Author's response to reviews

Title: Poor histological lesions in IgA nephropathy may be reflected in blood and urine peptide profiling

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Author's response to reviews: see over
RE: COMMENTS ON MANUSCRIPT Ms. No. 9398799598425947

Dear Dr. Henderson,

Thank you for your valuable and constructive comments. We would appreciate your reconsideration of the revised version of our manuscript, “Poor histological lesions in IgA nephropathy may be reflected in blood and urine peptide profiling.”

We have taken all of the referees’ comments and suggestions and the editorial points into account and produced a second revised version. We hope that these modifications will simplify our manuscript and render it more suitable for publication in BMC Nephrology.

We look forward to hearing from you.

Yours sincerely,

Ramón Romero-González, MD, PhD
Department of Nephrology
For easier reference, the points that have been raised by the reviewers are in **bold**, followed by our comments on the changes in the manuscript.

**Reviewer #1 - Comments**

The authors present the results of a prospective study on proteomic analysis of biofluids in 19 patients with IgA nephropathy (IgAN) and correlate peptide levels with the clinical, laboratory and morphological features identified as prognostically important in Oxford Classification of IgAN. This is quite a novel idea and has not been reported in the literature till date. The authors conclude that proteomic analysis of biofluids in IgAN patients may prove useful as non-invasive test for the diagnosis and prognosis. The study results are interesting. However, there are some minor points which need to be addressed before the manuscript may be accepted for publication. These are:

1. A standardized approach to abbreviations should be used. For example, following use of IgAN for IgA nephropathy initially in the manuscript, only IgAN should be used at all places. There are several places in introduction where full term is used again and again. Same applies to abbreviation of ESRD in the beginning of Introduction, but the full term is used again in discussion etc. Others include GKD.

   We agree with the reviewer’s comment. Accordingly, we have revised the entire manuscript to unify terms throughout the manuscript.

2. Other abbreviations appear without first fully spelling them out, eg. ACEi, ARBs, etc.

   We agree with the reviewer’s comment. Accordingly, we have revised the entire manuscript to unify terms throughout the manuscript.

3. Abstract: Methods: Line 2, remove “to” before 2006. Results: Tubulointerstitial area should be changed to “tubulointerstitial damage.” Glomerulosclerosis should be qualified as segmental glomerulosclerosis.
In agreement with the reviewer’s suggestion, we have removed “to” before 2006 from the text in ‘Abstract – Methods’, which now reads:

“We prospectively studied 19 patients with biopsy-proven IgAN and 14 healthy subjects from 2006 to 2009, excluding subjects with crescentic glomerulonephritis and collecting clinical and biochemical data at the time of diagnosis and during follow-up (24 months). Histological lesions were evaluated by Oxford classification. Proteomic analysis was performed by combining magnetic bead technology and mass spectrometry (MALDI-TOF MS) to obtain peptide profiles. Doubling of serum creatinine was considered a variable of poor renal prognosis.”

Similarly, we have reworded tubulointerstitial damage and segmental glomerulosclerosis in ‘Abstract – Results,’ which now reads:

“We identified 55 peptides—13 in serum, 26 in plasma, and 16 in urine—that differentiated IgAN patients from healthy subjects. A significant association was noted between serum/plasma and urine peptides and histological findings—ie, tubulointerstitial damage, segmental glomerulosclerosis, and endocapillary injury. We also identified 3 peptides—corresponding to bradykinin, uromodulin, and alpha-1-antitrypsin—that were associated with severity of lesions, such as tubulointerstitial damage and segmental glomerulosclerosis. Moreover, blood peptides with \( m/z \) 5337 and 9289 and urine peptides with \( m/z \) 1769, 1898, 1913, 1945, 2378, 2491, 2977, 3004, 3389, 3406, and 4752 correlated significantly with poor renal function.”

Accordingly, with the reviewer’s suggestion, we have revised the entire manuscript to unify these terms throughout the manuscript.

4. Main text: Results: The age of healthy subjects is given as mean, while in statistical section, it is mentioned that median with range is given.

We agree with the reviewer’s comment. We apologize for the misunderstanding regarding that mean data, and it has been removed from the text.

5. Table 1. Caption- remove “and healthy subjects.” as there is no information about controls in this table.
We agree with the reviewer’s comment. We apologize for the misunderstanding regarding information on the healthy subjects, and it has been removed from the text.

6. Table 2. Change the caption of the table to “Scores of histological lesions --- ---”. Give abbreviations for IFTA. “Score mesangial” should be changed to “mesangial hypercellularity score.”

We have improved the caption according to the reviewer’s suggestion, which now reads:

“Scores of histological lesions by Oxford classification in IgAN patients.”

