The relationship between haemoglobin level and type 1 diabetic nephropathy in Han patients in Anhui, China

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Key words type 1 diabetes mellitus, diabetic nephropathy, haemoglobin level.

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Received 4 December 2017; accepted 26 January 2018.

Abstract

Background/Aim: Recent studies have shown that low haemoglobin (Hb) levels promote the progression of chronic kidney disease. This study assessed the relationship between Hb level and type 1 diabetic nephropathy (DN) in Han patients in Anhui, China.

Methods: There was a total of 236 patients diagnosed with type 1 diabetes mellitus (T1DM) seen between January 2014 and December 2016 in our centre. Haemoglobin levels in patients with DN were compared with those without DN. The relationship between Hb level and the urinary albumin-creatinine ratio (ACR) was examined by Spearman’s correlational analysis and multiple stepwise regression analysis. The binary logistic multivariate regression analysis was performed to analyse the correlated factors for type 1 DN, calculate the odds ratio (OR) and 95% confidence interval (CI). The predicting value of Hb level for DN was evaluated by area under receiver operation characteristic curve (AUROC) for discrimination and Hosmer-Lemeshow goodness-of-fit test for calibration.

Results: The average Hb levels in the DN group (116.1 ± 20.8 g/L) were significantly lower than the non-DN group (131.9 ± 14.4 g/L), P < 0.001. Hb levels were independently correlated with the urinary ACR in multiple stepwise regression analysis. The logistic multivariate regression analysis showed that the Hb level (OR: 0.936, 95% CI: 0.910–0.963, P < 0.001) was inversely correlated with DN in patients with T1DM. In sub-analysis, low Hb level (Hb < 120 g/L in female, Hb < 130 g/L in male) was still negatively associated with DN in patients with T1DM. The AUROC was 0.721 (95% CI: 0.655–0.787) in assessing the discrimination of the Hb level for DN. The value of P was 0.593 in Hosmer-Lemeshow goodness-of-fit test.

Conclusions: In patients with T1DM, the Hb level is inversely correlated with urinary ACR and DN.

Introduction

Diabetic nephropathy (DN) is one of the most common chronic kidney diseases (CKD) and remains the leading cause of morbidity and mortality in type 1 diabetes mellitus (T1DM) patients. Previous studies have shown that anaemia is a frequent comorbidity of CKD and one of the risk factors for CKD progression. Meanwhile microalbumininuria can predict the progression of DN in T1DM patients. However, the association between haemoglobin (Hb) level and type 1 DN has not yet been studied. Therefore, the purpose of this research was to determine the correlation between Hb level and DN in T1DM patients.

Methods

Patients

We conducted a cross-sectional, patients-based study in a single medical centre in Anhui, which was to research the relationship between Hb level and DN. According to Standards of Medical Care in Diabetes-2007 of American Diabetes Association, T1DM and DN were diagnosed. A total of 236 hospitalised patients was recruited, who were considered to have T1DM presenting at the Department of Endocrinology in Anhui Provincial Hospital between January 2014 and December 2016, and the clinical data were collected in May 2017. The patients...
were excluded if they had the following reasons: age under 18 years, glomerulonephritis, or urinary tract infections. All participants signed informed consent and agreed to clinical data for clinical analysis at the time of admission. The research ethics committee of Anhui Provincial Hospital approved the study design.

Clinical and laboratory measurements

Using the hospital’s database, we retrospectively collected the demographic information, and clinical data, including duration of diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), haemoglobin (Hb) level, glycated haemoglobin A1c (HbA1c), albumin (ALB), total cholesterol (TC), triglyceride level (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid (UA), creatinine (Cr) and urinary albumin–creatinine ratio (ACR) in spot urine samples at the beginning of hospitalisation. But in premenopausal women, the Hb levels were checked at 1 week before or after menstruation.

The estimated glomerular filtration rate (eGFR) was estimated using the simplified modification of diet in renal disease equation: eGFR (mL/min/1.73m²) = 186 × serum creatinine (mg/dL)¹.¹³⁴ × age (year)⁻⁰.²⁰³ × 0.⁷⁴² (if female).

