

To: **Editor of *World Journal of Diabetes***

Re: Manuscript NO: 63902

Title: Association between admission hemoglobin level and prognosis in patients with type 2 diabetes mellitus

Thank you very much for giving us a precious opportunity to re-submit our manuscript. We appreciate the helpful comments and valuable suggestions from the reviewers and editors, which are very valuable and helpful for revising and improving the quality of our manuscript.

We carefully considered these comments and answered all the questions. We have also modified the manuscript accordingly and the changes in the text have been highlighted.

We very much hope the revised manuscript can be published in the World Journal of Diabetes.

Thank you very much for your consideration.

Sincerely yours,

Haofei HU

## The Point-by-Point responses

Thank you very much for the valuable questions and suggestions. We have carefully considered/answered all the points raised from the reviewers and modified the manuscript accordingly.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Reviewer's comments for authors (code; 05824426)

Title: Association between admission hemoglobin level and prognosis in patients with type 2 diabetes mellitus. Title number: 63902 Reviewer's comments for authors according to the checklist of the journals:

1. Title: Yes, the title reflects the hypothesis of the manuscript

**Response:** Thank you for the reviewer's comments.

2. The abstract was written interestingly and summarizes the works under manuscript, but it is better if the authors re-write the abstract in line with the scientific way of writing by separating different sections; like background; aims, methods, and materials, result, a conclusion with some implication. In addition, the purpose of the study (significance of the study) is not included in the abstract and introduction section too. Please incorporate the purpose of the study

**Response:** Thank you for the reviewer's comments. We have re-written the abstract in line with the scientific way which has been highlighted in the revised manuscript.

**Background:** Anaemia is common in chronic kidney disease (CKD) and is a major risk factor that contributes to mortality in CKD patients. Type 2 diabetes mellitus (T2DM) is one of the leading causes of CKD. The association between admission hemoglobin levels and renal damage in patients with T2DM remains unclear.

**Aims:** We aimed to evaluate the relationships between admission hemoglobin levels and prognosis in patients with T2DM.

**Materials and methods:** We performed a retrospective analysis of 265 consecutive presenting patients with T2DM between 2011 and 2015. The composite endpoint was end-stage renal disease (ESRD) or a 50% reduction in the estimated glomerular filtration rate (eGFR).

**Results:** In multivariable-adjusted Cox proportional hazards models, the adjusted hazard ratios (HRs) for the highest and middle tertiles compared to the lowest tertile of hemoglobin were 0.82 (95% CI, 0.11 to 6.26,  $p=0.8457$ ) and 0.28 (95% CI, 0.09 to 0.85,  $p=0.0246$ ), respectively, in the adjusted model (demographic factors, traditional risk factors, lipids). However, after further adjustment for glycaemia control, hemoglobin was positively related to the risk of the composite endpoint (HR: 1.05, 95% CI, 0.14 to 8.09,  $p=0.9602$ ) when the highest tertile was compared to the lowest tertile of hemoglobin. We found a U-shaped relationship between hemoglobin levels and the composite endpoint. The curve tended to reach the lowest level in an optimal hemoglobin level.

**Conclusion:** Among patients with T2DM, a U-shaped relationship between hemoglobin levels and renal damage was observed. A lower admission hemoglobin level (hemoglobin < 13.3 g/dl) is an independent predictor of renal damage.

The purpose of the study (significance of the study) was that we aimed to evaluate the relationships between admission hemoglobin levels and prognosis in patients with T2DM. Our hypothesis was that there may be an optimal hemoglobin level about the relationship between hemoglobin and renal progression in patients with T2DM. This study may help us to find the optimal hemoglobin level. And the optimal hemoglobin level may be an intervention target for patients with T2DM.

We have added the purpose of the study (significance of the study) in the abstract and introduction section and modified the manuscript accordingly and highlighted them in the text.

