Author's response to reviews

Title: Early accelerated senescence of circulating endothelial progenitor cells in premature coronary artery disease patients in a developing country- a case control study

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Author's response to reviews: see over
Dear Editor,

We thank the reviewers for their useful comments. Please find herewith response to specific comments raised by the reviewers.

Reviewer 1

Major Compulsory Revisions

1. Authors report an inverse correlation between EPCs and TG levels; however, their data shows that PCAD group had lower TGs and EPC number as compared to Control group.

Reply: Most of the PCAD patients in our study were on statins. Statins are known to influence triglycerides levels inversely and EPC numbers positively. Triglyceride levels were lower in PCAD group possibly because of statin use. Although the triglyceride levels were lower in patient group it significantly correlated with EPC numbers. An association between EPC and triglycerides have been reported by other authors also [Jialal I, Devaraj S, Singh U, Huet BA (2010) Decreased number and impaired functionality of endothelial progenitor cells in subjects with metabolic syndrome: implication for increased cardiovascular risk. Atherosclerosis 211: 297-302]. Triglyceride rich lipoproteins have been shown to increase senescence of endothelial progenitor cells via oxidative stress [Liu L, Wen T, Zheng XY, Yang DG, Zhao SP, Xu DY, LU GH (2009) Remnant like particle accelerate endothelial progenitor cells senescence and induce cellular dysfunction via oxidative mechanism. Atherosclerosis 202: 405-414]. Similar mechanism may be responsible for the inverse correlation between EPC and triglycerides in our patients. We have incorporated these points in the revised manuscript (pg 11). Since statins positively influence EPC numbers, we presume the correlation between triglycerides and EPC would have been even stronger in the absence of statin use in the patients. We have included these references in the revised manuscript (References 27, 28).

2. Authors have discussed that Increased TGs in PCAD may be contributing to senescence and reduced EPC in PCAD, but the PCAD
group has lower TGs than controls.

Reply: As explained in the response to comment 1, many of our subjects with PCAD were on statins, which is known to lower triglycerides [Branch A, Florenza AM, Rovellini A, Torri A, Muzio F, Macor S, Sommariva D (1999) Lowering effects of four statins on serum triglycerides. Eur J Pharmacol. 55: 499-502] (Reference 26 in the revised manuscript). The lower triglycerides observed in our PCAD patient group may be due to statin use.

3. Smoking has been shown to be a major independent predictor for the reduction of EPC levels, did authors examine this in their patient group?

Reply: The EPC numbers were lower in smokers (0.0138) as compared to non-smokers (0.0216). However the difference was not statistically significant (P=0.127). This sentence has been incorporated in the revised manuscript (pg 8).

4. Since most of the CAD patients were on Statins and these drugs are known to promote EPC mobilization, proliferation, migration, adhesion, differentiation and reduce senescence and apoptosis independent of their serum lipid-lowering effect. Authors need to discuss this vis-a-vis their results. Was there any correlation between duration or dose of Statin treatment and EPC number and senescence?

Reply: We did not find any correlation between dose of statins and EPC number and senescence. Most of our subjects were on low does of satins (<or=10mg). Inspite of statin use in PCAD patients EPC number was lower. We have included in the revised manuscript a paragraph on the effect of statins on EPC and our findings (pgs 11 and 12).

5. Is atherosclerosis the culprit for low EPC and increased senescence rather than age? If so, what are the factors contributing to this? Authors need to elaborate on this aspect.
Reply: Since the subjects in this study had a mean age of 43 we believe than age is not the culprit for low EPC in PCAD patients. Among the factors we studied we found significant association with only triglycerides. In total subjects (cases and control) EPC number was also associated with HDL. Although the EPC was lower in subjects who smoked the difference was not statistically significant. Physical activity is known to positively influence EPC numbers, which we have not assessed. We believe increased oxidative stress due to smoking and athrogenic lipoprotein phenotype characterized by high triglycerides and low HDL may be the culprit for low EPC and increased senescence. We have incorporated this explanation in the revised manuscript (pg 11).

Reviewer 2

The following revisions are necessary for improving this article.

Major Compulsory Revisions:
1. Since metabolic syndrome and diabetes have been reported to be one of the major factors to accelerate vascular aging, the authors should include medical history of diabetes in the confounding variables taken for adjustment in the statistical analyses of the number of EPCs, telomere length and telomerase activity.

Reply: Additionally adjusting for family history of diabetes did not change the results (The p value remained the same) because there were only 2 patients and 3 controls with family history of diabetes. We have incorporated these findings in the revised manuscript (pg 8).

Minor Essential Revisions:
2. The manuscript must be extensively edited. There are many wrong spacing in the sentences and spelling mistake. For example, in Abstract, L12, ?Result:-- and EPC TLwere? would be added a space as ?TL were?. In Discussion, L19,?patents? must be ?patients?.

Reply: We thank the reviewer for pointing out these errors. We have carefully gone through the manuscript and have made the necessary corrections.
3. In Background, L3, `vascular cellular senescence? should be `vascular cell senescence?.

Reply: The corrections have been incorporated.

4. Legends to Figures A and B are missing. They should be added for correct understanding.

Reply: Legend to figure has been added (pg 24).

5. References should be described according to Instruction to Authors, e.g. abbreviation of the Journals.
Reply: The references have been changed as per instruction to authors.

Discretionary Revisions:
6. The number of EPCs was reported to associate with the endothelial function. It is better to measure the flow-mediated brachial reactivity to validate the data

Reply: We agree with the author that measurement of flow mediated brachial reactivity measurement would have enriched the paper; it is difficult to carry out this now as the study was completed in 2010 and we have no follow-up with the patients.

7. Cellular senescence has several common features including morphological changes: cells flatten and enlarge, increased expression of cyclin-dependet kinase inhibitors such as p16, p21 and p53, decreased replicative capacity, shorter telomere and staining for beta-galactosidase at pH of 6.0: known as senescence-associated beta-galactosidase. The authors only reported the shortening of telomere length and the decrease of telomerase activity. In order to conclude the accelerated vascular senescence in PCAD patients, therefore, the authors should analyze at least another feature of cellular senescence of EPCs.
Reply: As mentioned in the response to previous comment, it is not possible to carry out these investigations now as the study was completed long back.

With regards,

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