Urine albumin excretions were assessed by urinary ACR in spot urine samples. The normal albuminuria (non-DN) was defined as a urinary ACR less than 30 mg/g, while DN was defined as a urinary ACR greater than or equal to 30 mg/g.¹⁴

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 20.0 version (IBM Corporation, Armonk, NY, USA). A normal distribution of continuous variables was summarised as means ± standard deviation (SD), while a skewed distribution of continuous variables was expressed as medians with interquartile ranges. For continuous data with a normal distribution and a skewed distribution between DN and non-DN patients, unpaired Student’s t-tests and nonparametric tests, respectively, were used for statistical analyses. The number (n) and percentage (%) in each category were calculated for categorical variables. The categorical variables were evaluated with chi-square test. The relationship between urinary ACR and Hb level was performed by Spearman’s correlational analysis and multiple stepwise regression analysis. The binary logistic multivariate regression analysis was performed to analyse the correlated factors for type 1 DN, calculate the odds ratio (OR) and 95% confidence interval (CI). A P-value <0.05 was considered statistically significant. The predicting value of Hb level for DN was evaluated by area under receiver operation characteristic curve (AUROC) for discrimination and Hosmer–Lemeshow goodness-of-fit test for calibration. The discrimination was used to evaluate the ability of a factor to identify disease occurrence or not. AUROC >0.70 implied that the factor had a higher discrimination. The calibration was used to evaluate the disease prediction accuracy of a factor. The calibration was higher when P > 0.05 in Hosmer–Lemeshow goodness-of-fit test.

Results

Patient characteristics and comparison of baseline covariates

The baseline characteristics of the 236 patients are shown in Table 1. No matter what gender, the average Hb levels in the non-DN group were significantly higher than the DN group. The urinary ACR levels in the DN group were significantly higher than the non-DN group (P < 0.05). The duration of diabetes, HbA1c, SBP, DBP, TC, TG, UA and Cr level was higher in DN patients (P < 0.05), whereas albumin levels in serum and eGFR were higher in the non-DN group (P < 0.05).

Correlations between ACR and other characteristics

To analyse the factors associated with albuminuria, the correlations between urinary ACR and other characteristics were assessed by the Spearman’s correlation analysis. Table 2 shows that urinary ACR is negatively correlated with eGFR, Hb levels and ALB, and positively correlated with the duration of diabetes, HbA1c, SBP, DBP, TC, TG, LDL-C, UA and Cr concentrations (all P < 0.05). After adjustment for those significant factors, stepwise multiple linear regression analysis showed an independent negative association between Hb level and urinary ACR.

The independent inverse association between Hb level and DN

Urinary ACR was correlated with eGFR, Hb levels, ALB, the duration of diabetes, HbA1c, SBP, DBP, TC, TG, LDL-C, UA and Cr concentrations in the Spearman’s correlation analysis. These ACR-correlated factors were included in the binary logistic regression multivariate analysis to identify the association between Hb level and DN. When DN was set as the dependent variable, the duration of diabetes, Hb level, eGFR, ALB, HbA1c, SBP,
Table 1 Patient demographics and laboratory data

