The Association Between Anion Gap and Diabetic Retinopathy: Based on Hospital Registry Data in Liaoning Diabetic Microvascular Complications Study

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Abstract

Background: Current evidences supported a highly relevant between acid-load and diabetes. The rise of serum anion gap (AG) has performed the significances in metabolic acidosis. Some factors correlated to diabetes might affect its complications such as diabetic retinopathy (DR).

Methods: A total of 3,411 Chinese adults with type 2 diabetes were selected form the hospital registry data on DR Sub-study of Liaoning Diabetic Microvascular Complications Study (LD-MCS), included 1,137 DR as the cases and 2,274 non-DR as the matching controls. Fasting venous blood test was applied to ascertain the ion levels, and serum AG (mmol/L) was computed by: AG = (sodium + potassium) – (chlorine + bicarbonate). According to Early Treatment for Diabetic Retinopathy Study standards, DR was diagnosed by two-field fundus photographs and classified as mild non-proliferative DR (NPDR), moderate NPDR, and vision-threatening DR (VTDR). Logistic regression models and linear regression models were used to analyze the relationships.

Results: In setting of the non-acidosis, higher AG was significantly associated with DR (P = 0.001), increased with aggravating retinopathy but decreased in VTDR level (P < 0.001). Multivariable-adjusted models showed that AG quartiles were independently linked with higher odds to occurrence (P for trend < 0.001) and severity (P for trend < 0.05) of DR, but with attention to the fluctuation of 75th AG quartiles. Linear logistic regression by stepwise method suggested the growth of age (P = 0.014), glycated hemoglobin (P = 0.018), and the homeostasis model assessment of insulin resistance index (P < 0.001) played an intimate role in the association between AG and DR.

Conclusions: Higher AG was independently related to the occurrence and progression of DR. Our findings suggested that serum AG might alter the risk of DR by affecting glucose metabolism and insulin sensitivity in patients with type 2 diabetes.

Backgroud

As one of the most common micro-vascular complications caused by hyperglycemia in diabetic patients, diabetic retinopathy (DR) has become the principal cause of visual impairments in working-age adults [1, 2]. Current evidences supported a highly relevant between acid-load or acidosis and the risk of diabetes. Clinical indicators for evaluating acid-base balance were proposed to relate to type 2 diabetes or insulin resistance, which is also the core of type 2 diabetes [3]. A large, population-based study involving 1,709 (60–84 years old) adults revealed that lactate levels were positively correlated with type 2 diabetes in the older adult population, and also associated with higher fasting plasma glucose (FPG) in non-diabetes [4]. Elevated levels of lactate were supposed as an early risk factor for males with diabetes in a cohort form Sweden [5]. Furthermore, a cross-sectional study of 1,496 non-diabetic adults in American National Health and Nutrition Examination Surveys concluded that the associations between higher serum anion gap (AG), lower bicarbonate (HCO_3^-) and insulin resistance were independently significant [6]. Interestingly, diabetic ketoacidosis (DKA), as one of the life-threatening acute complications of diabetes,

was also suggested to worsen insulin resistance [3] Other indicators of acidosis included blood ketone body [7] and related urine tests [8, 9] were suggested to alter the status of diabetes.

In view of the causality between diabetes and its complications, some status correlated to diabetic metabolism would be treated as essential conditions in the trigger or progression of retinopathy. Serum AG, as a frequently-used testing index in clinics, is served to evaluate the acid-base balance of human body, and its rise has performed the significant utility in the diagnosis of metabolic acidosis [10]. To date, the relationship between serum AG and DR has not yet been evaluated. Thus, this study designed a nested case-control model to analyze the relationship between serum AG and the presence as well as severity of DR based on the hospital-based population with type 2 diabetes.

Methods

Study subjects

Liaoning Diabetic Microvascular Complications Study (LD-MCS) is a serial epidemiological study of microangiopathy in type 2 diabetes from hospital registry in the First Affiliated Hospital of China Medical University, Liaoning Province, China. The whole study was approved by the Institutional Review Board (IRB) of the First Affiliated Hospital of China Medical University (AF-SOP-07-1.1-01/2019-13). LD-MCS includes three sub-studies: DR, diabetic peripheral neuropathy and diabetic nephropathy. LD-MCS established a retrospective hospital-based cohort database, which continuously recorded 5,066 Chinese subjects with type 2 diabetes aged over 18 from 2012 to 2018. More detailed rationale of LD-MCS was shown in Additional file (see Additional file 1). In current DR sub-study, designed as a nested case-control research, selected 3,411 subjects from LD-MCS. The selection method of the subjects was in accordance with Figure S1 (see Additional file 2). Firstly, 1,073 of 5,066 (21.2%) subjects were excluded for the following reasons: 1) diabetic ketoacidosis, hyperosmotic nonketotic diabetic coma and other diabetic acute metabolic disorders (n = 86, 1.7%); 2) the level of estimated glomerular filtration rate (eGFR) $\leq$ 60 ml/min, albuminuria or history of chronic kidney disease (n = 656, 12.9%); 3) pregnancy or lactation (n = 114, 2.3%); 4) major mental or physical illness for more than 3 years (n = 159, 3.1%), including myocardial infarction, cerebral infarction, malignant tumor, severe organ dysfunction and mental disorder; 5) incomplete information (n = 58, 1.1%). Then, after reviewing the pathological and clinical situations of the remaining 3,993 subjects, 1,137 (28.5%) patients with type 2 diabetes were diagnosed with DR, and they were defined as the case group. Two controls for each case (n = 2,274) were randomly sampled from the same hospitalized register database, and they were alive and no history of DR. All controls and cases were matched according to gender and age, and used the quotas performed on the diagnosis date. Finally, the complete medical records of 3,411 confirmed subjects was eligible for analyzing.

