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Vitamin D affects the neutrophil-to-lymphocyte ratio in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Chronic inflammation is an underlying feature of type 2 diabetes mellitus. Hypovitaminosis D is associated with type 2 diabetes mellitus, but whether it contributes to chronic inflammation is unclear. We examined the effects of vitamin D on various immune markers to evaluate its contribution to systemic inflammation in type 2 diabetes mellitus.

Materials and Methods: We retrospectively analyzed data from type 2 diabetes mellitus patients, people with prediabetes and control patients without diabetes (n = 9,746). Demographic and clinical variables were evaluated using descriptive statistics and generalized linear regression. A stratified analysis based on total serum vitamin D was also carried out.

Results: Neutrophil count was a significant predictor of 1,5-anhydroglucitol and glycated hemoglobin (HbA1c) in patients with prediabetes (1,5-anhydroglucitol: β = –0.719, P < 0.001 and HbA1c: β = –0.006, P = 0.002) and patients with diabetes (1,5-anhydroglucitol: β = 0.207, P = 0.004 and HbA1c: β = –0.067, P = 0.010). Lymphocyte count was a significant predictor of HbA1c in patients without diabetes (β = 0.056, P < 0.001) and patients with prediabetes (β = 0.038, P < 0.001). The neutrophil-to-lymphocyte ratio (NLR) was a significant predictor of HbA1c in patients without diabetes (β = –0.001, P = 0.032). No immune markers differed significantly based on vitamin D level among patients without diabetes (P> 0.05 for all). Among patients with prediabetes, those who were vitamin D-deficient had the highest NLR (P = 0.040). Among patients with diabetes, those who were vitamin D-deficient had the highest neutrophil count (P = 0.001), lowest lymphocyte count (P = 0.016) and highest NLR (P < 0.001).

Conclusions: The NLR is strongly influenced by serum vitamin D level. Given the high prevalence of hypovitaminosis D and elevated NLR among chronic disease patients and the elderly, our results suggest that clinical interpretation of NLR as a predictive marker of type 2 diabetes mellitus-related inflammation should consider vitamin D level, age and pre-existing morbidity.

INTRODUCTION

Insulin resistance and hyperglycemia in type 2 diabetes are associated with the induction of pro-inflammatory responses[1–3]. In β-cells of pancreatic islets, elevated blood glucose increases the levels of reactive oxygen species and induces endoplasmic reticulum stress, which in turn activates inflammasomes and induces interleukin-1beta expression[4,5]. A variety of immune cells are attracted to pancreatic islets by these pro-inflammatory processes and leukocyte infiltration contributes to low-grade inflammation, which correlates with the loss of both β-cell mass and function[6].

Obesity and adiposity are contributing factors to type 2 diabetes risk and progression[7]. In hypertrophic adipose tissue, glucotoxicity and lipotoxicity also cause adipocytes to secrete pro-inflammatory cytokines[8,9]. In type 2 diabetes patients, obesity-induced inflammation induces the expression of major histocompatibility complex II by adipocytes, which results in

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infiltration of adipose tissue by lymphocytes[10,11]. Although the mechanisms linking metabolic dysregulation with immunity induction remain largely unclear, these types of metabolism-related inflammatory processes are associated with the progression of type 2 diabetes and its complications[3].

Although epidemiological studies have shown that vitamin D deficiency is associated with type 2 diabetes[12,13], the findings of clinical studies investigating serum vitamin D levels and the effects of vitamin D supplementation on insulin sensitivity, hyperglycemia and type 2 diabetes risk have been conflicting[14,15]. The regulation of the immune process involved in innate, adaptive and autoimmunity is, however, affected by vitamin D levels[16,17]. Vitamin D produces anti-inflammatory effects on various immune cell functions, and a recent meta-analysis of randomized controlled trails found that vitamin D supplementation significantly improved serum levels of C-reactive protein, tumor necrosis factor-alpha and leptin in type 2 diabetes patients[18]. In addition, a previous study noted that vitamin D deficiency might lead to elevated mean platelet volume and neutrophil-to-lymphocyte ratio (NLR) levels[19].

Despite the lack of compelling evidence of a causal relationship between hypovitaminosis D and insulin resistance, defective insulin secretion or hyperglycemia, it is possible that vitamin D influences the risk of type 2 diabetes and its complications by modulating the contribution of inflammation to type 2 diabetes pathogenesis. To investigate this hypothesis, we examined serum levels of vitamin D in a large cohort of type 2 diabetes patients, prediabetes patients and control individuals, and compared various immune cell markers and leukocyte counts to determine whether serum vitamin D levels influenced these inflammation-related parameters. Our results show that vitamin D influences the relative proportions of lymphocytes and neutrophils in both prediabetes patients and diabetes patients, with a greater influence observed in diabetes patients, while showing no such effects in people without diabetes.

METHODS

Participants

We retrospectively analyzed data for type 2 diabetes patients, prediabetes patients and control patients without diabetes who were treated between May 2012 and December 2018 at Shanghai Xuhui Central Hospital, Shanghai, China. Control patients without diabetes were treated for various other conditions during the same period. Patients with hepatic failure, serum creatinine >120 μmol/L, hypothyroidism, hyperthyroidism, immune disorders, infection or receiving hormone therapy were excluded. According to the 2010 American Diabetes Association guidelines, type 2 diabetes was defined by a glycated hemoglobin (HbA1c) cut-off value of 6.5% and a fasting blood glucose concentration (FBG) >7.0 mmol/L or a post-prandial blood glucose level (PBG) >11.1 mmol/L. Prediabetes was defined as an FBG of 6.1–7.0 mmol/L or a PBG of 7.8–11.1 mmol/L. People without diabetes were defined as having an FBG <6.1 mmol/L or a PBG <7.8 mmol/L. The present study protocols were approved by the institutional review board of Shanghai Xuhui Central Hospital. The requirement of informed consent was waived, because all personal identifiers were removed before data collection. Our research was carried out according to the Declaration of Helsinki with regard to ethical principles for research involving human subjects.