3. Yes, the keywords were reflecting the focus of the study

**Response:** Thank you for the reviewer's comments.

4. Background: The authors described the backgrounds of the study concisely and adequately, however; the magnitudes of the problem and how either low or high

hemoglobin level was associated is not described well. Also, the purpose or significance of the study is not described.

**Response:** Thank you for the reviewer's comments. We have amended the manuscript accordingly and highlighted them in the text.

The magnitudes of the problem and how either low or high hemoglobin level was associated as follow:

Low hemoglobin level is one of the major risk factors for cardiovascular disease (CVD) and mortality and poor prognosis in CKD patients. However, some studies have shown that higher hemoglobin levels slightly increase the risk of death [5], and elevations in hemoglobin levels have been implicated in a higher risk of mortality and cardiovascular events. The admission hemoglobin levels would influence the prognosis in patients with T2DM.

The purpose of the study (significance of the study) was that we aimed to evaluate the relationships between admission hemoglobin levels and prognosis in patients with T2DM. Our hypothesis was that there may be an optimal hemoglobin level about the relationship between hemoglobin and renal progression in patients with T2DM. This study may help us to find the optimal hemoglobin level. And the optimal hemoglobin level may be an intervention target for patients with T2DM.

5. Methods: the method of the study is not described in an adequate way, the authors must amend the methodology sections of the study before acceptance of the manuscript for publication.

a. How they selected the participants, from where? (Criteria for selections of the participants)?.

**Response:** Thank you for the reviewer's comment. This was a retrospective cohort study. We used the database from the Shenzhen Second People's Hospital to analyze the association between admission hemoglobin level and prognosis in patients with type 2 diabetes mellitus. We selected consecutive patients for analysis. From January 2011 through December 2015, 265 patients who were enrolled in this study were diagnosed with T2DM at the Department of Nephrology and Endocrinology in Shenzhen Second People's Hospital. The patients were followed every 3 months for at least 3 months until the study endpoint or deadline. The deadline for the study was

June 30, 2016. The composite endpoint was an end-stage renal disease (ESRD) or a 50% reduction in the eGFR. We used the eGFR decline to reflect renal damage.

b. How they were excluded? Through physical examination, reviewing the records, interview of patients, or performing the different biochemical tests? It is not clear for readers?

**Response:** This was a retrospective cohort study. We used the database from the Shenzhen Second People's Hospital and reviewed the records. Patients with moderate to severe valvular disease, atrial fibrillation, other severe arrhythmias, congenital heart disease, or primary myocardial disease were excluded. The patients who had missing data for the admission hemoglobin levels and the composite endpoint were also excluded.

c. How those laboratory data like fasting glycemia, glycosylated hemoglobin (HbA1c), serum creatinine (Scr), 24-h urinary protein, hemoglobin, serum albumin (ALB), serum uric acid (SUA), blood urea nitrogen (BUN), total cholesterol (TC), and triglycerides (TG) were measured, and procedures, instruments used to measure those variables; how the performance of the instruments was checked is not described adequately?.

**Response:** All these laboratory tests were performed and checked at the Central Laboratory of Shenzhen Second People's Hospital. The blood and urine samples were collected when patients were on admission. And the blood samples were fasting venous blood. Urine samples are collected in one or more containers over a period of 24 hours. Reagent based method and automated analyzer were used for these laboratory variables. Fasting glycemia was measured by the Hexokinase method. Immunoassay method was used to measure HbA1c. 24-h urinary protein was measured by biuret method. Using Hemo-globincyanide (HiCN) Method and an automated hemoglobin analyzer measured hemoglobin level. Serum albumin is measured by the serum protein electrophoresis method. Enzymatic determination method was used to measure serum creatinine, serum uric acid, blood urea nitrogen, total cholesterol (TC), and triglycerides (TG). We have amended the manuscript, which is highlighted in the text (page 3 of the manuscript).

d. How the major causes of renal damages were excluded? Or confounders?

