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Journal Name:	<u>British Journal of Medicine and Medical Research</u>
Manuscript Number:	Ms_BJMMR_20784
Title of the Manuscript:	A pilot, randomized sham control trial of autologous bone marrow stem cells in acute ischemic central retinal vein occlusion (sic study)
Type of the Article	Original article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<p>Methods and results need a major and compulsory revision, as both sections lack a lot of details that make it impossible for others to benefit from this work , please find the following details:</p> <ol style="list-style-type: none"> 1) It is a little bit confusing when reading the methodology to know which type of cells has the authors used in there study. In line 79 : “Bone marrow stem cells were separated... “, should be changed to bone marrow mononuclear cells layer ,as yet at this stage stem cells are not separated (cleared in the following comments). 2) In line 80 ; “Stem cells were layered “, actually it’s the bone marrow sample that should be layered over the lymphocyte separation medium for the separation of the mononuclear cells layer that includes lymphocytes , monocytes and a heterogeneous population of 	<p>Thank you for your invaluable corrections.</p> <ol style="list-style-type: none"> 1. Change has been made (line 93) 2. Change has been made (line 94) 3. Change has been made (line 97) 4. Using flow cytometry, the mononuclear cells were characterized using the following antibodies- CD-34, CD-45, CD-3, CD-4 and CD-8 (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. These details are now shown in the text. (line 99-104) New reference from one of our earlier publications has also been added. (Reference 12) 5. Total cell count was done by counting in the Neubaur under 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. These details have now been added. (line 104-106) 6. Supplementary data sheet has been added. (line



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	<p>stem cells</p> <p>3) In line 83; “The harvested stem cells “, the harvested cells are the mononuclear cells and not the stem cells only , as stem cells are never separated using this physical method of separation but instead they can be separated using immunological methods or plastic adherence for the mesenchymal stem cells populations</p> <p>4) In line 84; authors mentioned that they evaluated the CD34+ count, please explain how this count was done, as for the CD34+ hematopoietic stem cells to be counted they have to be first isolated immunologically by using the antiCD34 monoclonal antibody by MACS or FACS technique .Authors didn’t mention to use any of these techniques and thus it is confusing whether they used the MNC layer or the purified CD34+ stem cells subpopulation???</p> <p>5) Authors mentioned that they evaluated the cells viability, count , morphology and purity using the</p>	<p>129)</p> <p>7. Supplementary data sheet has been added. (line 129)</p> <p>8. While it is true that some of the effect seen may be due triamcinolone, there is no literature that has actually studied the effect of such suboptimal doses of triamcinolone of any ocular pathology. Hence, we have already indicated this as a possible limiting factor in our discussion.</p> <p>9. The purpose of using 0.01mL triamcinolone along with 0.09mL was to suppress possible severe reaction to injection of autologous stem cells. Such severe reactions following autologous bone marrow stem cell injections have been observed in some other studies with stem cells conducted at our centre by other authors-unpublished data. These conditions included patients with retinitis pigmentosa and dry form of age related macular degeneration).</p> <p>10. All patients had an ischemia of more than 70% on fluorescein angiography. (line 83-84)</p> <p>11. The primary outcome measure in our study was development of anterior segment neovascularization. Improvement in macular edema and visual acuity were secondary outcome measures. Hence this patient was enrolled for the study.</p>
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	<p>Giemsa stain , the results of these evaluations for both cases, should be mentioned in the results of the study with figures if possible .</p> <p>6) In the results section, although the work was conducted in a very small number of patients, results were not thoroughly shown. For example, you mentioned in the methodology that ocular examination to the patients include the visual acuity, IOP, neovascularization, perfusion and central macular thickness, thus the results of such examination pre and post injection, at the follow-up intervals you mentioned, should be thoroughly presented in your results. So as long as there can't be any statistical analysis for your results due to the insufficient number of cases , at least comprehensive explanation of the ocular examinations done should be added to your manuscript.</p> <p>7) In case 2 , authors described some of the results after 6 month. The results should be shown after 12 month as in the first case and all examination</p>	
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	<p>results should be added in both cases in all follow up intervals.</p> <p>8) Authors mentioned in line 88 that the cells were mixed with triamcinolone acetonide . It is an anti inflammatory and an anti VEGF , thus although added in a very small dose but still can act as a confounding factor??? How can authors offend this?</p> <p>9) In line 89, authors said that the dose of triamcinolone acetonide given was to counter the possible immunogenic reaction in vitreous cavity. Cells were autologous together with very weak immune factors present in vitreous, the use of such drug seems to be unnecessary but instead it acted as a confounding factor to your results as mentioned in the pervious comment.</p> <p>10) The degree of ischemia must be added to your results</p> <p>11) In case 1, Please explain why this patient was still eligible for the study after you found the epi macular</p>	
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	membrane. According to my knowledge ,this membrane may hinder from the resolution of the macular edema	
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<p>Minor REVISION comments</p>	<ol style="list-style-type: none"> 1) In line 85, “..... stem cell isolation” , so if authors worked with the MNCs it should be addressed as MNCs in the manuscript and not stem cells , and if they used the CD34+ subpopulation it should be mentioned as such. I am almost sure that you injected MNCs as the number of cells you injected can never be obtained from primary culture(2hrs from isolation) if it is a purified stem cells subpopulation like CD34+ cells 2) Authors have used many abbreviations through the manuscript and didn’t mention its full text in the first time it was mentioned. Please revise the manuscript and correct accordingly 3) A figure for case 2 should be included in the manuscript as case 1 4) The (sic study) written in the title has no significant meaning so I think it is better to be deleted <p>Ethical issues:</p>	<ol style="list-style-type: none"> 1. Stem cells have been changed to CD34+ Mononuclear cells in the text. 2. All abbreviations have been expanded when they first appear in the text 3. Figure has been added. 4. The abbreviation has been deleted. <p>Ethical issues The normal recommendation for following up patients with central retinal vein occlusion is to follow up every 4 weeks. If during follow up, worsening is observed (by appearance of NVI, as in this patient) then panretinal photocoagulation is undertaken. When we started this trial the results of CRUISE trial were yet to be widely followed and so we did not immediately consider an intravitreal injection.</p>
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	<p>In Case 3, the patient's macular thickness was 1151μ , and was left as such till he suffered from ocular pain after 4 weeks .</p> <p>How ethical is to leave such patient without intervention to conduct the study?</p>	
<u>Optional/General</u> comments	Improvement of the grammar is required	Effort has been made to improve the grammar and we hope this is adequate.