Serum and urine analyses

Biochemical data included the serum levels of HbA1c, FBG, PBG, 1,5-anhydroglucitol (1,5-AG), triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, homocysteine, uric acid, creatinine, ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Data from urinalysis included the excreted albumin-to-creatinine ratio (UACR), 24-h urinary albumin (24-h UA) and estimated glomerular filtration rate (eGFR). Serum vitamin D levels were determined using liquid chromatography–tandem mass spectrometry, and samples were analyzed using an AB Sciex Pte API 4000 system (Framingham, MA, USA) equipped with a Shimadzu liquid chromatograph (Kyoto, Japan). Deuterated 26,26,26,27,27,27-d_6 and 6,19,19-d_3 internal standards (Merck, Darmstadt, Germany) were used. Data were recorded and analyzed using the Analyst 1.5 software (Applied Biosystems, Foster City, CA, USA). Control accuracies for low, medium and high concentrations were 85–115%, with a precision of <15% and intra- and interassay coefficients of variation of <10%. Serum 1,5-AG levels were measured using an enzymatic assay (Glycomark, New York, NY, USA) with sensitivity of 1.5 μg/mL, linearity <50 μg/mL and coefficients of variation of 2.3–4.8%, as described previously[20]. Other serum analytes were quantified using the Advia 2400 Clinical Chemistry System (Siemens Healthcare, Erlangen, Germany). Neutrophil and lymphocyte counts and the NLR were measured using a Sysmex XT-4000i hematology analyzer (Beckman Coulter, Fullerton, CA, USA).

Measurement of immunity-related indexes

Lymphocyte markers were quantified from peripheral venous whole blood samples within 2 h of collection. Staining was carried out using antibodies specific for CD4, CD8, CD3 or CD19 (BD Biosciences, San Jose, CA, USA), and the cells were sorted by flow cytometry in a FACS Aria Flow Cytometer (BD Biosciences) using appropriate isotype controls. The proportions of differentially stained cells were determined using the FlowJo software (Tree Star, Ashland, OR, USA), with the CD3 count representing total T lymphocytes and the CD19 count representing total B lymphocytes, as described previously[21].

Statistical analysis

The statistical analysis was carried out using the SPSS software (IBM, Armonk, NY, USA). Discrete data are presented as the number (n) and percentage. Categorical data were compared using a χ^2 analysis. Normally distributed continuous data were compared using an analysis of variance, and are presented as the mean ± standard deviation. Continuous data lacking a

normal distribution were compared using the Wilcoxon rank-sum test. The analyses of risk factors affecting 1,5-AG and HbA1c were carried out using generalized univariate and multivariate linear regression. The level of statistical significance was set at a two-sided $P < 0.05$.

RESULTS

Patient characteristics

The demographic, biochemical and immune-related variables are shown in Table 1. A total of 9,746 patients were included on the present study. These included 2,979 type 2 diabetes patients, 3,647 prediabetes patients and 3,120 control patients without diabetes. The majority of participants were men ($P = 0.017$). The largest difference between the percentages of men and women was observed in the type 2 diabetes group (54.2% vs 45.8%, respectively), with smaller differences in the prediabetes (50.8% vs 49.2%, respectively) and control patients (52.0% vs 48.0%, respectively), but no clear trend was observed with regard to the level of glycemic dysfunction. Diabetes patients were significantly older (aged 74.78 ± 13.45 years) than the prediabetes (73.70 ± 14.48 years) and control patients (66.76 ± 17.69 years, $P < 0.001$), with the trend reflecting the higher incidence of type 2 diabetes in older patients. Diagnostic categories for the control patients are shown in Table 2. The majority of the control patients received diagnoses related to coronary atherosclerosis ($n = 598$), cerebral infarction ($n = 298$), muscle strain injury ($n = 276$), hypertension ($n = 267$), pulmonary infection ($n = 208$) or angina ($n = 197$). A substantial number of control patients received only symptomatic treatment without diagnosis ($n = 133$), or had no available treatment or diagnostic data ($n = 687$).

Glycemic, renal, lipid and vitamin D profiles

The results of the serum and urine analyses are presented in Table 1. In diabetes patients, HbA1c (7.89% ± 1.74%), FBG (8.70 ± 3.32 mmol/L) and PBG (12.92 ± 4.66 mmol/L) were

Table 1 | Demographic, biochemical and immunological variables

Variable	*n*	Non-diabetes	*n*	Prediabetes	*n*	Diabetes	*P*-value
Age (years)	3,120	66.76 ± 17.69	3,647	73.70 ± 14.48	2,979	74.78 ± 13.45	<0.001
Sex							
Men	3,120	1,622 (52.0%)	3,647	1,851 (50.8%)	2,979	1,616 (54.2%)	0.017
Women		1,498 (48.0%)		1,796 (49.2%)		1,363 (45.8%)	
HbA1c (%)	1,640	5.32 ± 0.29	3,327	6.01 ± 0.31	2,519	7.89 ± 1.74	<0.001
FBG (mmol/L)	2,192	4.91 ± 0.61	1,810	5.50 ± 0.86	1,865	8.70 ± 3.32	<0.001
PBG (mmol/L)	243	6.03 ± 0.95	730	7.50 ± 1.69	1,260	12.92 ± 4.66	<0.001
1,5-AG (μg/mL)	318	19.83 ± 10.74	862	19.02 ± 10.81	1,082	8.76 ± 7.66	<0.001
Triglyceride (mmol/L)	2,423	1.26 ± 0.97	3,391	1.30 ± 0.84	2,644	1.56 ± 1.25	0.155
Cholesterol (mmol/L)	2,423	4.18 ± 1.21	3,391	4.12 ± 1.11	2,644	4.12 ± 1.15	<0.001
HDL (mmol/L)	2,420	1.20 ± 0.37	3,385	1.17 ± 0.35	2,644	1.09 ± 0.33	0.705
LDL (mmol/L)	2,423	2.22 ± 0.85	3,389	2.20 ± 0.81	2,644	2.21 ± 0.80	0.023
Uric acid (mmol/L)	3,083	0.31 ± 0.13	3,611	0.32 ± 0.11	2,945	0.32 ± 0.13	<0.001
Creatinine (μmol/L)	3,083	73.70 ± 73.22	3,611	75.93 ± 57.98	2,945	81.71 ± 74.13	<0.001
UACR (μg/mg)	504	531.32 ± 4,009.40	1,130	234.97 ± 936.41	1,306	369.25 ± 1,028.50	<0.001
24-h UA (g)	79	0.12 ± 0.21	173	0.11 ± 0.29	609	0.07 ± 0.16	0.002
eGFR (mL/min/1.73 m^2)	2,229	84.02 ± 31.96	3,172	78.86 ± 29.36	2,546	76.08 ± 31.01	<0.001
Vitamin D_2 (ng/mL)	627	1.07 ± 2.77	1,469	1.53 ± 3.68	1,459	1.32 ± 3.44	0.389
Vitamin D_3 (ng/mL)	627	12.67 ± 7.02	1,469	12.27 ± 7.20	1,459	12.21 ± 7.14	0.758
Total vitamin D (ng/mL)	627	13.71 ± 7.44	1,469	13.78 ± 7.95	1,459	13.57 ± 7.65	0.011
Homocysteine (μmol/L)	769	16.77 ± 9.67	1,749	16.25 ± 8.44	1,568	15.58 ± 6.93	0.015
Neutrophil (10^6/mL)	3,102	4.47 ± 3.66	3,613	4.51 ± 2.82	2,959	5.02 ± 3.46	0.004
Lymphocyte (10^6/mL)	3,102	1.47 ± 1.87	3,613	1.53 ± 0.72	2,959	1.63 ± 2.77	0.115
NLR	3,102	4.76 ± 21.61	3,613	4.07 ± 10.14	2,959	4.58 ± 6.27	<0.001
CD19 (%)	1,030	8.68 ± 6.51	1,400	10.46 ± 7.04	1,350	11.47 ± 8.24	<0.001
CD3 (%)	1,030	72.01 ± 11.97	1,400	69.70 ± 11.20	1,350	69.66 ± 11.46	0.051
CD4 (%)	1,030	42.68 ± 11.79	1,400	43.05 ± 11.06	1,350	43.77 ± 10.92	0.119
CD8 (%)	1,030	26.94 ± 11.52	1,400	24.90 ± 10.47	1,350	24.07 ± 10.16	0.073
CD4/CD8	1,030	2.07 ± 2.39	1,400	2.15 ± 1.33	1,350	2.22 ± 1.22	<0.001

