results please refer to supplementary data sheet.

3. RESULTS

3.1 Case 1 (Intervention Group)

41 year old female with no systemic risk factors and symptoms of 2 weeks duration, baseline BCVA of 1/60 Snellen equivalent and central macular thickness of 764 µ. On first post-operative day there were 2+ cells in AC, which resolved by 2 weeks with topical prednisolone acetate eye drops. At 6 months follow up there was no evidence of anterior segment neovascularization and patient had a BCVA of 6/12 Snellen equivalent and central macular thickness of 262 µ (Fig. 1) Intraocular pressure was within the normal range at all follow-ups.

3.2 Case 2 (Intervention Group)

64 year old hypertensive female with symptoms of 12 week duration, baseline BCVA of 3/60 Snellen equivalent and presence of fine epimacular membrane. On first postoperative day patient had central vitreous haze, which persisted till 4 weeks. It soon cleared spontaneously without the need for any additional therapy. At the end of 12 months patient had no evidence of anterior segment neovascularization with BCVA of 6/60 and pseudohole with pre-existing epimacular membrane (Fig. 2). Intraocular pressure remained normal throughout the follow up.

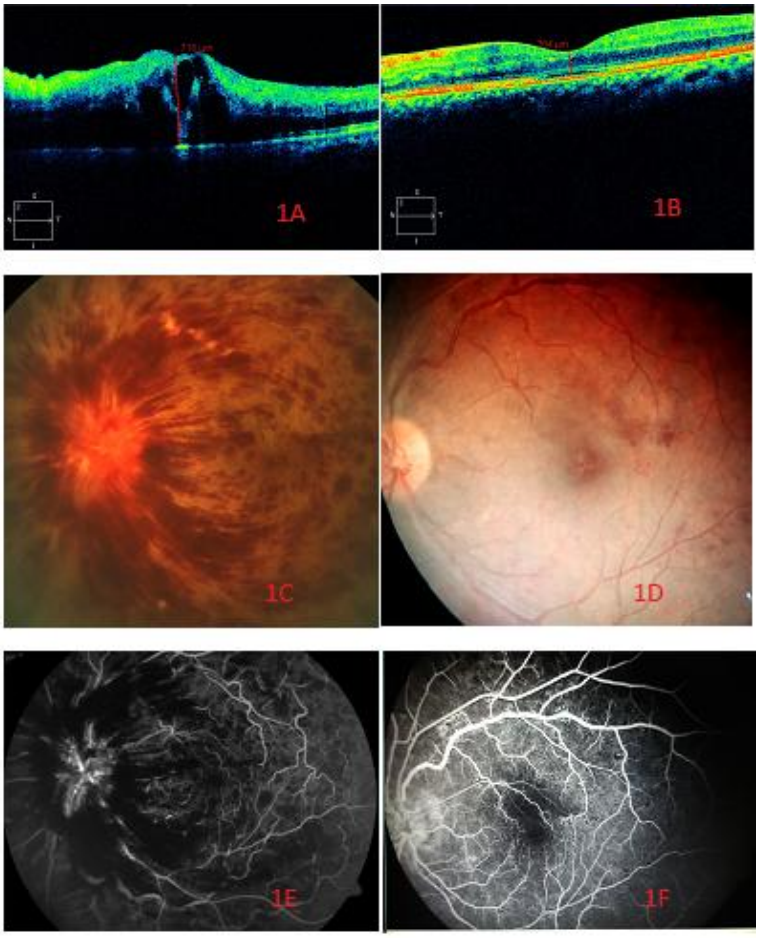

Fig. 1. 1A- Pre intervention SD-OCT showing macular edema with cystic spaces. 1B- 24 week post intervention SD-OCT showing resolution of macular edema. 1C- Pre-intervention fundus photograph. 1D- 24 week post-intervention fundus photograph. 1E- Pre intervention fundus fluorescein angiography. 1F- 24 week post-intervention fundus fluorescein angiography

