

Relationship between anemia, serum bilirubin concentrations, and diabetic retinopathy in individuals with type 2 diabetes

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Abstract

This study sought to assess the hypothesis that anemia is associated with diabetic retinopathy in type 2 diabetes mellitus (DM) and investigate the factors mediating the relationship between anemia and diabetic retinopathy.

In total, 1637 individuals with type 2 DM were examined in a cross-sectional study. Anemia was defined as hemoglobin level <120 g/L in women and <130 g/L in men. A logistic regression model was used to determine the association between anemia and diabetic retinopathy.

Anemia was more prevalent in individuals with diabetic retinopathy. Logistic regression analysis found a statistically significant association between anemia and diabetic retinopathy after adjustment for traditional risk factors (odds ratio, 1.44; 95% confidence interval, 1.10–1.89, $P = .009$). Further adjustment for serum bilirubin levels removed the statistically significant association.

In individuals with type 2 DM, anemia is related to diabetic retinopathy, and its association may be mediated by a correlated change in serum bilirubin levels.

Abbreviations: ACR = albumin excretion rate; A1C = glycated hemoglobin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence intervals; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; NPDR, non-proliferative diabetic retinopathy; OR = odds ratio; PDR = proliferative diabetic retinopathy; VEGF = vascular endothelial growth factor.

Keywords: anemia, bilirubin, diabetic retinopathy

1. Introduction

Diabetic retinopathy is a common complication in individuals with diabetes mellitus (DM), leading to visual impairment and

blindness.^[1] Aside from its detrimental impact on vision, diabetic retinopathy might be associated with increased systemic vascular complications in individuals with type 2 DM.^[1,2] Even though chronic hyperglycemia, diabetes duration, and hypertension have been well recognized as crucial risk factors for diabetes retinopathy, the risk of diabetic retinopathy could not be completely eliminated by controlling for blood glucose and blood pressure.^[1] Hence, other factors may also be involved in the pathogenesis of diabetic retinopathy.

Anemia is frequently found in individuals with type 2 DM.^[3] Many previous studies have shown a close link between anemia and hypoxia-induced organ damage, including heart failure, angina, and pedal ulceration, as well as increased mortality.^[4,5] An increasing body of evidence also implicates anemia in diabetes-related organ damage.^[6,7] Retinal hypoxia is also suggested to be associated with diabetic retinopathy.^[5]

Although bilirubin, a metabolite of heme, was once considered to be a toxic waste product, it has recently emerged as an antioxidant and cytoprotectant.^[8] Previous clinical studies have shown a protective role of bilirubin in diabetic microangiopathy.^[9–11] Low serum bilirubin levels are reported to be associated with an increased risk of diabetic retinopathy.^[12,13] As serum bilirubin is generated from the degradation of hemoglobin,^[14] the effect of anemia on the retina might be related to a reduction in serum bilirubin levels with antioxidant activities. However, the relationship among anemia, bilirubin, and diabetic retinopathy has not yet been clarified.

The purpose of the current study was, therefore, to explore the associations between anemia, serum bilirubin concentrations, and diabetic retinopathy in individuals with type 2 DM.