Data presented as the number (percentage) or mean ± standard deviation. Vitamin D detected as 25-hydroxy vitamin D. 1,5-AG, 1,5-anhydroglucitol; 24-h UA, 24-h urinary albumin; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NLR, neutrophil-to-lymphocyte ratio; PBG, post-prandial blood glucose; UACR, urinary albumin-to-creatinine ratio.

Table 2 | Numbers and percentages of non-diabetic control patients in the diagnostic categories

Diagnosis	*n*	Percentage (%)
Heart failure	33	1.06
Acute coronary syndrome	19	0.61
Angina	197	6.31
Auricular fibrillation	17	0.54
Arrhythmia	22	0.71
Coronary atherosclerosis	598	19.17
Rheumatic heart disease	11	0.35
Hypertension	267	8.56
Cerebral ischemia	111	3.56
Cerebral infarction	298	9.55
Cerebral hemorrhage	19	0.61
Dizziness and vertigo	13	0.42
Acute exacerbation of chronic bronchitis	20	0.64
Chronic obstructive pulmonary disease	110	3.53
Pulmonary infection	208	6.67
Pneumonia	34	1.09
Thyroid cancer	11	0.35
Nodular goiter	13	0.42
Chronic kidney disease, stage 5	23	0.74
Muscle strain injury	276	8.85
Symptomatic treatment	133	4.26
No diagnosis or treatment	687	22.02

highest ($P < 0.001$ for all), whereas levels were intermediate in prediabetes patients (6.01 ± 0.31%, 5.50 ± 0.86 mmol/L, 7.50 ± 1.69 mmol/L, respectively) and lowest in patients without diabetes (5.32 ± 0.29%, 4.91 ± 0.61 mmol/L, 6.03 ± 0.95 mmol/L, respectively). The 1,5-AG level was highest in controls (19.83 ± 10.74 μg/mL), lower in prediabetes patients (19.02 ± 10.81 μg/mL) and lowest in diabetes patients (8.76 ± 7.66 μg/mL, $P < 0.001$), which suggested poor dietary habits and/or insulin management among diabetes during the 2 weeks before blood sample collection.

The uric acid level was significantly lower in patients without diabetes (0.31 ± 0.13 mmol/L, $P < 0.0001$) than in diabetes patients (0.32 ± 0.13 mmol/L) or prediabetes patients (0.32 ± 0.11 mmol/L), whereas the serum creatinine level was significantly higher in diabetes patients (81.71 ± 74.13 μmol/L, $P < 0.001$) than in patients without diabetes (73.70 ± 73.22 μmol/L) and prediabetes patients (75.93 ± 57.98 μmol/L). Both the UACR (531.32 ± 4009.40 μg/mg, $P < 0.001$) and the 24-h UA (0.12 ± 0.21 g, $P = 0.002$) were significantly higher in patients without diabetes, compared with those in prediabetes patients (234.97 ± 936.41 μg/mg and 0.11 ± 0.29 g, respectively) and diabetes patients (369.25 ± 1028.50 μg/mg and 0.07 ± 0.16 g, respectively). In diabetes patients, eGFR was 76.08 ± 31.01 mL/min/1.73 m^2, which was significantly lower ($P < 0.0001$) than that of prediabetes patients (78.86 ± 29.36 mL/min/1.73 m^2) and patients without diabetes (84.02 ± 31.96 mL/min/1.73 m^2). The high incidence of renal disease in type 2 diabetes patients explains this trend toward lower eGFR with greater glycemic dysfunction, and eGFR <90 mL/min/1.73 m^2 for all three study groups reflects the relative old age of the cohort.

Levels of triglycerides ($P = 0.155$) and HDL ($P = 0.705$) did not differ significantly between the study groups. In patients without diabetes, levels of total cholesterol (4.18 ± 1.21 mmol/L,$P < 0.001$) and low-density lipoprotein (2.22 ± 0.85, $P = 0.023$) were significantly higher than those with prediabetes (4.12 ± 1.11 and 2.20 ± 0.81 mmol/L, respectively) and diabetes (4.12 ± 1.15 and 2.21 ± 0.80 mmol/L, respectively). Homocysteine (16.77 ± 9.67 μmol/L, $P = 0.015$) was also significantly higher in patients without diabetes, compared with that in prediabetes (16.25 ± 8.44 μmol/L) and diabetes patients (15.58 ± 6.93 μmol/L). The differences in vitamin D levels were modest, but the total vitamin D level in diabetes patients (13.57 ± 7.65 ng/mL, $P = 0.011$) was significantly lower than that in prediabetes patients (13.78 ± 7.95 ng/mL) and patients without diabetes (13.71 ± 7.44 ng/mL). There were no significant differences in the levels of vitamin D_2 ($P = 0.389$) or vitamin D_3 ($P = 0.758$).

Immune cell indices

As shown in Table 1, the number of neutrophils significantly increased with advancing glycemic dysfunction, with 4.47 ± 3.66 × 10^6 cells/mL in patients without diabetes, 4.51 ± 2.82 × 10^6 cells/mL in prediabetes patients and 5.02 ± 3.46 × 10^6 cells/mL in diabetes patients ($P = 0.004$). The number of lymphocytes was also highest in diabetes patients and lowest in patients without diabetes, but the differences in lymphocyte numbers between patients without diabetes, prediabetes patients and diabetes patients were not statistically significant ($P = 0.115$). The NLR in patients without diabetes was significantly higher (4.76 ± 21.61, $P < 0.001$) than that in prediabetes patients (4.07 ± 10.14) and diabetes patients (4.58 ± 6.27).

