Author’s response to reviews

Title: A case of insulin-like growth factor 2-producing gastrointestinal stromal tumor with severe hypoglycemia.

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Author’s response to reviews:

9th March, 2020

Re: Manuscript BEND-D-19-00536
Dear Dr. CHII-MIN HWU,

We appreciate the suggestions and comments made by the Editorial Committee on our manuscript entitled ‘’A case of insulin-like growth factor 2-producing gastrointestinal stromal tumor with severe hypoglycemia.’’. We wish to submit the revised manuscript along with the attached responses to the Editorial Committee’s comments.

   Again, we thank you for your consideration and patience in reviewing this manuscript and hope that you will now find it suitable for publication in BMC Endocrine Disorders.

Sincerely,

Miwa Morita, MD, PhD
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Editor’s Comments

Both reviewers suggested that the current manuscript needs English editing. Please follow their suggestions.

----- We thank Prof. Chii-Min Hwu. Prior to initial submission, our manuscript was edited by native English speaker of “Springer Nature Author Service” (Submission ID: 2LXYFWZB). In revised manuscript, we carefully checked grammar and spellings again.

Comments of Reviewer: 1

In their manuscript titled "A case of insulin-like growth factor 2-producing gastrointestinal stromal tumor with severe hypoglycemia" Yamasaki et al. describe a rare case of big-IGF2 secreting gastric GIST with initial presentation of hypoglycemia. However, there are some important issues that should be addressed.
We thank Prof. Liang-Yu Lin for the insightful suggestion on our manuscript and constructive comments. Now we successfully answered all questions that Prof. Lin asked except for new western blot analysis by technical issue.

Comment 1: The authors should provide the source of primary antibody for big IGF2 and IGF2 in Western blotting examination. Furthermore, any specific preparation was required before blood sample for Western blotting. The authors should provide better figure in Western blotting exam.

Comment 2: The authors should explain why the IHC stain for IGF2 of tumor is strong positive but negative in Western blotting (Figure 4)?

We appreciate the Reviewer’s superb comments. In IHC analysis, we cannot distinguish the difference between normal IGF2 and a high molecular weight IGF2 (a big-IGF2 or pro-IGF-2E (68-88)), since both are containing same amino acid sequence as normal IGF2 region. Therefore, IHC analysis on tumor samples positive for IGF2 staining is indeed results of immunoreaction of antibody on the normal IGF2 sequence of big-IGF2. As reviewer suggested, Western blot analysis for tumor sample did not display normal IGF2 (7.5 kDa) (if any, very tiny levels) but with very high levels of big IGF2, suggesting that majority of IGF2 produced in tumor is big-IGF2.
In the revised version, we added the following sentences to Discussion and Conclusions parts (red).

Added parts: (Page5, line 140-146)

Although the IHC stain for normal IGF2 of tumor is strong positive, a normal IGF2 was not detected in Western blotting in Figure 4. A high molecular weight IGF2, known as a big-IGF2 or pro-IGF-2E (68-88), contained same amino-acid sequence with normal IGF2 region, therefore it is impossible to distinguish large molecule IGF2 from normal IGF2 by IHC. Western blot analysis for tumor sample did not display normal IGF2 (7.5 kDa) (if any, very tiny levels) but with very high levels of big IGF2, suggesting that majority of IGF2 produced in tumor is big-IGF2.

Comment 3: What is "CPR" in the table 1? Is it a typing error?

-----We appreciate the Reviewer’s comments. CPR is an abbreviation for C peptide. In the revised version, we changed CPR to C peptide in Table 1.

Comment 4: Arrowheads or arrows are required in figure 1, 2 and 3 in order to point out the tumor mass and IHC staining.

-----We appreciate the Reviewer’s comments. In the revised version, we added triangular arrow in Figure 1 and 3. Since, histological analysis, cell morphological/staining patterns are quite diffuse and is difficult to indicate specific cell or cite, therefore we added parts of the Titles and legend (red), as shown below.

Added parts: (Page12)

Figure 2. Pathological findings of endoscopic ultrasound-guided fine-needle aspiration biopsy

A. Hematoxylin-eosin (HE) staining shows hyperplastic spindle cell. (X400)

B. Immunostaining for c-KIT is diffuse positive in tumor cytoplasm. (X400)

C. Immunostaining for Discovered on DOG-1 is diffuse strongly positive in tumor cytoplasm. (X400)

D. Immunostaining for ki-67 is positive in nucleus. (X400)

Figure 3. Pathological findings of the resected specimen
A. Hematoxylin-eosin (HE) staining shows that tumor is primary muscular coat of gastric. (X100)

B. Immunostaining for IGF2 is diffuse positive in tumor cytoplasm. (X100)

Comment 5: The authors should ask native English speaker to check the manuscript.

-----Prior to initial submission, our manuscript was edited by native English speaker of “Springer Nature Author Service” (Submission ID: 2LXYFWZB). In revised manuscript, we carefully checked grammar and spellings again.

Comment 6: The Information on outcome is very sparse. The authors described that patient did not require to maintain the glucose level only.

-----We appreciate the Reviewer’s comments. We added parts of the Case presentation (red), as shown below.

Added parts: (Page5, line 127-128)

Since then, he has been monitored by CT in our hospital, and is free from relapse of tumor or hypoglycemia for 2.5 years.

Comments of Reviewer: 2

A very interesting case report of an 82 year old man with insulin-like growth factor 2 (IGF2) producing gastrointestinal stromal tumor causing severe hypoglycemia.

-----We thank Prof. Ting-I Lee for positive evaluation of our manuscript.

