Association between hemoglobin levels and non-alcoholic fatty liver disease in patients with young-onset type 2 diabetes mellitus

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Abstract. This retrospective study aimed to investigate the relationship between hemoglobin (Hb) levels and non-alcoholic fatty liver disease (NAFLD) in patients with young-onset type 2 diabetes mellitus (T2DM). Data were collected for 296 patients with young-onset T2DM admitted to the first Affiliated Hospital of Guangxi Medical University from May 2017 to January 2020. Subjects were divided into NAFLD (n = 186) and non-NAFLD groups (n = 110). Patients with NAFLD had significantly higher Hb levels (p = 0.001). According to logistic regression analysis, Hb levels were significantly correlated with NAFLD after adjusting for confounding factors [odds ratio (OR) = 1.024, 95% confidence interval = 1.003–1.046, p = 0.028]. Subjects were also grouped according to Hb quartiles. After adjusting for sex and body mass index (BMI), the OR (95%CI) for NAFLD significantly increased with increasing Hb levels (p for trend = 0.009). Patients were also divided into lean (BMI <25 kg/m², n = 139) and overweight/obese groups (BMI ≥25 kg/m², n = 157), with adjusted ORs (95%CI) for the highest quartiles of 1.797 (0.559–5.776) and 6.009 (1.328–27.181), respectively. Further quartile classification of Hb according to sex showed adjusted OR (95%CI) for the highest compared with the lowest quartile of 2.796 (1.148–6.814) for males and 2.945 (0.482–17.997) for females. In conclusion, high Hb levels were associated with the presence of NAFLD in patients with young-onset T2DM, especially in males and overweight/obese patients.

Key words: Young-onset, Type 2 diabetes mellitus, Non-alcoholic fatty liver disease, Hemoglobin

THE PREVALENCE of type 2 diabetes mellitus (T2DM) has been increasing rapidly worldwide. The age at which diabetes mellitus (DM) is diagnosed is falling and the prevalence of young-onset T2DM (defined as <40 years at diagnosis) has been increasing [1, 2]. In China, approximately 5.7% of diabetic patients aged ≥18 years are <40-years-old at diagnosis [3]. Young-onset T2DM is related to longer disease exposure and elevated risks of chronic complications, associated with a more aggressive phenotype of DM [1, 2].

Non-alcoholic fatty liver disease (NAFLD) is a metabolic liver disorder that occurs in people with low levels of alcohol consumption [4]. About 25% of the population are affected by NAFLD worldwide [5], but the prevalence is >50% among patients with T2DM [6]. In a 20-year follow-up study, 6% of adolescents with NAFLD died or required liver transplants and the standardized morality rate was 13.6 [7], suggesting that the coexistence of NAFLD and young-onset T2DM is associated with substantial morbidity and mortality.

Routine blood examination is currently widely used in clinical practice. Previous studies demonstrated that serum hemoglobin (Hb) levels were strongly associated with NAFLD [8-16]; however, there is currently limited evidence regarding the relationship between Hb levels and NAFLD in patients with T2DM. The present study aimed to investigate the association between Hb levels and NAFLD in patients with young-onset T2DM.

Materials and Methods

Research subjects

This was a retrospective case-control study. A total of 1,621 inpatients with T2DM admitted to the Department of Endocrinology in the first Affiliated Hospital of Guangxi Medical University from May 2017 to January 2020 were reviewed. The inclusion criteria were: (1) phenotype of DM was T2DM (based on the diagnosis criteria of the American Diabetes Association [17]); (2) patients <40-years-old when diagnosed with T2DM; and (3) no alcohol consumption or ≤140 g ethanol per week for males and ≤70 g for females. The exclusion criteria...
were: (1) positive hepatitis B surface antigen or the presence of hepatitis C virus antibody; (2) clinical symptoms or signs of liver or renal disorders; (3) diseases that may influence serum Hb levels, such as chronic lung diseases, erythropoietin/steroid-producing tumors, sleep apnea syndrome, and regular administration of diuretics; (4) inflammation, malignancy, or other critical diseases; (5) lactation or pregnancy for women; (6) acute diabetic complications such as diabetic ketoacidosis and hyperglycemic hyperosmolar status within 1 month; and (7) lack of necessary laboratory or physical examination data. Overall 296 patients were eligible and enrolled in the study (Fig. 1). The current study was conducted in accordance with the principles of Declaration of Helsinki, and was reviewed and approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University [2020 (KY-E-035)].

