



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2013_IJBcRR_7264
Title of the Manuscript:	Can Total Antioxidant Capacity Be A Surrogate Marker In Chronic Obstructive Pulmonary Disease ?
Type of the Article	Original Research Article

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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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SDI Review Form 1.6

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	1. Overall it is hard to follow the manuscript. There is a considerable amount of results and methods information in the discussion that needs to be placed in the appropriate sections of the manuscript. Based on the discussion section it appears there is a lot of emphasis on methods but this is a clinical research manuscript and not a methods paper. 2. The analysis done for Table 4 is not clear. First, why was the mean difference between groups used? It would be better to use "fold change" from the non-smoker control group for all comparisons. Then the appropriate statistical analyses are not just an ANOVA but ANOVA plus a post-hoc type of analysis with Bonferroni or other post-hoc analyses. It is also strange that standard errors are the same for each group of comparisons. This analysis needs to be re-done and then some corresponding graphical representation of key significant results would be helpful. 3. Figures 1 and 2 show no correlation, but actually raise questions about the data set. The results for albumin and bilirubin are relatively "tight" within the patients, but there is huge variability in	I agree with the reviewer. Suggestions incorporated As suggested Statistical section is completely revised. ANOVA plus , post -hoc BONFERRONI as suggested has been applied. Regarding the standard errors same for each group of comparisons. I just want to bring to notice that the formula used in statistic for SE is $S.E. = \sqrt{\text{Error Mean Square} \times \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}$ Where ni, nj are the number of subject in ith jth group. In the four groups ni=nj=nk=nl=43. Therefore SE was same. Anyway the table is now modified in revised manuscript As these figures 1 and 2 of old manuscript were not showing any correlation, therefore on suggestion of other reviewer it has been deleted but the results are discussed in result and



SDI Review Form 1.6

	the urate results. What accounts for this dramatic variability? 4. Figures 3 and 4 do not add anything at all and actually confused the reviewer. What's their purpose? Trolox has not be defined at all in the manuscript. The information presented in figure 4 is not described in the methods. Are these method related figures, but not patient data? 5. It is important for COPD projects to know the GOLD classification of the patient population. How many COPD patients from each GOLD stage – (I-IV)? If this information is available, then if possible, statistical analyses based on GOLD stages would be important. As in other published papers, you can group them as mild (Gold I-II) to moderate/severe (Gold III-IV). 6. The statistical methods section needs to be more specific. If certain software was used, what statistical analyses tests were performed and what is the P value considered significant for each analysis? If T-tests were used were they one-tail or two-tail? If ANOVA was used, what were the post-hoc analyses to assess differences between groups? 7. Glutathione is the major endogenous antioxidant in the body. There is no discussion how this will be factored into the analysis of TAC or considered as a reason for changes in TAC between groups.	discussion section (section no3 of manuscript made as per <u>SDI Paper Template Version 1.6 Date 11.10.2012.</u>) This confusion is now cleared, in materials and method section. Former fig 3 and 4 are presently fig 1 and 2 and are in method section and has been discussed in result and discussion section.. Fig 4 (now fig 2) is definitely the TAC of COPD patient data. I fully agree with the reviewer. Out of 86 COPD patients the gold stage I, II, III and IV are as 8, 29, 33 and 30% which further gets categorised into smokers and non-smokers. Clubbing the data without considering the smoking status leads to 37% mild COPD and 63% moderate/severe COPD. Data was tried statistically but did not yield any appreciable result. I fully agree with the reviewer . Statistical section is now completely revised, which has been meticulously done with help of another scientist in biostatistics who did MSc statistics with specialisation in operation research from IIT, Powai, Mumbai. ANOVA plus , post –hoc BONFERRONI as suggested has been applied. All the suggestions have been incorporated. I fully agree with the reviewer. As this analyte was not estimated in this study it was not discussed.
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SDI Review Form 1.6

	8. Since spirometry was collected from all the patients, can any correlations with the TAC or ceruloplasmin be made based on spirometry results? 9. There is no mention of other medications that the COPD patients might be taking or diet of any of the subjects. Corticosteroids and foods rich in antioxidants, red meat dominant diets might all influence the results. 10. Based on the results presented, this reviewer does not agree with the conclusions. In fact, no clear cut conclusions can be made whether TCA or CP has clinical usefulness for COPD diagnosis.	All the spirometry details and their correlation with number of aspects are being studied by separate team. So it is ethically improper to make any comments. I am looking after the biochemical alterations Dietary pattern was not looked into this study Now completely modified and revised as suggested by this reviewer, the reviewer will hopefully agree
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SDI Review Form 1.6

<u>Minor</u> REVISION comments	1. What do the authors mean by surrogate in the title and in other places in the manuscript? Surrogate is defined as replacement or substitute. What is the substitution? Did the authors mean to say biomarker or clinical marker? 2. Please provide the gender make-up of the smokers vs. non-smokers. How many females and how many males in each group? Does one gender predominate in the smokers for controls and/or COPD? 3. The ferroxidase activity assay description is confusing. There is no information provided to describe the principle of the assay. If this is a published assay, then the specifics of the assay do not need to be included in this manuscript, but only how they may have modified the assay for their individual needs. The principle of the assay is critical for the reader to understand what was done.	I fully agree with the reviewer on the concept of surrogate. Revision on this aspect has been carried out . Yes , correctly, biomarker I agree with the reviewer . one gender predominate but distribution is same in controls and COPD. That has now been incorporated in the manuscript. Principle of ceruloplasmin assay as suggested is now incorporated . It is absolute a new assay for which Indian patent has been granted, and has been used by number of researcher. It has been quoted in national and international journals. Refer N Am J Med Sci. 2013 ; 5(1): 41-46, J Indian Soc Periodontol. 2013; 17(3): 333-337
<u>Optional/General</u> comments	1. In the United States, ceruloplasmin is a standard clinical lab test. There are also ELISA and activity assay kits commercially available which would then allow for better control on the assays. 2. Also measuring C-reactive protein might be beneficial as an overall measure of the inflammatory status of the patient. 3. There are several places where words such as “the” are missing, there are inconsistent use of periods at the end of each reference, and incorrect words used (e.g. “Their instead of The”). It would be helpful to have someone review the manuscript for typos, missing or extra letters, correct word usage and punctuation.	Thanks for the information. Majority of kits including ELISA are based on ceruloplasmin as molecule and doesnot give the idea about its ferroxidase activity. Our method, which costs just 50 paisa (0.5 rupee, reagent cost) gives the more relevant enzymatic activity known as ferroxidase activity and measures in just 30 seconds with 10µL serum. Yes I agree with the reviewer CRP definitely adds English is comparatively polished now, wherever required. Finally, I sincerely thank the reviewer for meticulously going through the manuscript and giving extremely valuable suggestions