February 28th, 2016

Dear Editor,

World Journal of Gastroenterology

Please find enclosed the edited manuscript in Word format (file name: CHi3L1PlasmaLeve-Manuscript revision-ESPS Manuscript NO: 21417).

Title: Plasma Chitinase 3-Like 1 is persistently elevated During First Month after Minimally Invasive Colorectal cancer Resection

Authors: H.M.C Shantha Kumara, David Gaita, Hiromichi Miyagaki , Xiaohong Yan ,Sonali AC Hearth, Linda Njoh,Vesna Cekic, Richard L Whelan

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 21417

The manuscript has been improved according to the suggestions of all reviewers:

Manuscript NO: 21417

WJSO Reviewers Comments and replies to comments;

Reviewer 1:

Intriguing article indeed. In the research paper "Plasma Chitinase 3-Like 1 persistently elevated During First Month after Minimally Invasive Colorectal cancer Resection" authors describe a results of a small study in which they assessed blood levels of CHI3L1 after minimally invasive colorectal resection. The paper is written in a good language, the logic is clear and the subject and results are discussed graphically and meaningfully. However, it can be advised to authors to address a few issues before this paper will be published

Comment 1: The title is probably missing the "is": "…is persistently elevated…"

Response: Manuscript title is updated as per the comment.

Comment 2: The Conclusion in the Abstract should follow obviously from the results obtained in this particular study; now it's more describing the authors' hypothesis on the pathogenesis of colorectal cancer recurrence, as the levels of other proangiogenic proteins were not studied here, as well as no experiments have been done on how exactly CHI3L1 promotes the growth of residual tumor. It should be reformulated so that it follows directly from the findings of the research, and does not include guesses arisen after the literature analysis.

Response: Conclusion of the abstract is updated as per comment.

Comment 3: The introduction is well written but sometimes it reminds more a discussion. If possible, it should be made shorter and express more of a clear statement of why this research has been done and what authors wanted to investigate.

Response: The manuscript introduction was updated as per this comment. We have edited the introduction and eliminated some parts of it. The authors believe that it is important to explain briefly the biochemical function of CHI3L1, and how it contributes to enhance the blood angiogenic properties after surgery. The introduction and the discussion is also geared to the surgical readership (who are not likely to have much knowledge of CHI3L1). We left important explanatory sections of the introduction that described underlying principles behind this line of research (similar persistent long duration protein elevations have been noted and that postop plasma has been shown to stimulate endothelial cell proliferation, migration and invasion) so that the reader will understand why the study may have relevance. We apologize for the length of the introduction but hope it will be acceptable.

Comment 4: Concerning statistical analysis in the paper: it is known that the human populations are usually highly heterogeneous, and unless the sample sizes are large enough (200 and more) the normality of distribution should be checked prior to making any comparisons. It can be advised to authors that they perform this analysis (for example, Shapiro-Wilks test or any other); if the data in all groups is distributed normally then the current analysis and description can be retained. But if the data is not distributed normally, neither T-test can be used, nor can the results be described as means ± standard deviation; instead, medians and quartiles should be used, the comparisons should be performed with non-parametric tests, and the figure should rather show box plots or dot plots of data, not the bars of mean with standard deviation.

Response: Statistical analysis was carried out as per reviewer's comments. Since preoperative and corresponding postoperative CHI3L1 values were not normally distributed the comparison of CHI3L1 levels for the Pre vs. Postoperative CRC was performed with the use of non parametric (Wilcoxon signed rank) test and the data were reported as Median and CI values. Also the data is depicted in a bar graph expressing CHI3L1 levels as median and 75% quartile range. We believe this graph is more suitable to explain the CHI3L1 levels difference at each post operative time points.

Reviewer 2: Authors analyzed CRC patients in an IRB approved data/plasma bank who underwent elective MICR. They conclude that persistently elevated CHI3L1 levels, tmay promote the growth of residual tumor after MICR for cancer. Minor recommendation

Comment 1: Please, defined IRB - The introduction is too long (2.5 pages). Some sentences of the introduction are more appropriate for the discussion

Response: Manuscript was updated as per comment. IRB term is defined. The introduction is revised. Please see the response to the similar comment form reviewer 1.