Measurements

Each participant from DR Sub-study of LD-MCS carried out a comprehensive clinical assessment and her/his basic characteristics were recorded. This study extracted the following information, included

gender, age, height, weight, body mass index (BMI), heart rate (HR), blood pressure (BP, included systolic BP [SBP], diastolic BP [DBP], and pulse pressure [PP]), tobacco and alcohol use, use of antidiabetic drugs and duration of diabetes. Oral anti-hyperglycemic drugs or insulin injections were considered to use of antidiabetic drugs. Smoker or drinker was defined as those who self-described past or present use of tobacco or alcohol, regardless of the frequency and quantity. Venous blood test with fasting ≥ 8 hours summarized the concentration of the following items: hemoglobin A1c (HbA1c), FPG, insulin (baseline), potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), chlorine (Cl^-), magnesium (Mg^{2+}), phosphorus (P), HCO_3^-, AG and serum creatinine (Scr). Insulin resistance was calculated based on homoeostasis model assessment (HOMA-IR) by: (baseline insulin [mU/L] × FPG [mmol/L] / 22.5) [11]. The level of eGFR was estimated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [12].

Definition

Type 2 diabetes was defined following the standards of American Diabetes Association (ADA) [13]. Fundus assessments depended on two-field colorful retinal images, and the diagnosis of DR was confirmed from the worse eye abiding by the criterion of Early Treatment for Diabetic Retinopathy Study (ETDRS) [14]. DR was rated as 3 severity scales, included mild non-proliferative DR (NPDR), moderate NPDR, and vision-threaten DR (VTDR) [14, 15]. The whole process of fundus photographic and diagnosis was completed by trained ophthalmologists in a double-blind condition. Serum AG was defined as the concentration difference between undetermined anions (UA) and undetermined cations (UC), which was generally calculated as $Na^+ - (Cl^- + HCO_3^-)$. Because of the concentration of K^+ in blood was much smaller compared to Na^+, Cl^- or HCO_3^-, K^+ was often omitted in clinical calculation of AG. However, this research computed AG (mmol/L) using the following equation: $AG = (Na^+ + K^+) - (Cl^- + HCO_3^-)$ [16].

Statistical analysis

All epidemiological analysis was completed by Statistical Product and Service Solutions (IBM, version 24), and EmpowerStats (www.empowerstats.com, X&Y Solutions, inc. Boston MA). The subjects were described as mean ± SD (measurement data), or quantity and percentage (counting data). The level of AG was analyzed as a continuous variable, while its quartiles were treated as categorized variables and represented by letter Q for each grade, namely Q1 (0–25%): < 14.69; Q2 (25% – 50%): 14.69–16.36; Q3 (50% – 75%): 16.37–17.92; Q4 (75% – 100%): ≥ 17.93. Kolmogorov-Smirnov test was used to verify the normal distribution of data. The continuous variables were tested by one-way ANOVA or nonparametric test, and Chi-square analysis was applied to categorical variables. Further, this research exploited 3 confounder models to investigate the relationships between AG and the occurrence as well as severity of DR, as model 1 adjusted by gender and age; model 2 adjusted by gender, age, duration of diabetes, antidiabetic agents use, smoker, drinker, height, SBP, PP, HbA1c and Mg^{2+} levels; model 3 adjusted by above variables as well as HOMA-IR and eGFR. Multi-factor logistic regression models were studied to clarify the associations, and trend test basing on logistic regression was performed by modeling the AG quartile categories as a continuous variable. The expression of logistic regression based on odds ratios

(OR) and 95% confidence intervals (CI). Linear regression models were performed to explore the correlations between the level of AG and potential DR-related factors, using entering and stepwise methods to select variables, respectively. In addition, a smooth curve-fitting adjusted by confounding factors was used to visualize the dose-response between DR and serum AG. All statistical analysis regarded non-DR of the control group or Q1 of AG quartiles as the references. $P < 0.05$ indicated that statistical differences were significant.

Results

Table 1 listed basic characteristics of all participants in this study, which included 1,137 (33.3%) DR patients, and the number of mild NPDR, moderate NPDR and VTDR was 772 (22.6%), 124 (3.6%) and 241 (7.2%), respectively. Duration of diabetes, use of antidiabetic agents, SBP, PP and AG were significantly associated with both presence and severity of DR, while HCO_3^- level was associated with its presence, and height, alcohol and tobacco use, HbA1c and Mg^{2+} levels were associated with DR severities (all $P < 0.05$). With the aggravating of retinopathy, the levels of AG were 16.18 ± 2.46, 16.42 ± 2.46, 16.77 ± 2.10 and 16.48 ± 2.35, respectively, showing a rising trend, but decreased in VTDR group ($P < 0.001$).

Table 1
Basic characteristics of study population.

	Non-DR	DR		Mild NPDR	Moderate NPDR	VTDR	
Variables	N = 2274	N = 1137	P	N = 772	N = 124	N = 241	P
Gender (male, %)	1294 (56.9)	647 (56.9)		445 (57.6)	60 (48.4)	142 (58.9)	0.237
Age (year)	57.37 ± 11.74	57.37 ± 11.74		57.21 ± 11.97	59.74 ± 11.81	56.66 ± 10.83	0.106
Duration of diabetes (year)	6.59 ± 5.95	9.06 ± 6.65	< 0.001	8.32 ± 6.47	10.84 ± 7.40	10.50 ± 6.43	< 0.001
Antidiabetic agents (yes, n, %)	2132 (93.8)	1106 (97.3)	< 0.001	747 (96.8)	121 (97.6)	238 (98.8)	< 0.001
HR (bmp)	80.82 ± 10.67	81.83 ± 11.44	0.050	81.86 ± 11.57	81.72 ± 12.16	81.81 ± 10.65	0.090
SBP (mmHg)	134.02 ± 18.31	136.80 ± 20.21	< 0.001	136.84 ± 20.25	137.60 ± 20.07	136.23 ± 20.22	0.001
DBP (mmHg)	82.87 ± 11.07	82.85 ± 11.89	0.963	82.78 ± 11.98	83.18 ± 11.59	82.89 ± 11.79	0.987
PP (mmHg)	51.15 ± 14.46	53.95 ± 16.32	< 0.001	54.06 ± 16.35	54.43 ± 15.90	53.34 ± 16.49	< 0.001
Height (cm)	168.31 ± 8.11	167.76 ± 8.10	0.064	167.84 ± 7.97	166.11 ± 8.52	168.38 ± 8.21	0.018
Weight (kg)	69.76 ± 12.59	69.23 ± 12.48	0.248	69.44 ± 12.43	67.50 ± 12.23	69.48 ± 12.73	0.261
BMI (kg/m^2)	24.52 ± 3.40	24.50 ± 3.46	0.885	24.55 ± 3.45	24.35 ± 3.35	24.41 ± 3.56	0.904
Smoker (yes, n, %)	601 (26.4)	317 (27.9)	0.368	194 (25.1)	40 (32.3)	83 (34.4)	0.016
Drinker (yes, n, %)	456 (20.1)	242 (21.3)	0.401	140 (18.1)	35 (28.2)	67 (27.8)	0.002