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2. Patients and methods

2.1. Participants

We conducted a cross-sectional study in 1637 randomly chosen individuals with type 2 DM who visited the diabetes clinic of our hospital from January 2017 to December 2018. Based on the “Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus”,^[15] we made the diagnosis of type 2 DM. We defined the presence of anemia as a hemoglobin level <120 g/L in women and <130 g/L in men.^[16] Anemia was categorized into two groups: grade I anemia with hemoglobin levels ≥ 110 g/L and grade II anemia with hemoglobin levels <110 g/L. Hypertension was defined as a blood pressure $\geq 140/90$ mmHg or taking anti-hypertensive drugs. An individual was considered to have hyperlipidemia if total cholesterol levels were ≥ 6.5 mmol/L and/or triglycerides levels ≥ 2.3 mmol/L or if anti-hyperlipidemic agents were prescribed. We gathered information regarding smoking status, diabetes duration, and other health-associated parameters through a standardized questionnaire. Individuals with a history of pancreatitis, chronic liver disease (including hepatitis B or C), liver cirrhosis, glucocorticoid use, advanced renal dysfunction (serum creatinine more than 177 mmol/L), alcoholism, malignancy, infection, hemolysis, acute or chronic blood loss, hemoglobinopathies, or red blood cell transfusion were excluded from this study. In addition, individuals with serum bilirubin concentrations greater than the upper limit of normal (>22.2 mmol/L) and those with serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels more than twice the upper limit of normal (>74 U/L) were excluded. The study was approved by the ethics committee of Chonnam National University Hospital, with informed consent provided by all participants.

2.2. Measurement

After overnight fasting, venous blood samples were collected. We measured glycated hemoglobin (A1C) level using ion exchange liquid chromatography with a model HLC-723-GHbV apparatus (Tosoh, Tokyo, Japan). We determined hemoglobin levels using cyanmethemoglobin spectrophotometry (Beckman-Coulter Inc., Miami, FL). We quantified serum levels of AST and ALT using the AU5407 analyzer (Olympus, Tokyo, Japan). We measured serum bilirubin levels with an enzymatic method using bilirubin oxidase on a model AU5407 analyzer (Olympus, Tokyo, Japan) and assessed urinary albumin excretion using the urinary albumin: creatinine ratio (ACR) in random urine samples. We determined estimated glomerular filtration rate (eGFR) by the Chronic Kidney Disease Epidemiology Collaboration equation.^[17] Nephropathy was categorized as a urinary ACR ≥ 300 mg/gCr or eGFR <60 mL/min/1.73 m². To diagnose diabetic retinopathy, funduscopy was conducted by ophthalmologists after pupil dilation. Study participants were categorized into three groups: no diabetic retinopathy, non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR). In this study, diabetic retinopathy included NPDR and PDR.

2.3. Statistical analyses

Data are presented as mean \pm standard deviation or median (interquartile range). The Mann–Whitney *U* test or Student's *t* test was used for continuous variables, and the Chi-squared test

was used for categorical variables. To explore the relationship between anemia and diabetic retinopathy, multivariable analysis was conducted using logistic regression models with identified factors and previously recognized risk factors. Logarithmic transformation was conducted prior to analysis for parameters with skewed distribution. Model 1 included sex and age as covariates. Model 2 was adjusted by Model 1 variables plus smoking habits, body mass index, ALT levels, hyperlipidemia, hypertension, diabetes duration, A1C levels, and nephropathy. Further, bilirubin level was entered as a covariate (Model 2a). SPSS version 20.0 (SPSS, Chicago, IL) was used for statistical analyses. A *P* value $<.05$ indicated statistical significance.

3. Results

Characteristics of individuals with type 2 DM are presented in Table 1. Individuals with diabetic retinopathy were older and had longer diabetes duration, lower body mass index, lower eGFR, higher urinary ACR, and lower serum bilirubin, ALT, and hemoglobin levels than those without diabetic retinopathy. Individuals with diabetic retinopathy had higher systolic blood pressures and higher A1C levels than those without diabetic retinopathy. Individuals with diabetic retinopathy were associated with a higher prevalence of hypertension and nephropathy than those without diabetic retinopathy. In addition, anemia was significantly more common in individuals with diabetic retinopathy than in those without diabetic retinopathy. Furthermore, the

Table 1
Characteristics of subjects with Type 2 diabetes mellitus.