[The manuscript introduction was updated as per this comment. We have edited the introduction and eliminate some parts of it. The authors believe that it is important to explain briefly the biochemical function of CHI3L1 and how it contributes to enhance the blood angiogenic properties after surgery. The introduction and the discussion also focused to the surgical readership (who is not likely to have much knowledge of CHI3L1). We left important explanatory sections of the introduction that described underlying principle behind this line of research (similar persistent long duration protein elevations have been noted and that postop plasma has been shown to stimulate endothelial cell proliferation, migration and invasion) so that the reader will understand why the study may have relevance. We apologize for the length of the introduction but hope it will be acceptable.]

Comment 2: . It would be interesting to compare these data in patients whom underwent an invasive colorectal surgery. Own data or literature.

Response: The number of samples and the remaining volumes of banked plasma per sample in our tissue bank from open colorectal resection patients will not permit us a CHI3L1 study presently. We agree that it would be interesting to see if the abdominal access method utilized impacts the postoperative plasma values. Of note, in the current study, when the CHI3L1

results of the laparoscopic-assisted (n=47) and hand-assisted laparoscopic (n=33) subgroups were compared no significant differences were noted at any postoperative time point despite the fact that the hand-assisted groups mean incision length was at least 4 cm longer than that of the laparoscopic group.

Please note that we have done a comparison of open and laparoscopic colorectal resection groups in regards to plasma VEGF levels, a key plasma protein play an important role in angiogenesis. In this comparison we determined plasma VEGF levels on POD 1 and 3 and compared them to their respective preoperative baselines. On POD 1 a significant increase in the mean plasma VEGF level for the open group was noted; no difference was noted in the laparoscopy group at that time point (Belizon A et al.; Ann Surg. 2006, 244:792-8). On POD 3 both surgical methods were associated with significant increases from baseline although the increase was larger in the open group. We subsequently have done studies that determined blood VEGF levels during weeks 2, 3, and 4 after minimally invasive surgery (MIS) for both colorectal malignancies and benign pathology. A persistent increase was noted during weeks 2 and 3. Unfortunately, we have not done an open vs. laparoscopic comparison for the 2nd, 3rd, and 4th weeks after resection (again because of insufficient available plasma). We have been able to evaluate the impact of pre and postoperative plasma on endothelial cell proliferation in vitro. (1 study of MIS and 1 of open surgery) (Shantha Kumara HMC et al. Surg Endosc. 2012 Mar;26(3):790-5 and Shantha Kumara, H M C et al, Ann Surg. 2009 Jun;249(6):973-7.) These studies demonstrated that postop plasma (from the second and third postop weeks) after both types of surgery stimulated in vitro EC proangiogenic behavior in a similar fashion and to a similar level. We believe that the intra-abdominal wounds (and not the abdominal wounds) are the main source of the added proangiogenic proteins found in the blood. Thus, we do not think that the late postop plasma levels of CHI3L1 would be different in open vs MIS patients although we have no data to support this position presently.

Comment 3: The paragraph starting by "The authors conducted a small randomized clinical study ..." and finalizes with "which makes them safe for the early postop period", is too

speculative. Please, explain some mechanisms or detail the relatiunship that authors tried to establish or delete this part.

Response: The section was deleted and the manuscript title is updated as per the comment.

Reviewer 3 ; Dear authors I read with interest the manuscript n.21417. IT is a well written manuscript about an interesting topic, but I have some minor remark:

Comment 1: Please spell IRB in the abstract

Response: Manuscript was updated as per comment. IRB term in the abstract is defined.

Comment 2: Please make shorter the introduction

Response: The introduction is revised. Please see the response to the similar comment from reviewer 1.

[The manuscript introduction was updated as per this comment. We have edited the introduction and eliminate some parts of it. The authors believe that it is important to explain briefly the biochemical function of CHI3L1 and how it contributes to enhance the blood angiogenic properties after surgery. The introduction and the discussion also focused to the surgical readership (who is not likely to have much knowledge of CHI3L1). We left important explanatory sections of the introduction that described underlying principle behind this line of research (similar persistent long duration protein elevations have been noted and that postop plasma has been shown to stimulate endothelial cell proliferation, migration and invasion) so that the reader will understand why the study may have relevance. We apologize for the length of the introduction but hope it will be acceptable.]