Research methods

Patient information was collected from electronic medical records. Demographic and anthropometric data included name, age, sex, onset age of DM, drinking and smoking history, height, weight, systolic blood pressure, and diastolic blood pressure. Body mass index (BMI) was calculated according to the formula weight (kg)/height$^2$ (m$^2$). Laboratory data including Hb, neutrophil count, lymphocyte count, liver function, renal function, lipid profile, and glucose metabolism indexes, as well as abdominal ultrasonography results were also collected. All laboratory parameters were obtained at the same time as the NAFLD diagnosis following fasting for $>8$ h. In patients with repeated hospitalization, the first medical record was used.

Hepatic steatosis index is a simple and efficient screening tool for NAFLD, calculated using the following formula: hepatic steatosis index = 8 × [alanine aminotransferase (ALT)/aspartate aminotransferase (AST) ratio] + BMI (+2, if female; +2, if DM) [18]. An index $<30.0$ or $>36.0$ ruled out NAFLD with a sensitivity of 93.1%, or detected NAFLD with a specificity of 92.4%, respectively [18]. The diagnosis of NAFLD in this study was achieved based on the presence of fatty liver detected by abdominal ultrasonography after excluding competing hepatic disease etiologies and significant alcohol consumption [19]. Insulin resistance was quantified according to a computerized updated version of the homeostasis model assessment 2 insulin resistance (HOMA2-IR), which has been validated as an accurate method for detecting insulin resistance [20]. Fasting plasma glucose and fasting C-peptide were used to calculate HOMA2-IR in the present study.

Statistical analysis

Statistical analyses were performed using SPSS software, version 17.0. The normality of the distribution was tested by the Kolmogorov–Smirnov test. Continuous variables, with a normal distribution were expressed as mean ± SD and analyzed by independent-sample t-tests or one-way analysis of variance (ANOVA), while data with non-normal distributions were expressed as median (percentile 25, percentile 75) and analyzed with Mann-Whitney U tests or Kruskal-Wallis tests. Data for categorical variables were expressed as frequency and compared with $\chi^2$ tests. Odds ratios (ORs) and 95% confidence interval (CIs) for NAFLD were calculated by logistic regression analysis. A two-sided $p$-value $<0.05$ was considered statistically significant.

Results

Of the 296 patients enrolled, 235 (79.4%) were males (mean age: 36.98 ± 6.21 years, range: 19–48 years). The patients were divided into two groups based on the presence of NAFLD: a NAFLD group ($n = 186$) and a non-
NAFLD group (n = 110). Patients in the NAFLD group had significantly higher weight, BMI, Hb, neutrophil count, lymphocyte count, gamma-glutamyl transferase (GGT), AST, ALT, serum uric acid, triglycerides, fasting plasma glucose, fasting C-peptide, postprandial 2 h C-peptide, hepatic steatosis index, and HOMA2-IR, but lower age, diabetic duration and high-density lipoprotein cholesterol, compared with the non-NAFLD group (all p < 0.05) (Table 1). However, sex distribution, onset age, smoking proportion, height, blood pressure, total bilirubin, direct bilirubin, blood urea nitrogen, serum creatinine, endogenous creatinine clearance rate, total cholesterol, low-density lipoprotein cholesterol, homocysteine, postprandial 2 h plasma glucose and glycosylated hemoglobin (HbA1c) were similar in both two groups (all p > 0.05) (Table 1).