Abbreviations: AG, anion gap; BMI, body mass index; Ca^{2+}, calcium; Cl^-, chlorine; DBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCO_3^-, bicarbonate; HOMA-IR, the homeostasis model assessment of insulin resistance index; HR, heart rate; K^+, potassium; Mg^{2+}, magnesium; Na^+, sodium; NPDR, non-proliferative diabetic retinopathy; P, phosphorus; PP, pulse pressure; SBP, systolic blood pressure; Scr, serum creatinine; VTDR, vision-threaten diabetic retinopathy.

	Non-DR	DR		Mild NPDR	Moderate NPDR	VTDR	
HbA1c (%, mmol/mol)	8.49 ± 2.25 (69.29 ± 24.59)	8.64 ± 2.29 (70.89 ± 25.02)	0.074	8.54 ± 2.26 (69.89 ± 24.71)	8.58 ± 2.31 (70.30 ± 25.22)	8.96 ± 2.35 (74.40 ± 25.72)	0.025
FPG (mmol/L)	9.15 ± 3.57	9.33 ± 3.51	0.158	9.20 ± 3.48	9.14 ± 3.18	9.82 ± 3.76	0.051
Insulin (mU/L)	12.45 ± 7.80	12.65 ± 8.29	0.502	12.90 ± 9.06	11.92 ± 4.71	12.22 ± 7.06	0.418
HOMA-IR	5.18 ± 4.37	5.27 ± 4.25	0.559	5.34 ± 4.63	4.80 ± 2.35	5.30 ± 3.72	0.091
K^+ (mmol/L)	3.94 ± 0.38	3.94 ± 0.41	0.927	3.94 ± 0.39	3.95 ± 0.34	3.94 ± 0.49	0.993
Na^+ (mmol/L)	141.18 ± 2.76	141.15 ± 2.73	0.735	141.22 ± 2.71	141.45 ± 2.08	140.79 ± 3.06	0.629
Ca^{2+} (mmol/L)	2.27 ± 0.15	2.27 ± 0.18	0.503	2.26 ± 0.16	2.29 ± 0.14	2.29 ± 0.23	0.707
Cl^- (mmol/L)	104.01 ± 3.10	103.88 ± 3.22	0.267	103.99 ± 3.20	103.91 ± 2.85	103.54 ± 3.44	0.174
Mg^{2+} (mmol/L)	0.86 ± 0.08	0.85 ± 0.09	0.111	0.86 ± 0.09	0.86 ± 0.08	0.83 ± 0.08	< 0.001
P (mmol/L)	1.16 ± 0.20	1.16 ± 0.19	0.540	1.16 ± 0.19	1.16 ± 0.16	1.18 ± 0.21	0.700
HCO_3^- (mmol/L)	24.93 ± 2.42	24.74 ± 2.36	0.023	24.74 ± 2.39	24.73 ± 2.21	24.71 ± 2.38	0.156
AG (mmol/L)	16.18 ± 2.46	16.47 ± 2.40	0.001	16.42 ± 2.46	16.77 ± 2.10	16.48 ± 2.35	< 0.001
Scr (umol/L, mg/dL)	58.92 ± 14.63 (0.67 ± 0.17)	59.18 ± 14.94 (0.67 ± 0.17)	0.625	59.06 ± 14.29 (0.67 ± 0.16)	57.80 ± 12.64 (0.65 ± 0.14)	60.28 ± 17.77 (0.68 ± 0.20)	0.686
eGFR (mL/min)	102.56 ± 16.66	102.48 ± 17.46	0.901	102.76 ± 17.20	100.99 ± 16.68	102.34 ± 18.68	0.754

Abbreviations: AG, anion gap; BMI, body mass index; Ca^{2+}, calcium; Cl^-, chlorine; DBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCO_3^-, bicarbonate; HOMA-IR, the homeostasis model assessment of insulin resistance index; HR, heart rate; K^+, potassium; Mg^{2+}, magnesium; Na^+, sodium; NPDR, non-proliferative diabetic retinopathy; P, phosphorus; PP, pulse pressure; SBP, systolic blood pressure; Scr, serum creatinine; VTDR, vision-threaten diabetic retinopathy.

Table 2 showed the variations of each index based on AG quartiles. There were positive relationships between HbA1c, FPG, insulin, HOMA-IR, Ca^{2+}, P and AG quartiles, and the negative ones were between Cl^{-}, HCO_3^{-} and AG quartiles (all $P < 0.001$). In addition, the serum levels of Na^{+} ($P = 0.008$) and Scr ($P = 0.027$) were also significantly correlated with AG quartiles. Moreover, the rising of AG quartiles was significantly associated with the proportion of DR ($P = 0.001$) and its severe cases ($P < 0.001$), respectively.

Table 2
Comparisons of population characteristics based on AG quartiles.