Comment 3: Please compare data from scientific literature about the levels of chi3l1 in invasive surgery; IT should be helpful to add a table about

Response: Please see our below response to the similar comment by reviewer 2.

[The number of samples and the remaining volumes of banked plasma per sample in our tissue bank from open colorectal resection patients will not permit us a CHI3L1 study presently. We agree that it would be interesting to see if the abdominal access method utilized impacts the postoperative plasma values. Of note, in the current study, when the CHI3L1 results of the laparoscopic-assisted (n=47) and hand-assisted laparoscopic (n=33) subgroups were compared no significant differences were noted at any postoperative time point despite the fact that the hand-assisted groups mean incision length was at least 4 cm longer than that of the laparoscopic group.

Please note that we have done a comparison of open and laparoscopic colorectal resection groups in regards to plasma VEGF levels, a key plasma protein pay an important role in angiogenesis. In this comparison we determined plasma VEGF levels on POD 1 and 3 and compared them to their respective preoperative baselines. On POD 1 a significant increase in the mean plasma VEGF level for the open group was noted; no difference was noted in the laparoscopy group at that time point (Belizon A et al.; Ann Surg. 2006, 244:792-8). On POD 3 both surgical methods were associated with significant increases from baseline although the increase was larger in the open group. We subsequently have done studies that determined blood VEGF levels during weeks 2, 3, and 4 after minimally invasive surgery (MIS) for both colorectal malignancies and benign pathology. A persistent increase was noted during weeks 2 and 3. Unfortunately, we have not done an open vs. laparoscopic comparison for the 2^{nd}, 3^{rd}, and 4^{th} weeks after resection (again because of insufficient available plasma). We have been able to evaluate the impact of pre and postoperative plasma on endothelial cell proliferation in vitro. (1 study of MIS and 1 of open surgery) (Shantha Kumara HMC et al. Surg Endosc. 2012 Mar;26(3):790-5 and Shantha Kumara, H M C et al, Ann Surg. 2009 Jun;249(6):973-7.) These studies demonstrated that postop plasma (from the second and third postop weeks) after both types of surgery stimulated in vitro EC proangiogenic behavior in a similar fashion and to a similar level. We believe that the intra-abdominal wounds (and not the abdominal wounds) are the main source of the added proangiogenic proteins found in the blood. Thus, we do not think

that the late postop plasma levels of CHI3L1 would be different in open vs MIS patients although we have no data to support this position presently].

Comment 4: Please compare the clinical implications of the results about chi3l1 with those arising from the other available aforementioned markers

Response: As mentioned in the manuscript (introduction and discussion), in addition to promoting angiogenesis in general there is strong in vivo evidence that Chi3L1 promotes tumor angiogenesis in particular. Kawda M *et all* [Kawada M, et al Oncogene 2012; 31:3111-3123] showed that the CHI3L1 increased the secretion of inflammatory chemokines, IL-8 and monocyte chemoattractant protein-1 (MCP-1) from SW480 cells through mitogen-activated protein kinase (MAPK) signaling pathway in vitro. Furthermore, Chi3L1 expressing colon cancer cells significantly increased macrophage recruitment in xenograft mice, as well as tumor growth and angiogenesis. Macrophages are known to promote cancer progression by producing a number of growth and proangiogenic factors [Pollard JW 2004; 4: 71-78]. We have reported significant long persistent elevation of IL8 and MCP-1 [Shantha Kumara HMC et al World J Gastrointest Oncol 2014; 6: 413-419 Shantha Kumara HMC et al SAGES 2013, April 17-20, Annual Meeting abstract no: 46101- poster] Baltimore, MD USA] in CRC patients after MICR. Persistently elevated levels of plasma CHI3L1 levels together with IL8 and MCP-1 may collectively enhance the bloods angiogenic property after MICR. The manuscript discussion is updated as per the comment.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology.*

Sincerely yours,

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