	Without DR	With DR	<i>P</i> value
N	1067	570	
Age (yr)	58.1 \pm 13.3	61.5 \pm 12.7	<.001
Men (%)	529 (49.6)	268 (47.0)	.323
Alcohol, n (%)	208 (19.5)	108 (18.9)	.790
Current smoking, n (%)	159 (14.9)	70 (12.3)	.145
Hypertension, n (%)	521 (48.8)	351 (61.6)	<.001
Hyperlipidemia, n (%)	528 (49.5)	297 (52.1)	.312
Diabetes duration (years)	5.0 (9.0)	15.0 (12.0)	<.001
Body mass index (kg/m ²)	24.5 \pm 3.7	23.8 \pm 4.6	.003
Systolic BP (mmHg)	126.6 \pm 16.9	129.9 \pm 17.1	<.001
Diastolic BP (mmHg)	77.1 \pm 11.0	77.8 \pm 11.3	.226
A1C (%)	8.5 \pm 2.4	9.0 \pm 2.2	<.001
A1C (mmol/mol)	69 \pm 26	75 \pm 24	<.001
Total cholesterol (mmol/L)	4.67 \pm 1.16	4.68 \pm 1.22	.880
LDL cholesterol (mmol/L)	2.83 \pm 0.98	2.83 \pm 1.03	.963
HDL cholesterol (mmol/L)	1.19 \pm 0.34	1.16 \pm 0.37	.083
Triglyceride (mmol/L)	1.45 (1.05)	1.39 (1.13)	.544
Hemoglobin (g/L)	132.6 \pm 18.5	123.8 \pm 18.7	<.001
Total bilirubin (μ mol/L)	12.8 \pm 4.0	11.2 \pm 3.8	<.001
AST (U/L)	22.0 (11.0)	21.0 (9.0)	.071
ALT (U/L)	22.0 (17.0)	19.0 (14.0)	.001
Urinary ACR (mg/gCr)	10.3 (36.3)	49.3 (240.4)	<.001
eGFR (mL/min/1.73 m ²)	95.8 (23.0)	87.3 (36.3)	<.001
Anemia, n (%)	305 (28.6)	274 (48.1)	<.001
Nephropathy, n (%)	137 (12.8)	211 (37.0)	<.001
Use of oral hypoglycemic agents, n (%)	799 (74.9)	418 (73.3)	.494
Use of insulin, n (%)	267 (25.0)	154 (27.0)	.379

Data are represented as the mean \pm standard deviation or median (interquartile range).

Numbers in parentheses indicate percentage.

ACR = albumin excretion rate, A1C = glycated hemoglobin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BP = blood pressure, eGFR = estimated glomerular filtration rate, HDL cholesterol = high-density lipoprotein cholesterol, LDL cholesterol = low-density lipoprotein cholesterol.