| Characteristics | Total participants (n = 236) | Patients with DN (n = 96) | Patients without DN (n = 140) | P-value |
|-----------------|-----------------------------|--------------------------|-------------------------------|---------|
| Male/Female     | 118:118                     | 37:59                    | 81:59                         | 0.004   |
| Age (years)     | 33.9 ± 11.6                 | 34.6 ± 11.6              | 33.5 ± 11.6                   | 0.50    |
| Duration of diabetes (years) | 6.9 ± 6.3                | 9.6 ± 7.0                | 5.1 ± 5.0                     | <0.001  |
| BMI (Kg/m²)     | 20.3 ± 3.3                  | 20.4 ± 3.3               | 20.1 ± 3.2                    | 0.46    |
| HbA1C (%)       | 9.6 ± 2.8                   | 10.2 ± 3.3               | 9.1 ± 2.4                     | 0.005   |
| SBP (mmHg)      | 123.3 ± 17.6                | 128.8 ± 21.8             | 119.6 ± 12.9                  | <0.001  |
| DBP (mmHg)      | 78.8 ± 11.1                 | 82.1 ± 12.9              | 76.6 ± 9.0                    | <0.001  |
| Hb (g/L)        | 125.5 ± 18.9                | 116.1 ± 20.8             | 131.9 ± 14.4                  | <0.001  |
| Male            | 134.1 ± 15.6                | 125.5 ± 17.3             | 138.0 ± 13.0                  | <0.001  |
| Female          | 116.8 ± 18.1                | 110.2 ± 20.8             | 125.5 ± 11.7                  | <0.001  |
| TC (mmol/L)     | 4.3 ± 1.1                   | 4.5 ± 1.3                | 4.2 ± 0.9                     | 0.03    |
| TG (mmol/L)     | 1.2 ± 1.1                   | 1.6 ± 1.4                | 0.9 ± 0.7                     | <0.001  |
| HDL-C (mmol/L)  | 1.2 ± 0.4                   | 1.2 ± 0.4                | 1.3 ± 0.4                     | 0.21    |
| LDL-C (mmol/L)  | 2.4 ± 0.9                   | 2.5 ± 1.0                | 2.3 ± 0.8                     | 0.18    |
| ALB (g/L)       | 38.8 ± 5.3                  | 37.1 ± 6.4               | 40.0 ± 4.1                    | <0.001  |
| UA (μmol/L)     | 282.9 ± 112.9               | 317.0 ± 126.6            | 260.3 ± 96.9                  | <0.001  |
| Cr (μmol/L)     | 78.6 ± 60.9                 | 98.7 ± 89.2              | 64.9 ± 19.1                   | <0.001  |
| eGFR (ml/min/1.73m²) | 118.1 ± 51.8               | 104.1 ± 62.6             | 127.7 ± 40.4                  | 0.001   |
| urinary ACR (mg/g) | 24.5 (11.3, 69.5)          | 121 (45, 975.5)          | 13 (8, 21)                    | <0.001  |

ACR, albumin–creatinine ratio; ALB, albumin; BMI, body mass index; Cr, creatinine; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HbA1c, glycated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride level; UA, uric acid.

Table 2 The risk factors of urinary ACR in patients with T1DM

| Parameter | Simple linear regression | Stepwise multiple linear regression |
|-----------|--------------------------|-----------------------------------|
|           | r                        | P-value | β Coefficient ± SE | P-value |
| Age (years) | 0.07                     | 0.30    |                   |         |
| Duration of diabetes (years) | 0.22                     | 0.001   |                   |         |
| BMI (Kg/m²)  | 0.003                    | 0.97    |                   |         |
| HbA1C (%)    | 0.15                     | 0.02    |                   |         |
| SBP (mmHg)   | 0.34                     | <0.001  | 12.9 ± 3.1         | <0.001  |
| DBP (mmHg)   | 0.19                     | 0.003   |                   |         |
| Hb (g/L)     | −0.47                    | <0.001  | −13.3 ± 3.1        | <0.001  |
| TC (mmol/L)  | 0.20                     | 0.003   | 135.4 ± 50.7       | 0.008   |
| TG (mmol/L)  | 0.15                     | 0.03    |                   |         |
| HDL-C (mmol/L) | −0.013                   | 0.85    |                   |         |
| LDL-C (mmol/L) | 0.20                     | 0.004   |                   |         |
| ALB (g/L)    | −0.38                    | <0.001  | −35.5 ± 10.8       | 0.001   |
| UA (μmol/L)  | 0.31                     | <0.001  | 1.3 ± 0.6          | 0.033   |
| Cr (μmol/L)  | 0.62                     | <0.001  | 7.7 ± 1.1          | <0.001  |
| eGFR (ml/min/1.73m²) | −0.38                   | <0.001  |                   |         |

ALB, albumin; BMI, body mass index; Cr, creatinine; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HbA1c, glycated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride level; UA, uric acid.

Table 3 Logistic regression multivariate analysis of independent related factors for the diabetic nephropathy in 236 patients with type 1 diabetes

| Variable | OR (95% CI) | P |
|----------|-------------|---|
| Duration of diabetes (years) | 1.126 (1.047–1.209) | 0.001 |
| HbA1C (%) | 1.459 (1.194–1.783) | <0.001 |
| DBP (mmHg) | 1.071 (1.013–1.133) | 0.017 |
| Hb (g/L) | 0.936 (0.910–0.963) | <0.001 |

CI, confidence interval; DBP, diastolic blood pressure; Hb, haemoglobin; HbA1c, glycated haemoglobin A1c; OR, odds ratio.
independent risk factor associated with the presence of DN in patients with T1DM.