7. Table 3. Caption: between “double of creatinine” may be changed to “doubling of serum creatinine”

We have improved the caption according to the reviewer’s suggestion, which now reads:

“Relationship between doubling of serum creatinine and peptide peaks.”

8. Additional table 2. What are the units of the values of different peptides?

The peak area values of different peptides have been represented in arbitrary units (AU). In Additional Table 2, we have added a description of the units of the values of different peptides.

9. Figure legends: Figure 2 legend: remove “mesangial proliferation”

In agreement with the reviewer’s suggestion, we have removed “mesangial proliferation” from the Figure 2 legend, which now reads:

“Figure 2– Differentially expressed peptide peaks in urine by segmental glomerulosclerosis lesion.

Box plot of differential urine expression of \textit{m/z} 1945 (a), \textit{m/z} 2392 (b), \textit{m/z} 4013 (c), and \textit{m/z} 3389 (d) in patients with IgAN patients without (S 0) or with (S1) segmental glomerulosclerosis lesions. Outliers are open circles.”
Reviewer #2 - Comments

1. I think serum and urine proteomic can provide useful information with non-invasive, safe method in IgA nephropathy patients. However, my main concern in this article is these peptides (bradykinin, UMOD, A1AT...) are specific for IgA nephropathy. Is there any data of serum and urine proteomic data for other kidney disease?

We agree with the reviewer’s comments; hence, UMOD and A1AT have been related to several disease processes, such as diabetic nephropathy in urine. Otherwise, the bradykinin has been described as having a crucial role in the pathogenesis of focal segmental glomerulosclerosis [45].

We acknowledge the importance of addressing the specificity of these peptides in diagnosis. Hence, in our previous report, we suggested that the composite of different associations of UMOD and A1AT peptides allowed us to differentiate between proliferative and nonproliferative forms of GKD. Nevertheless, in the present study, we propose that our peptide profile can be used as a potential biomarker predictor of renal outcome in IgAN.

2. Second, is there follow-up sample of serum and urine? Follow-up results of serum and urine proteomic result would be helpful to this article.

We appreciate the reviewer’s suggestion; we agree with the reviewer that it would be very interesting to assess the follow-up results of serum and urine proteomic data. Yet, our study cohort comprises only proteomics samples collected at time of diagnosis from subjects who underwent a renal biopsy; we did not perform a follow-up.

The main objective of our study was to find a peptide profile that could be used as a diagnostic and prognostic tool. Nevertheless, we appreciate the reviewer’s comment, which could be considered in future studies to assess several features, such as therapeutic effects and renal disease progression.
3. There are too many paragraphs in introduction, result, and discussion. If possible, put together similar contents and discuss more detail of this study.

We agree with the reviewer’s comment. Accordingly, we have revised the entire manuscript to unify the manuscript.

In agreement with the reviewer’s suggestion, we have removed the following paragraph in ‘Methods – Renal biopsy’:

“We obtained a single renal biopsy specimen from each patient at the beginning of the study before initiating immunosuppressive treatment.”

Similarly, we have removed the following paragraphs in ‘Discussion’ (page 15, lines 1-4 and lines 15-19):

“The significance of IgA nephropathy is based on its incidence and prognosis: 15% to 40% of patients develop end-stage renal disease after 20–25 years of follow-up [1]. For this reason, methods must be developed to make an early diagnosis.”

“Tubular atrophy, interstitial fibrosis, and glomerulosclerosis have been implicated as the most powerful histological predictors of outcome [3, 4]. Also, several clinical risk factors, such as male gender, hypertension, proteinuria, absence of hematuria, and increased serum creatinine levels, are associated with a poor outcome [1].”

In addition, we have improved the following paragraphs under ‘Background’ (page 5, lines 1-9 and lines 18-21):

“IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide and is a significant cause of renal disease, leading to end-stage renal disease (ESRD) in 15% to 40% of patients after 20–25 years of follow-up [1]. For this reason, methods must be developed to make an early diagnosis.

Several clinical risk factors, such as male gender, hypertension, increased serum creatinine level, proteinuria >1 gr/day, and absence of hematuria, are associated with a poor prognosis [2]. Further, histopathological findings at the time of diagnosis, such as glomerulosclerosis and chronic tubulointerstitial damage, are also predictors of poor renal outcome [3-5].”
"In the past decade, proteomics has been applied extensively to various fields of medicine, including nephrology [10-15]. Particularly, in urine, because it can be obtained noninvasively, allowing one to identify glomerular kidney disease (GKD)-related markers [16-26]."

Similarly, we have included the following paragraph under 'Discussion' (page 18, lines 9-11):

"Recently, upregulation of bradykinin receptor has been described to mediate the progression of focal segmental glomerulosclerosis [45]."