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Tile: **Insulin-like growth factor-1, IGF binding protein-3 and the risk of esophageal cancer in a nested case-control study**

Manuscript Type: Case Control Study

Dear Professor Ma:

We appreciate you for considering our manuscript entitled " **Insulin-like growth factor-1, IGF binding protein-3 and the risk of esophageal cancer in a nested case-control study** " by Adachi et al. as a publication in in *World Journal of Gastroenterology.* We revised our paper, in which we changed words in blue when we corrected, and answered reviewer's comment.

We sincerely hope that reviewers will accept the manuscript. Thank you very much.

Sincerely yours,

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Answering letter for reviewer

Manuscript NO: 32275
Column: Case Control Study

Title: Insulin-like growth factor-1, IGF binding protein-3, and the risk of esophageal cancer in a nested case-control study

This is an interesting and valuable article in exploring the association between esophageal carcinoma, IGF1 and IGFBP3. This is the merit and value of the paper that can be referred and cited by other studies in future when the results are found.

However, there are several concerns that should be clarified. It is the concern on the mater for the interested readers who can be involved in this research and can repeatedly practice it in future. I here illustrate some that are unclear, non-understandable, and non-readable letting shortcomings clearly limit the contribution of the paper.

Major concerns:

1. In Discussion (page 10), authors addressed that In this study, neither serum levels of IGF1 nor IGFBP3 were related to the OR for esophageal cancer. However, in Results(page 8), I see that the mean serum level of IGFBP3 was significantly lower in the cancer group than in the controls, see Table 1.

→ We thank the reviewer's comment. The original Table 2 is both IGF1 and IGFBP3 had been adjusted each other. As the reviewer commented, each serum concentration without adjustments showed some relationship to odds ratio. However, if each parameter was adjusted each other, these relations were not seen. Thus, we changed and fixed new Table 2.

2. A flow chart regarding the research method is required for readers to take a quick

glance at the overall research perspective so as to know the nested exploration researches in this study. For instance, I summarized the four tables as below:

Table 1: Significance found in IGFBP3 between groups

Table 2: In overall perspective, we have not found any significance among three tertile groups including those adjusted life styles and using single variable logistic regression.

Table 3: Ratio method was not significant in tertile groups, but significance was found using the subtraction method.

Table 4. Male and age below 65 were found in difference using subtraction method.

→ According to the reviewer's advice, we added Figure 1 "Flow chart for selection of cases and control".

3. Authors described in page 11 that as serum IGF1 levels were higher in viscerally obese patients with esophageal cancer than non-obese patients[21, 37], visceral obesity may influence the IGF axis. In page 10, I also see that the molar level of IGFBP3 is higher than that of IGF1. Why we cannot assume that the two have the BMI-like relation. That is to study whether the value of IGF1/ squared (IGFBP3/100) can predict the risk (or cancer development) of esophageal carcinoma.

   That is, we expected the relation such as using the formula of IGF1/ squared (IGFBP3/100) to test whether any significance is existed between groups.

→ According to the reviewer's suggestion, we analyzed the relation between the formula of IGF1/squared(IGFBP3/100) and odds ratios of esophageal cancer. As we showed the results of Table alpha, the formula was not related to the risk of esophageal carcinoma. Thus, we did not added these data in the revised manuscript.

Table alpha. Odds ratios and 95% confidence intervals for esophageal cancer according to a molar formula of IGF1 and IGFBP3

	Tertile			
	1 (referent)	2	3	p for trend

molar IGF1/ squred(IGFBP3/100)	< 10.980	10.980 - 15.996	> 15.996	
No. of case / control	7 / 29	11 / 28	13 / 29	
OR (95% CI)	1	2.608 (0.600 - 11.340)	3.546 (0.735 - 17.110)	0.125
OR adjusted (95% CI)	1	2.512 (0.532 - 11.860)	3.210 (0.625 - 16.480)	0.181

adjusted, adjusted for cigarette smoking, BMI, and alcohol intake

4. Usually, we test variables that are statistically significance first, and then put them into study. That is, we see the two variables should be significant in Table 1. If so, we can confirm and be confident that any changes we design in a study, no mater we use subtraction or ratio method or even the formula of IGF1/ squared (IGFBP3/100), should be reached at a common conclusion that a significance is found.

   As for the study, we have not seen all variables that are together with a statistical significance between groups. The following studies or explorations are usually not important as we expected. The findings in this study, such as male and age under 65 or the subtraction method between IGFBP3 and IGF1, are significant and meaningful, are the occasional result of exploration, not in certainty. When we apply it to other samples or with different sample size, the result will be different. That is no enough to make any inference.

→ According the reviewer's opinion, we changed Table 2. Serum concentration of IGF1 includes both binding and free forms of IGF1. Anyway, the former is inactive form and the latter is active form of IGF1. Many papers reported that free IGF1, the molar ratio of IGF1/IGFBP3, showed the risk of several cancers, even if total IGF1 did not show any risk of cancers.

5. The baseline survey was conducted between 1988 and 1990. Whether any institute review board (Ethics approval and consent to participate) was involved in this study is required to declare.

→ The original survey was approved by the Ethical Board of the Nagoya University School of Medicine, as several papers of JACC study have mentioned. We added the sentence that the Ethical Board of the Nagoya University School of Medicine approved this study in Materials and Methods.

6. A total of 31 cases and 86 control subjects were eligible for the present analysis. Availability of data and materials are required to disclose in an appendix because data are small or are drawn with a scatter plot (IGF1 on X axis and IGFBP3 on Y axis) classified with two groups to see the relation of both variables. Readers can see whether IGF and IGFBP form a complex in a 1:1 molar ratio, or the molar level of IGFBP3 is higher than that of IGF1.

→ According to the reviewer's suggestion, we added Figure 2 of a scatter plot of IGF1 and IGFBP3.

Minor concerns:

1. The first paragraph In Results(in page 8) revealed that there are three terms regarding groups of case, cancer, and control. Authors should identify them in consistence across all content of the manuscript.

→ According to the reviewer's advice, we fixed those terms.

2. Two Figures(flowchart and scatter plot) are required to let readers more clearly understanding the study.

→ According to the reviewer's advice, we added 2 figures.

3. Whether the value of IGF1/ squared (IGFBP3/100) can predict the risk (or cancer development) of esophageal carcinoma is expected in the manuscript.

→ Although we analyzed the formula of IGF1/sequared(IGFBP3/100), it is not associated the risk of esophageal cancer. So we did not added these informations.

4. Thanks for this interesting read. Good luck in the further work on this topic!

→ We appreciate the reviewer's advice.