To investigate further the predicting value of Hb level for DN, Hosmer–Lemeshow goodness-of-fit test was used to value the calibration and area under AUROC was used to assess the discrimination. Figure 1 shows that AUROC is $0.721$ (95% CI $0.655–0.787$) in assessing the discrimination of the Hb level for DN. The value of $P$ was $0.593$ in Hosmer–Lemeshow goodness-of-fit test. The prediction of Hb level for type 1 DN has good calibration and discrimination.

Discussion

Previous studies have accounted that anaemia was a frequent comorbidity of CKD and one of the risk factors for CKD progression.4–10 We went into a study to verify if Hb level was related to the prevalence of type 1 DN. We discovered that Hb level was independently and inversely related to albuminuria, and was an independent risk factor for DN in patients with T1DM. We identified that low Hb level (Hb < 120 g/L in female, Hb < 130 g/L in male) may be an independent risk factor associated with the presence of DN in patients with T1DM.

Although there has been a lot of research, the exact mechanism that low Hb level may influence DN remains unknown. It is generally known that the haemoglobin is the transporter of oxygen in the body, and kidneys are in high demand for oxygen supply and always maintain active metabolic status. Therefore it is so essential to keep balance between kidney oxygen consumption and supply, and keep a rational range of Hb level in blood. The persistent low Hb level associated with impaired oxygen delivery is the main cause of chronic hypoxia, which has been considered to be closely associated with CKD.15–25

Hypoxia inducible factor 1 (HIF-1) plays an important role in the transcriptional responses to hypoxia which may be closely associated with chronic renal injuries, including DN.26–28 Previous studies show that in a streptozotocin-induced diabetes mouse model, HIF-1 has an important role in the early response to prevent diabetes-induced tissue damage and renal injury is accelerated by hypoxia-inducible factor 1α deficiency.29 The HIF-1α Pro582Ser polymorphism also has an effect on DN, by conferring a relative resistance to the repressive effect of glucose on HIF-1α.30 Similarly, pharmacologic activation of the HIF system may prevent development of DN.31 But Nayak BK et al. revealed that YC-1, an anti-HIF-1 agent, reduces renal hypertrophy, extracellular matrix protein accumulation, oxidative stress, and urine albumin excretion in type 1 diabetic OVE26 mice, they provided strong evidence that HIF-1 may prompt diabetes-induced renal injury in vivo.28 The impact of HIF-1 on the DN is not exactly the same in present studies, still needs further research.

However, this research had several limitations. First, our study was just a single-centre, cross-sectional

| Model   | Hb (g/L)   | Unadjusted OR (95% CI) | P-value | Adjusted OR (95% CI) | P-value |
|---------|------------|------------------------|---------|----------------------|---------|
| Model 1 | Male       | <130                   | 1 (Reference) | 0.001 | 0.247 (0.084–0.726) | 0.011 |
|         | ≥130       | 0.260 (0.114–0.595)    | 0.001   | 0.247 (0.084–0.726) | 0.011 |
| Female  | <120       | 1 (Reference)          | 0.004   | 0.321 (0.133–0.773) | 0.011 |
|         | ≥120       | 0.329 (0.155–0.696)    | 0.004   | 0.321 (0.133–0.773) | 0.011 |
| Model 2 | Hb (continuous) | 0.949 (0.932–0.966) | <0.001 | 0.936 (0.910–0.963) | <0.001 |

CI, confidence interval; Hb, haemoglobin; OR, odds ratio; adjustment for the duration of diabetes, glycated haemoglobin A1c, diastolic blood pressure.
observational research, we could not get a causal conclusion between Hb level and DN in patients with T1DM. Second, the conclusions could not exclude the influence of the confounding factors (e.g. antihypertensive medication and oral hypoglycaemic agents), and our study did not analyse the relationship between the trend of Hb level and DN. Third, whether our research findings suit for other groups remains uncertain further studies are needed to confirm whether our research findings are suitable for all populations.

Conclusion

In patients with T1DM, the Hb level is inversely correlated with urinary ACR and DN.

Acknowledgement

We express sincere gratitude to our colleagues who contributed to the research, although their names do not appear on the paper.

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