**Response:** Thank you for the reviewer's comments. In this study, we used statistical methods to retrospectively examine the relationship between the admission hemoglobin levels and renal damage in patients with type 2 diabetes mellitus. Cox proportional hazards models were used to evaluate these relationships. The traditional risk factors for renal damage include age, gender, obesity, serum creatinine, blood pressure, and proteinuria. ACEI and (or) ARB use can improve survival and renal prognosis of patients with Type 2 diabetes [1]. And some studies also showed that serum albumin, serum triglyceride, serum total cholesterol, hyperglycemia were risk factors for renal damage [2,3,4,5,6]. All of these factors were confounders. We evaluated the relationship between the admission hemoglobin levels and the composite endpoint with adjustment for these confounding variables.

e. How GFR was measured? Is not described in an appropriate way

**Response:** We used the database from the Shenzhen Second People's Hospital to get the general clinical data, including age, gender, body mass index (BMI; kg/m<sup>2</sup>), and the serum creatinine value. The enzymatic determination method was used to measure serum creatinine at the Central Laboratory of Shenzhen Second People's Hospital. The estimated glomerular filtration rate (eGFR) was calculated using the CKD epidemiology collaboration equation, which is shown on page 3 of the manuscript.

f. As a retrospective design of the study; is that really possible to controls those confounding factors appropriately. Maybe the authors got some variable of the patients from an electronic source or medical records and take us confounding variable? How was the quality of the data maintained? Because at the end of the days finding of the study was concluded based on consideration of controlling those variables.

**Response:** This was a retrospective cohort study. We used the database from the Shenzhen Second People's Hospital and reviewed the records to get the general clinical data and the laboratory data. The Shenzhen Second People's Hospital which is the First Affiliated Hospital of Shenzhen University is Guangdong Province High-level Hospital. All electronic medical records are followed national standards. Two doctors in this study cross checked these data to maintain the quality of the data.

The traditional risk factors for renal damage include age, gender, obesity, serum creatinine, blood pressure, and proteinuria. ACEI and (or) ARB use can improve survival and renal prognosis of patients with Type 2 diabetes [1]. And some studies also showed that serum albumin, serum triglyceride, serum total cholesterol, hyperglycemia were risk factors for renal damage [2,3,4,5,6]. We evaluated the relationship between the admission hemoglobin levels and the composite endpoint with adjustment for these confounding variables. Whether the variables were adjusted determined by the following principle: when added to this model, changed the matched hazard ratio by at least 10% [7].

g. How the distribution of the data was tested, or by which method? It is not described well?

**Response:** The Shapiro–Wilk test was used to test the distribution of the data. Continuous variables were expressed as the means  $\pm$  standard deviations (normal distribution) or medians (quartiles) (skewed distribution), and categorical variables were expressed as a frequency or percentages. The one-way ANOVA (normal distribution), Kruskal-Wallis H (skewed distribution) test and chi-square test (categorical variables) were used to determine any significant differences between the means and proportions of the groups.

h. How does the stage of the CKD categorize?

**Response:** The stages of CKD are classified as follows [8] :

Stage 1: Kidney damage with normal or increased eGFR ( $>90$  mL/min/1.73 m<sup>2</sup>)

Stage 2: Mild reduction in eGFR (60-89 mL/min/1.73 m<sup>2</sup>)

Stage 3: Moderate reduction in eGFR (30-59 mL/min/1.73 m<sup>2</sup>)

Stage 4: Severe reduction in eGFR (15-29 mL/min/1.73 m<sup>2</sup>)

Stage 5: Kidney failure (eGFR  $< 15$  mL/min/1.73 m<sup>2</sup> or dialysis)

i. How the quality of data was maintained (performance of analyzers)? Especially for Hgb, glucose, lipid profile, proteins, enzymes?. Please amend all those points raised here?