Serum concentrations of GGT, AST, and ALT were strongly associated with NAFLD and may serve as serum biomarkers for the severity of hepatic damage in patients with NAFLD [10]. Analysis of the association between Hb levels and GGT, AST, and ALT levels may therefore indirectly reflect the association between Hb and NAFLD. All patients were classified into quartiles by Hb levels: quartile1 (Q1, <131 g/L), quartile2 (Q2, 131–146 g/L), quartile3 (Q3, 146–155 g/L), and quartile4 (Q4, ≥155 g/L). Serum GGT, AST, and ALT concentrations all increased significantly in line with increases in Hb level (all p < 0.05) (Table 2). BMI also showed increasing trend, though the difference was not significant (p = 0.05) (Table 2).

We identified variables that were independently associated with NAFLD by binary logistic regression, with NAFLD as the dependent variable. Eighteen variables were included in the logistic regression model (Table 3). BMI and Hb level were significantly associated with NAFLD (p = 0.034, 0.028, respectively) (Table 3). We determined the risk of NAFLD among patients with different Hb levels in different models (Table 4). After adjusting for sex and BMI, the OR (95%CI) for NAFLD increased significantly in line with increasing Hb quartile (p for trend = 0.009). Patients were also divided into a lean group (BMI <25 kg/m², n = 139) and an overweight/obese group (BMI ≥25 kg/m², n = 157) according to BMI. The adjusted OR (95%CI) for the highest quartile compared with the lowest quartile was 1.797 (0.559–5.776) in the lean group and 6.009 (1.328–27.181) in the overweight/obese group.

We performed further logistic regression analysis after quartile classification of Hb separately by sex (for men: Q1 (<139 g/L, n = 58), Q2 (139–150 g/L, n = 66), Q3 (150–156 g/L, n = 55), and Q4 (≥156 g/L, n = 56); for women: Q1 (<115 g/L, n = 15), Q2 (115–128 g/L, n = 16), Q3 (128–137 g/L, n = 16), and Q4 (≥137 g/L, n = 14)). After adjustment for BMI, the ORs (95% CIs) of NAFLD for patients in Q2, Q3, and Q4 were 1.411 (0.602–3.305), 1.783 (0.769–4.134), and 2.796 (1.148–6.814), respectively, in men (p for trend = 0.020), and 1.603 (0.301–8.532), 11.938 (1.619–88.012), and 2.945 (0.482–17.997), in women, compared with patients in Q1 (p for trend = 0.075).

Discussion

The results of this study suggested that there could be a significant correlation between Hb levels and NAFLD in patients with young-onset T2DM. First, patients with NAFLD had higher Hb levels. Second, serum transaminase concentrations all tended to be higher in patients with higher Hb levels, indirectly supporting the relationship between Hb levels and NAFLD. Furthermore, Hb was also identified to be significantly associated with the presence of NAFLD by logistic regression analysis, after adjusting for confounding factors.

Previous research suggested that the prevalence of NAFLD in patients with T2DM ranged from 5%–87%, differed by some factors such as lipid profile, BMI, and glucose metabolism [6]. The prevalence of NAFLD in patients with young-onset type 2 diabetes in the current study was approximately 62.8%, which was in agreement with a previous cross-sectional study of 1,217 inpatients with T2DM, which showed that approximately 61% of the patients had NAFLD [21]. However, another cross-sectional study performed in China found that only 50.6% of 8,571 inpatients with T2DM were diagnosed with NAFLD, which was lower than our results [22], possibly because of the higher BMI of patients in our research [6].

