A pilot, randomized sham control trial of autologous bone marrow stem cells in acute ischemic central retinal vein occlusion (sic study)

Summary

In this pilot, sham controlled RCT in patients with ischemic CRVO we studied the safety and efficacy of intravitreal injection of autologous bone marrow derived stem cells and found that both patients who received stem cell injections did not develop anterior segment neovascularization at 1 year follow up. No injection related SAEs were observed. Based on our observations we recommend a larger, multicentric study to establish the efficacy of this treatment in patients with ischemic CRVO.

Abstract

Purpose: To study the safety and efficacy of autologous bone marrow derived stem cells injected intravitreally in patients with ischemic CRVO

Study design: Randomized sham controlled trial

Method: 4 cases with ischemic CRVO were recruited into the study. 2 cases were randomized into intervention group and 2 into control group. After baseline investigations including BCVA, intra ocular pressure, fundus fluorescein angiography, OCT, patients in intervention group received intra vitreal injection of autologous bone marrow derived stem cells and 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 the patients in intervention group did not develop anterior segment neovascularization over a follow up period of 1 year. 1 patient in control group developed

neovascularization of iris and raised intra ocular pressure over a follow up period of 6 weeks requiring trabeculectomy for control of intra ocular pressure. Another patient in control group was lost to follow up after 2 weeks.

Conclusions: Though the numbers are very small, our initial observations suggest that intravitreal injection of stem 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.

Key words

Stem cells, vascular occlusion, retina, intravitreal injection, neovascular glaucoma

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 flourescein angiography.[5] Major complications of ischemic CRVO are macular edema and anterior segment neovascularisation with subsequent neovascular glaucoma. Treatment options so far used for the management of ischemic CRVO include pan retinal photocoagulation, intravitreal anti-VEGF agents and steroids. CRVO study has shown that pan retinal photocoagulation causes regression of neovascularisation in 56% of cases if instituted after early evidence of 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 intra vitreal 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 neovascularisation 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 intravenous 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 neurotropic factors like insulin like growth factor, basic fibroblast growth factor, 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]

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. Four cases with ischemic CRVO confirmed by fundus fluorescein angiography without evidence of anterior segment neovascularisation or glaucoma or other concurrent ocular pathology such as cataract or diabetic retinopathy were recruited into the study. Two cases were randomized into intervention group and 2 into study group. All the patients underwent a thorough ocular examination including best corrected measurement of visual acuity and intra ocular pressure by Goldmann applanation tonometer, anterior segment evaluation with slit lamp and Goldmann single mirror gonioscopy to rule out presence of anterior chamber

neovascularisation. All the patients also underwent fundus fluorescein angiography to note the perfusion status and SD-OCT to note central macular thickness.

Patient 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. Patient in control group underwent sham procedure where in patients were 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 stem cells were separated by Ficoll density separation method. Stem cells were 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 heparinised normal saline to remove the traces of Ficoll. All the procedures were done under strict aseptic condition. The harvested stem cells were evaluated for viability, CD 34+ count, total count, morphology and Giemsa staining.

Intravitreal injection was given within 2 hours of stem cell isolation. Patient's pupil were dilated with tropicamide 0.5% and conjunctiva anaesthetized with proparacaine 0.5%. Eye was cleaned with povidone iodine and draped. Stem cell preparation of 0.09 ml containing 6-8 million stem cells was mixed with 0.01 ml of triamcinolone acetonide containing 0.04 mg to counter the possible immunogenic reaction in vitreous cavity. The mixture was injected at a distance of 3.5-4.0 mm from limbus in the inferotemporal qaudrant with 26G needle on tuberculin syringe. AC paracentesis was done. Topical Moxifloxacin 0.5% was instilled for a week after the procedure. For patients in control group, eye was cleaned, draped and the globe was pressed with hub of syringe to produce sensation of pain and similar post-procedure topical drops were prescribed. 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 week, 4 week, 8 week, 12 week and 24 week 36 weeks and 48 weeks, with periodic evaluation of best corrected visual acuity, intra ocular pressure, slit lamp examination for evidence of intra ocular inflammation, iris neovascularization, gonioscopy for neovascularization of angle. SD-OCT was done in every follow up and fundus fluorescien angiography was done at 4, 12 and 24 week.

Results

Case 1 (Intervention group): 64 year old female with symptoms of 12 week duration with baseline BCVA of 3/60 snellen equivalent and presence of epi macular membrane with history of hypertension. On first post op day patient had dense vitreous haze in centre which persisted till 4 weeks. 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.

