Answers to the REVIEWERS

December 18, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15022-review.doc).

Title: Severe increased albuminuria as a marker of arterial stiffness in CKD stage 1-2 hypertensive non-diabetic patients

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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

Responses to Reviewer # 1:

The study is well designed and presented; however, it has a few limitations

1. Cross sectional design, as acknowledged by the authors, does not allow to established cause effect relationships;

Response 1:
We have already recognized the problem with the cross sectional design of the study and thus we have also added a comment on the limitations regarding cause-effect relationships (page 12, L 10) and now reads :

The cross sectional design, which does not allow to establish cause-effect relationships as well as the small number of patients involved are potential limitations of our study.

2. The rational for including only patients treated with RAS-blockers should explained more clearly

Response 2:

Thank you for the comment. We have now made it clearer and added a comment regarding rational for including only patients treated with RAS-blockers (Material and methods, 2nd paragraph) and now reads:

All the patients were treated with RAAS agents (ACE inhibitors or angiotensin type 1(AT1) receptor blockers) plus other antihypertensive drugs as needed. Our choice for the first agent was a RAAS blocker based on the favorable effects of these agents on albuminuria and AS reduction as well as for their anti-fibrotic and anti-inflammatory effects [17, 18]. Furthermore the reno-and cardio-protective effects beyond their hypotensive effects were also well established [19] and for our patients with moderately or severely albuminuria this treatment was already applied for at least 6 months.

3. CKD should be defined in greater detail: which was/were the patients' nephropathy? Were other sings(beyong albuminuria) of renal damage present?

Response 3

We appreciated the comment and we have added more details regarding CKD and albuminuria and nephropathy (Material and methods, 1st paragraph) and now reads:

We included 115 consecutive hypertensive patients with CKD stages 1-2 (Stage 1: kidney damage with normal or increased GFR, Stage 2: kidney damage with mild reduced GFR 60-89ml/min). None of our patients had albuminuria > 1g/24h. Albuminuria levels were stable for the past 6 months. Patients with known glomerulopathy proven by biopsy were excluded. Most of our patients had hypertensive nephosclerosis with duration of hypertension for more than 10 years and none of them had diabetes mellitus.

ADDITIONAL COMMENTS

1 Please indicate the patients' smoking status, whether any diabetics included

Response:

We added smoking status at the end of the section with material and methods and in the table 1. However the number of smokers was too small to include smoking habit in the multivariate analysis and we have already mentioned that we did not included diabetic individuals.

2 The terms atherosclerosis and arterial stiffness cannot be used as synonymous

Response:

Thank you for the comment. We deleted the word atherosclerosis from the abstract (page 3, line 3)

3 The decrease in HB levels is not likely to be attributable to renal causes at that (near normal) degree of renal function

Response:

We appreciated this comment and to be more precise we have now rephrased it (page 11, last line) as follows:

In this group of patients, despite the near normal degree of Ht levels along with increased inflammatory indices, such as fibrinogen and hsCRP, Ht might be related to the kidney dysfunction as suggested by Hiramoto at al [35].

4 In this population the CKD-EPI equation is preferable for estimating glomerular filtration rate

Response:

We have included the suggestion for the CKD-EPI formula in the table 1.

Responses to Reviewer #2:

Major 1: According to the present data the author cannot state "worsering arterial stiffness " in the title because of cross sectional study

Response 1:

We apologize for the statement. We change the title and now reads: Severe increased albuminuria as a marker of arterial stiffness in CKD stage 1-2 hypertensive non-diabetic patients

2 As mentioned in Discussion section longitudinal study should be done to address whether severely increased albuminuria is a factor of AS elevation and it's progressive deterioration, in non-diabetic hypertensive patients treated with renin angiotensin aldosterone blockade agents(RAAS)(P5,L3-5 in the third paragraph). Then the authors can discuss the mechanisms linking AS and albuminuria (P11,the second paragraph)

Response 2:

We appreciated this comment and to be more precise we change the aforementioned (P5, second paragraph) and now reads:

However, limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents (RAAS). It has been suggested that endothelial dysfunction could be a possible mechanism involved in the remodeling of the arterial wall affecting AS and modifying glomerular permeability leading to increased albumin excretion [16, 17]. In addition, AS could influence glomeruli function through an increased pulsatile stress, causing glomerular damage [18].

3 How long did patients have the duration of hypertension and receive RAAS

3. We appreciate the comment; however we have already given the appropriate response: (reviewer #1 response 2 & 3)

4 How did the patients exclude patients with glomerulonephritis? If the amount of albuminuria is extremely large, the cause of albuminuria should be considered to be the primary glomerulopathy. Then the magnitude of albuminuria in such patients may not be directly associated with AS.

Response: We totally agree with this comment. However we have given these information's in the reviewer comment #1, answer 3. However, it is impossible, to exclude the diagnosis of glomerulopathy since we haven't performed a kidney biopsy in the study population.

Minor"Fifth-eight patients" should read "Fifty-eight patients"(P3 abstract)

Response: Thank you. We corrected the mistake

Responses to Reviewer #3

1 The authors should clarify the dose of ARB/ACEi, prescribed for the patients. Otherwise, how about were the CCDs used in the patients?

Response 1: We apologize for the statement. We have added the necessary information's regarding the medications received by the study population: page 6, end of the paragraph.

Ramipril and quinapril were prescribed at the doses of 20mg OD and valsartan and olmesartan at the doses of 320 and 20mg OD respectively. Six patients received valsartan 160mg OD and 2 patients received quinapril 40 mg OD. A very small number of patients received amlodipine at a dose of 10 mg OD (Table 1).

2. Cardiorenal syndrome could be important for the patients' outcome. The authors should be clarify did these patients have any heart disease?

Response 2: We also apologize for the statement. We added these data (Material and Methods, page 6, first paragraph):

Furthermore, we excluded patients with acute myocardial infraction, unstable angina, stroke, heart failure or transient ischemic attack within the past year.

3. In multivariate linear regression analysis of the parameters associated with the absolute values of PWV (table 4), the authors showed albumin in the covariates panel. Serum or urine albumin?

Response 3: Thank you for the comment: it is urine albumin and it is written as Ualb in the table 4

3 References and typesetting were corrected

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

Sincerely yours,

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