1,5-AG risk factors

The demographic and clinical variables were evaluated as predictors of 1,5-AG level using generalized linear regression models. The results of the univariate analysis are shown in Table 3. In prediabetes patients, the sex (β = 2.772, $P < 0.001$), PBG (β = –0.956, $P = 0.004$), uric acid (β = 0.016, $P < 0.001$), creatinine (β = –0.016, $P = 0.013$), 24-h UA (β = –0.961, $P = 0.012$), UACR (β = –0.002, $P < 0.001$), eGFR (β = 0.039, $P = 0.006$), neutrophil count (β = –0.631, $P < 0.001$), NLR (β = –0.226, $P = 0.013$), CD19 (β = 0.245, $P = 0.002$) and CD4 (β = 0.0972, $P = 0.036$) were significant predictors of 1,5-AG. The significant predictors of 1,5-AG in diabetes patients included age (β = 0.124, $P < 0.001$), HbA1c (β = –2.413, $P < 0.001$), FBG (β = –0.311, $P = 0.005$), PBG (β = –0.438, $P < 0.001$), triglyceride (β = –0.509, $P = 0.008$), uric acid (β = 0.007, $P < 0.001$), UACR (β = –0.001, $P = 0.004$), eGFR (β = –0.028, $P = 0.001$), neutrophil count (β = 0.361, $P < 0.001$), lymphocyte count (β = –0.715, $P = 0.040$) and NLR (β = 0.250, $P < 0.001$).

Table 3 | Demographic, biochemical, and immunological variables as predictors of 1,5-anhydroglucitol.

Variable	Non-diabetes			Prediabetes			Diabetes		
	n	β	*P*-value	*n*	β	*P*-value	*n*	β	*P*-value
Sex (male vs female)	318	6.633	<0.001	862	2.772	<0.001	1,082	0.230	0.621
Age	318	−0.053	0.218	862	−0.042	0.155	1,082	0.124	<0.001
HbA1c	300	5.287	0.014	857	−2.093	0.073	1,065	−2.413	<0.001
FBG	156	0.234	0.879	423	−1.149	0.062	575	−0.311	0.005
PBG	133	−0.771	0.458	419	−0.956	0.004	787	−0.438	<0.001
Triglyceride	315	0.072	0.950	845	−1.024	0.052	1,055	−0.509	0.008
Cholesterol	315	−0.395	0.556	845	0.027	0.941	1,055	−0.411	0.067
HDL	315	3.796	0.030	845	1.099	0.294	1,055	1.233	0.102
LDL	315	−1.158	0.217	845	0.029	0.952	1,055	−0.524	0.108
Homocysteine	248	−0.134	0.168	689	−0.050	0.237	874	0.065	0.107
Uric acid	317	0.008	0.111	854	0.016	<0.001	1,070	0.007	<0.001
Creatinine	317	−0.031	0.001	854	−0.016	0.013	1,070	−0.007	0.065
24-h UA	45	−2.041	0.010	124	−0.961	0.012	457	−0.130	0.532
UACR	236	−0.001	<0.001	641	−0.002	<0.001	890	−0.001	0.004
eGFR	315	0.087	<0.001	846	0.039	0.006	1,052	−0.028	0.001
Vitamin D_2	287	−0.150	0.566	778	−0.078	0.378	962	−0.043	0.477
Vitamin D_3	287	0.440	<0.001	778	0.069	0.194	962	0.028	0.407
Total vitamin D	287	0.351	<0.001	778	0.035	0.450	962	0.015	0.633
Neutrophil	316	−0.547	0.005	856	−0.631	<0.001	1,073	0.361	<0.001
Lymphocyte	316	0.339	0.720	856	0.170	0.750	1,073	−0.715	0.040
NLR	316	−0.654	0.003	856	−0.226	0.013	1,073	0.250	<0.001
CD19	156	0.027	0.872	497	0.245	0.002	703	−0.076	0.089
CD3	156	0.138	0.070	497	0.019	0.679	703	−0.002	0.955
CD4	156	0.062	0.493	497	0.097	0.036	703	−0.002	0.942
CD8	156	0.100	0.222	497	−0.087	0.052	703	0.027	0.373
CD4/CD8	156	−0.444	0.507	497	0.202	0.555	703	0.106	0.658

1,5-AG, 1,5-anhydroglucitol; 24-h UA, 24-h urinary albumin; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NLR, neutrophil-to-lymphocyte ratio; PBG, post-prandial blood glucose; UACR, urinary albumin-to-creatinine ratio. Total n = 3,120.

Significant predictors of 1,5-AG among prediabetes and diabetes patients in the univariate analysis were evaluated in multivariate models. Data for 236 patients without diabetes, 635 prediabetes patients and 1,031 diabetes patients met the dataset requirements for the multivariate models. As shown in Table 4, the multivariate analysis showed that significant predictors of 1,5-AG in prediabetes patients included the sex (β = 2.985, P = 0.001), uric acid (β = 0.021, P < 0.001), creatinine (β = −0.021, P = 0.010), UACR (β = −0.001, P = 0.002) and neutrophil count (β = −0.719, P < 0.001). In diabetes, significant predictors of 1,5-AG included age (β = 0.065, P = 0.001), HbA1c (β = −2.275, P < 0.001), uric acid (β = 0.008, P < 0.001), eGFR (β = 0.025, P = 0.005) and neutrophil count (β = 0.207, P = 0.004).

HbA1c risk factors

The demographic and clinical variables were also evaluated as potential predictors of HbA1c using linear regression models. In the univariate analysis of HbA1c, data for men and women were analyzed separately. The results of the univariate analysis are shown in Table 5. In prediabetes patients, female sex (β = 0.001, P = 0.003), age (β = 0.001, P = 0.002), FBG (β = 0.017, P = 0.020), triglyceride (β = 0.033, P < 0.001), HDL (β = −0.049, P = 0.001), creatinine (β = −0.008, P < 0.001), neutrophil count (β = 0.047, P < 0.001), lymphocyte count (β = −0.002, P = 0.003), NLR (β = −0.001, P = 0.027), CD3 (β = −0.002, P = 0.007) and CD8 (β = −0.003, P = 0.002) were significant predictors of HbA1c. Significant predictors of HbA1c in diabetes patients included both sexes (men: β = 0.150, P = 0.031; women: β = −0.027, P < 0.001), age (β = −0.098, P = 0.025), 1,5-AG (β = −2.413, P < 0.001), FBG (β = 0.121, P < 0.001), PBG (β = 0.189, P < 0.001), triglyceride (β = −0.101, P < 0.001), HDL (β = 0.230, P < 0.001), uric acid (β = −0.001, P = 0.001), creatinine (β = −0.001, P = 0.010), 24-h UA (β = 0.289, P < 0.001), eGFR (β = 0.011, P < 0.001), vitamin D_2 (β = −0.027, P < 0.001), vitamin D_3 (β = −0.035, P = 0.015), neutrophil count (β = −0.051, P < 0.001), lymphocyte count (β = 0.268, P < 0.001), NLR (β = −0.046, P < 0.001), CD3 (β = 0.027, P < 0.001), CD4 (β = 0.015, P = 0.002) and CD4/CD8 (β = 0.020, P < 0.001).