Comment 1: In your case presentation, describing contrasted abdominal CT (Figure 1) please indicate whether this is figure 1A, B, or C? Additionally, kindly add which figure described "A retrospectively unclassified ~20mm mass…”

-----We appreciate the reviewer’s comments. We changed figure number and added parts of the Case presentation (red), as shown below.

Added parts: (Page4, line 108-109)
Contrasted abdominal CT revealed a large lobulated mass of 116 x 70 x 72 mm with central necrosis and heterogeneous enhancement around the gastric corpus (Fig 1 A and B). A retrospectively unclassified ~ φ20 mm mass was found in the same location on a CT (Fig 1C) for follow-up of regular IPMN (1.5 years ago).

Comment 2: After the operation, IGF2 was decreased, and IGF1 and ratio of IGF2/IGF1 remained elevated. You only mentioned that IGF1 was not yet normalized. But why is there a gradual elevation of the IGF1 levels? This point should be further discussed.

-----We appreciate the reviewer’s comments. We added following sentences in the discussion. We added parts of the Discussion and Conclusions (red), as shown below.

Added parts: (Page6, line 165-171)

The reason why the restoration of IGF1 levels was not completed in our patients after operation was not clear yet. The big IGF2 is thought to suppress GH secretion and thereby reduces IGF1, therefore it could be possible that GH-IGF1 axis is not normalized yet at 1 week after operation. Also, we could not measure the levels of IGFBPs, by which IGFs levels and turn over were significantly modified (Yakar S, et al, FASEB J, 2009). Therefore, the potential contribution of IGFBPs level alteration before and after operation on the levels of IGF1 cannot be excluded.

Comment 3: In table 1, what is the reference range of IGF1? The whole words of the abbreviations should be written in the footnotes such as Alb: albumin (or you write the whole word in the table)….You also mentioned Glucagon was measured by radioimmunoassay, how about the rest of the exams how was they measures? by enzyme-linked immunosorbent assay or what methods?

-----We appreciate the reviewer’s comments. The reference range of IGF1 is differ by age and sex, only up to 77 years old. As a reference, we added a reference range of 77 years old man. We modified table 1 to avoid using abbreviations and added Examination method in the footnotes as shown below.

Added parts: (Page11-12)

Glucagon and IGF1 were measured by radioimmunoassay (RIA); ACTH, cortisol, and growth hormone were measured by chemiluminescent enzyme immunoassay (CLEIA); adrenalin, noradrenalin, and dopamine were measured by High Performance Liquid Chromatography (HPLC).
Reference range of Insulin growth factor 1 (IGF1) for 77 years old male. The reference range of IGF1 is differ by age and sex, only up to 77 years old.

Comment 4: Table 2, abbreviations of the whole words should be written in the footnotes such as IGF1: insulin growth factor 1… What is the normal range of IGF1?

-----We appreciate the reviewer’s comments. We added the whole words for IGF1, IGF2 in the footnotes. For the above reasons (please see the Reply for comment 3), we added a normal range of 77 years old man in the footnotes.

Comment 5: Figure 1 CT scan of the abdomen. Summary description of significant findings and arrows on the significant finding. "B, C; at the time of admission does this means B and C at the time of admission?

-----We appreciate the Reviewer’s comments. In the revised version, we added triangular arrow in Figure 1. We changed figure number and modified parts of the Titles and legend (red), as shown below.

Added parts: (Page12)

Figure 1. CT scan of the abdomen.

A and B; at the time of admission
C; one and a half years ago of admission

Comment 6: Figure 2. The magnifications of HE stain 100X? Kindly described clearly where immunostaining analysis for c-KIT, DOG-1, Ki-67 are positive?

-----We appreciate the Reviewer’s comments. Since, histological analysis, cell morphological/staining patterns are quite diffuse and is difficult to indicate specific cell or cite, therefore we added parts of the Titles and legend (red), as shown below.

Added parts: (Page12)

Figure 2. Pathological findings of endoscopic ultrasound-guided fine-needle aspiration biopsy

A. Hematoxylin-eosin (HE) staining shows hyperplastic spindle cell. (X400)
B. Immunostaining for c-KIT is diffuse positive in tumor cytoplasm. (X400)
C. Immunostaining for Discovered on DOG-1 is diffuse strongly positive in tumor cytoplasm. (X400)

D. Immunostaining for ki-67 is positive in nucleus. (X400)

Comment 7: Figure 3. The magnification of the HE stain? Kindly described clearly where immunostaining analysis for insulin growth factor 2 (IGF2) is positive

-----We appreciate the Reviewer’s comments. The magnification of the HE staining is 100X. Therefore, it is difficult to indicate IHC staining, we added parts of the Figure legend (red), as shown below.

Added parts: (Page12)

Figure 3. Pathological findings of the resected specimen

A. Hematoxylin-eosin (HE) staining shows that tumor is primary muscular coat of gastric. (X100)

B. Immunostaining for IGF2 is diffuse positive in tumor cytoplasm. (X100)

Comment 4: Figure 4. On the first abbreviation of IGF2 write the whole word: insulin growth factor 2 (IGF2). The description should be rewritten as "Big IGF2 (11-18kDA) was detected only in the pre-operative serum and tumor samples.

-----We appreciate the Reviewer’s comments. We modified parts of the Titles and legend (red), as shown below.

Added parts: (Page12)

Figure 4. Western immunoblot analysis of serum and tumor insulin growth factor 2 (IGF2).

Big IGF2 (11-18kDA) was detected only in the pre-operative serum and tumor samples.

Comment 8: The manuscript would benefit from substantial editing for grammar and style.

-----Prior to initial submission, our manuscript was edited by native English speaker of “Springer Nature Author Service” (Submission ID: 2LXYFWZB). In revised manuscript, we carefully checked grammar and spellings again.