	AG quartiles				
	Q1 (< 14.69)	Q2 (14.69–16.36)	Q3 (16.37–17.92)	Q4 (≥ 17.93)	
Variables	N = 851	N = 850	N = 856	N = 854	P
Gender (male, %)	486 (57.1)	492 (57.9)	478 (55.8)	485 (56.8)	0.863
Age (year)	56.73 ± 11.95	56.92 ± 11.61	58.07 ± 11.57	57.74 ± 11.79	0.054
Duration of diabetes (year)	7.53 ± 6.32	7.23 ± 6.07	7.55 ± 6.42	7.35 ± 6.39	0.674
Antidiabetic agents (yes, n, %)	813 (95.5)	798 (93.9)	819 (95.7)	808 (94.6)	0.289
HR (bmp)	81.39 ± 11.47	81.65 ± 10.51	80.92 ± 11.16	80.69 ± 10.58	0.251
SBP (mmHg)	134.94 ± 19.06	134.77 ± 19.40	135.20 ± 19.35	134.88 ± 18.23	0.970
DBP (mmHg)	82.78 ± 11.18	82.35 ± 11.16	82.98 ± 11.21	83.32 ± 11.83	0.346
PP (mmHg)	52.15 ± 15.34	52.42 ± 14.99	52.22 ± 15.33	51.55 ± 15.00	0.668
Height (cm)	168.35 ± 8.02	168.46 ± 8.13	167.97 ± 8.02	167.80 ± 8.26	0.248
Weight (kg)	69.48 ± 12.63	70.04 ± 12.67	69.53 ± 12.64	69.29 ± 12.29	0.645
BMI (kg/m^2)	24.42 ± 3.55	24.56 ± 3.35	24.55 ± 3.44	24.52 ± 3.34	0.825
Smoker (yes, n, %)	239 (28.1)	228 (26.8)	233 (27.2)	218 (25.5)	0.689
Drinker (yes, n, %)	176 (20.7)	174 (20.5)	169 (19.7)	179 (21.0)	0.935

Abbreviations: AG, anion gap; BMI, body mass index; Ca^{2+}, calcium; Cl^-, chlorine; DBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCO_3^-, bicarbonate; HOMA-IR, the homeostasis model assessment of insulin resistance index; HR, heart rate; K^+, potassium; Mg^{2+}, magnesium; Na^+, sodium; NPDR, non-proliferative diabetic retinopathy; P, phosphorus; PP, pulse pressure; SBP, systolic blood pressure; Scr, serum creatinine; VTDR, vision-threaten diabetic retinopathy.

	AG quartiles				
HbA1c (%, mmol/mol)	8.35 ± 2.29 (67.78 ± 25.04)	8.53 ± 2.14 (69.71 ± 23.43)	8.52 ± 2.23 (69.60 ± 24.39)	8.76 ± 2.37 (72.19 ± 25.88)	< 0.001
FPG (mmol/L)	8.93 ± 3.59	9.14 ± 3.55	9.11 ± 3.27	9.65 ± 3.73	< 0.001
Insulin (mU/L)	11.33 ± 7.85	12.48 ± 8.51	12.73 ± 7.11	13.52 ± 8.19	< 0.001
HOMA-IR	4.56 ± 4.10	5.21 ± 4.59	5.20 ± 3.68	5.88 ± 4.77	< 0.001
K^+ (mmol/L)	3.95 ± 0.41	3.92 ± 0.38	3.94 ± 0.35	3.74 ± 0.41	0.773
Na^+ (mmol/L)	141.21 ± 2.84	141.34 ± 2.63	141.25 ± 2.51	140.90 ± 2.97	0.008
Ca^{2+} (mmol/L)	2.24 ± 0.20	2.25 ± 0.14	2.27 ± 0.13	2.31 ± 0.16	< 0.001
Cl^- (mmol/L)	105.18 ± 3.08	104.51 ± 2.86	103.81 ± 2.83	102.37 ± 3.07	< 0.001
Mg^{2+} (mmol/L)	0.86 ± 0.09	0.86 ± 0.08	0.85 ± 0.08	0.85 ± 0.09	0.305
P (mmol/L)	1.14 ± 0.20	1.16 ± 0.18	1.17 ± 0.18	1.17 ± 0.21	< 0.001
HCO_3^- (mmol/L)	26.90 ± 2.22	25.19 ± 1.78	24.26 ± 1.86	23.13 ± 1.98	< 0.001
Scr (umol/L, mg/dL)	60.15 ± 15.47 (0.68 ± 0.18)	58.55 ± 14.02 (0.66 ± 0.16)	59.20 ± 14.44 (0.67 ± 0.16)	58.13 ± 14.90 (0.66 ± 0.17)	0.027
eGFR (mL/min)	101.86 ± 17.29	103.35 ± 16.60	101.84 ± 16.94	103.08 ± 16.87	0.129
DR (yes, n, %)	251 (29.5)	260 (30.6)	310 (36.2)	316 (37.0)	0.001
DR level (yes, n, %)					
Mild NPDR	183 (21.5)	178 (20.9)	190 (22.2)	221 (25.9)	< 0.001

Abbreviations: AG, anion gap; BMI, body mass index; Ca^{2+}, calcium; Cl^-, chlorine; DBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCO_3^-, bicarbonate; HOMA-IR, the homeostasis model assessment of insulin resistance index; HR, heart rate; K^+, potassium; Mg^{2+}, magnesium; Na^+, sodium; NPDR, non-proliferative diabetic retinopathy; P, phosphorus; PP, pulse pressure; SBP, systolic blood pressure; Scr, serum creatinine; VTDR, vision-threaten diabetic retinopathy.

	AG quartiles			
Moderate NPDR	19 (2.2)	35 (4.1)	33 (3.9)	37 (4.3)
VTDR	49 (5.8)	47 (5.5)	87 (10.2)	58 (6.8)

Abbreviations: AG, anion gap; BMI, body mass index; Ca^{2+}, calcium; Cl^{-}, chlorine; DBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCO_3^{-}, bicarbonate; HOMA-IR, the homeostasis model assessment of insulin resistance index; HR, heart rate; K^{+}, potassium; Mg^{2+}, magnesium; Na^{+}, sodium; NPDR, non-proliferative diabetic retinopathy; P, phosphorus; PP, pulse pressure; SBP, systolic blood pressure; Scr, serum creatinine; VTDR, vision-threaten diabetic retinopathy.