Table 4 | Predictors of 1,5-anhydroglucitol according to multivariate models

Predictor	Non-diabetes n = 236		Prediabetes n = 635		Diabetes n = 1,031	
	β	*P*-value	β	*P*-value	β	*P*-value
Sex (male vs female)	6.165	<0.001	2.985	0.001	—	—
Age	—	—	—	—	0.065	0.001
HbA1c	—	—	—	—	−2.275	<0.001
Uric acid	—	—	0.021	<0.001	0.008	<0.001
Creatinine	—	—	−0.021	0.010	—	—
UACR	−0.001	<0.001	−0.001	0.002	—	—
eGFR	—	—	—	—	0.025	0.005
Neutrophil	—	—	−0.719	<0.001	0.207	0.004

1,5-AG, 1,5-Anhydroglucitol; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; UACR, urinary albumin-to-creatinine ratio.

Table 5 | Demographic, biochemical and immunological variables as predictors of glycated hemoglobin

Variable	Non-diabetes			Prediabetes			Diabetes		
	n	β	*P*-value	*n*	β	*P*-value	*n*	β	*P*-value
Male	866	−0.006	0.687	1,673	0.005	0.614	1,347	0.150	0.031
Female	774	0.001	0.262	1,654	0.001	0.003	1,172	−0.027	<0.001
Age	1,640	−0.001	0.842	3,327	0.001	0.002	2,519	−0.098	0.025
1,5-AG	300	0.046	0.025	857	−0.010	0.405	1,065	−2.413	<0.001
FBG	734	0.001	0.967	1507	0.017	0.020	1,416	0.121	<0.001
PBG	213	0.004	0.014	698	−0.002	0.073	1,231	0.189	<0.001
Triglyceride	1,576	0.001	0.885	3220	0.033	<0.001	2,422	−0.101	<0.001
Cholesterol	1,576	−0.395	0.556	3220	0.027	0.941	2,422	−0.411	0.067
HDL	1,573	0.102	<0.001	3215	−0.049	0.001	2,422	0.230	<0.001
LDL	1,576	0.022	0.009	3219	−0.002	0.745	2,422	−0.005	0.963
Homocysteine	674	0.001	0.740	1722	−0.001	0.256	1,536	−0.075	0.159
Uric acid	1,629	−0.001	0.188	3301	0.001	0.625	2,498	−0.001	0.001
Creatinine	1,629	−0.008	<0.001	3301	−0.008	<0.001	2,498	−0.001	0.010
24-h UA	61	−0.026	0.246	167	0.001	0.985	598	0.289	<0.001
UACR	429	−0.001	0.034	1110	−0.001	0.068	1,256	−0.002	0.798
eGFR	1,434	5.287	0.014	3028	−2.093	0.073	2,349	0.011	<0.001
Vitamin D_2	555	0.004	0.322	1452	0.003	0.161	1,438	−0.027	<0.001
Vitamin D_3	555	0.003	0.042	1452	−0.001	0.603	1,438	−0.035	0.015
Total vitamin D	555	0.003	0.035	1452	0.001	0.896	1,438	0.007	0.286
Neutrophil	1,637	0.062	<0.001	3303	0.047	<0.001	2,502	−0.051	<0.001
Lymphocyte	1,637	−0.001	<0.001	3303	−0.002	0.003	2,502	0.268	<0.001
NLR	1,637	0.001	0.055	3303	−0.001	0.027	2,502	−0.046	<0.001
CD19	600	0.008	0.001	1302	0.001	0.280	1,226	0.001	0.464
CD3	600	−0.002	0.165	1302	−0.002	0.007	1,226	0.027	<0.001
CD4	600	0.002	0.173	1302	0.001	0.476	1,226	0.015	0.002
CD8	600	−0.003	0.005	1302	−0.003	0.002	1,226	0.082	0.059
CD4/CD8	600	0.029	0.012	1302	0.016	0.027	1,226	0.020	<0.001

1,5-AG, 1,5-Anhydroglucitol; 24-h UA, 24-h urinary albumin; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NLR, neutrophil-to-lymphocyte ratio; PBG, post-prandial blood glucose; UACR, urinary albumin-to-creatinine ratio.

Significant predictors of HbA1c among prediabetes and diabetes patients in the univariate analysis were evaluated in multivariate models. Data for 1,637 patients without diabetes, 3,206 prediabetes patients and 625 diabetes patients were sufficient for use in the multivariate models. As shown in Table 6, the multivariate analysis showed that significant predictors of

Table 6 | Predictors of glycated hemoglobin according to multivariate models

Predictor	Non-diabetes *n* = 1,637		Prediabetes n = 3,206		Diabetes *n* = 625	
	β	*P*-value	β	*P*-value	β	*P*-value
FBG	—	—	—	—	0.142	<0.001
PBG	—	—	—	—	0.157	<0.001
Triglyceride	—	—	0.026	<0.001	—	—
Uric acid	—	—	0.001	0.042	—	—
eGFR	—	—	—	—	0.009	<0.001
Neutrophil	–0.005	0.016	–0.006	0.002	–0.067	0.010
Lymphocyte	0.056	<0.001	0.038	<0.001	—	—
NLR	–0.001	0.032	—	—	—	—

eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; NLR, neutrophil-to-lymphocyte ratio; PBG, post-prandial blood glucose.

HbA1c in prediabetes patients included triglyceride (β = 0.026, $P < 0.001$), uric acid (β = 0.001, $P = 0.042$), neutrophil count (β = –0.006, $P = 0.002$) and lymphocyte count (β = 0.038, $P < 0.001$). Among diabetes patients, significant predictors of HbA1c in the multivariate models included FBG (β = 0.142, $P < 0.001$), PBG (β = 0.157, $P < 0.001$), eGFR (β = 0.009, $P < 0.001$) and neutrophil count (β = –0.067, $P = 0.010$).

Stratified analysis

Previous studies reported that vitamin D supplementation reduces type 2 diabetes risk[22], and hypovitaminosis D is associated with the incidence of type 2 diabetes complications[23–26]. Therefore, we carried out a stratified analysis of the demographic and clinical variables based on total vitamin D levels, for which data were sufficient to include 627 controls without diabetes, 1,469 prediabetes patients and 1,459 diabetes patients. As shown in Table 7, vitamin D levels differed significantly based on sex in prediabetes ($P = 0.005$) and diabetes patients ($P = 0.002$), with more men in the low vitamin D category than women among both prediabetes (53.4%) and diabetes patients (53.3%). Vitamin D differed significantly according to age in all three study groups (patients without diabetes: $P = 0.049$; prediabetes patients: $P = 0.001$; and diabetes patients: $P = 0.025$), but the only clear trend in age was observed among patients without diabetes, with the patients in the low vitamin D category (79.47 ± 15.51 years) being older than those with moderate vitamin D levels (75.64 ± 14.73 years) or high vitamin D levels (75.38 ± 15.54 years).

Although FBG differed significantly based on vitamin D level among diabetes patients ($P = 0.001$), no clear trend in FBG was observed. The 1,5-AG differed significantly based on vitamin D among controls ($P = 0.033$). The eGFR differed significantly based on vitamin D level among both patients without diabetes ($P = 0.001$) and prediabetes patients ($P = 0.005$), but no clear trends were observed. Differences in serum lipids according to vitamin D level varied between patients without diabetes, prediabetes patients and diabetes patients, with significant differences in HDL among patients without diabetes ($P = 0.011$) and prediabetes patients ($P < 0.001$), significant differences in total cholesterol among prediabetes ($P = 0.001$) and diabetes patients ($P < 0.001$), and significant differences in low-density lipoprotein ($P < 0.001$) and triglycerides ($P = 0.028$) among diabetes patients only. Homocysteine differed significantly based on vitamin D in diabetes patients only ($P = 0.019$), with progressively higher homocysteine levels in type 2 diabetes patients with lower vitamin D.

No significant differences in leukocyte counts or immune cell markers were observed among patients without diabetes ($P > 0.05$ for all). Neutrophil count ($P = 0.001$), lymphocyte count ($P = 0.016$) and NLR ($P < 0.001$) differed significantly based on vitamin D level among diabetes patients, whereas only NLR differed significantly based on vitamin D level in prediabetes patients ($P = 0.040$). Significant differences in lymphocyte markers were observed among prediabetes patients only. In prediabetes patients, the CD19 and CD8 counts varied significantly according to vitamin D level ($P = 0.050$ and $P = 0.040$, respectively), but a clear trend was observed for CD8 only, with progressively higher CD8 counts in prediabetes patients with lower vitamin D. These data suggest that vitamin D influences the NLR in diabetes patients by altering neutrophil and lymphocyte numbers, but might not alter the relative proportions of lymphocyte subpopulations. However, the lowest number of neutrophils and highest number of lymphocytes were observed in type 2 diabetes patients with only moderate vitamin D levels. These results suggest that the effects of vitamin D on these parameters are not linear in nature and/or are influenced by other factors.

DISCUSSION

Vitamin D deficiency leads to impaired insulin secretion and glucose intolerance[27], which contributes to type 2 diabetes. Chronic low-grade inflammation is a key underlying feature of type 2 diabetes and its complications, and is thought to be caused by the effects of hyperglycemia on immune cells and lymphoid tissues[28]. In turn, the resulting localized and systemic

Table 7 | Stratified analysis based on total vitamin D level in patients without diabetes, prediabetes patients and patients with diabetes

Variable	Low vitamin D (<20.0 ng/mL)	Moderate vitamin D (20 – 30 ng/mL)	High vitamin D (>30 ng/mL)	*P*-value
Patients without diabetes (n = 627)				
Sex				
Men	262 (51.5%)	52 (53.6%)	7 (33.3%)	0.232
Women	247 (48.5%)	45 (46.4%)	14 (66.7%)	
Age (years)	79.47 ± 15.51	75.64 ± 14.73	75.38 ± 15.54	0.049
HbA1c (%)	5.33 ± 0.29	5.36 ± 0.20	5.38 ± 0.30	0.571
FBG (mmol/L)	4.79 ± 0.55	4.82 ± 0.57	4.79 ± 0.72	0.955
PBG (mmol/L)	6.06 ± 0.93	6.10 ± 0.88	5.13 ± 0.48	0.219
1,5-AG (μg/mL)	19.09 ± 10.45	23.78 ± 10.62	23.10 ± 11.56	0.033
Triglyceride (mmol/L)	1.09 ± 0.63	1.13 ± 0.58	1.33 ± 1.17	0.235
Cholesterol (mmol/L)	3.91 ± 1.02	3.96 ± 0.86	3.85 ± 0.80	0.871
HDL (mmol/L)	1.20 ± 0.36	1.31 ± 0.34	1.30 ± 0.34	0.011
LDL (mmol/L)	2.02 ± 0.72	1.97 ± 0.62	1.86 ± 0.59	0.481
Homocysteine (μmol/L)	17.14 ± 11.25	15.03 ± 5.53	17.07 ± 8.74	0.297
Uric acid (mmol/L)	0.31 ± 0.12	0.33 ± 0.11	0.29 ± 0.07	0.356
Creatinine (μmol/L)	80.26 ± 72.15	82.66 ± 84.45	77.05 ± 58.76	0.937
UACR (μg/mg)	48.59 ± 223.46	105.01 ± 334.31	182.01 ± 414.89	0.439
24-h UA (g/day)	0.12 ± 0.22	0.13 ± 0.30	0.13 ± 0.30	0.195
eGFR (mL/min/1.73 m^2)	69.66 ± 28.02	81.82 ± 31.94	77.79 ± 25.77	0.001
Neutrophil (10^6/mL)	4.28 ± 3.29	4.12 ± 2.30	4.08 ± 1.95	0.867
Lymphocyte (10^6/mL)	1.51 ± 1.85	1.53 ± 0.61	1.35 ± 0.62	0.911
NLR	3.67 ± 4.02	3.38 ± 3.45	4.25 ± 5.13	0.625
CD19 (%)	8.88 ± 5.17	9.15 ± 5.02	11.25 ± 3.30	0.634
CD3 (%)	69.11 ± 10.79	70.78 ± 9.89	66.25 ± 6.95	0.550
CD4 (%)	43.44 ± 9.27	44.98 ± 11.55	41.75 ± 9.11	0.593
CD8 (%)	23.94 ± 9.71	23.93 ± 9.83	21.50 ± 0.58	0.882
CD4/CD8	2.23 ± 1.28	2.29 ± 1.20	1.95 ± 0.41	0.866
Prediabetes patients (n = 1,469)				
Sex				
Men	629 (53.4%)	104 (44.3%)	22 (38.6%)	0.005
Women	548 (46.6%)	131 (55.7%)	35 (61.4%)	
Age (years)	79.68 ± 13.78	76.20 ± 13.37	81.77 ± 12.68	0.001
HbA1c (%)	6.02 ± 0.29	6.00 ± 0.31	6.06 ± 0.27	0.308
FBG (mmol/L)	5.35 ± 0.79	5.38 ± 0.75	5.50 ± 0.70	0.641
PBG (mmol/L)	7.46 ± 1.69	7.36 ± 1.58	7.01 ± 1.56	0.438
1,5-AG (μg/mL)	19.01 ± 11.11	20.24 ± 10.49	17.08 ± 6.93	0.288
Triglyceride (mmol/L)	1.27 ± 0.74	1.36 ± 0.79	1.26 ± 0.62	0.259
Cholesterol (mmol/L)	3.94 ± 1.03	4.19 ± 1.04	4.20 ± 0.90	0.001
HDL (mmol/L)	1.14 ± 0.32	1.23 ± 0.33	1.32 ± 0.32	<0.001
LDL (mmol/L)	2.09 ± 0.75	2.21 ± 0.75	2.14 ± 0.67	0.074
Homocysteine (μmol/L)	16.72 ± 9.24	15.48 ± 6.66	15.83 ± 9.72	0.190
Uric acid (mmol/L)	0.33 ± 0.12	0.34 ± 0.10	0.32 ± 0.11	0.530
Creatinine (μmol/L)	79.20 ± 50.79	74.91 ± 42.42	93.81 ± 110.63	0.055
UACR (μg/mg)	228.25 ± 971.89	138.79 ± 422.52	264.62 ± 624.43	0.526
24-h UA (g/day)	0.11 ± 0.29	0.04 ± 0.07	0.18 ± 0.25	0.309
eGFR (mL/min/1.73 m^2)	71.33 ± 27.96	77.76 ± 26.39	69.61 ± 27.31	0.005
Neutrophil (10^6/mL)	4.22 ± 2.15	4.09 ± 1.95	4.40 ± 2.75	0.545
Lymphocyte (10^6/mL)	1.55 ± 0.72	1.66 ± 0.66	1.61 ± 0.49	0.073
NLR	3.51 ± 3.61	2.93 ± 2.36	3.03 ± 2.07	0.040
CD19 (%)	10.03 ± 6.34	11.33 ± 6.16	7.84 ± 4.18	0.050
CD3 (%)	69.73 ± 10.23	69.77 ± 9.93	67.68 ± 9.75	0.687
CD4 (%)	43.41 ± 10.43	45.61 ± 11.65	43.79 ± 11.57	0.185
CD8 (%)	25.02 ± 10.51	23.58 ± 7.28	22.14 ± 8.75	0.040
CD4/CD8	2.17 ± 1.32	2.51 ± 1.40	2.14 ± 1.28	0.076