**Response:** All the laboratory data were tested and checked at the Central Laboratory of Shenzhen Second People's Hospital. The blood and urine samples were collected when patients were on admission. And the blood samples were fasting venous blood. Urine samples were collected in one or more containers over a period of 24 hours. Reagent based method and automated analyzer were used to these laboratory variables. Fasting glycemia was measured by Hexokinase method. Immunoassay method was used to measure HbA1c. 24-h urinary protein was measured by biuret method. Using Hemo-globincyanide (HiCN) Method and an automated hemoglobin analyzer measured hemoglobin level. Serum albumin are measured by serum protein electrophoresis method. The enzymatic determination method was used to measure serum creatinine, serum uric acid, blood urea nitrogen, total cholesterol (TC), and triglycerides (TG). These measurements are relatively mature methods. And the accuracy of the data is guaranteed.

6. Results: The author describes the result coherently and logically; the objectives of the study were achieved well, the study was made a good substantial contribution to the progress of fields.

**Response:** Thank you for the reviewer's comments.

7. Discussion: the discussion section was written in a concise, clear, and logical way. The finding of the study was interpreted and reported appropriately and adequately. It describes scientific significance and relevance to clinical practices sufficiently. However, authors should remove p-value and HR from the discussion sections

**Response:** Thank you for the reviewer's comments. We have removed p-value and HR from the discussion sections.

8. Tables were properly illustrated content of the papers and prepared in good quality.

**Response:** Thank you for the reviewer's comments.

9. How normality of the data checked is not described well? Including the methods. The measurement method of some variables was not clearly indicated?



**Response:** The Shapiro–Wilk test was used to test the normality of the data. All the laboratory data were tested and checked at the Central Laboratory of Shenzhen Second People’s Hospital. The blood and urine samples were collected when patients were on admission. And the blood samples were fasting venous blood. Urine samples were collected in one or more containers over a period of 24 hours. Reagent based method and automated analyzer were used to these laboratory variables. Fasting glycemia was measured by Hexokinase method. Immunoassay method was used to measure HbA1c. 24-h urinary protein was measured by biuret method. Using Hemo-globincyanide (HiCN) Method and an automated hemoglobin analyzer measured hemoglobin level. Serum albumin are measured by serum protein electrophoresis method. Enzymatic determination method was used to measure serum creatinine, serum uric acid, blood urea nitrogen, total cholesterol (TC), and triglycerides (TG). We have amended the manuscript which is highlighted in the text.

10. The authors used appropriate SI units, but not clarified the abbreviation section/Foote notes of the tables

**Response:** We have added the abbreviation section/Foote notes of the tables as follow: kg/m<sup>2</sup>: kilogram / square meter; mmHg: millimeter of mercury; g/L: gram per litre; g/dl: grams per decilitre; mmol/L: millimolar per litre; µmol/L: micromolar per litre; mg/d: milligrams per day. We have amended the manuscript which is highlighted in the text.

11. Authors used latest and updated reference, cited properly and listed in a correct order

**Response:** Thank you for the reviewer’s comments.

12. The manuscript is written concisely and coherently and well organized and presented

**Response:** Thank you for the reviewer’s comments.

13. the author prepared the manuscript in accordance with the guideline of basic study, however, the abstract, method, and materials section is not written in a sufficient way

**Response:** Thank you for the reviewer's comments. We have re-written the abstract, method, and materials section. We have amended the manuscript which is highlighted in the text.

14. the manuscripts meet ethical requirements and got approval from the review committee

**Response:** Thank you for the reviewer's comments.

### **References:**

- [1] Improved survival and renal prognosis of patients with type 2 diabetes and nephropathy with improved control of risk factors. Andrésdóttir G, Jensen ML, Carstensen B, Parving HH, Rossing K, Hansen TW, et al. *Diabetes Care*. **37**,1660-1667(2014).
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- [3] Syrjänen J, Mustonen J, Pasternack A. Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. *Nephrol Dial Transplant*; **15**, 34(2000).
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