To further investigate the relationship between AG and DR, Table 3 showed logistic regression outcomes based on the three models, in which non-DR group was treated as a reference. Except VTDR in model 1 (OR 1.05, 95% CI 1.00-1.11; P = 0.063), the correlations between AG and the presence as well as severity of DR were totally significant after eliminating confounding interferences in both model 1 and model 2 (all P < 0.05). In model 3 adjusted potential confounders, AG levels were significantly correlated with DR (1.06, 1.02–1.09; P < 0.001), mild NPDR (1.05, 1.01–1.08; P = 0.011), moderate NPDR (1.12, 1.03–1.21; P = 0.005) and VTDR (1.06, 1.00-1.12; P = 0.037), respectively.

Table 3
Logistic regression models for association between AG and DR.

	DR		Mild NPDR		Moderate NPDR		VTDR	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Model 1	1.05 (1.02, 1.08)	0.001	1.04 (1.01, 1.08)	0.019	1.10 (1.02, 1.19)	0.013	1.05 (1.00, 1.11)	0.063
Model 2	1.06 (1.02, 1.09)	< 0.001	1.05 (1.01, 1.08)	0.009	1.11 (1.03, 1.20)	0.007	1.06 (1.00, 1.12)	0.045
Model 3	1.06 (1.02, 1.09)	< 0.001	1.05 (1.01, 1.08)	0.011	1.12 (1.03, 1.21)	0.005	1.06 (1.00, 1.12)	0.037

Abbreviation: AG, anion gap; CI, confidence interval; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; OR, odds ratio; VTDR, vision-threatening diabetic retinopathy.

Model 1 adjusted by gender and age; Model 2 adjusted by gender, age, duration of diabetes, antidiabetic agents, SBP, PP, height, smoker, drinker, HbA1c and Mg^{2+}; Model 3 adjusted by gender, age, duration of diabetes, antidiabetic agents, SBP, PP, height, smoker, drinker, HbA1c, Mg^{2+}, HOMA-IR and eGFR.

Figure 1 summarized the trend test results of multivariable-adjusted models based on logistic regression. In all three models, the trend relationships between AG quartiles and DR presences (all P for trend < 0.001) as well as its severity (all P for trend < 0.05) were all significant. Model 3 adjusted for gender, age, duration of diabetes, antidiabetic agents, SBP, PP, height, smoker, drinker, HbA1c, Mg^{2+}, HOMA-IR and eGFR, the OR values monotonically increased with the increasing AG quartiles (Q2: OR 1.09, 95% CI 0.88–1.35; Q3: 1.39, 1.13–1.71; Q4: 1.46, 1.18–1.80). The similar positive trends were observed in mild NPDR group, but those were not monotonous in moderate NPDR (Q3: 1.98, 1.10–3.55) and VTDR (Q3: 2.04, 1.40–2.98) groups because of Q3 fluctuating. When comparing horizontally, AG quartiles showed an inversely U-shape with the aggravation of retinopathy. In addition, multivariate smoothing spline plots was used to investigate the correlations between serum AG and the cases of each severity of DR (exposure) (see Figure S2 in Additional file 3). With the aggravation of retinopathy, the dose-responses between serum AG and the exposure changed from a nearly straight liner to a curve (P = 0.0005 for any-severity of DR; P = 0.0115 for mild NPDR; P = 0.0151 for moderate NPDR; P = 0.0205 for VTDR). Furthermore, an optimal cut-off for AG level at 16.00 was identified as the best value for increasing the risk for presence of DR.

Table 4 employed a linear regression analysis of AG and DR-related factors involved in model 3, and the variable selection methods were entered and stepwise. Among all variables, male, drinker, older, high levels of SBP, HbA1c, HOMA-IR and eGFR had positive effects on AG, while use of antidiabetic agents, duration, PP, height, smoking and Mg^{2+} had the reverse ones. The links between age (β = 0.042; P = 0.014), HbA1c (β = 0.042; P = 0.018), HOMA-IR (β = 0.082; P < 0.001) and AG were the most convincing after excluding insignificant variables.

Table 4
Linear regression model between AG and DR-related factors.

Variables	β	P
Gender (male, n)	0.021	0.445
Age (year)	0.062	0.003
Duration of diabetes (year)	-0.019	0.278
Antidiabetic agents (yes, n)	-0.003	0.878
SBP (mmHg)	0.046	0.108
PP (mmHg)	-0.064	0.033
Height (cm)	-0.046	0.081
Smoker (yes, n)	-0.030	0.148
Drinker (yes, n)	0.014	0.488
HbA1c (%, mmol/mol)	0.035	0.055
Mg^{2+} (mmol/L)	-0.002	0.926
HOMA-IR	0.083	< 0.001
eGFR (mL/min)	0.027	0.207
Combine of variables [a]		
Age (year)	0.042	0.014
HbA1c (%, mmol/mol)	0.042	0.018
HOMA-IR	0.082	< 0.001

Abbreviation: AG, anion gap; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HOMA-IR, the homeostasis model assessment of insulin resistance index; Mg^{2+}, magnesium; PP, pulse pressure; SBP, systolic blood pressure.

[a] Using stepwise method to select variables. Stepwise method ordinally included the most significant variable ($P < 0.05$) and then excluded the most indistinctive variable ($P > 0.10$), which was aimed to obtain an optimal combine of variables based on Forward method and Backward method.

Discussion

We conducted a large sample of nested case-control study to explore the relationship between DR and serum AG, in which all participants were hospitalized patients with comprehensive data. A survey exceeding 6,000 sets of inpatients' subjects showed a 37% increase in their levels of serum AG, which meant that hospitalized patients, more comorbidities compare to general public probably, had a relatively

raised serum AG in most cases [17]. In the setting of non-acidosis, the results of this study considered that the level of AG was associated with the presence and severity of DR. With the aggravation of DR severity, the level of AG remained rising but decreased in VTDR level. Multi-factor logistic regression models showed that AG quartiles were independently linked with higher odds to the incidence and severity of DR, but with premise of the fluctuation on Q3 of AG quartiles. The adjusted smooth curve-fitting visualized that serum AG formed a nearly positive linear correlation with the severity of DR. Linear regression models were applied between the level of AG and DR-related factors in order to investigate the potential mechanism of this correlation. When using stepwise method to search the most optimal combination of variables, the growth of age, HbA1c and HOMA-IR played an intimate role in the increase of serum AG. Therefore, we surmised that serum AG might alter the risk of retinopathy by affecting glucose metabolism and insulin sensitivity in patients with type 2 diabetes. However, it should be noted that HOMA-IR was generally at a higher level in DR patients in this study, although the differences were not statistical.