Table 7 (*Continued*)

Variable	Low vitamin D (<20.0 ng/mL)	Moderate vitamin D (20 – 30 ng/mL)	High vitamin D (>30 ng/mL)	*P*-value
Patients with diabetes (n = 1,459)				
Sex				
Men	628 (53.3%)	102 (44.9%)	18 (33.3%)	0.002
Women	550 (46.7%)	125 (55.1%)	36 (66.7%)	
Age (years)	77.89 ± 12.75	75.54 ± 12.43	79.17 ± 11.02	0.025
HbA1c (%)	8.11 ± 1.91	8.12 ± 1.86	7.56 ± 1.11	0.107
FBG (mmol/L)	8.51 ± 3.23	7.37 ± 2.55	8.66 ± 2.51	0.001
PBG (mmol/L)	13.11 ± 4.81	13.83 ± 4.74	12.08 ± 3.12	0.074
1,5-AG (μg/mL)	8.63 ± 7.67	8.62 ± 6.91	10.41 ± 9.31	0.304
Triglyceride (mmol/L)	1.58 ± 1.29	1.71 ± 1.52	2.04 ± 1.37	0.028
Cholesterol (mmol/L)	4.05 ± 1.09	4.32 ± 1.05	4.61 ± 1.13	<0.001
HDL (mmol/L)	1.09 ± 0.33	1.09 ± 0.30	1.06 ± 0.25	0.703
LDL (mmol/L)	2.15 ± 0.74	2.33 ± 0.69	2.55 ± 0.77	<0.001
Homocysteine (μmol/L)	15.59 ± 6.77	14.45 ± 5.93	13.59 ± 6.34	0.019
Uric acid (mmol/L)	0.32 ± 0.13	0.32 ± 0.10	0.33 ± 0.12	0.750
Creatinine (μmol/L)	81.71 ± 63.98	76.47 ± 48.53	74.96 ± 55.44	0.397
UACR (μg/mg)	418.16 ± 1145.26	314.95 ± 907.51	169.19 ± 301.35	0.198
24-h UA (g/day)	0.73 ± 1.58	0.50 ± 1.20	0.27 ± 0.30	0.192
eGFR (mL/min/1.73 m^2)	73.59 ± 31.17	78.03 ± 30.19	75.36 ± 26.85	0.145
Neutrophil (10^6/mL)	4.82 ± 3.10	4.05 ± 2.08	4.13 ± 1.83	0.001
Lymphocyte (10^6/mL)	1.55 ± 0.99	1.75 ± 0.75	1.62 ± 0.58	0.016
NLR	4.33 ± 6.01	2.80 ± 2.30	3.02 ± 2.21	<0.001
CD19 (%)	11.59 ± 6.97	12.64 ± 6.33	10.81 ± 6.55	0.237
CD3 (%)	69.99 ± 10.41	71.03 ± 8.49	71.73 ± 8.51	0.422
CD4 (%)	44.4 ± 10.34	45.46 ± 9.00	47.31 ± 9.35	0.224
CD8 (%)	23.85 ± 9.70	23.63 ± 8.98	22.31 ± 8.92	0.712
CD4/CD8	2.24 ± 1.19	2.28 ± 1.13	2.50 ± 1.23	0.509

Data presented as number (percentage) or mean ± standard deviation. 1,5-AG, 1,5-Anhydroglucitol; 24-h UA, 24-h urinary albumin; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NLR, neutrophil-to-lymphocyte ratio; PBG, post-prandial blood glucose; UACR, urinary albumin-to-creatinine ratio.

inflammation further contributes to impaired glycemic control and reduced insulin sensitivity in both normal weight and obese people[11]. A number of different drugs and antibodies that block the action of pro-inflammatory targets, such as interleukin-1, tumor necrosis factor and monocyte chemoattractant protein 1, have been shown to improve glycemic control[29]. Vitamin D has also been reported to have beneficial effects on glycemic outcomes and type 2 diabetes-associated inflammation, which suggests a nutritional component in type 2 diabetes pathogenesis, but similar studies of the effects of vitamin D have yielded conflicting results[30,31].

