Reviewer's report

Title: Association of plasma endotoxin, inflammatory cytokines and risk of colorectal adenomas.

Version: 2 Date: 9 October 2012

Reviewer: Ahmed Abdulamir

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Major compulsory
1. The background focused on the role of the Gram negative bacteria in the dysbiosis associated to CRC while it has been shown in numerous studies that S. bovis and later S. gallolyticus are most strongly associated with CRC and both of them are Gram positive. They need to mention this as it is related to their research.

Methods
2. For ELISA, although they referred to a previous study, there is a need to mention the minimal detectable values for each cytokines detected by the kit used in this study depending on their own standard curve. Personally I prefer they explain the whole procedure again in this study in order to be more readily available for the readers.

3. Again for the RT qPCR, authors are encouraged to mention the whole procedure. They did not mention whether they took into consideration the real duplication factor of their PCR reaction, or have they assumed it was $x^2$, ie. They need to measure the efficiency of PCR reaction. Any RT qPCR without calculating the PCR efficiency is considered invalid.

4. Here I want to ask that RNA quality measurement by Agilent bioanalyzer was directly done after extraction or there was an intervening storage period. Authors need to mention that how long they stored RNA, if any, and was stored at what temp till being converted to cDNA. This is important as RNA is not as table as cDNA, therefore, long periods of storage might affect the RNA integrity and quality. The quality of RNA must be mentioned at least briefly.

Results
5. Authors did not mention the limiting and exclusion criteria of their cases. The medical and infection history of the cases were not shown at all. This is a serious drawback in this study. How can we be sure that the claimed increase in inflammatory cytokines is not due other reasons rather than a possible colonic neoplasm and in the current shortage of patients history and exclusion criteria, how we can trust the correlations done with plasma endotoxin

6. The general characteristics of the study population mentioned in table (1) are not justified within the rationale of the study. Moreover, authors did not get use of
these data for linking with the levels of endotoxin and inflammatory cytokines. As we know obesity, race, fat intake etc might have sort of relation to the studied targets of the study. Therefore, table 1 has lost its core importance. It is strongly advised that authors correlate or associate these criteria with the obtained values of the study.

7. Authors did not classify histologically the adenoma cases. It is well known for the experts in the field that benign adenomas are largely different in terms of prognosis, histopathology, aggressiveness, and transformation rate when compared to non-benign. And adenomas in this study must be classified into tubular, villous and tubule-villous and authors must try to find associations with inflammatory cytokines and endotoxin separately as well.

8. The other thing, authors did not mention the locations of adenomas and did not do statistics about their location, is it in the colon and which part or in the rectum?

9. Taking rectal samples of mucosa from the anal verge is not necessarily a good choice for adenoma. Rectal samples might not reflect the inflammatory state at or near adenomatous lesions especially we do not know the location of adenomas. Second, why authors did not take mucosal samples adjacent or near adenomas (<2cm) in order to detect the local mucosal inflammation at or near to adenomas which is much more precise than taking rectal mucosa from the anal verge or at least presenting 2 lines of investigation; distant mucosal and adjacent or close to adenoma mucosal investigation.

10. Moreover, since colonoscopy was done for detecting adenomas, I suppose adenomas were removed. Hence, why authors did not measure the cytokine levels at adenomatous tissue and relate it to the distant mucosal and plasma levels of cytokines and endotoxin, they need to justify why they didn't do so

Minor

1. -In the abstract, the relationships among plasma endotoxin and plasma and mucosal cytokines expression is expressed in a not clear way, please clarify in more detail. Any abbreviation mentioned in the abstract must be defined.

2. -The conclusion part of abstract seems not clear and not well written

3. -In the keywords must add Limulus Amebocyte Lysate

4. -Many abbreviations are not defined at the first time of appearance such as IBD or UNC.

5. -For the data analysis: the tests used are sound but authors need to justify why they used Spearman's rank test and Mann Whitney tests instead of the parametric counterparts such as t-test. Were their data classified as non-parameteric? if so, what tests did they use to determine the normality of their data.

6. Discussion & conclusion: are good and authors smartly avoided
I think the paper has a strong potential and impact and is important in its field. The paper language is well-written. However, the paper has serious drawbacks which need serious editing.

The current status of the paper is not suitable for publishing, I recommend that the paper is extensively edited to meet the requests of the reviewers before being considered for publication.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests