

## ANSWERING REVIEWERS



August 1, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11748-edited.doc).

**Title:** Clinical significance and usefulness of soluble HB-EGF in gastric cancer

**Author:** Hye Won Chung, Hoon Young Kong, Jong-Baeck Lim

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 11748

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

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) **Accept**

**Comments to Authors** – no

(2) **Accept**

**Comments to Authors**

Interesting manuscript with innovative endpoint and potential clinical impact. It would be interesting if there was a fifth group assessing sHB-EGF levels in post-treatment GC patients and investigate the potential usefulness of this biomarker in disease monitoring as well (e.g. if sHB-EGF levels are reduced after gastric cancer management and rebound in disease recurrence). Nevertheless, it could be the subject of another study. Mixing control group patients with normal mucosa and chronic superficial gastritis (CSG) may cloud the results, since gastric inflammation status may affect (increase) sHB-EGF levels comparing to normal mucosa. It would be preferable to distinct these two sub-groups of patients, although the clinical significance of this study is between non-cancer and cancer patients.

**Response)**

Regretfully, we could not assess sHB-EGF levels in post-treatment GC patients because we do not have the serum sample of post-treatment GC patients. As reviewer pointed, it is the subject of another study. Thus, we could not approve to use the serum sample of these subjects from IRB. However, we will evaluate the serum sHB-EGF levels in post-treatment GC patients in further another study in future as reviewer's recommendation.

For the sHB-EGF levels between normal mucosa and chronic superficial gastritis (CSG), we could access and compare the sHB-EGF levels between normal mucosa and CSG as reviewer's recommendation. They were described in Supplementary Table S1. To conclude, there was no significant difference between normal mucosa (n=15; mean  $\pm$  SD, 83.1  $\pm$  59.0) and CSG (n=15; 89.7  $\pm$  87.6) by independent sample t-test ( $p = 0.811$ ).

### (3) Major Revision

#### Comments to Authors

In this manuscript authors studied the clinical usefulness of soluble heparin-binding EGF-like growth factor as a biomarker for gastric cancer. HB-EGF is overexpressed in several cancer cell lines and cancer tissues including human gastric cancer tissues. HB-EGF is also reported to be involved in malignant phenotype and chemo-resistance of cancer cells. Therefore this study would be useful to develop a useful biomarker for gastric cancer diagnosis. The major concern of this manuscript is the value of HB-EGF in serum. This reviewer has an experience to measure the serum HB-EGF level using the ELISA kit purchased from R & D which is identical to the kit used by these authors. This reviewer's personal data indicated that HB-EGF level in serum was almost 100-1,000 pg/ml in both normal volunteers and cancer patients. The paper published recently also indicated that serum HB-EGF level in ovarian cancer patient was 10-100 pg/ml (Am J Transl Res 2012, 4:415-421). However, in this manuscript authors indicated that average serum HB-EGF levels were about 94.7 and 314.4 ng/ml in normal control group and AGC group, respectively. These values are about thousand times higher than that of reported previously. How did authors determine the value of HB-EGF concentration? At the beginning, this reviewer strongly recommends authors to check the experimental methods, data and evaluation of the data whether they were correctly conducted. The manufacture is not warranting measuring HB-EGF concentration in serum with ELISA kit used for the authors. Therefore, in the second authors should indicate the standard curve for the determination of HB-EGF concentrations by this ELISA method and the method correctly measure serum HB-EGF level.

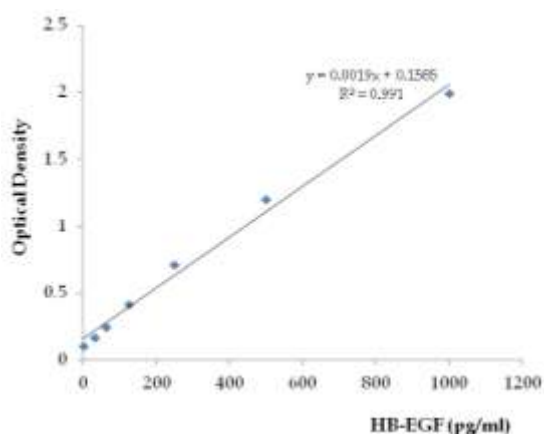
#### Response)

We are afraid that we misdescribed the unit of serum sHB-EGF. The unit of serum sHB-EGF is 'pg/ml'. But, we misdescribed it as 'ng/ml.' Thus, real our results of average serum HB-EGF levels are **between 94.7 pg/ml (normal control) and 314.4 pg/ml (AGC group)**, not between 94.7 ng/ml and 314.4 ng/ml, respectively.

Because 'ng/ml' is thousand times of 'pg/ml,' corrected our results (between 94.7 pg/ml and 314.4 pg/ml) are consistent with the results of the reviewer's personal data (almost 100-1,000 pg/ml) and not thousand times higher than that of reported previously.

We also rechecked the experimental methods, data and evaluation of the data. Here, we showed one example of the standard curve for the determination of HB-EGF concentrations by ELISA method (R & D) among tested triplicate of HB-EGF ELISA results (we tested its levels in triplicate).

Please, see the following figure.



(Standard Curve for HB-EGF)

3 References and typesetting were corrected

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

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Jong-Baeck Lim', enclosed in a thin black rectangular border.

Jong-Baeck Lim, MD, PhD

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