Because of the potential increased risk of cardiovascular diseases among diabetic patients with concurrent NAFLD [23, 24], there is a clinical need to identify the potential risk factors for NAFLD. Obesity has been reported to be significantly associated with NAFLD [25, 26], and a meta-analysis of 21 cohort studies suggested that obesity independently resulted in 3.5-fold elevated risk of developing NAFLD compared with normal weight [26]. BMI is a common grouping indicator for obesity, and is also commonly used to evaluate the extent of obesity [27]. The meta-analysis also showed that each unit increment in BMI led to a 20% increase in the relative risk of NAFLD [26]. Consistently, we also identified BMI as a significant factor for NAFLD. Notably, however, we also found a significant relationship between Hb levels and NAFLD in patients with young-onset T2DM, and this relationship was independent of BMI.

Hb has been reported to be associated with NAFLD in the non-DM population [8-16]. Proteomic analysis by
| Variable                | T2DM without NAFLD (n = 110) | T2DM with NAFLD (n = 186) | p value |
|-------------------------|------------------------------|---------------------------|---------|
| Age (years)             | 38.00 ± 6.02                 | 36.38 ± 6.25              | 0.029   |
| Male [n (%)]            | 88 (80%)                     | 147 (79%)                 | 0.842   |
| Onset age (years)       | 33.22 ± 5.97                 | 32.66 ± 5.68              | 0.420   |
| Diabetic duration (years)| 4.79 ± 2.58                  | 3.72 ± 1.98               | 0.043   |
| Smoking [n (%)]         | 41 (37.3%)                   | 69 (37.1%)                | 0.976   |
| Height (m)              | 1.67 ± 0.07                  | 1.68 ± 0.10               | 0.312   |
| Weight (kg)             | 65.90 ± 13.75                | 79.66 ± 17.08             | 0.000   |
| BMI (kg/m^2)            | 23.50 ± 3.94                 | 28.09 ± 5.20              | 0.000   |
| SBP (mmHg)              | 127.37 ± 21.94               | 131.70 ± 17.68            | 0.064   |
| DBP (mmHg)              | 83.86 ± 16.20                | 85.45 ± 12.65             | 0.349   |
| Hemoglobin (g/L)        | 137.36 ± 21.11               | 145.26 ± 16.56            | 0.001   |
| Male                    | 141.45 ± 18.57               | 149.88 ± 13.81            | 0.000   |
| Female                  | 120.98 ± 23.08               | 128.00 ± 14.56            | 0.207   |
| Neutrophil (×10^9/L)    | 4.09 ± 1.24                  | 4.50 ± 1.81               | 0.020   |
| Lymphocyte (×10^9/L)    | 2.16 ± 0.62                  | 2.48 ± 0.75               | 0.000   |
| GGT (U/L)               | 26 (18, 44.25)               | 38.15 (27.15, 59.25)      | 0.000   |
| AST (U/L)               | 22.37 ± 11.22                | 27.42 ± 12.84             | 0.001   |
| ALT (U/L)               | 21 (15, 32)                  | 31 (20, 46)               | 0.000   |
| TBIL (μmol/L)           | 10.04 ± 6.21                 | 10.73 ± 5.13              | 0.305   |
| DBIL (μmol/L)           | 2.6 (2.1, 3.53)              | 2.6 (2.3, 3.5)            | 0.349   |
| BUN (mmol/L)            | 5.14 ± 2.17                  | 4.73 ± 1.40               | 0.077   |
| Scr (μmol/L)            | 74.65 ± 34.08                | 69.69 ± 19.22             | 0.110   |
| SUA (μmol/L)            | 324.57 ± 106.9               | 394.02 ± 99.56            | 0.000   |
| Ccr (mL/min)            | 107.4 ± 36.44                | 112.99 ± 34.59            | 0.188   |
| TC (mmol/L)             | 5.07 ± 1.78                  | 5.07 ± 1.60               | 0.997   |
| Triglyceride (mmol/L)   | 1.32 (0.99, 2.23)            | 1.98 (1.35, 3.38)         | 0.000   |
| HDL-C (mmol/L)          | 1.10 ± 0.34                  | 0.99 ± 0.31               | 0.010   |
| LDL-C (mmol/L)          | 3.12 ± 1.43                  | 2.94 ± 0.96               | 0.265   |
| Homocysteine (μmol/L)   | 11.06 ± 4.46                 | 10.53 ± 3.26              | 0.294   |
| FPG (mmol/L)            | 6.99 ± 2.99                  | 7.79 ± 2.98               | 0.025   |
| 2hPG (mmol/L)           | 13.89 ± 5.87                 | 14.63 ± 4.64              | 0.266   |
| HbA1c (%)               | 9.43 ± 2.83                  | 9.54 ± 2.27               | 0.732   |
| FCP (ng/mL)             | 1.34 (0.44, 2.33)            | 2.5 (1.73, 3.46)          | 0.000   |
| 2hCP (ng/mL)            | 3.86 (2.5, 5.52)             | 6.19 (3.67, 9.89)         | 0.000   |
| HOMA2-IR                | 1.20 (0.38, 2.00)            | 2.10 (1.40, 2.90)         | 0.000   |
| Hepatic steatosis index | 34.97 ± 5.45                 | 40.97 ± 6.55              | 0.000   |

NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GGT, gamma-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; BUN, blood urea nitrogen; Scr, serum creatinine; SUA, serum uric acid; Ccr, endogenous creatinine clearance rate; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; 2hPG, postprandial 2 h plasma glucose; HbA1c, glycosylated hemoglobin; FCP, fasting C-peptide; 2hCP, postprandial 2 h C-peptide; HOMA, homeostasis model assessment; IR, insulin resistance.
therefore further investigated the association between clinical trial revealed that regular phlebotomy to reduce NAFLD is primarily related to overweight/obesity, it can also occur in lean individuals with a BMI <25 kg/m² [30]. However, evidence suggests that lean subjects with overweight/obese patients but not in lean patients. The results of subgroup analyses by sex and BMI thus suggested the need to pay particular attention to males and overweight/obese individuals with NAFLD [31]. In our study, there was a significant association between Hb and NAFLD in overweight/obese patients but not in lean patients. The results of subgroup analyses by sex and BMI thus suggested the need to pay particular attention to males and overweight/obese patients with high Hb levels, in order to prevent NAFLD.

### Table 2  Comparison of transferase concentrations and BMI grouped by hemoglobin quartiles

| Variable       | Q1            | Q2            | Q3            | Q4            | p value |
|----------------|---------------|---------------|---------------|---------------|---------|
| GGT (U/L)      | 35.85 ± 25.51 | 39.08 ± 18.88 | 41.72 ± 20.06 | 49.73 ± 28.66 | 0.030   |
| AST (U/L)      | 23.32 ± 10.32 | 25.47 ± 10.04 | 26.21 ± 15.35 | 30.20 ± 11.48 | 0.007   |
| ALT (U/L)      | 27.12 ± 17.08 | 29.31 ± 16.46 | 33.32 ± 20.65 | 39.35 ± 22.71 | 0.001   |
| BMI (kg/m²)    | 24.93 ± 4.16  | 26.73 ± 5.90  | 26.90 ± 4.88  | 27.01 ± 5.75  | 0.050   |

GGT, gamma-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index. Hemoglobin quartiles: Q1 (<131 g/L), Q2 (131–146 g/L), Q3 (146–155 g/L), Q4 (≥155 g/L).