We retrospectively analyzed various demographic and clinical variables, including vitamin D levels, in a large Chinese cohort (n = 9,746) of type 2 diabetes patients, prediabetes patients and control patients without diabetes (Table 1). We examined whether these variables were risk factors for elevated HbA1c and/or reduced 1,5-AG level, both of which are major indicators of glycemic dysfunction in the progression of type 2 diabetes (Tables 4,6). To evaluate the effects of vitamin D on inflammation, we also carried out a stratified analysis based on serum vitamin D level to determine whether the study variables, which included immune cell counts and lymphocyte markers, varied significantly between diabetes patients, prediabetes patients , and control patients without diabetes.

The present results showed that total vitamin D was lowest in diabetes patients (P = 0.011), but there were no significant differences between the three study groups in the levels of vitamin D_2 (P = 0.389) or vitamin D_3 (P = 0.758). These results are consistent with the high prevalence of hypovitaminosis D among patients with type 2 diabetes[32,33]. However, vitamin D deficiency is also highly prevalent among patients with other chronic diseases, including pulmonary and cardiovascular diseases[34]. A substantial number of the control patients without diabetes in the present study were diagnosed with pulmonary or cardiovascular morbidities (Table 2), and were therefore more likely to be vitamin D-deficient than healthy people without diabetes. A higher rate of hypovitaminosis D among the controls without diabetes might thus have confounded our comparison of vitamin D_2 and D_3 levels between the study groups.

We observed a clear trend toward greater numbers of peripheral blood leukocytes in patients with an increasing level of glycemic dysfunction, with the highest neutrophil and lymphocyte counts occurring in diabetes patients, and the lowest counts of each occurring among the control patients without diabetes ($P = 0.004$ and $P = 0.115$, respectively). By contrast, the NLR was highest in patients without diabetes ($P < 0.001$). This result is inconsistent with previous reports that elevated NLR is associated with insulin resistance and glucose intolerance[35–37], and is a reliable indicator of neurological, vascular and renal complications in type 2 diabetes patients[35,38–40], whereas the predictive value of neutrophil and lymphocyte counts alone as risk factors for hyperglycemia has not been shown. However, similar to hypovitaminosis D, elevated NLR is also highly prevalent among patients with various chronic diseases other than type 2 diabetes, such as hypertension[41], chronic kidney disease[42], severe chronic obstructive pulmonary disease[43], and cardiovascular diseases, including cardiac arrhythmias and acute coronary syndrome[44,45]. A substantial portion of our controls without diabetes were diagnosed with hypertension ($n = 267$), chronic kidney disease ($n = 23$), chronic obstructive pulmonary disease ($n = 110$) or cardiovascular diseases (combined total, $n > 1,000$), which might have influenced the present results.

We then used linear regression models to further investigate the relationship between vitamin D levels, immune markers and glycemic indicators. In our multivariate analyses of risk factors for reduced 1,5-AG and elevated HbA1c, we found that neutrophil count was a significant predictor of 1,5-AG and HbA1c in both prediabetes (1,5-AG: $\beta = -0.719$, $P < 0.001$ and HbA1c: $\beta = -0.006$, $P = 0.002$) and diabetes patients (1,5-AG: $\beta = 0.207$, $P = 0.004$ and HbA1c: $\beta = -0.067$, $P = 0.010$). The lymphocyte count was a significant predictor of HbA1c in patients without diabetes ($\beta = 0.056$, $P < 0.001$) and prediabetes patients ($\beta = 0.038$, $P < 0.001$) only, and NLR was a significant predictor of HbA1c in patients without diabetes only ($\beta = -0.001$, $P = 0.032$). Therefore, we were unable to detect a strong relationship between NLR and glycemic dysfunction in diabetes or prediabetes patients . A previous large-scale prospective study in China reported that both elevated neutrophil count and elevated lymphocyte count were independently associated with type 2 diabetes incidence[46], and neutrophil count has also been proposed as a marker of type 1 diabetes[47]. Given the relatively high prevalence of elevated NLR among patients diagnosed with other chronic diseases, many of which are often manifested as complications in diabetics, it is possible that neutrophil count might be a more reliable indicator of type 2 diabetes risk, especially among patients with a greater risk of chronic diseases, such as the elderly. In a previous study, comparing vitamin D levels between relatively well-regulated type 2 diabetes (HbA1c <8%) with other poorly controlled type 2 diabetes patients, vitamin D correlated significantly and inversely with HbA1c[48], which is somewhat contrary to the present findings. However, also in the present study, HbA1c values were higher (>8%) in the low and moderate vitamin D groups than in the high vitamin D patients (<8%), although without statistical significance.

Our stratified analysis based on serum vitamin D level showed that none of the immune markers differed significantly among patients without diabetes ($P > 0.05$ for all; Table 7). In prediabetes patients, the NLR differed significantly ($P = 0.040$). Although no clear trend in NLR was observed in prediabetes patients, those who were vitamin D deficient had the highest NLR (Table 7). In diabetes patients, neutrophil count ($P = 0.001$), lymphocyte count ($P = 0.016$) and NLR ($P < 0.001$) differed significantly based on vitamin D level, with the highest number of neutrophils, lowest number of lymphocytes and highest NLR in vitamin D-deficient diabetes patients (Table 7). Given the high prevalence of hypovitaminosis D and elevated NLR among chronic disease patients and the elderly[49,50], these results suggest that clinical interpretation of elevated NLR as a predictive marker of type 2 diabetes-related inflammation should carefully consider vitamin D level, age and pre-existing morbidity.

The present findings are, however, subject to certain limitations, some of which are related to study design. Despite the large sample size, the single-center design of the present study might serve to limit the extension of our findings to other populations due to differences in ethnicity and environmental factors. The retrospective nature of our analysis might introduce the risk of selection bias. The diabetes group in the present study was significantly older than the control group without diabetes (74.78 ± 13.45 years vs 66.76 ± 17.69 years, $P < 0.001$), which might have contributed to differences in vitamin D level, NLR and type 2 diabetes risk, as discussed above. In addition, a substantial number of patients in all three of the groups lacked complete datasets for the serum biochemical variables and immune markers, and therefore could not be included in the multivariate and stratified analyses. Large-scale longitudinal studies of vitamin D level and inflammation markers in an aging cohort are warranted to evaluate each as risk factors for type 2 diabetes.

DISCLOSURE

The authors declare no conflict of interest.

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