Case 2 (intervention group): 41 year old female with symptoms of 2 weeks duration with baseline BCVA of 1/60 snellen equivalent and central macular thickness of 764µ with no systemic risk factors. On first post operative day there was 4+ cells in AC which resolved by 2 weeks with topical prednisolone acetate. 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 262µ. (Figure. 1)

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 0.02% mitomycin c was done to control intra ocular pressure as adequate control was not achieved with medical measures.

Case 4 (sham group): 70 year old male with baseline BCVA of 1/60 snellen equivalent with central macular thickness of 760µ 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.

Discussion

Ischemic CRVO is a major cause of neovascular glaucoma. Pan retinal photocoagulation after the development of significant anterior chamber neovascularization involving 2 clock hours of iris is the current standard of care. Intravitreal injection of bone marrow derived stem cells has shown some benefit in mouse models of inherited retinal degenerations and retinitis pigmentosa.[12,13] But these are chronic disease processes and an end point cannot be determined. In contrast CRVO is an acute event, involving inner retina and an end point can be determined. Human studies involving intravenous injection of autologous bone marrow derived stem cells have been done in patients with ischemic stroke[10] which is also an acute event and of vascular origin.

Both the patients who received intravitreal injection of stem 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 stem cells is less likely. The immediate post-injection sterile reaction was well controlled due to concurrent injection of a microdose of triamcinolone. Thus the combined dose of 0.09mL of

autologous bone marrow stem cells and 0.01mL of triamcinolone was found to be safe and well tolerated.

In both the 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 2 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 akin to that well established for stroke victims.

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.[14]

Conclusion In this pilot study we found intravitreal injection of autologous bone marrow cells to be safe and efficacious in patients with acute onset CRVO. Multicentric and large clinical trials are suggested to add further evidence to our initial observations.

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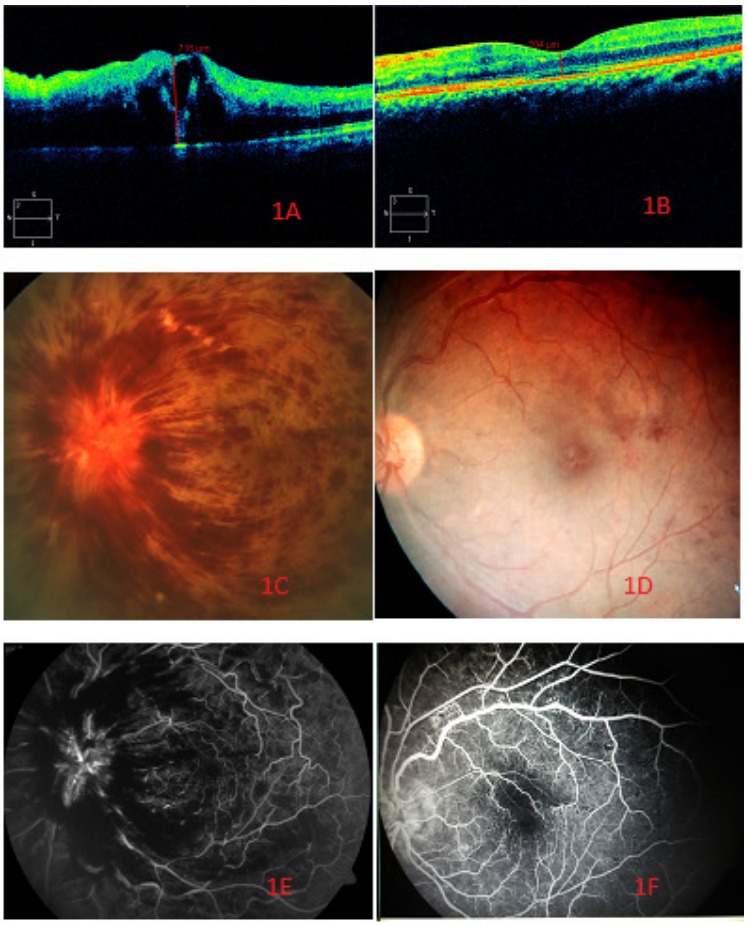
Figure legend

Figure 1: 1A- Pre intervention SD-OCT showing macular edema with cystic spaces. 1B- 6 week post intervention SD-OCT showing resolution of macular edema. 1C- Pre-intervention fundus photograph. 1D- 6 week post-intervention fundus photograph. 1E- Pre intervention fundus fluorescien angiography. 1F- 6 week post-intervention fundus fluorescien angiography.



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