### Table 3  Logistic regression analysis of NAFLD risk factors in patients with young-onset T2DM

| Variable                  | p value   | OR (95%CI) |
|---------------------------|-----------|------------|
| Age (years)               | 0.552     | 1.020 (0.956–1.087) |
| Sex                       | 0.173     | 0.464 (0.154–1.399) |
| Diabetic duration (years) | 0.721     | 1.016 (0.931–1.109) |
| Weight (kg)               | 0.600     | 0.983 (0.923–1.047) |
| BMI (kg/m²)               | 0.034     | 1.271 (1.018–1.586) |
| Hemoglobin (g/L)          | 0.028     | 1.024 (1.003–1.046) |
| Neutrophil (>10⁹/L)       | 0.559     | 1.063 (0.866–1.304) |
| Lymphocyte (>10⁹/L)       | 0.181     | 1.391 (0.858–2.257) |
| GGT (U/L)                 | 0.094     | 1.014 (0.998–1.030) |
| AST (U/L)                 | 0.487     | 0.982 (0.931–1.034) |
| ALT (U/L)                 | 0.062     | 1.028 (0.999–1.058) |
| SUA (µmol/L)              | 0.842     | 1.000 (0.997–1.004) |
| Triglyceride (mmol/L)     | 0.238     | 1.125 (0.925–1.368) |
| HDL-C (mmol/L)            | 0.078     | 0.319 (0.090–1.137) |
| FPG (mmol/L)              | 0.171     | 1.200 (0.924–1.559) |
| FCP (ng/mL)               | 0.408     | 2.579 (2.073–24.328) |
| 2hCP (ng/mL)              | 0.174     | 1.090 (0.963–1.235) |
| HOMA2-IR                  | 0.456     | 0.347 (0.021–5.599) |

NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; BMI, body mass index; GGT, gamma-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SUA, serum uric acid; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; FCP, fasting C-peptide; 2hCP, postprandial 2 h C-peptide; HOMA, homeostasis model assessment; IR, insulin resistance.
The relationship between NAFLD and Hb level remains poorly understood, but there are several possible explanations. First, insulin resistance plays a pivotal role in the progression of NAFLD [32, 33], and Hb has been reported to be significantly associated with insulin resistance [34, 35]. It could therefore be speculated that insulin resistance might provide a bridge between high Hb and NAFLD. Second, as an iron-containing metalloprotein, Hb reserves most of the systemic iron, and Hb levels are closely related to iron levels [36]. Iron is a highly reactive element, and the accumulation of iron in excess of physiological requirements disrupts redox homeostasis and catalyzes the generation of reactive oxygen species, resulting in oxidative stress [37], which is in turn one of the vital pathophysiological mechanisms underlying NAFLD [38]. Third, hepatocellular steatosis can give rise to sinusoidal distortion and decreased intrasinusoidal volume [39]. As a result, the sinusoids become inefficient blood conduits with impaired tissue perfusion, potentially leading to hypoxia [39], which may in turn trigger compensatory erythropoiesis and augmented Hb levels. Hb levels could also be influenced by diet, and the consumption of more animal product could be related to higher Hb levels [8]. Diet is also a risk factor for NAFLD [40], and excessive intake of animal products may therefore lead to elevated Hb levels and a high prevalence of NAFLD.

The study had several limitations. First, it was a retrospective case-control study with low power to address the causality of the relationship between Hb and NAFLD. Second, all the patients were inpatients and most were young men without advanced complications, leading to inevitable bias. The study results may thus fail to reflect the overall picture for young-onset T2DM. Third, the lack of certain data meant that some parameters of hematopoiesis (e.g., red blood cell count, reticulocyte count, hematocrit, leukocyte count, platelet count) and possible confounders (e.g., dietary habit, waist circumference, physical activity, antidiabetic drugs, diabetic duration, diabetic complications) were not considered in this study.

In conclusion, patients with young-onset T2DM and concurrent NAFLD exhibited higher Hb levels than patients without concurrent NAFLD. Serum Hb levels may thus be associated with the presence of NAFLD in young-onset T2DM. Prospective large-scale studies are needed to confirm the roles of serum Hb in the occurrence of NAFLD in patients with young-onset T2DM.

Acknowledgment

The authors gratefully appreciated the team of the Department of Medical Statistics in Guangxi Medical University for their work in the area of statistics in this study.

Disclosure

All authors declare that they have no conflict of interest.

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