Author’s response to reviews

Title: Serum vaspin levels are reduced in Japanese chronic hemodialysis patients

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Author’s response to reviews: see over
June 1, 2012

Dear, Prof. Jigisha Patel, MRCP, Ph.D., Editor, *BMC Nephrology*

We would like to submit the revised version of manuscript entitled ‘Serum vaspin levels are reduced in Japanese chronic hemodialysis patients’. We hope we addressed all the issues, which reviewers raised.

Thank you for the consideration of publication.

Sincerely yours,

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**Point-by-point response to reviewers**

**Referee 1**

**Abstract**

1. **You need to rewrite the background. It’s confused: visceral adipose tissue-derived serine is an adipokine identified from visceral adipose tissues?**
   We recently published ref#26 and revised the background as referee recommended in page 2 lines 2-6, ‘**Background**: Visceral adipose tissue-derived serine proteinase inhibitor (vaspin) is an adipokine identified from genetically obese rats and it correlates with insulin resistance and obesity in human. Recently, we found that 7% of Japanese population with minor allele sequence (A) of rs77060950 revealed higher levels of serum vaspin and we investigated the serum vaspin levels in chronic hemodialysis patients.’

2. **It’s strange use CD for HD patients.**
   We replaced CD as HD throughout the revised manuscript by indicating red font.

3. **It doesn’t make sense: “vaspin levels were reported to negatively correlate with glomerular filtration rate” in HD patients its levels should be high.**
We completely agreed the referee and we deleted the sentence from the background.

4. Normal subjects? Why? The HD patients are abnormal?
We agreed with the referee. We presented as ‘control healthy volunteers or subjects’ throughout the manuscript by indicating red font.

5. Human vaspin RIA system is obvious to measure vaspin levels.
We revised the methods in abstract in page 2 lines 7-9, ‘Methods: Japanese control healthy volunteers (control; n=95, 49.9 ± 6.91 years) and patients undergoing hemodialysis therapy (HD; n=138, 51.4 ± 10.5 years) were enrolled into this study and serum samples were subjected to human vaspin RIA system.’

6. How many patients?
We indicate the number of the total control subjects and patients and also the number of VaspinHigh group, ‘Methods: Japanese control healthy volunteers (control; n=95, 49.9 ± 6.91 years) and patients undergoing hemodialysis therapy (HD; n=138, 51.4 ± 10.5 years) were enrolled into this study and human vaspin RIA system was used. Results: The measurement of serum vaspin levels demonstrated that a fraction of control subjects (n=5) and HD patients (n=11) revealed much higher levels, > 10 ng/ml (VaspinHigh group).’

7. Do you believe that statistical analysis in all subjects is correct?
In previous study such as ref#14, they also analyzed control, HD patients and all subjects. Analyzing the all subjects including control and HD patients may be suffered from various confounding factors associated with renal failure. However, multivariate linear analysis revealed that TG and serum creatinine significantly associated with serum vaspin levels after adjusting age, BMI, hemoglobin, albumin and blood glucose. The result is one of the important messages and we would like to remain it in our manuscript.

Introduction/methods
1. Vaspin levels increase according to obesity degree?
As the referee suggested we revised the introduction in page 3 lines 7-10, ‘In obese adults[3-6] and children[6, 7], serum vaspin levels increased according to the degree of obesity and insulin resistance, and serum levels were reduced by the weight reduction by lifestyle modification and bypass surgery.’

2. Vaspin is dialyzable?
We discussed the dialyze of the vaspin in page 12 lines 12-14, ‘Since molecular mass of vaspin is ~50 kDa[1], it may not be efficiently eliminated by hemodialysis.’

3. How we can do correlation between vaspin and GFR in HD patients?
We deleted the GFR from the manuscript and we correlated the serum creatinin levels and vaspin.

4. **You had measured waist circumference. So, you need to describe in methods.**
   
   We described the measurement of waist in page 5 lines 15-16, ‘The waist circumference was measured midway between the lower rib margin and the iliac crest.’

**Discussion**

1. **Why did you discuss a lot about leptin and adiponectin? You need to discuss about vaspin.**

   During the review process, we published ref#26 and #27 and we now can discuss more about vaspin in page 11 line 10 to page 12 line, ‘Actually, we demonstrated that a significant subpopulation (7%) of both the subjects with normal fasting glucose level (n=259) and patients with type 2 diabetes (n=275) displayed much higher levels of 10-40 ng/ml (Vaspin\textsubscript{High} group), while the serum vaspin levels of 93% of the samples varied from 0.2 to 3 ng/ml in Vaspin\textsubscript{Low} group. By genotyping, rs77060950 tightly linked to serum vaspin levels, i.e. CC (0.6 ± 0.4 ng/ml), CA (18.4 ± 9.6 ng/ml) and AA (30.5 ± 5.1 ng/ml) (p < 2 \times 10^{-16})[26]. In current study, we demonstrated that similar frequency of Vaspin\textsubscript{High} group was also observed in HD patients and they seemed to be genetically defined. Furthermore, the population with higher vaspin levels more than 10 ng/ml is different in ethnic groups, i.e. ~1% in European population in previous report[14]. We also demonstrated that ~1% of Danish twin population revealed higher vaspin levels more than 10 ng/ml by using human vaspin RIA system[27].’

2. **It’s not necessary to show all graphics.**

   We deleted Figure 1 and added Table 2.

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**Referee 2**

We appreciate for the comments from referee 2. During the review process, we published ref#26 and #27 and we now can discuss more about vaspin in page 11 line 10 to page 12 line, ‘Actually, we demonstrated that a significant subpopulation (7%) of both the subjects with normal fasting glucose level (n=259) and patients with type 2 diabetes (n=275) displayed much higher levels of 10-40 ng/ml (Vaspin\textsubscript{High} group), while the serum vaspin levels of 93% of the samples varied from 0.2 to 3 ng/ml in Vaspin\textsubscript{Low} group. By genotyping, rs77060950 tightly linked to serum vaspin levels, i.e. CC (0.6 ± 0.4 ng/ml), CA (18.4 ± 9.6 ng/ml) and AA (30.5 ± 5.1 ng/ml) (p < 2 \times 10^{-16})[26]. In current study, we demonstrated that similar frequency of Vaspin\textsubscript{High} group was also observed in HD patients and they seemed to be genetically defined. Furthermore, the population with higher vaspin levels more than 10 ng/ml is different in ethnic groups, i.e. ~1% in European population in previous report[14]. We also demonstrated that...
~1% of Danish twin population revealed higher vaspin levels more than 10 ng/ml by using human vaspin RIA system[27].'

Editorial corrections
1. We described ethics committee names in page 5 lines 9-12, ‘The study protocol was approved by the ethics committee of Okayama Southern Institute of Health, Okayama University Hospital and Shigei Medical Research Hospital, and the written informed consent was obtained from all participants.’

2. We added the Disclosure and Authors’ contributions in page 14 lines 4-11, ‘Disclosures; The authors declare that they have no competing interests. Authors’ contributions: JI, JW, YT, SK, SA, KN, and HM participated in the design of the study and recruitment of the patients. JI, ST and JFM established the RIA assay system and carried out all the measurements. JW, KH, and AN participated in the cloning of vaspin gene and production of recombinant vaspin protein. JI, JW and HM conceived of the study, participated in coordination, and performed the statistical analysis. All authors read and approved the final manuscript.’