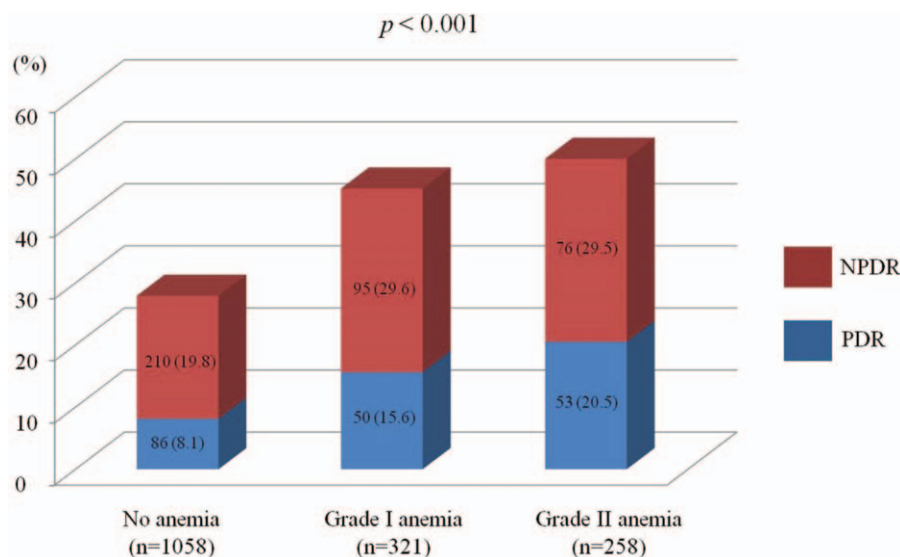


Figure 1. Diabetic retinopathy prevalence according to anemia grade. Diabetic retinopathy severity increased with anemia grade ($P < .001$). Data are represented as frequencies (percentages). NPDR=non-proliferative diabetic retinopathy, PDR=proliferative diabetic retinopathy

severity of diabetic retinopathy increased with the grade of anemia ($P < .001$, Fig. 1).

To explore the effects of anemia on diabetic retinopathy, we performed multivariable analyses using logistic regression models (Table 2, Fig. 2). The relationship between anemia and

diabetic retinopathy was statistically significant after adjusting for sex, age, smoking habits, body mass index, ALT level, hypertension, hyperlipidemia, diabetes duration, A1C level, and nephropathy (odds ratio [OR]: 1.44; 95% confidence interval [CI]: 1.10–1.89, $P = .009$; Model 2). The addition of serum

Table 2

Odds ratio for diabetic retinopathy in individuals with Type 2 diabetes mellitus.

	Diabetic Retinopathy			
	Anemia (yes vs no)	P value	Bilirubin, $\mu\text{mol/L}$	P value
Unadjusted	2.31 (1.87–2.86)	<.001	—	—
Model 1	2.18 (1.76–2.70)	<.001	—	—
Model 2	1.44 (1.10–1.89)	.009	—	—
Model 2a	1.30 (0.98–1.72)	.074	0.96 (0.92–0.99)	.009

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex, smoking, body mass index, ALT, hypertension, hyperlipidemia, DM duration, A1C, and nephropathy

Model 2a: model 2 + bilirubin

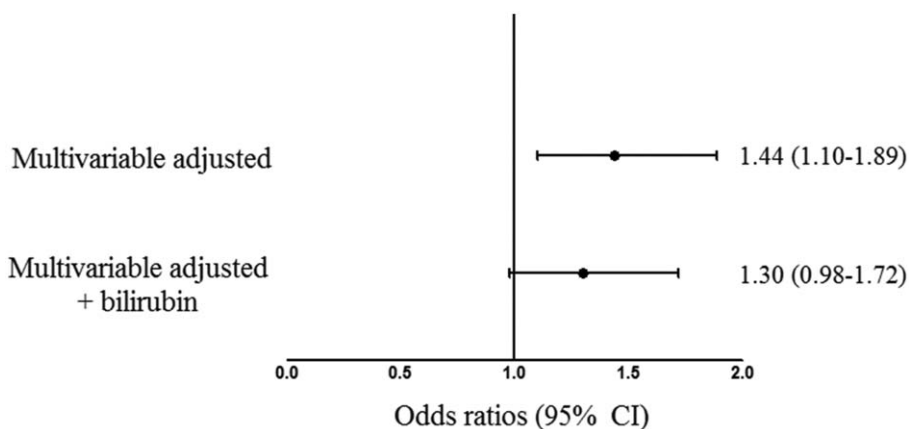


Figure 2. Odds ratios for diabetic retinopathy with anemia as an independent variable after adjusting for confounding factors. Odds ratios were adjusted for age, sex, smoking, body mass index, ALT, hypertension, hyperlipidemia, DM duration, A1C level, and nephropathy.

bilirubin concentrations to this model attenuated this association (OR: 1.30, 95% CI: 0.98–1.72, $P = .074$; Model 2a).

4. Discussion

We found a positive relationship between anemia and diabetic retinopathy in individuals with type 2 DM after adjusting for conventional risk factors including diabetes duration, A1C, and hypertension. Moreover, our findings suggest that the association between anemia and diabetic retinopathy might be partly mediated by a correlated change in serum bilirubin concentrations.