Recent research suggested that participants with insulin resistance or type 2 diabetes were considered to relate to rising serum ketone bodies and declining urine PH [7, 9], which were relevant indexes of metabolic acidosis. In order to controlled the complicated influences of renal excretion and insulin status imposing on serum AG, this study excluded participants with the level of eGFR $\leq$ 60 ml/min or renal disfunction, and still obtained the statistical correlations of AG and DR. Further, a more significant association was developed after adding two variables as HOMA-IR and eGFR in model 3. They all meant that there were interlaced contacts among insulin resistance, acid load and renal function in DR or diabetic patients. Patients suffering from type 2 diabetes would develop a higher level of renal acid excretion and organic acid production [6]. Owing to the direct consequence of insulin resistance or long-term dysglycemia metabolism, incomplete renal function impeded ammonia production in proximal convoluted and potassium ion excretion in distal convoluted tubules [18–21]. Further, the functional defect of diabetic nephron was more prominent when suffering type 4 renal tubular acidosis, and the confusion of acid-base would be exacerbated [21]. But it is worthy to note that non-diabetic people with greater insulin resistance were also statistically accompanied with hypocapnia and low levels of citrate in the urine [8].

As the dysfunction of glycometabolism, diabetes causes multiple organ system diseases, and gradually forms a series of vascular or nonvascular complications. Insulin resistance is the main representation of type 2diabetes, although its pathogenesis of insulin has been widely concerned, it is not totally clear. Previous studies have shown that inflammatory reactions, the disturbance of fatty acid metabolism and mitochondrion function are all implicated in insulin resistance [22]. Metabolic acidosis obstructs the binding of insulin to receptor [23, 24], or decreases the sensitivity of tissue to insulin [25], which has been observed in human studies and rats models. Cell culture study revealed that excessive acid blocked receptor phosphorylation and insulin signal transduction [26]. Clinical tests of acid-base metabolism such as decreasing plasma HCO_3^- and increasing AG, have been proposed to induce insulin resistance in non-diabetic population, even these indicators were still at normal levels [6]. While in female patients with

type 2 diabetes, the similar negative correlation between HCO_3^- and the risk of diabetes remained significant after controlling for C-reactive protein [3]. In addition, multiple studies have pointed that organic anion as one of the sources of endogenous acids, the level of its generation rate was significantly rising in patients with diabetes [27]. However, higher level of AG is also a result of the accumulation of endogenous acids. Population-based epidemiologic investigations among African American [4] and Swedish male [5] demonstrated a positive link between serum lactate (a component of AG) and the prevalence of type 2 diabetes, probably acting as the signal of impaired glucose tolerance and hyperinsulinism. The findings in this study suggested that increasing serum AG was independently related to the occurrence and progression of DR. Therefore, the higher serum AG was not only one of the feedbacks of acid overload in clinic, its monitoring might also act as a potential warning signal for type 2 diabetes and diabetic complications including DR.

Moreover, heavier acid loading would promote the rising of blood pressure [27, 28], as well as stimulate cortisol [29–31] and growth hormone [32] performing insulin anti-regulatory, then further triggered inflammatory response and protein consumption. Insulin resistance added the risks of type 2 diabetes and its complications, as well as poor blood pressure control and inflammation were also important mechanisms of DR [1, 33]. The high level of AG probably affected the trigger and progression of retinopathy through various channels. In this study, the level of AG increased with the worsen of DR levels. According to the adjusted smoothing spline plots, the findings suggested that serum AG should be controlled at a low-normal level to prevent diabetic patients from developing DR. Interestingly, among diabetic patients with serum AG levels between 16.00 to 18.00 may have a high risk for presence of moderate-DR and VTDR, and further prospective clinical studies are needed to validate our findings.

This study was one of the series projects of DR Sub-study of LD-MCS, which firstly investigated the association between serum AG, and presence as well as severity of DR in the setting of non-acidosis. However, the following limitations need to be mentioned: First, the proportion of mild NPDR, moderate NPDR and VTDR in this study was 67.9% (n = 772), 10.9% (n = 124), and 21.2% (n = 241) respectively. A non-linear fluctuation between AG and DR levels might be induced by the inclusion of less moderate NPDR or more VTDR. Second, the research results stemmed from cross-sectional subjects, since the basic characteristics of participants were recorded only once, it was still unclear how the level of AG changes the presence of DR and its severity over time, as well as the causal link of this relativity. Third, the serum AG is defined as the concentration of major cation (Na^+) minus the major anion (Cl^- and HCO_3^-), and its existence is mostly implicated the net anionic valence of plasma proteins (primarily albumin) [34]. The worries in this study when using AG was that the level of albumin had not been corrected. Fourth, we did not have records of blood PH or plasma ketone body, which limited the procedures of detailed reviewing participants' acid-base metabolism. But patients with acidosis have been excluded according to the historical diagnosis, and the average values of all ions and acid-base indexes were at normal ranges, so we believed that there was unlikely a highly bias in the results. Similarly, the diet habits and nutrition of study participants was not traced, which played a partial role in acid-base physiology [35]. Further studies are still warranted to complement the above limitations.

Conclusions

In this nested case-control study, our findings from hospitalized patients with type 2 diabetes illustrated an independent association between serum AG and the severity of DR using multivariable-adjusted models. The positive linear increase of AG could be observed in the presence of DR, as well as in mild NPDR and moderate NPDR levels, but not in VTDR level. The existing results suggested that serum AG as a biomarker of excessive acid and acidosis, might alter the status of insulin resistance, further promote the appearance and progression of DR. The monitoring of serum AG would provide a new approach for early waring and prevention of retinopathy.