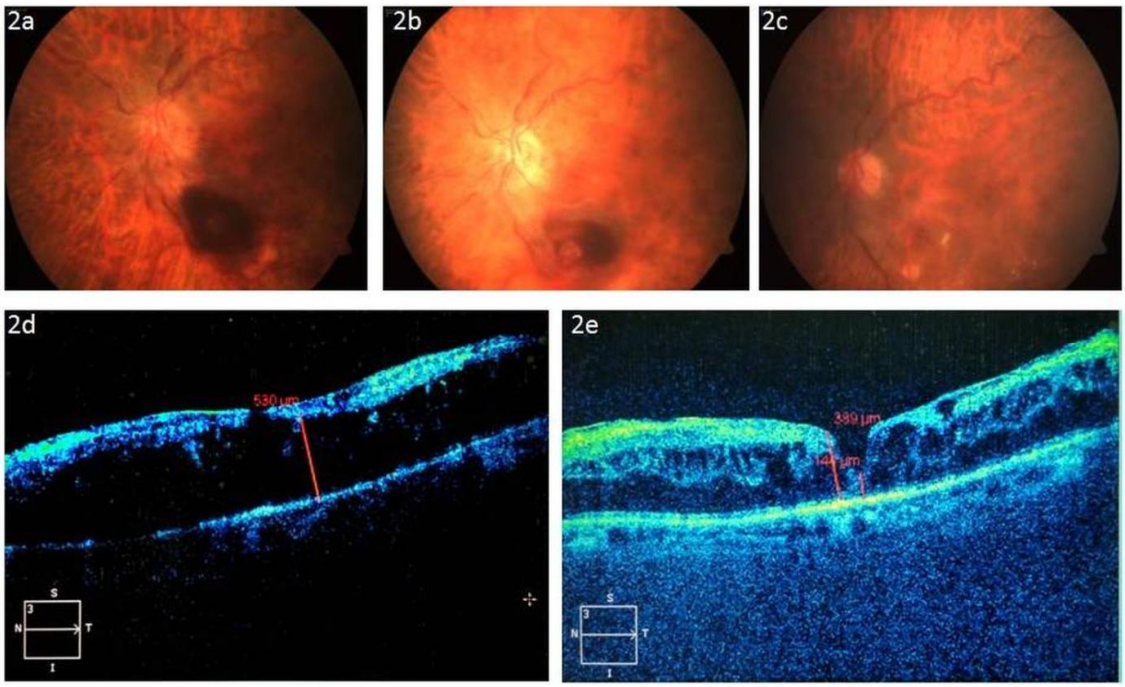

Fig. 2. 2a- Pre intervention fundus photograph. 2b- 4 week post-intervention fundus photograph. 2c- 24 week post-intervention fundus photograph. 2d- Pre intervention SD-OCT showing macular edema with cystic spaces. 2e- 24 week post intervention SD-OCT showing pseudohole with pre-existing epi macular membrane

3.3 Case 3 (Sham Group)

74 year old male with symptoms of 12 week duration with hypertension with baseline visual acuity of 1/60 Snellen equivalent and central macular thickness of 1151µ. There was no evidence of anterior segment neovascularization on 4 week follow up but patient presented at 6 week with complaints of ocular pain. Intra ocular pressure was recorded to be 42 mm of Hg on Goldmann applanation tonometer. There was no evidence of NVI but gonioscopy revealed presence of NVA. Patient underwent pan retinal photocoagulation. Trabeculectomy with Mitomycin C 0.02% was done to control intraocular pressure, as adequate control was not achieved with medical measures.

3.4 Case 4 (Sham Group)

70 year old male with baseline BCVA of 1/60 Snellen equivalent with central macular thickness of 760 µ and history of 10 weeks. Patient was lost to follow up after 2 weeks. At 2 weeks there was no evidence of anterior segment neovascularization and intraocular pressure was normal.

4. DISCUSSION

Ischemic CRVO is a major cause of neovascular glaucoma. Pan retinal photocoagulation after the appearance of anterior chamber neovascularization involving 2 clock hours of iris is the current standard of care. Intravitreal injection of bone marrow derived mononuclear cells has shown some benefit in mouse models of inherited retinal degenerations and retinitis pigmentosa [13,14]. These are chronic disease processes and an end point is difficult to establish. In contrast, CRVO is an acute event that has a well established natural history which evolves over the initial one year. Human studies involving intravenous injection of autologous bone marrow derived stem cells have been done in patients with ischemic stroke, which is also an acute event, and of vascular origin.

Both patients who received intravitreal injection of mononuclear cells had minimal intraocular inflammation in the first week which resolved without any complication. So, it gives a little evidence that the risk of severe intraocular inflammation after intravitreal injection of bone marrow derived mononuclear cells is less likely.

The immediate post-injection sterile reaction was minimal and well controlled due to concurrent injection of microdose of triamcinolone. Thus the combined dose of 0.09 mL of autologous bone marrow derived mononuclear cells and 0.01 mL of triamcinolone was found to be safe and well tolerated.

In both patients in intervention group, anterior segment neovascularization did not develop over a follow up period of 12 months. The best result was observed in case 1 in whom the injection was given within 2 weeks of developing symptoms. Visual acuity in this patient improved from 1/60 to 6/12. This suggests that early intervention with stem cells may be able to aid better functional recovery. Better visual recovery following early intervention has also been observed following intravitreal anti-VEGF injection [15]. In animal models of stroke and some initial stem cell trial in patients with neuronal stroke too, it is believed that early intervention with stem cells is likely to improve functional recovery [16].

An important limitation of our study is the small sample size owing to which it would be difficult to ascertain if the absence / presence of anterior segment neovascularization was a result of the known natural history of CRVO [17].

5. CONCLUSION

In this pilot study we found that a single intravitreal injection of autologous bone marrow derived mononuclear cells produces an early sterile reaction. However, in the long term it was well tolerated and may help to reduce the risk of anterior segment neovascularization in patients with acute onset CRVO. Multicentric and large clinical trials are suggested to add further evidence to our initial observations.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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