Individuals with type 2 DM commonly have anemia.^[3] Regardless of the presence of nephropathy, hemoglobin levels continue to decline over time in individuals with type 2 DM.^[3,18] Evidence points to anemia as a risk factor for adverse cardiovascular events including coronary heart disease, heart failure, and cardiovascular mortality.^[4,19] In addition, anemia might be implicated in diabetes-related organ damage.^[5] Qiao et al^[20] reported that anemia is associated with an increased risk for diabetic retinopathy. Ito et al^[6] showed a close relationship between anemia and diabetic retinopathy in individuals with type 2 DM. Furthermore, our study showed that anemia was positively associated with diabetic retinopathy after adjusting for sex, age, smoking habits, body mass index, ALT, hypertension, hyperlipidemia, diabetes duration, A1C, and nephropathy (Model 2 in Table 2). Our data therefore support the preceding findings that anemia is implicated in retinal damage in diabetes.^[5,6,20]

With regards to underlying factors linking anemia with organ damage, previous studies have focused on hypoxia. Anemia causes tissue hypoxia, which is a critical etiology of diabetic retinopathy.^[5] Retinal hypoxia is proposed to stimulate synthesis of vascular endothelial growth factor (VEGF), a strong stimulant of neovascularization.^[5,21] This factor also increases vascular permeability and retinal exudates.^[5,21] Individuals with anemia have been reported to have increased systemic levels of VEGF.^[22] Therefore, decreased oxygen delivery due to anemia might have detrimental effects on the retina of individuals with diabetes. Additionally, anemia might contribute to increased oxidative stress due to a decrease in the absolute number of red blood cells with antioxidant defense and enhanced free radical production.^[23,24]

Furthermore, anemia might be linked to serum bilirubin levels. Bilirubin is generated from the sequential catalytic degradation of the heme in hemoglobin by heme oxygenase and biliverdin reductase.^[25] Thus, a reduction in hemoglobin levels might be implicated in a change in physiological serum bilirubin levels. Bilirubin has been recently recognized as a natural antioxidant that scavenges free radicals.^[8] Experimental studies have shown that all types of bilirubin, including unconjugated bilirubin, conjugated bilirubin, free bilirubin, and albumin-bound bilirubin, have effective antioxidant capacities.^[8,26,27] Bilirubin is suggested to inhibit the formation of advanced glycation end products and protein kinase C^[28] and to be involved in inflammatory processes.^[29] All of these pathways have been implicated in the pathogenesis of diabetic retinopathy.^[30] Further support comes from clinical studies showing that serum bilirubin levels might play a protective role against DM and cardiovascular disease.^[31,32] Furthermore, previous clinical studies have reported close associations between serum bilirubin levels and diabetic microangiopathy.^[10,11] Yasuda et al^[12] reported an

inverse relationship between total serum bilirubin levels and diabetic retinopathy. Sekioka et al^[13] showed that total serum bilirubin levels were negatively related to the severity of diabetic retinopathy.^[13] Thus, we hypothesized that the relationship between anemia and diabetic retinopathy might be linked to a correlated change in serum bilirubin levels. In the current study, a statistically significant association between anemia and diabetic retinopathy was abolished when serum bilirubin level was further entered into Model 2, which adjusted for other confounders in the multiple regression model (Model 2a in Table 2). Our findings, therefore, indicate that the proposed induction of diabetic retinopathy by anemia might be partly mediated by a change in serum bilirubin levels.

In conclusion, our study shows a positive relationship between anemia and diabetic retinopathy in individuals with type 2 diabetes, which might be partly attributed to a correlated decline in total serum bilirubin levels. Further prospective investigations are necessary to explore the causal associations among anemia, bilirubin, and diabetic retinopathy.

Author contributions

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Writing – review & editing: Seon-Young Park, Dong Jin Chung, Min Young Chung.

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