Abbreviations

ADA, American Diabetes Association; AG, anion gap; BMI, body mass index; BP, blood pressure; Ca^{2+}, calcium; CIconfidence intervals; Cl^-, chlorine; DBP, diastolic blood pressure; DKA, diabetic ketoacidosis; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; ETDRS, Early Treatment for Diabetic Retinopathy Study; FPG, fasting plasma glucose; HbA_1c, glycated hemoglobin; HCO_3^-, bicarbonate; HOMA-IR, the homeostasis model assessment of insulin resistance index; HR, heart rate; IRB, Institutional Review Board; K^+, potassium; LD-MDS, Liaoning Diabetic Microvascular Complications Study; Mg^{2+}, magnesium; Na^+, sodium; NPDR, non-proliferative diabetic retinopathy; OR, odds ratios; P, phosphorus; PP, pulse pressure; SBP, systolic blood pressure; Scr, serum creatinine; UA, undetermined anions; UC, undetermined cations; VTDR, vision-threaten diabetic retinopathy.

Declarations

Ethics approval and consent to participate

The whole study was approved by the Institutional Review Board (IRB) of the First Affiliated Hospital of China Medical University (AF-SOP-07-1.1-01/2019-13). Written informed consents were achieved for the entire study population included in this study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Xinyu Wang: Formal analysis, Writing-Original Draft; Litong Yao: Conceptualization, Writing-Original Draft; Xiang Li: Software, Investigation; Yifan Zhong: Methodology, Writing-Review and Editing; Yan Wang: Validation, Software; Jingyang Wu: Visualization, Investigation; Jin Geng: Formal analysis; Yun Zhou: Data Curation; Jiahua Zhang: Data Curation; Jun Chen: Data Curation; Yingying Xu: Conceptualization, Supervision; Yudong Hu: Methodology, Supervision; Lei Liu: Supervision, Project administration, Funding acquisition. Lei Liu is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Authors' information

Not applicable.

References

1. Wong TY, Cheung CM, Larsen M, Sharma S, Simó R. Diabetic retinopathy. Nat Rev Dis Primers. 2016;2:16012.
2. Yamada M, Hiratsuka Y, Roberts CB, Pezzullo ML, Yates K, Takano S, et al. Prevalence of visual impairment in the adult Japanese population by cause and severity and future projections. Ophthalmic Epidemiol. 2010;17(1):50–7.
3. Mandel EI, Curhan GC, Hu FB, Taylor EN. Plasma bicarbonate and risk of type 2 diabetes mellitus. CMAJ. 2012;184(13):E719-25.
4. Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, et al. Association of blood lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. Int J Epidemiol. 2010;39(6):1647–55.

5. Ohlson LO, Larsson B, Björntorp P, Eriksson H, Svärdsudd K, Welin L, et al. Risk factors for type 2 (non-insulin-dependent) diabetes mellitus. Thirteen and one-half years of follow-up of the participants in a study of Swedish men born in 1913. Diabetologia. 1988;31(11):798–805.
6. Farwell WR, Taylor EN. Serum bicarbonate, anion gap and insulin resistance in the National Health and Nutrition Examination Survey. Diabet Med. 2008;25(7):798–804.
7. Avogaro A, Crepaldi C, Miola M, Maran A, Pengo V, Tiengo A, et al. High blood ketone body concentration in type 2 non-insulin dependent diabetic patients. J Endocrinol Invest. 1996;19(2):99–105.
8. Cupisti A, Meola M, D'Alessandro C, Bernabini G, Pasquali E, Carpi A, et al. Insulin resistance and low urinary citrate excretion in calcium stone formers. Biomed Pharmacother. 2007;61(1):86–90.
9. Maalouf NM, Cameron MA, Moe OW, Adams-Huet B, Sakhaee K. Low urine pH: a novel feature of the metabolic syndrome. Clin J Am Soc Nephrol. 2007;2(5):883–8.
10. Emmett M. Anion-gap interpretation: the old and the new. Nat Clin Pract Nephrol. 2006;2(1):4–5.
11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
12. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12.
13. (2) Classification and diagnosis of diabetes. Diabetes Care. 2015;38 Suppl:S8-s16.
14. Grading diabetic retinopathy. from stereoscopic color fundus photographs–an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology. 1991;98(5 Suppl):786–806.
15. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677–82.
16. Kraut JA, Madias NE. Serum anion gap: its uses and limitations in clinical medicine. Clin J Am Soc Nephrol. 2007;2(1):162–74.
17. Lolekha PH, Vanavanan S, Lolekha S. Update on value of the anion gap in clinical diagnosis and laboratory evaluation. Clin Chim Acta. 2001;307(1–2):33–6.
18. Abate N, Chandalia M, Cabo-Chan AV Jr, Moe OW, Sakhaee K. The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. Kidney Int. 2004;65(2):386–92.
19. Electrolyte. and Acid-Base Disturbances in Patients with Diabetes Mellitus. N Engl J Med. 2019;381(16):1598.
20. Fuster DG, Bobulescu IA, Zhang J, Wade J, Moe OW. Characterization of the regulation of renal Na+/H + exchanger NHE3 by insulin. Am J Physiol Renal Physiol. 2007;292(2):F577-85.
21. Karet FE. Mechanisms in hyperkalemic renal tubular acidosis. J Am Soc Nephrol. 2009;20(2):251–4.

22. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. Physiology (Bethesda). 2004;19:183–90.
23. Igarashi M, Yamatani K, Fukase N, Daimon M, Ohnuma H, Ogawa A, et al. Effect of acidosis on insulin binding and glucose uptake in isolated rat adipocytes. Tohoku J Exp Med. 1993;169(3):205–13.
24. Whittaker J, Cuthbert C, Hammond VA, Alberti KG. The effects of metabolic acidosis in vivo on insulin binding to isolated rat adipocytes. Metabolism. 1982;31(6):553–7.
25. DeFronzo RA, Beckles AD. Glucose intolerance following chronic metabolic acidosis in man. Am J Physiol. 1979;236(4):E328-34.
26. Hayata H, Miyazaki H, Niisato N, Yokoyama N, Marunaka Y. Lowered extracellular pH is involved in the pathogenesis of skeletal muscle insulin resistance. Biochem Biophys Res Commun. 2014;445(1):170–4.
27. Khairallah P, Scialla JJ. Role of Acid-Base Homeostasis in Diabetic Kidney Disease. Curr Diab Rep. 2017;17(4):28.
28. Pohl MA, Blumenthal S, Cordonnier DJ, De Alvaro F, Deferrari G, Eisner G, et al. Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. J Am Soc Nephrol. 2005;16(10):3027–37.
29. Maurer M, Riesen W, Muser J, Hulter HN, Krapf R. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. Am J Physiol Renal Physiol. 2003;284(1):F32–40.
30. Esche J, Shi L, Sánchez-Guijo A, Hartmann MF, Wudy SA, Remer T. Higher diet-dependent renal acid load associates with higher glucocorticoid secretion and potentially bioactive free glucocorticoids in healthy children. Kidney Int. 2016;90(2):325–33.
31. Buehlmeier J, Remer T, Frings-Meuthen P, Maser-Gluth C, Heer M. Glucocorticoid activity and metabolism with NaCl-induced low-grade metabolic acidosis and oral alkalization: results of two randomized controlled trials. Endocrine. 2016;52(1):139–47.
32. Brüngger M, Hulter HN, Krapf R. Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis: new cause of growth hormone insensitivity in humans. Kidney Int. 1997;51(1):216–21.
33. Wong TY, Cheung N, Tay WT, Wang JJ, Aung T, Saw SM, et al. Prevalence and risk factors for diabetic retinopathy: the Singapore Malay Eye Study. Ophthalmology. 2008;115(11):1869–75.
34. Feldman M, Soni N, Dickson B. Influence of hypoalbuminemia or hyperalbuminemia on the serum anion gap. J Lab Clin Med. 2005;146(6):317–20.
35. Fagherazzi G, Vilier A, Bonnet F, Lajous M, Balkau B, Boutron-Rualt MC, et al. Dietary acid load and risk of type 2 diabetes: the E3N-EPIC cohort study. Diabetologia. 2014;57(2):313–20.

Figures

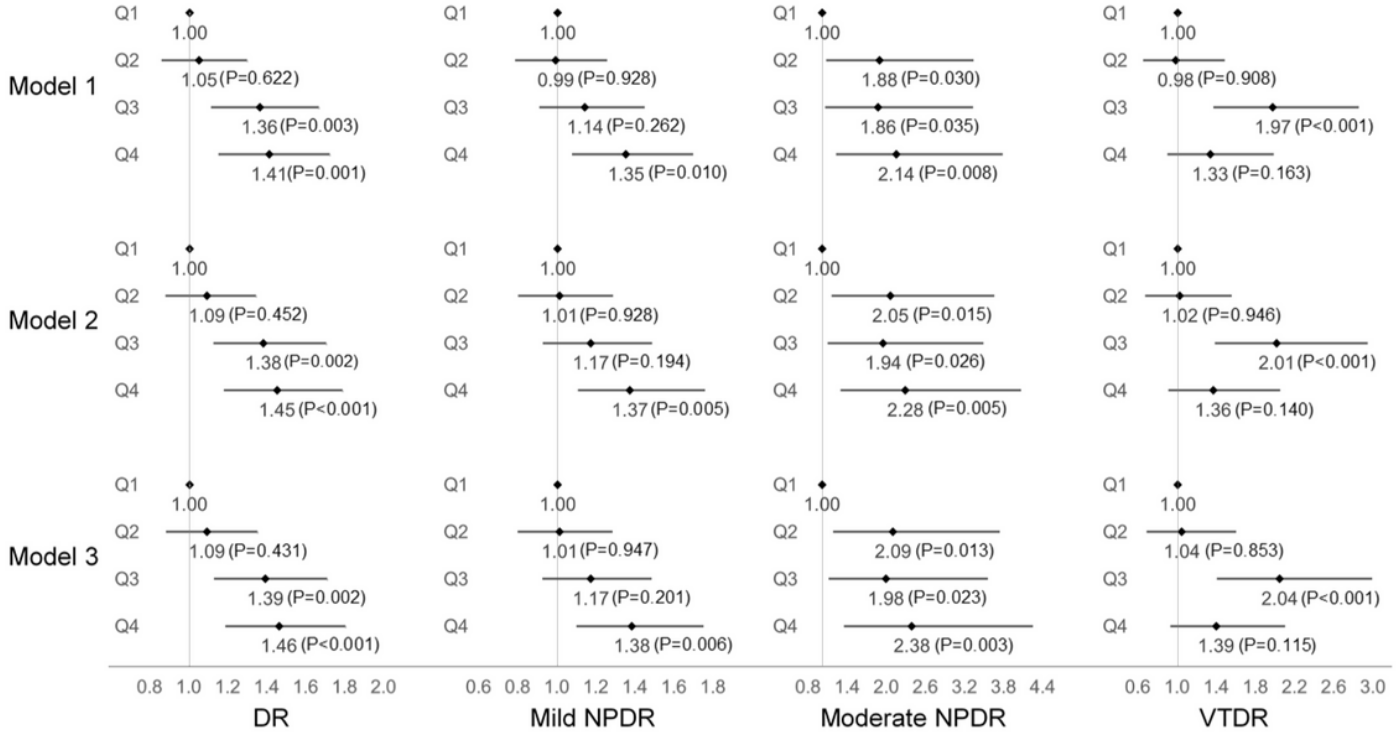

Figure 1

Multivariable-adjusted models between AG quartiles and DR based on logistic regression. Figure 1 legend: AG, anion gap; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy. The results of Figure 1 were based on binary and multivariate logistic regression analysis, and error bars represented 95% confidence interval. AG quartiles were treated as categorized variables and represented by letter Q for each grade. Model 1 adjusted by gender and age; Model 2 adjusted by gender, age, duration of diabetes, antidiabetic agents, SBP, PP, height, smoker, drinker, HbA1c and Mg2+; Model 3 adjusted by gender, age, duration of diabetes, antidiabetic agents, SBP, PP, height, smoker, drinker, HbA1c, Mg2+, HOMA-IR and eGFR. In all three models, P value for trend in presence of DR was < 0.001, and P value for trend in mild NPDR, moderate NPDR and VTDR was < 0.05.

Supplementary Files

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